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Review

Developments in the use of soluble ionic polymers as pseudo-stationary phases for electrokinetic chromatography and stationary phases for electrochromatography

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Abstract

This article reviews the development, characterization and application of soluble ionic polymeric materials as pseudo-stationary phases for electrokinetic chromatography and as stationary phases for electrochromatography since 1997. Polymeric pseudo-stationary phases for electrokinetic chromatography, including cationic polymers, anionic siloxane and acrylamide polymers, polymerized surfactants (micelle polymers), and chiral polymers are reviewed. Also reviewed are suspended molecularly imprinted polymer micro-particles. Application of polymeric pseudo-stationary phases with electrospray ionization mass spectrometric detection is presented. Recent progress in the development and characterization of physically adsorbed stationary phases for electrochromatography using polymers of the same or similar chemistry is also reviewed.

Keywords: Reviews; Electrokinetic chromatography; Electrochromatography; Stationary phases, electrochromatography; Pseudo-stationary phases; Polymers

Contents

Abbreviations: EKC, electrokinetic chromatography; PSP, pseudo-stationary phase; OT-CEC, open tubular capillary electrochromatography; CMC, critical micelle concentration; MIP, molecularly imprinted polymer; PAH, polynuclear aromatic hydrocarbon; PCB, polychorinatedbiphenyl; BOH, 1,1′-binaphthol; AMPS, 2-acrylamido-2-methyl-1-propanesulfonic acid; AGENT, allyl glycidyl ether-*N*-methyltaurine; AGESS, allyl glydicyl ether-sulfonate modified siloxane; PAA, poly(allylamine); OMAt, octyl methacrylate; LMAt, lauryl methacrylate; SMAt, stearyl methacrylate; LAt, lauryl acrylate; LMAm, lauryl methacrylamide; DHCHAt, dihydrocholesteryl acrylate; pEI, poly(ethyleneimine); *t*OAm, *tert*-octyl acrylamide; LSER, linear solvation energy relationship; pDADMA, poly(diallyl dimethyl ammonium); pSUS, poly(sodium undecenyl sulfate); pAAU, poly(sodium 11-acrylamidoundecanoate); pSUA, poly(sodium undecylenate); pSUP, poly(sodium undecenoyl aminoethyl phosphonate); pSUT, poly(sodium undecenoyl taurinate); pSUL, poly(sodium undecenoyl leucinate); SDS, sodium dodecyl sulfate; THF, tetrahydrofuran; GPC, gel permeation chromatography; ACN, acetonitrile; EPA, US Environmental Protection Agency; PTH, phenylthiohydantoin; MIP, molecularly imprinted polymer; DOPA, 3,4-dihydroxyphenylalanine

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1. Introduction

Micellar electrokinetic chromatography (MEKC) is a powerful technique for the separation of a variety of compounds [1–4]. Analytes are separated in MEKC based on their relative affinity for a micellar pseudo-stationary phase (PSP).

The PSP migrates through the capillary, typically in the same direction as the analytes but at a rate slower than electroosmotic flow, with a migration time t_{psp}. This results in limited migration range for separation of the analytes between t_0 and t_{psp}, and modified equations for the retention factor, k:

$$k = \frac{t_r - t_0}{t_0(1 - t_r/t_{psp})} \quad (1)$$

and for the resolution between two analytes, R_s:

$$R_s = \frac{\sqrt{N}}{4}\frac{\alpha - 1}{\alpha}\frac{k_m}{k_m + 1}\left[\frac{1 - (t_0/t_{psp})}{1 + (k_m t_0/t_{psp})}\right] \quad (2)$$

In both equations t_0 is the migration time of a nonionic species that does not interact with the PSP and t_r is the migration time of the analyte. In Eq. (2), α is the chemical selectivity between two analytes (defined as the ratio of the retention factors), N is the number of theoretical plates, and k_m is the mean retention factor for the two analytes. Both equations reduce to the conventional equations when the t_{psp} becomes infinite, corresponding to a conventional stationary phase.

Fig. 1 illustrates the effect of the mobility of the PSP on resolution. The product of the two retention factor terms in Eq. (2) ($f(k)$, see Eq. (3)) are plotted as a function of the

$$f(k) = \frac{k_m}{k_m + 1}\left[\frac{1 - (t_0/t_{psp})}{1 + (k_m t_0/t_{psp})}\right] \quad (3)$$

retention factor k for various ratios of t_{psp}/t_0. For t_{psp}/t_0 values of 4–5, the resolution suffers dramatically relative to conventional chromatography at all retention factors, and especially at high retention factors. Relative to conventional HPLC, this is more than compensated by the improvement in efficiency, except for very highly retained hydrophobic compounds. Relative to capillary electrochromatography (CEC), for which efficiency is often equivalent with EKC, this is a significant disadvantage of EKC.

Study of Fig. 1 and Eq. (2) leads to the conclusion that PSPs should have several properties if they are to provide high resolution separations. They should be stable and soluble under a range of analytical conditions such that the retention factor can be adjusted to within a fairly narrow optimum range. They should have high electrophoretic mobility to provide a wide migration range. Phases should be available with a wide range of chemical structures to provide varied chromatographic selectivity. They should have very low or zero critical micelle concentration (CMC) to permit efficient application of secondary media such as cyclodextrins and to minimize Joule heating. PSPs should have high electrophoretic mobility such that they provide a wide migration range. They should be monodisperse, at least with respect to chemical interactions with solutes and electrophoretic mobility, and should allow for fast mass transfer of analytes between the PSP and buffer medium so that high efficiency separations can be achieved.

Conventional micellar PSPs have several significant limitations as PSPs [5–7]. These limitations are primarily the result of the fact that micelles are equilibrium self-assemblies of low molecular weight surfactants. The self-assembly equilibrium limits the choice of analytical conditions, re-

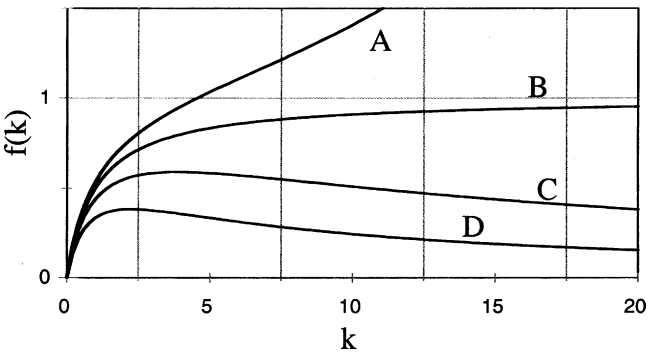

Fig. 1. Plot of the product of the retention factor terms in Eq. (2) as a function of the retention factor. (A) $t_{mc}/t_0 = -30$ (negative value indicates that pseudo-stationary phase has net velocity opposite to electroosmotic flow), (B) $t_{mc}/t_0 = \infty$ (infinite value is equivalent to conventional chromatography), (C) $t_{mc}/t_0 = 15$, (D) $t_{mc}/t_0 = 5$.

quires relatively high concentration of surfactant, and limits the variety of chemical structures that can be employed as PSPs. Additionally, the use of relatively high concentrations of conventional low molecular weight surfactants makes it very difficult to employ mass spectrometric detection with MEKC.

Mindful of these requirements and limitations, there has been significant effort toward the development and characterization of novel PSPs for electrokinetic chromatography (EKC). A variety of surfactants have been studied, as have monomolecular and polymeric PSPs that do not require self-assembly into micelles. The use of polymeric PSPs eliminates the requirement of self-association, thus addressing the problems noted above. The polymers provide stable PSPs for which the primary covalent structure and concentration does not change with changes in the analytical conditions. Polymers can be synthesized and employed with virtually any selectivity or electrophoretic mobility. The polymers have zero CMC, meaning that there is no free surfactant to interfere with separations or detection. Polymeric PSPs can thus be employed with mass spectrometric detection. In short, these compounds have the potential to provide many properties desirable in a PSP. Limitations are that they may not be monodisperse, and the mass transfer kinetics may be slower than with conventional micelles, both of which could lead to diminished plate counts relative to conventional micelles.

The use of polymeric materials of similar chemistry in electrokinetic separations has recently been extended to the use of micro-particle suspensions and the immobilization of the polymers by physical adsorption to create truly stationary phases. Both of these approaches, which are at the interface between electrokinetic chromatography and electrochromatography, are reviewed in this article. For the purposes of this review, all separations in which the polymers are dissolved or suspended in solution and are thus mobile will be defined as EKC, while those separations in which the polymer is physically immobilized and stationary will be considered electrochromatography.

Similar polymeric materials have also been used in solution or immobilized on surfaces for ion exchange separations. While this is an area of significant interest and development in recent years, it is not the subject of the current review. Readers interested in ion exchange electrokinetic separations are referred to a recent review in this journal [8].

The introduction and characterization of polymeric PSPs for EKC has been reviewed in detail [5–7,9,10]. Two reviews by this author in this journal concentrated on studies of the performance and selectivity of polymeric PSPs from their introduction in 1992 until 1997 [5,6]. Since that time, several other reviews have detailed the development and selectivity of polymeric PSPs [10–14]and the development and characterization of chiral polymeric PSPs. [9] The current review will consider those developments and studies with polymeric and dendrimeric PSPs since the previous reviews by this author in this journal in 1997.

2. Electrokinetic chromatography

2.1. Achiral polymers

A significant number of studies concerning the development and characterization of achiral polymeric PSPs have been reported. Polymerizable surfactants based on vinyl and acrylamide chemistry have been applied and characterized. Several linear copolymers based on siloxane and acrylamide or acrylate chemistry have been introduced and characterized in detail. Cationic polymers have been studied and their performance compared to similar low molecular weight additives. The generalized structures of the polymers are presented in Figs. 2, 5 and 10.

Linear solvation energy relationship (LSER) studies have been employed to characterize the chemical selectivity of many of these polymers. The results of these studies are summarized in Table 1 and are discussed in each of the sections regarding the individual polymers and in a summary at the end of this section. Comparisons of the LSER coefficients should be made with caution since different test solutes were used for the various studies. The results of the various studies for SDS micelles are remarkably similar, however, leading to the conclusion that the results can be cautiously compared.

2.1.1. Polymerized surfactants

During the review period polymerized surfactants (also called micelle polymers) with the structures shown in Fig. 2 have been studied and applied as PSPs. The selectivity of Poly(sodium undecylenate) (pSUA, Fig. 2A) has been studied, and the polymer has been applied to polynuclear aromatic hydrocarbon (PAH) separations. The effect of the chemical structure of the ionic head group (Fig. 2B and C) on chemical selectivity has been studied. Poly(sodium undecenyl sulfate) (pSUS, Fig. 2D) was introduced and characterized several years ago, and has continued to be applied to separations of hydrophobic compounds. Poly(sodium 11-acrylamidoundecenoate) (pAAU, Fig. 2E) was introduced and characterized more recently.

The chemical selectivity of pSUA (Fig. 2A) polymerized using three different chemical initiators with different hydrophobicity was studied by Palmer and Tellman [15]. Initiation with very hydrophobic 2,2′-azobis(2,4-dimethylvaleronitrile) produced polymers with significantly lower molecular weight that were not soluble in aqueous buffers. The other two initiators produced polymers with no significant differences in selectivity, perhaps because steric restriction prevents interaction of solutes with the end groups in the interior of the polymer.

Moy et al. reported that pSUA outperformed other PSPs for the separation of PAHs [16]. Using THF as an organic modifier, they were able to separate all sixteen priority pollutant PAHs. They predicted that they would be able to separate up to 1000 compounds by using the pSUA electrokinetic chromatography as the second dimension in a GPC–EKC

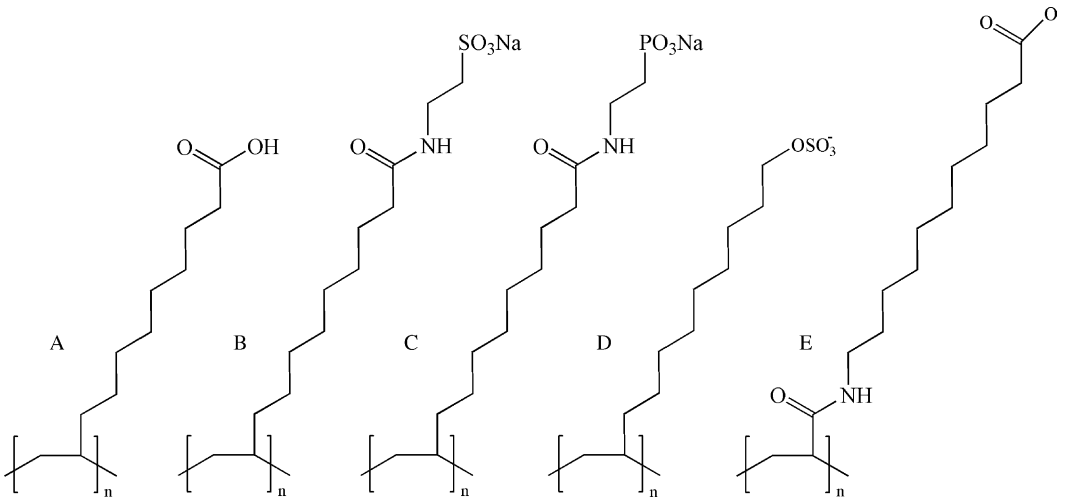

Fig. 2. Structures of polymerized surfactants. (A) pSUA, (B) pSUT, (C) pSUP, (D) pSUS, (E) pAAU.

apparatus. The system was applied to a soil extract with good results. The polymer could not be used, however, with laser induced fluorescence detection at 257 nm.

Tellman and Palmer also investigated the effect of the ionic head group on the selectivity of the micelle polymers. They synthesized and characterized analogs with amide bonds and sulfonate and phosphonate ionic groups (pSUT, pSUP, Fig. 2B and C) [17]. The sulfonate analog did provide significantly higher electrophoretic mobility, and both of the polymers could be employed in low pH (2.5) buffers. Very little difference in selectivity was observed between the pSUA, pSUS, pSUT and pSUP analogs for the separation of the majority of substituted aromatic compounds studied, although the selectivity of the phosphonate phase was significantly different from that of the sulfonate phase for particular classes of compounds. Linear solvation energy relationship studies also showed some significant differences in the cohesiveness of the phosphonate and sulfonate polymeric phases, but otherwise no significant differences were observed. Both polymeric phases were found to be more cohesive than SDS micelles (Table 1). While the ionic head group does have some effect

Table 1
LSER results for selected polymeric pseudo-phases

PSP	*m*	*r*	*s*	*a*	*b*	*c*	*n*
SDS[25]	2.74 (0.11)	0.27 (0.08)	−0.37 (0.07)	−0.23[a] (0.13)	−1.82 (0.16)	−1.65 (0.11)	18
pSUA[25]	2.11 (0.09)	0.26 (0.06)	−0.16 (0.06)	−0.27 (0.11)	−1.05 (0.13)	−1.86 (0.09)	18
pSUT[17]	2.85 (0.73)	[b]	0.561[a] (0.54)	0.71[a] (0.56)	−1.53 (0.61)	−2.61 (0.58)	13
pSUP[17]	3.86 (0.91)	[b]	−0.44[a] (0.66)	0.11[a] (0.70)	−1.39 (0.75)	−2.79 (0.72)	13
pAAU[25]	1.64 (0.11)	0.18 (0.08)	0.45 (0.08)	−0.15[a] (0.13)	−1.18 (0.17)	−2.28 (0.11)	18
2% Elvacite 2669 + 40 mM SDS[33]	2.96 (0.15)	0.44 (0.10)	−0.42 (0.12)	[b]	−2.74 (0.19)	−1.56	22
PDADMA[52]	[a]	0.75 (0.06)	−0.35 (0.07)	0.19 (0.05)	[a]	−0.81	15
Polybrene[52]	[a]	1.56 (0.13)	−0.91 (0.15)	[a]	[a]	0.09	15
AGENT[29]	2.1 (0.2)	0.76 (0.1)	−0.07[a] (0.1)	0.45 (0.09)	−1.9 (0.2)	−2.8 (0.2)	40
C_8AGENT-20[29]	2.2 (0.3)	0.49 (0.2)	−0.90 (0.2)	0.11[a] (0.1)	−2.5 (0.2)	−1.5 (0.2)	40
C_{12}AGENT-10[29]	1.3 (0.3)	0.58 (0.2)	−1.0 (0.2)	0.51 (0.1)	−2.0 (0.2)	−1.3 (0.3)	40
C_{12}AGENT-15[29]	2.5 (0.3)	0.32 (0.2)	−0.86 (0.2)	0.21 (0.1)	−2.4 (0.3)	−1.8 (0.3)	40
C_{12}AGENT-20[29]	2.4 (0.2)	0.59 (0.1)	−0.78 (0.1)	0.23 (0.07)	−2.4 (0.2)	−2.0 (0.2)	40
C_{18}AGENT-20[29]	2.5 (0.3)	0.32 (0.2)	−1.1 (0.2)	0.33 (0.1)	−2.6 (0.3)	−1.8 (0.3)	40
C_{12}AGESS-8[30]	2.04 (0.2)	0.40 (0.1)	−0.17 (0.1)	0.24 (0.08)	−2.09 (0.2)	−2.50 (0.2)	38
C_{12}AGESS-13[30]	2.72 (0.1)	0.46 (0.06)	−0.43 (0.08)	0.27 (0.04)	−2.46 (0.09)	−2.40 (0.1)	38
pOMAt-21[35]	3.56 (0.16)	0.470 (0.094)	−0.60 (0.14)	−0.407 (0.074)	−3.75 (0.20)	−2.66 (0.14)	20
pLMAt-15[35]	3.65 (0.18)	0.43 (0.11)	−0.67 (0.16)	−0.274 (0.083)	−3.70 (0.22)	−2.84 (0.16)	20
pSMAt-16[35]	3.78 (0.21)	0.65 (0.12)	−0.85 (0.18)	−0.495 (0.098)	−3.83 (0.26)	−2.73 (0.19)	20
pLAt-13[35]	3.58 (0.26)	0.39 (0.15)	−0.40 (0.22)	−0.02[a] (0.12)	−3.52 (0.32)	−2.96 (0.23)	20
pLMAm-19[35]	2.88 (0.13)	0.374 (0.075)	−0.32 (0.11)	0.254 (0.059)	−2.45 (0.16)	−2.69 (0.11)	20
pDHCHAt-33[35]	3.40 (0.20)	0.65 (0.12)	−0.46 (0.17)	0.241 (0.093)	−3.20 (0.25)	−2.68 (0.18)	20
ptOAm-49[35]	3.36 (0.16)	0.333 (0.096	−0.44 (0.14)	0.434 (0.075)	−3.22 (0.20)	−2.86 (0.14)	20

n is the number of solutes used. The number in parentheses is the standard error.
[a] Not significantly different from zero.
[b] Parameter not included in the model.

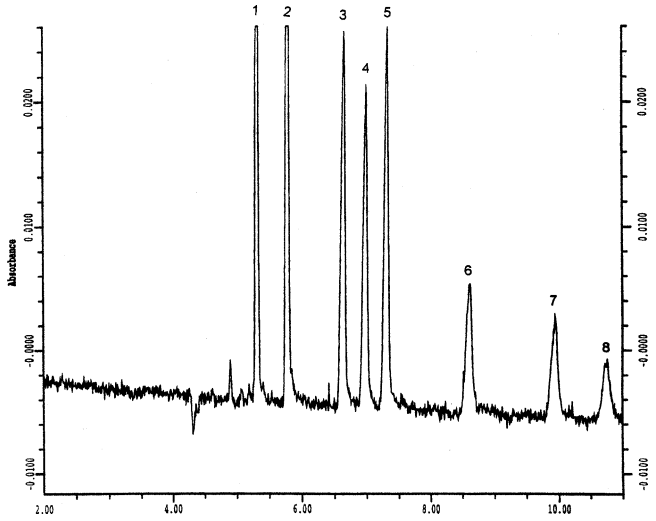

Fig. 3. Electropherogram for the separation of eight PCB congeners in EPA PCB 525.1 test mixture. 0.5% (w/v) pSUS, 40% acetonitrile, pH 9.2. (1) PCB 1, (2) PCB 5, (3) PCB 29, (4) PCB 47, (5) PCB 98, (6) PCB 154, (7) PCB 171, (8) PCB 200. From ref. [22] with permission.

on performance, the effect on the selectivity appears to be minor.

The SUS polymer (Fig. 2D) has been studied for a variety of separations, often of hydrophobic analytes in organic-modified buffers. The polymer has been utilized for the separation of PAHs, monomethylbenz[*a*]anthracenes and methylated isomers of benzo[*a*]pyrene [18–21]. Shamsi et al. separated all sixteen polynuclear aromatic hydrocarbons using pSUS in 57% acetonitrile [18]. Akbay et al. attempted to correlate retention of monomethylbenz[*a*]anthracene isomers with the length and length-to-breadth ratio (*L*/*B*). Unlike liquid chromatography, they found a better correlation with length than with *L*/*B* [20]. These authors also observed no significant change in the partial specific volume of the phase in 35% acetonitrile relative to water, indicating that any change in the conformation of the polymer in acetonitrile-modified buffers must be subtle. Norton et al. were able to separate seven of twelve methyl benzo[*a*]pyrene isomers in approximately 26 min using pSUS in 35% ACN [19]. Many of the peaks showed significant fronting, but the separation efficiency was still good.

A separation of polychlorinated biphenyl (PCB) congeners in 40% acetonitrile using pSUS was also reported [22]. A separation of an EPA PCB test mix is shown in Fig. 3. The separation of natural pyrethrum extracts using pSUS was optimized and the results compared with separations using SDS micelles and liquid chromatography [23]. The EKC method using pSUS gave an optimum separation of six extracts in less than 15 min, which was faster than that achieved with SDS micelles (25 min) or HPLC (50 min). Peak splitting would result in both of these applications unless the sample was dissolved in an appropriate solvent.

The SUS polymer was also used in aqueous buffers modified with millimolar concentrations of ionic liquids for the separation of alkyl–aryl ketones and chlorophenols [24]. At these concentrations, the modifiers are better described as nonmicellar hydrophobic ions. The modifiers influenced the elution time and separation efficiency, and did in some cases enhance resolution when used with pSUS. No improvement in resolution was observed when the modifiers were used with SDS micelles. The optimum concentration of the modifiers depended on their structure, with some modifiers not providing enhanced separations at any concentration.

Polymers of sodium 11-acrylamidoundecanoate (pAAU, Fig. 2E) were synthesized and characterized by size exclusion chromatography and multiple angle laser light scattering, and as PSPs for EKC [25,26]. A remarkably high molecular weight of two or three million Daltons is reported, as well as a narrow polydispersity index of 1.3–1.5. The polymer was shown to have selectivity considerably different from SDS micelles, and was less hydrophobic than SDS micelles. The polymerized surfactant was able to be used in buffers modified with 30% acetonitrile for the separation of PAHs. Linear solvation energy relationships were used to characterize the selectivity of pAAU [25]. A representative separation of the probe solutes is shown in Fig. 4. These results, as well as results from the same report for SDS micelles and for pSUA are presented in Table 1. Relative to SDS and pSUA, pAAU is a remarkably polar PSP. The polymer is better able to interact with polar and polarizable groups than water, making it much more polar than any other polymeric phases studied to date. The source of this highly polar nature is not discussed by the author, but may stem from the presence of amide functionality at the normally non-polar tail end of the surfactant. Linear acrylamido copolymers of 2-acrylamido-2-methyl-1-propane sulfonic acid are also more polar than the acrylate counterparts, but not to the same extent as reported for pAAU.

2.1.2. Siloxane polymers

The siloxane polymers shown in Fig. 5A and B have been studied extensively as PSPs for electrokinetic chromatography [27–29]. The phases have the advantage that the final molecular mass is known because they are synthesized from hydrosiloxane polymers of known nominal molecular weight. Using this approach, polymers with a wide variety of ionic head group and pendant group chemistries could be easily synthesized with the same backbone chemistry. Siloxane polymers of this type could provide a vehicle for application of the wide variety of silicon-based chemistries developed for gas and liquid chromatography over the past few decades.

The siloxane chemistry most studied is that shown in Fig. 5A, with octyl-, dodecyl- or octadecyl-pendant groups at varying degrees of substitution. The polymers were given the acronym AGENT because hydrosiloxanes were modified with allyl glycidyl ether followed by *N*-methyl taurine. This configuration provides polymers of sufficient aqueous solubility when the fraction of silicon centers modified with the ionic group exceeds 70% [27].

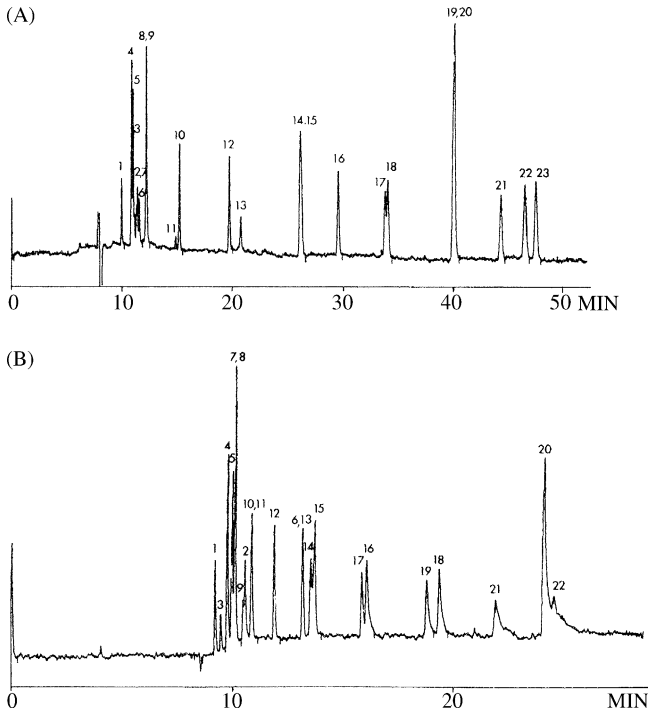

Fig. 4. Electrokinetic Chromatograms for the separation of substituted aromatic compounds used for LSER analysis using (A) pSUA and (B) pAAU. 1.2% (w/v) polymer, 12.5 mM Na_2HPO_4 and $Na_2B_4O_7$ pH 9.2, 13 kV, UV detection at 254 nm, 50 cm effective, 65 cm total length capillary. (1) benzyl alcohol, (2) 4-nitrobenzyl alcohol, (3) 2-ohenylethanol, (4) benzaldehyde, (5) benzonitrile, (6) 4-nitroaniline, (7) nitrobenzene, (8) acetophenone, (9) indole, (10) propiophenone, (11) bromobenzene, (12) *n*-butyrophenone, (13) naphthalene, (14) valerophenone, (15) propyl benzoate, (16) biphenyl, (17) *n*-hexanophenone, (18) fluorene, (19) *n*-heptaphenone, (20) phenanthrene, (21) *n*-octaphenone, (22) *n*-nonanophenone, (23) *n*-decanophenone. From ref. [25] with permission.

The hydrophobicity of these polymers can be varied from being less than SDS micelles to greater than SDS micelles by varying the density and the length of the alkyl chain [27]. Both electrophoretic mobility and separation efficiency pass through a maximum at 10–20% substitution with alkyl chains [27]. The polymers provided very different selectivity from SDS micelles, but selectivity did not vary greatly between polymers with different alkyl chain length or extent of substitution [27]. The dodecyl modified polymer with 15–20% substitution provided the best overall performance in terms of electrophoretic mobility, solubility, and efficiency.

AGESS polymers (Fig. 5B) have also been studied, and were found to provide significantly different chemical selectivity from AGENT polymers of similar structure [30]. Linear solvation energy relationship studies were used to characterize the selectivity of the siloxanes in greater detail [29,30]. Selected results are presented in Table 1 and are labeled C_nAGENT-# or C_nAGESS-# where n refers to the alkyl chain length and # refers to the percentage substitution with the alkyl chain. A striking feature of the AGENT materials relative to most other PSPs is their very low propensity for interaction with polar or polarizable compounds (s-term). This is not entirely due to the non-polar nature of the siloxane backbone, because it is not apparent in the results for AGESS materials. It is possible that the shorter linker arm between the backbone of the siloxane and the ionic head group in the case of AGESS leads to a more polar phase. Another interesting feature is the ability of both of the siloxane polymers to interact strongly with hydrogen bond donors (a-term). This can not be explained by the presence of the tertiary amine on AGENT, as it is absent in AGESS polymers. This behavior may thus be attributed to the backbone chemistry. Finally, the siloxanes are more cohesive (m-term) than might have been expected given the relatively flexible siloxane backbone.

Siloxane polymers were applied to the separation of hydrophobic compounds in buffers modified with organic solvents [28]. C_8AGENT-20, C_{12}AGENT-15 and C_{12}AGENT-25 were used in buffers containing up to 50% acetonitrile or 60% methanol for the separation of alkyl–aryl ketones and PAHs. The polymers maintain large migration windows and high methylene selectivities in the organic-modified buffers, and addition of organic solvents also the separation efficiency for all but the most hydrophobic compounds. The siloxanes were used to separate 12 of 14 PAHs in acetonitrile-modified buffers, but separation of the PAHs could not be achieved in methanol-modified buffers. The performance of these polymers in organic modified buffers was not as good as that of other polymeric PSPs.

2.1.3. Acrylamide and acrylate copolymers

A variety of polymers based on either acrylate, acrylamide, or mixed backbones have been introduced and studied as PSPs. The advantage of many of these polymers is that either the polymers or the monomers are commercially available, often with significant variations in structure.

Several reports have appeared utilizing or characterizing Elvacite 2669 (Fig. 5C). This polymer was studied for the separation of organophosphorus pesticides in methanol and acetonitrile modified buffers [31]. Plate numbers and resolution were lower with the polymer than with cholate micelles. Retention factors were lower, and resolution was the same or less than with SDS micelles. Overall, the polymer was not competitive with the cholate phase.

Wiedmer et al. have studied the behavior of Elvacite 2669 for the separation of hydrophobic compounds in buffers modified with methanol [32]. The viscosity, the migration times of some neutral hydrophobic compounds, and the light scattering properties of polymer solutions were studied as a function of the concentration of methanol and the polymer. The results showed the structure and behavior of Elvacite 2669 to be highly dependent on the methanol–water ratio. Significant intermolecular aggregation was observed. Significant interaction of the Elvacite 2669 with the capillary wall was also observed at polymer concentrations in excess of 0.5%.

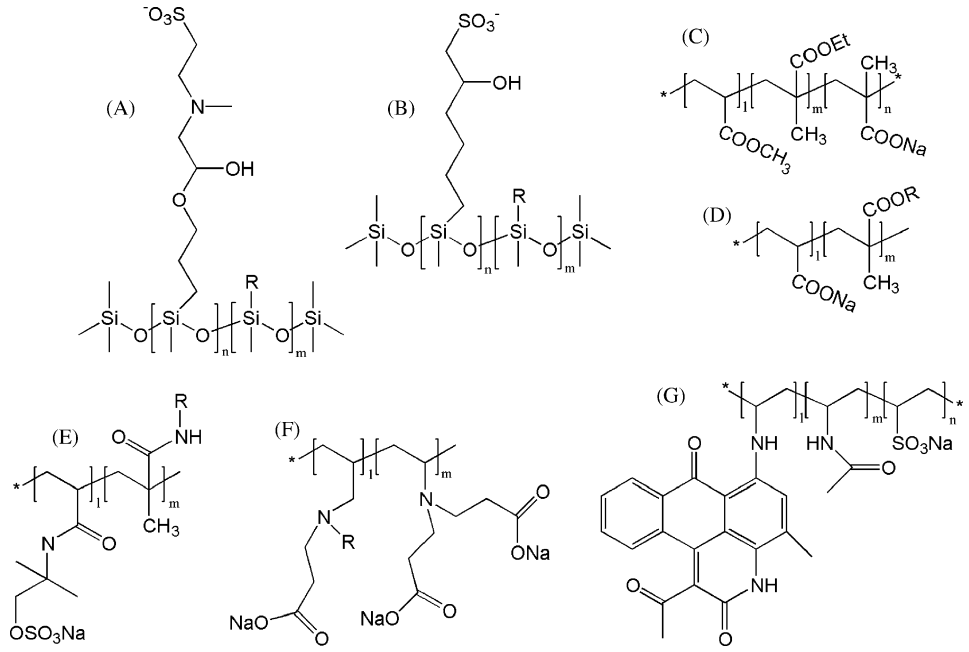

Fig. 5. Structures of linear ionic polymers. (A) AGENT, (B) AGESS, (C) Elvacite 2669, (D) acrylate copolymers, (E) AMPS copolymers, (F) polyallylamine, (G) polymeric dye.

Leonard and Khaledi used mixed phases of Elvacite 2669 and SDS for the separation of 22 substituted benzene and naphthalene compounds, and conducted studies into the chemical selectivity of the mixed phase [33]. The elution window (t_{psp}/t_0) increases from 1.99 to 3.60 as the SDS concentration is increased from 0 to 100 mM. Retention, surface tension, and conductivity data imply a single SDS/polymer aggregate structure in solution with a CMC of 2 mM SDS in 2% polymer solution. Fluorescence and EKC studies indicate that the polymer/SDS costructure is more micelle-like (less polar and less cohesive) than the polymer alone.

A similar study used complexes of polyacrylic acid and polymethacrylic acid with cationic surfactants dodecyltrimethylammonium bromide [14]. Highly efficient separations of phenols and derivatized amino acids are reported. Somewhat surprisingly, these complexes of an anionic polymer and a cationic surfactant appear to provide broad migration windows, and the addition of the cationic surfactant does not reverse the electroosmotic flow.

A series of acrylate copolymers with differing alkyl chain lengths and molecular weights have been studied as PSPs [34]. The polymers have the structure shown in Fig. 5D. All of the polymers had the same acrylate/alkyl acrylate mole ratio and approximately the same molecular mass, but were substituted with alkyl chain lengths of nine (C_9), thirteen (C_{13}) and eighteen (C_{18}) carbons. 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 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. Further work has shown that mixtures of the C_9 and C_{18} phases provide intermediate and predictable selectivity [7]. Using a simple model based on no intermolecular aggregation, it was possible to predict the mobility of analytes when using mixed phases to within 10% absolute error.

Several reports have detailed the development and characterization of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) copolymers as PSPs (Fig. 5E) [35–39]. Copolymers are synthesized by copolymerization of AMPS with a variety of (meth)acrylate and (meth)acrylamide comonomers. The effect of the mole fraction of comonomer was studied in detail using copolymers of AMPS and lauryl methacrylamide made up of from 0.6 to 1 mole fraction AMPS [37]. The electrophoretic mobility of the polymers increased, the hydrophobicity decreased, and peak symmetries for more hydrophobic solutes decreased as the mole fraction of AMPS increased. An optimum balance of selectivity, separation efficiency and electrophoretic mobility were obtained with an AMPS mole fraction of 0.80.

AMPS was copolymerized with octyl methacrylate (OMAt), lauryl methacrylate (LMAt), stearyl methacrylate (SMAt), lauryl acrylate (LAt), lauryl methacrylamide (LMAm), stearyl amide, dihydrocholesteryl acrylate (DHCHAt) and *tert*-octyl acrylamide (*t*OAm) and the phases were characterized using LSER [35,39]. Selected LSER results are presented in Table 1 under the acronyms above followed by the mole percentage of the nonionic comonomer. Significant differences in the selectivity of the

AMPS copolymers and SDS micelles were observed. With few exceptions, the AMPS copolymers are the least cohesive of the polymeric phases studied to date, with most being less cohesive than SDS micelles. This is unusual, as the covalent stabilization of polymeric PSPs has generally been observed to result in more cohesive phases. Acrylamide copolymers are better able to donate and accept hydrogen bonds, and are more polar than their acrylate counterparts [35]. This is very much apparent for the hydrogen bond accepting ability, for which methacrylates are weaker bases than water, while acrylamides are stronger bases than water. Increases in the fraction of amide monomer also appear to increase the cohesiveness of the phases, possibly due to hydrogen bonding along the backbone of the polymers [35]. Increases in comonomer fraction and pendant chain length decrease the hydrogen bond accepting and donating ability of the polymers, and reduce the cohesiveness of the polymers, but this did not result in significant changes in the overall selectivity of the polymers. The semiplanar DHCHAt and tertiary *t*OAm comonomers did not show dramatic differences in the LSER parameters, although DHCHAt was the only acrylate AMPS copolymer with better hydrogen bond accepting strength than water, and *t*OAm was the only AMPS copolymer more cohesive than SDS micelles [39]. Where DHCHAt did appear to provide unique selectivity was in the separation of planar PAHs from non-planar alkyl–aryl ketones. Although the selectivity differences were not dramatic, the performance of the DHCHAt/AMPS copolymer was very impressive, with separation efficiencies in excess of 190 000 plates in 10 min or less. Representative separations utilizing the DHCHAt copolymer in acetonitrile-modified buffers are presented in Fig. 6.

The strongly acidic sulfonic functionality, relatively high electrophoretic mobility, low cohesiveness and low polarity of the AMPS copolymers makes them ideal candidates as agents to effect online preconcentration of solutes by sweeping. In the sweeping technique, analytes to be preconcentrated and separated are injected in a large plug of buffer containing no PSP [40,41]. The pH is adjusted to a low value, such that electroosmotic flow is suppressed. When the potential is applied, analytes are “swept” into a relatively narrow zone of high concentration as the PSP migrates through the immobile sample zone. Using a combination of sweeping from a sample solvent of low organic modifier content and separation in a zone of high organic modifier content, the separation and detection of quinine and progesterone was achieved at concentrations as low as 12.5 ppb [36].

The relatively low conductivity and high separation efficiency of the DHCHAt copolymer make it an ideal candidate as PSP for high-speed separations by EKC. As shown in Fig. 7, Shi et al. have achieved the separation of 12 of 15 PAHs in less than 2.5 min using DHCHAt/AMPS copolymer in an acetonitrile-modified buffer using a 23 cm capillary and an applied voltage of 30 kV [38]. Plate numbers ranged from 20 000 for the most hydrophobic solutes to 113 000 for the less hydrophobic solutes.

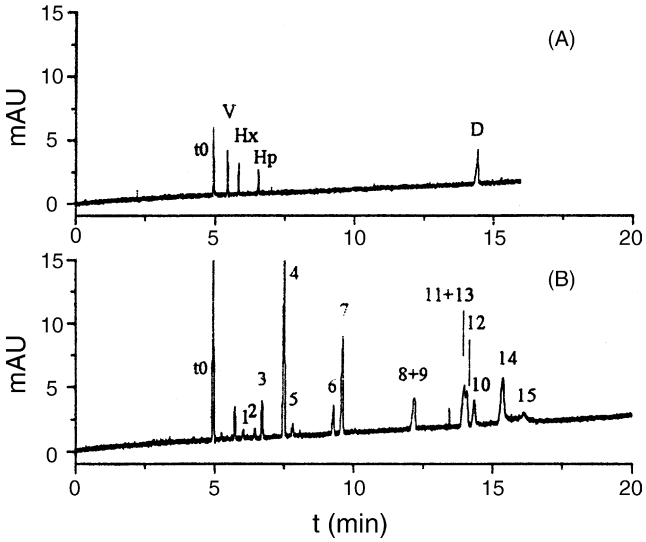

Fig. 6. Separations by pDHCHAt-58. Polymer concentration: 0.72%, ACN = 30% (v/v), borate buffer: 35 mM, (pH = 9.2 before adding ACN). Column effective/total length: 45.5 cm/53.9 cm, voltage: 20 kV, current: 11 μA. Column temperature: 25.0 °C. UV: 254 nm. (A) Alkyl-phenyl ketones, V: valerophenone, HX: hexanophenone, HP: heptanophenone, D: *n*-dodecanophenone. Injection: 1 s at 1500 Pa. (B) PAHs, (1) acenaphthylene, (2) acenaphthene, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) fluoranthene, (7) pyrene, (8) chrysene, (9) benz[*a*]anthracene, (10) benzo[*a*]pyrene, (11) benzo[*e*]pyrene, (12) benzo[*k*]fluoranthene, (13) benz[*e*]acephenanthrylene, (14) benzo[*ghi*]perylene, (15) dibenz[*a,h*]anthracene, injection: 3 s at 5000 Pa. From ref. [39] with permission.

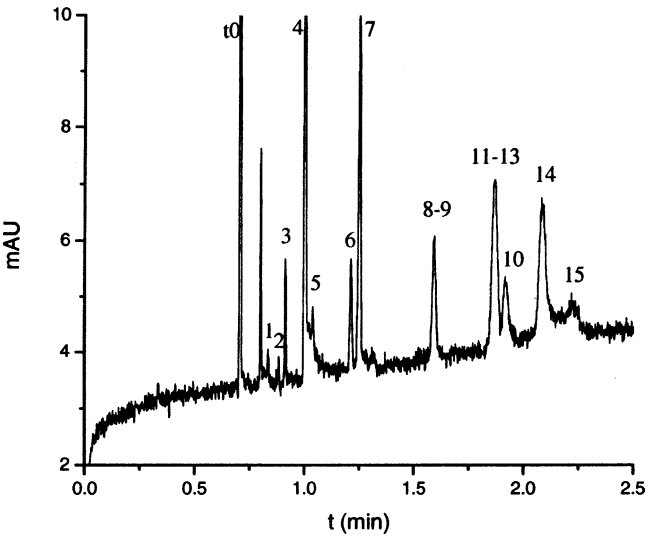

Fig. 7. High-speed separation of 15 PAHs. (1) Acenaphthylene, (2) acenaphthene, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) fluoranthene, (7) pyrene, (8) chrysene, (9) benz[*a*]anthracene, (10) benzo[*a*]pyrene, (11) benzo[*e*]pyrene, (12) benzo[*k*]fluoranthene, (13) benz[*e*]acephenanthrylene, (14) benzo[*ghi*]perylene, (15) dibenz[*a,h*]anthracene. The peak immediately after t_0 is an impurity. Separation conditions: pDHCHAt-58 (0.72%, w/v), sodium borate, 35 mM, ACN, 29.6% (v/v), pH of the buffer before adding ACN was 9.2, column effective/total length, 23.0 cm/31.2 cm, voltage, 30 kV, current, 38 μA, column temperature, 35.0 °C, UV, 254 nm, injection, 2 s at 2500 Pa. From ref. [38] with permission.

2.1.4. Polyallylamine-supported phases

Tanaka et al. have studied polyallylamine (PAA) supported pseudo-stationary phases with varying alkyl chain lengths, and different degrees of alkylation in methanol-modified buffers for the separation of alkyl–aryl ketones and PAHs (Fig. 5F) [42–44]. The hydrophobicity of PAA modified with dodecyl chains is similar to that of SDS micelles in both aqueous media and 60% methanol while the hydrophobicity of hexadecyl modified PAA is higher than that of SDS in both 20 and 60% methanol. Plots of log k versus carbon number for the alkyl–aryl ketones are not always linear, especially at intermediate methanol concentrations. The non-linearity 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. 8, the selectivity of hexadecyl-modified PAA is very similar to that for dodecyl-modified PAA for the separation of PAHs and alkyl–aryl ketones in 40% methanol, but rather different in 60% methanol. 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. As was observed with pSUA in acetonitrile-modified buffers, 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. Additionally, as was observed with pSUA, plots of log k versus percent methanol were non-linear, indicating a change in the retention mechanism. These results can be correlated with the results of dynamic light scattering studies, which have shown that there is a bimodal distribution of relaxation times in aqueous solutions, indicating intermolecular association [42]. In 40% methanol, a narrow and presumably unimolecular distribution is observed.

The degree of substitution of the backbone with dodecyl (10–20%) and hexadecyl (5–25%) chains was also studied [42]. As with AMPS copolymers, greater substitution leads to a narrower migration range, greater sample capacity, and greater methylene selectivity.

Mixtures of PAA polymers with decyl and hexadecyl chains were used to modify the migration window and the selectivity of separation of PAHs in methanol and acetonitrile modified buffers [44]. An example of the results is presented in Fig. 9. The mixture of two phases provided adequate peak capacities for both early and late eluting compounds. A model was presented which assumed independent contribution of the two phases to solute partition. This model adequately predicted the observed selectivity, but gave up to 40% error in the t_r/t_0 values for later eluting compounds.

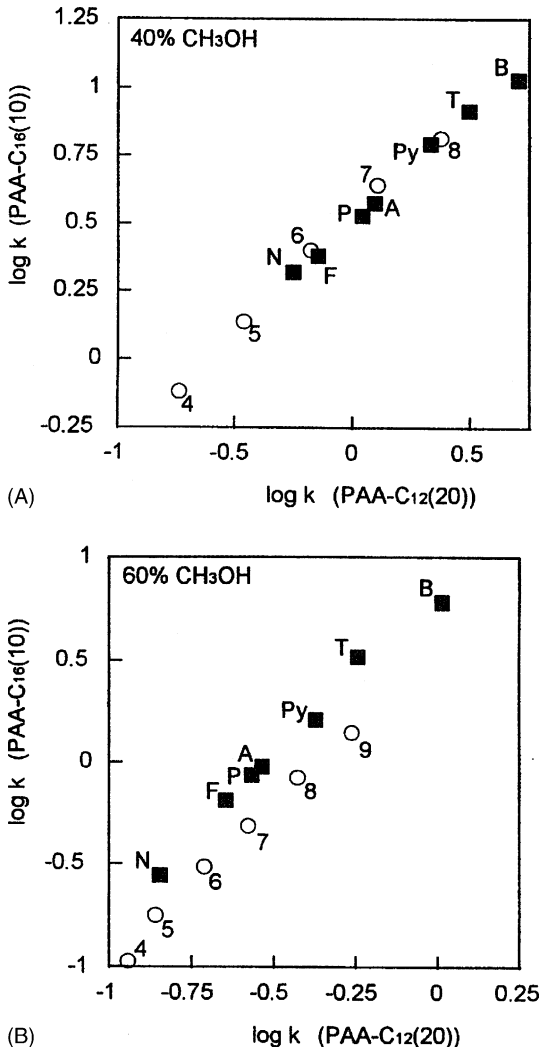

Fig. 8. Selectivity comparison of PAA-C_{16} with PAA-C_{12} in (A) 40% methanol and (B) 60% methanol for alkyl phenyl ketones and PAH. 4–9 refers to the number of carbons in the alkyl phenyl ketone chain and N: naphthalene, F: fluorene, P: phenanthrene, A: anthracene, Py: pyrene, T: triphenylene, and B: benzo[*a*]pyrene. From ref. [5] with permission.

2.1.5. Polymeric dye

An aromatic polymeric dye (Fig. 5G) has been employed for the separation of nitro and amino aromatic compounds [45]. The dye showed significantly different chemical selectivity from SDS micelles, but due to strong UV absorbance, partial filling and counter pressure to reduce flow had to be used. This led to poor efficiency for early eluting compounds, but improved resolution for late eluting compounds. The results indicate that polymers with aromatic functionality could be used to provide unique selectivity with alternative modes of detection.

2.1.6. Cationic polymers

Several studies have also been published detailing the characterization and application of the cationic polymers shown in Fig. 10. These cationic polymers adsorb to the capillary walls, causing a reversal in the direction of elec-

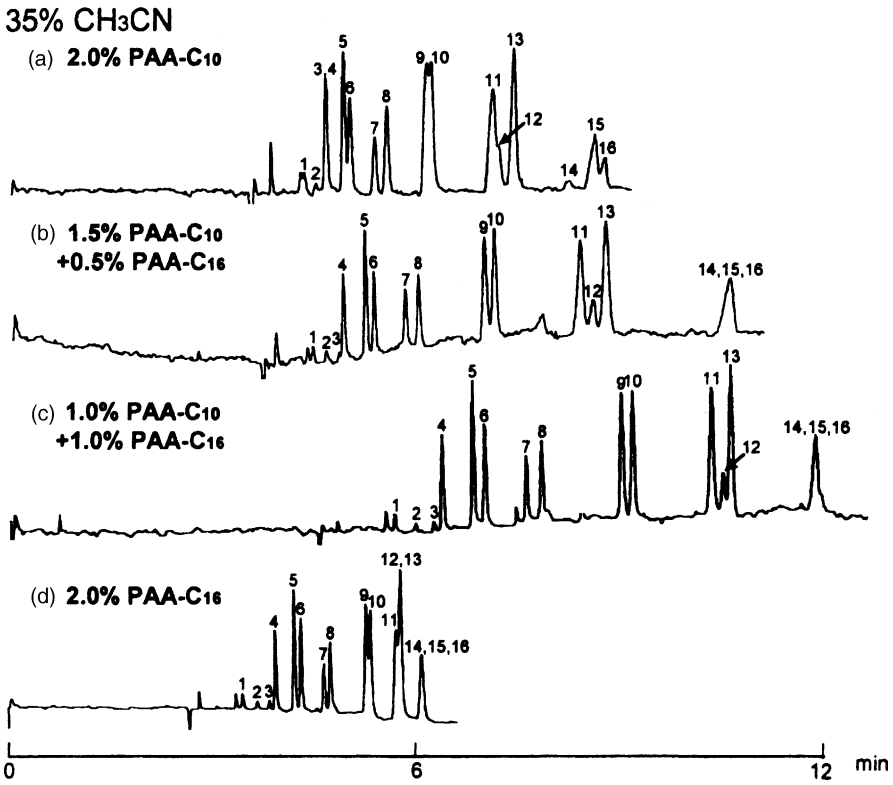

Fig. 9. Separation of 16 PAH with (a) PAA-C_{10}, 2% (w/v), (b) PAA-C_{10}, 1.5% (w/v) + PAA-C_{16}, 0.5% (w/v), (c) PAA-C_{10}, 1.0% (w/v) + PAA-C_{16}, 1.0% (w/v), and (d) PAA-C_{16}, 2.0% (w/v). (1) Naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) chrysene, (10) benz[*a*]anthracene, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) benzo[*a*]pyrene, (14) dibenz[*a*,*h*]anthracene, (15) indeno[1,2,3-*cd*]pyrene, and (16) benzo[*ghi*]perylene. From ref. [44] with permission.

troosmotic flow. In many cases, this also stabilizes the electroosmotic flow such that more reproducible migration times are obtained. The polymers have electrophoretic mobility counter to the electroosmotic flow, permitting EKC separations. A small fraction of the analytes studied may interact with polymers at the capillary wall, but for most solutes this is not a significant contribution to retention [46].

Polyethyleneimine (pEI, Fig. 10A) was first employed as a pseudo-stationary phase for the separation of phenols in 1997 [47]. Strong interactions were observed between the phenols and pEI at high and low pH. The selectivity of pEI is very different from that of other pseudo-stationary phases studied [48]. The migration time of phenols depended on the number of hydroxyl groups, but no selectivity was observed between mono-, di- and trimethyl substituted phenols. This demonstrates a lack of selectivity based on lipophylicity. Addition of methanol or acetonitrile to the separation buffer reduced the affinity of the phenols for the phase, which appears contrary to interactions based on polarity alone [49]. Both organic solvents were found to be detrimental to separations of phenols with PEI pseudo-stationary phase.

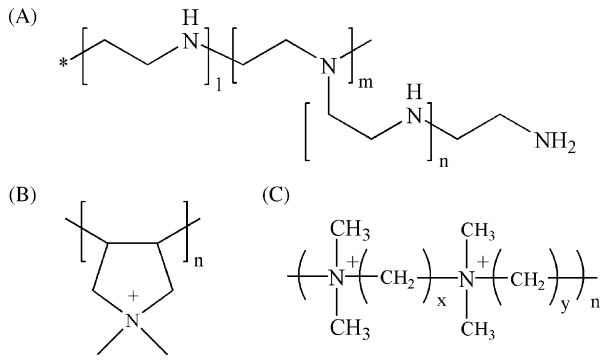

Fig. 10. Structures of cationic polymers. (A) Polyethyleneimine (pEI), (B) poly(diallyldimethylammonium bromide) (pDADMA), (C) ionenes.

Polydiallyldimethylammonium bromide (pDADMA, Fig. 10B) has been studied as a PSP and its performance has been compared to low-molecular-mass cationic additives of similar structure [46,50]. The monomeric additives were found to introduce retention into the EKC system, although the strength of interaction of solutes with the monomeric additives was significantly less than that with the polymer. The separation selectivity was found to be similar for the various additives, although in some cases reversal of solute migration order was observed between monomeric and polymeric additives. For the monomeric materials the mobility of the additive–solute complex is different from that of the additive alone. This leads to some complications with calculation of retention factors and selectivity, and to a reduced retention window for the monomeric additives. The overall performance of the polymeric additive was deemed

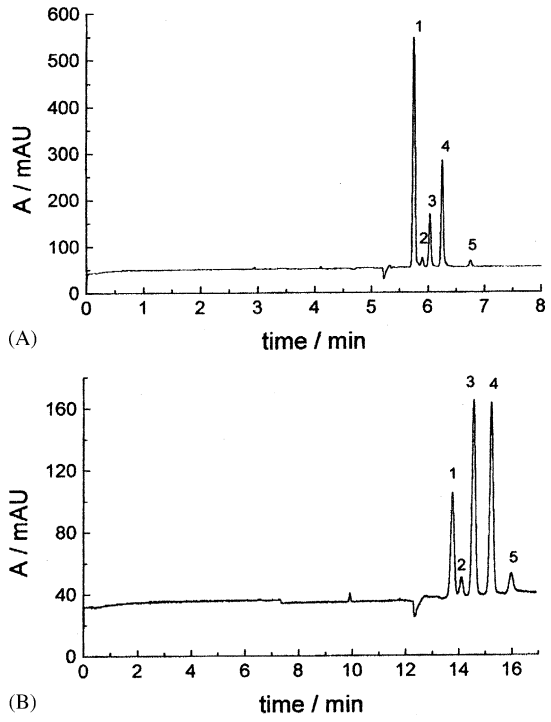

Fig. 11. Separation of a mixture of analytes using (A) 1.8% (w/w) 2,10-ionene and (B) 1.8% 2,5-ionene in acetate buffer at pH 5.2. (1) Dimethyl sulfoxide, (2) benzyl alcohol, (3) phenol, (4) resorcinol, (5) 2-naphthol. From ref. [53] with permission.

superior due to increased retention and wider migration window.

pDADMA was also studied for application to the separation of olanzapine, carbamazepine, and their major metabolites [51]. The separation was not successful because the polymer provided insufficient hydrophobic interactions to resolve carbamazepine and neutral metabolites even at a polymer concentration of 4% (w/w).

Ionenes (Fig. 10C) with varied structure have also been studied, with polybrene (3,6-ionene) being the most frequently reported. Ionenes have the advantage over the more hydrophilic pDADMA that they can provide stronger hydrophobic interactions. Additionally, their charge density and the density of hydrophobic groups can be adjusted by selection of the monomers.

The performance and selectivity of polybrene was compared with that of pDADMA and low molecular weight cationic additives [52]. In the polybrene system, the analytes with the highest retention factors are oligophenols. 1- and 2-naphthol had high retention factors in all of the systems studied, possibly due to interaction with additives adsorbed to the capillary walls. The selectivity for most solutes was significantly different with polybrene relative to the monomeric additives.

The chromatographic behavior of 2,5-, 2,10- and an aromatic ionene has been studied using alkyl–aryl ketones and phenols as analytes [53,54]. Fig. 11 shows representative separations using the two alkyl ionenes. The results indicate that hydrophobic interactions play the most important role in the separations, and that changing the length of the chains or insertion of an aromatic group significantly effect performance and selectivity. Because of its higher hydrophobicity, 2,10-ionene showed the greatest promise as a PSP.

LSER analysis of the cationic polymers polybrene and pDADMA showed that these polymers have cohesivity (m-term) not significantly different from water, making them much more cohesive than SDS micelles and the most cohesive polymers studied to date [50,52]. The polymers are also not significantly different from water in their ability to donate hydrogen bonds, making them the most acidic polymers studied to date. Finally, polybrene shows a very strong tendency for interaction with the nonbonding electrons of solutes (r-term). These results indicate that the chemical selectivity of these polymers should be very much different from that of micelles and other polymeric phases.

2.1.7. Linear solvation energy relationship characterization

LSER studies have shown some significant differences in the solvation properties of the various polymers. The results also indicate that the solvation properties of polymeric phases are not a subset of those of micellar phases, but are highly unique and divergent. The cationic phases, for example, are very cohesive, having values for m not significantly different from water. The AMPS copolymers, on the other hand, have low cohesivity, with values for m greater than SDS micelles. The parameter that shows the greatest variation among the polymers is the ability to accept hydrogen bonds, as SDS micelles and the polymerized surfactants are less basic than water, while the siloxane polymers are more basic than water. Copolymers of AMPS with methacrylates are less basic than water, while copolymers of AMPS with acrylamides are more basic than water. pDADMA is more basic than water, while polybrene is not significantly different from water. Significant differences in other parameters such as the ability to donate hydrogen bonds and the ability to interact with nonbonding and Π electrons are also observed, particularly for the cationic PSPs. These differences in the LSER parameters indicate significant differences in chemical selectivity and provide motivation for the continued development of polymeric phases with unique chemical selectivity. Elimination of the need for micellization permits application of polar polymers which have very different chemical selectivity from that of conventional micelles.

2.2. Chiral pseudo-stationary phases

Work has continued in the development of chiral polymeric PSPs. The general structures of the phases reported are presented in Fig. 12. The greatest number of reports concerns polymerized surfactants having amino acid head group chemistry (Fig. 12A and B). Newly introduced polymers include linear polymers containing amino acid moieties, and a linear copolymer incorporating Pirkle-type chiral selectors.

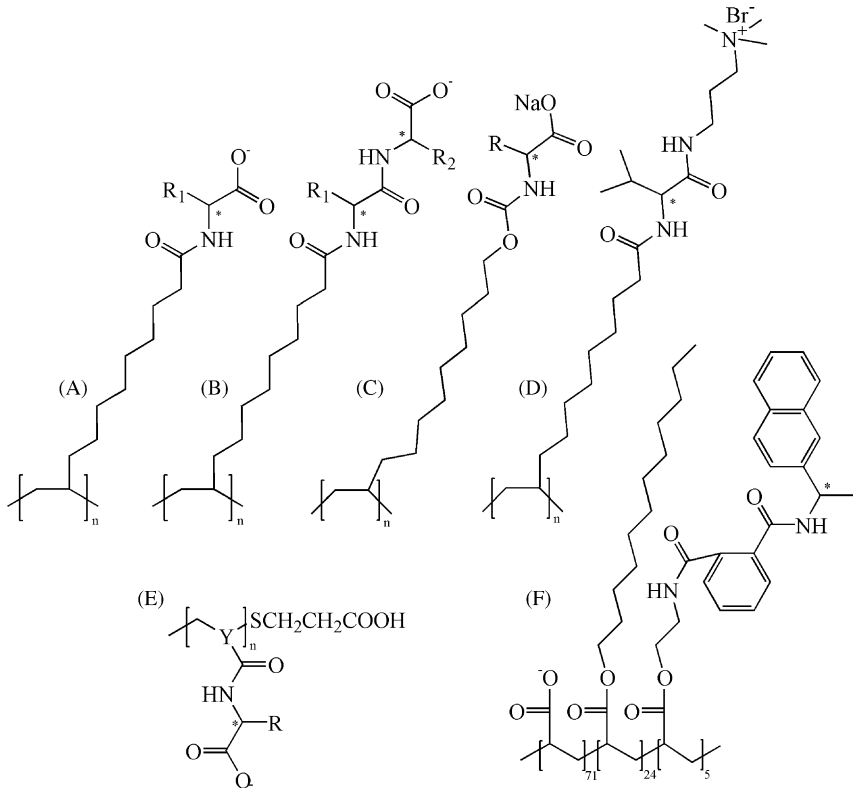

Fig. 12. Structures of chiral polymeric PSPs. (A) Poly(sodium undecenoyl aminoacidate), (B) poly(sodium undecenoyl diaminoacidate), (C) poly(sodium *N*-undecenoxy carbonyl-aminoacidate), (D) cationic chiral polymers, (E) linear amino acid acrylamides, (F) Pirkle type chiral polymer.

Finally, molecularly imprinted polymer particles have been introduced and shown to be useful for chiral separation.

2.2.1. Polymerized amino acid surfactants

There is a large body of work with polymers of the general structure presented in Fig. 12A and B. This work includes applications and fundamental studies of the polymer structure and the nature of the chemical interactions responsible for chiral separation. The results of these studies prior to 2000 have been comprehensively reviewed [9].

An extensive study has compared the performance of polymerized surfactants to micellar solutions of their monomer counterparts [55]. Eighteen surfactants with one or two amino acids on the head group were studied for the separation of five different chiral solutes. Typically, the monomer surfactant must be used at much higher equivalent monomer concentration than the polymerized surfactant to achieve separation. This, and greater conductivity of monomer solutions, means that the current is higher when monomeric surfactants are used. Joule heating may thus limit the efficiency of separations using the monomers. Whether or not the polymerized surfactant provided better chiral selectivity than its monomer counterpart was dependent on the chiral solute and the surfactant head group structure. In just over 60% of the cases studied, the polymeric surfactant provided better selectivity than its monomeric counterpart. For the non-ionic analytes studied, the polymers outperformed the monomers 85% of the time. The monomer surfactant micelles performed better in cases where penetration into the micelle core was important, implying that the more cohesive covalent structure of the polymer limits penetration. For the one cationic solute investigated, the monomeric phases always provided better or equal selectivity than the polymeric materials. The authors suggest that this may also be a result of the looser configuration of the monomer micelles. The monomers provide better or equal selectivity for an anionic solute half of the time.

An extensive study of the performance of L,L-leucyl-valinate using 75 cationic, neutral and anionic racemic compounds showed that the chiral polymer was able to provide some level of resolution for 58 of the compounds [56]. Anionic compounds are more difficult to resolve, presumably due to ionic repulsion.

The depth of penetration of the solutes and the effect of temperature on the depth of penetration was studied using three chiral solutes and L,L-leucyl-leucinate and L,D-leucyl leucinate monomer and polymer PSPs [57]. Two of the solutes studied were found to interact with the inner (N-terminal) amino acid for both the polymer and the monomer phases, under all conditions. A third solute, (±)-1,1′-binaphthyl-2,2′-dihydrogen phosphate, interacted with both amino acids to approximately the same extent with the polymer. The depth of penetration of this solute in the polymeric PSP was affected by temperature: at 12 °C the solute

interacts with the inner amino acid, while at 55 °C it interacts with the exterior amino acid. This led to a reversal in migration order of the enantiomers between 12 and 55 °C.

The effect of the steric factors and hydrophobicity at the ionic head group was studied in some detail. A glutamic acid substituted polymer was synthesized, and the second carboxyl esterified with methyl, ethyl or *tert*-butyl groups [58]. The hydrophobic ester create steric hindrance for solutes and cause the head group to be oriented differently. The change in orientation or ionization of the head group was found to enhance most of chiral separations studied, but the bulky nature of the glutamic acid group and particularly the bulky *tert*-butyl group prevented good chiral separations for some solutes. However, for other solutes, the *tert*-butyl group was found to be essential to resolve the enantiomers. In a second study, polymers with leucine, norleucine, *tert*-butyl leucine, isoleucine, valine, norvaline, and proline head groups were compared for the separation of five enantiomeric compounds [59]. The steric hindrance of the *tert*-butyl group and the four carbon chain on norleucine resulted in lower chiral resolution, while a side chain length of one or two carbons had no significant effect on resolution. The rigid proline head group was conducive for some separations, but resolution was often limited by the lack of a H-bonding site near the chiral center.

Tarus et al. polymerized sodium undecenyl leucinate in the presence of hexanol and undecylenyl alcohol and investigated the polymers for the chiral separation of coumarinic and benzoin derivatives [60]. It is unclear whether the undecylenyl alcohol is incorporated into the polymer backbone. Polymerization in the presence of the alcohols was found to give larger and lower polarity polymers than polymerization in aqueous environments. The resolution of comarinic derivatives was improved using either hexanol or undecylenyl alcohol, while the resolution of benzoin derivatives was only improved using hexanol.

Poly(undecenyl leucinate) with different monovalent counterions was studied for the separation of benzoin and binapthyl compounds [61]. Counterions larger than Na^+ (K^+, Rb^+, Cs^+) favored higher resolution of all analytes studied.

Several studies have been reported utilizing NMR and fluorescence spectroscopy, often in combination with EKC studies, to characterize the polymers and the chemical interactions between the polymers and solutes [58–65]. The binding strength of two chiral solutes with poly(sodium undecenyl valinate) and poly(sodium undecenyl isoleucinate) were studied by EKC, NMR and fluorescence and the results were consistent with the EKC studies. One exception was 2,2′-dihydroxy-1,1′-binaphthyl, which was not separated by EKC with poly(sodium undecenyl valinate), but showed significantly different interaction strengths for the two enantiomers by fluorescence. NMR study of the interaction of (*S*)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate with sodium undecenyl-L-valine-L-leucine and sodium undecenyl-L-leucine-L-valine and their polymeric counterparts indicated that while the site of interaction was the same, the strength of the interaction is weaker with the polymeric surfactants [63]. The fluorescence anisotropy of solutes interacting with polymeric PSPs was found to correlate well with the EKC selectivity of the solutes [64]. The aggregation behavior of the monomeric and polymeric chiral surfactants was studied by steady-state fluorescence quenching techniques. The degree of polymerization for most of the polymer surfactants (18–62) studied was one half to one third of the aggregation number of the corresponding monomer surfactants (38–74) [62]. In five cases, the aggregation number of the monomeric surfactants was found to be very high (110–380), but the degree of polymerization of the corresponding polymers was not different from the other polymers.

Poly(sodium undecenyl-L-valinate) and poly(sodium undecenoyl L-norvalinate were studied for the separation of seven phenylthiohydantoin (PTH) amino acid pairs [66]. Significant tailing of the peaks was observed in these separations, possibly due to adsorption of the polymers on the capillary walls. The problem was not solved by the addition of methanol, SDS or urea. Poly(sodium undecenyl-L-valinate) provided better separations at low pH, while poly(sodium undecenyl-L-norvalinate) provided better separations at high pH, where a more open polymer structure is proposed to enhance selectivity. Using a combination of poly(sodium undecenyl-D-valinate) and hydroxypropyl cyclodextrin, 5 PCB pairs were resolved in less than 40 min [67].

In an effort to improve the solubility of the amino acid modified polymers, Akbay et al. copolymerized sodium undecenoyl-L-leucinate (SUL) with sodium undecenyl sulfate (Fig. 2D) [68]. The strongly acidic sulfate groups should improve the solubility of this polymer at pH below 7, although this was not demonstrated in the report. The copolymers were found to have a higher specific volume than the SUL homopolymers, implying a more open, flexible structure. The copolymers were used for the separation of aryl-alkyl ketones and benzodiazepines. The electrophoretic mobility and hydrophobicity of the copolymers were higher than the SUL homopolymers. The enantioselectivity of the polymers decreases as the SUL content is reduced, and at least 60% SUL was required to achieve chiral separations of benzodiazepines.

Sodium oleyl-leucyl-valinate was synthesized, polymerized, and used as a PSP [69]. This polymer is more hydrophobic than the undecenyl counterparts, and it is suggested that this may lead to better separations of hydrophobic enantiomers. It was demonstrated that comparable chiral resolution of binapthyl, benzoin, and warfarin compounds could be achieved with lower concentrations of this polymer than of its undecenyl counterpart, and that the separations were faster using the oleyl polymer.

Using chemistry that has been shown to work well with monomeric surfactants [70], Rizvi et al. synthesized and polymerized sodium *N*-undecenoxy carbonyl-L-

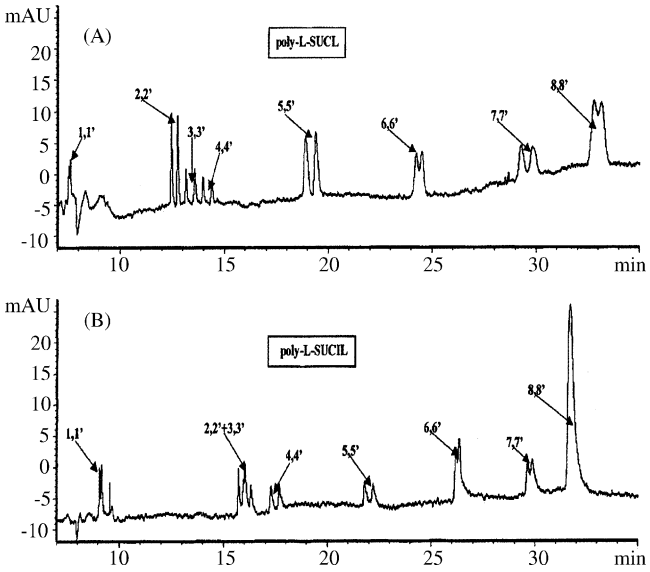

Fig. 13. Comparison of (A) poly(sodium *N*-undecenoxy carbonyl-L-leucinate) (poly-L-SUCL) and (B) poly(sodium *N*-undecenoxy carbonyl-L-isoleucinate) (poly-L-SUCIL) for simultaneous separation and enantioseparation of β-blockers: 1,1′-atenolol; 2,2′-carteolol; 3,3′-metoprolol; 4,4′-pindolol; 5,5′-oxprenolol; 6,6′-talinolol; 7,7′-alprenolol; 8,8′-propranolol at equivalent monomer concentration (50 mM) CHES/TEA buffer, pH 8.8. From ref. [71] with permission.

leucinate (Fig. 12C) and sodium *N*-undecenoxy carbonyl-L-isoleucinate and studied the polymers for the chiral separation of β-blockers [71]. Representative separations using these polymers are shown in Fig. 13. The alkenoxy surfactants were demonstrated to have dramatically higher chiral resolving ability for the β-blockers than their amide counterparts, suggesting that the presence of an additional oxygen near the polar head group provides a significant contribution to chiral recognition of β-blockers.

Dobashi et al. synthesized a cationic chiral polymeric surfactant with the structure shown in Fig. 12D and compared its performance to monomeric surfactants of similar structure [72]. The polymer did not perform as well as similar monomeric surfactants. Elimination of the dynamic association–dissociation equilibrium by polymerization did not enhance the separation. It is proposed that the lower selectivity observed may be due to spaces between the surfactant monomers and the greater penetration of water into the micellar core.

2.2.2. Linear chiral polymers

Linear acrylamide polymers containing amino acid moieties (Fig. 12E) have been synthesized and characterized [73]. These polymers have the same chiral selectors as many of the polymerized chiral surfactants described above. An L-alanine PSP of this structure did not provide chiral selectivity for 3,5-dinitrobenzoyl amino acid isopropyl esters. An L-valine phase did provide reasonable selectivity for the same solutes, but the resolution was not sufficient due to weak interactions of the solutes with the polymer. It is thought that the very hydrophilic nature of these polymers, confirmed by pyrene fluorescence studies, limits the extent of interaction of solutes with the phase. Synthesis of more hydrophobic copolymers containing methylene linker arms and *N*-methyl amide comonomers did not yield stronger interactions or better separations.

2.2.3. Pirkle type copolymers

Chiral separations have also been reported utilizing a copolymer that is 24% *n*-dodecylacrylate, 71% acrylic acid and 5% of an acrylate modified with a π-basic Pirkle-type chiral selector, *N*-*S*-[1-(1-naphthyl)ethyl]phthalamic acid (Fig. 12F) [74]. The proposed mechanism of separation is that the solutes partition into the hydrophobic domain created by the polymeric PSP, where they interact with the chiral selector. The authors note the very high efficiency afforded by the polymers, but do not rule out that this may be the result of a focusing mechanism. Good resolution of dinitrobenzoyl-derivatized amino acid enantiomers was obtained using the polymer. The polymers are UV absorbing, however, which may lead to problems with UV detection unless a partial filling approach is utilized.

2.2.4. Molecularly imprinted polymer particles

Several very interesting reports of the use of molecularly imprinted polymer (MIP) micro-particles as a PSPs have appeared [75–83]. The microspheres are synthesized by precipitation polymerization, giving a narrow distribution of particle sizes of 0.2–0.5 μm diameter that are free of stabilizing surfactants. The microspheres are used as a slurry or suspension in aqueous buffers. The suspended particles do scatter light, such that a partial-filling protocol [84] must be used when UV detection is utilized. The advantage of the approach is the highly tuned separation selectivity that the MIPs afford. The success of these reports is very promising, and it is hoped that this will lead to the further development of molecularly imprinted PSPs.

Following the initial report of MIP suspension templated with S-propanolol in which a chiral separation of propanolol in less than 1.5 min was demonstrated [83], Spégel et al. investigated the effect of the amount of template, use of weakly interacting monomers, and separation conditions [78,82]. They found that the amount of template has a dramatic effect on the size (and by inference possibly the porosity and pore size distributions) of the particles, that the use of weakly interacting monomers improved the efficiency but reduced the selectivity of the separations, and that the crosslinker ethyleneglycol dimethacrylate provided higher efficiency separations than two other commonly used crosslinkers. In order to maintain a stable suspension of particles, 90% acetonitrile was used. In this buffer, hydrophobic interactions between the particles and solutes are minimized, leaving electrostatic interactions as the most important contributor to retention. The particles were templated for *s*-propanolol, but were found to provide enantiomeric selectivity for pindolol and atenolol as

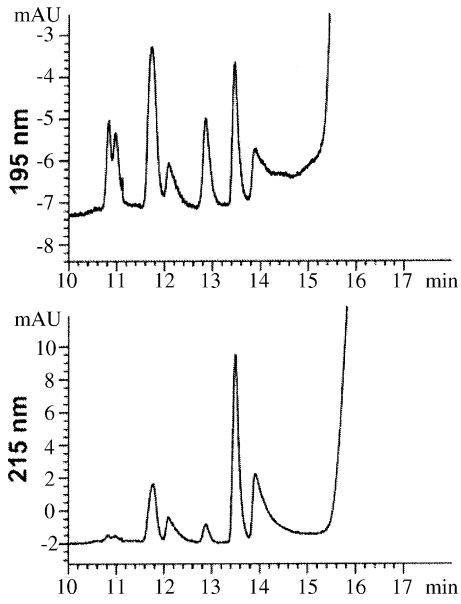

Fig. 14. Simultaneous enantiomer separation of the analogues (1) atenolol, (2) pindolol, and (3) propranolol utilizing the cross-selectivity of (*S*)-propranolol MIP nanoparticles in a partial-filling application of CEC using 195 and 215 nm detection wavelengths. The electrolyte was 90% acetonitrile and 10% acetic acid/triethenolamine at pH 3.5. From ref. [76] with permission.

well (Fig. 14). Although the particles and separation conditions were optimized (including conducting separations at 60 °C), significantly lower efficiency and peak tailing was still observed for the more retained analyte (Fig. 14).

Two methods were investigated to achieve MIP separations with selectivity toward more than one predetermined target [77]. In the first method, a mixture of particles templated to two different solutes was utilized. In the second approach, a single MIP was synthesized using two templates. Both approaches were successful, but the mixed-MIP approach proved easier to optimize.

Uncharged spherical particles imprinted with (+)− ephedrine and with radii on the order of 100–200 nm were used as a nonionic PSP for the separation of the cationic ephedrine enantiomers at a low pH where electroosmotic flow was minimized [75].

2.3. Dendrimers

Relatively few reports of the use of dendrimers as PSPs have appeared during the review period. Stathakis et al. used anionic and cationic poly(amidoamine) dendrimers as buffer additives to alter the selectivity of separations of chicken sarcoplasmic proteins [85]. Very low concentrations (10^{-4}%, w/v) of anionic dendrimer were observed to impove resolution substantially. A cationic dendrimer also improved resolution, but at the cost of very long analysis times. The improvement in separation was attributed to either ionic interactions or hydrophobic partitioning. In either case, separation efficiency was rather poor.

Commercial dendrimers modified with sulfonic acid terminal groups have been used to separate two dimethylphenol isomers [86]. The optimal separation using dendrimers was better than with sodium dodecyl sulfate micelles. Better separation efficiency was observed with higher concentrations of dendrimer.

Gao et al. have studied the properties and utility of commercially available poly(amidoamine) dendrimers with ethylene diamine cores for the separation of phenylglycine, tyrosine, phenylalanine, DOPA, homophenylalanine and methyl-DOPA [87]. Characterization of the materials by capillary electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry showed that the dendrimer materials were actually a complex mixture rather than uniform monomolecular materials. Relatively poor selectivity and high baseline noise was observed at pH 7. At pH 2.5 the dendrimers were still anionic and caused the cationic analytes to migrate toward the anode with strong ionic association and better selectivity. Reduced background absorbance and better baselines were also observed at the lower pH.

Castagnola et al. have reviewed the characterization and application of dendrimers as PSPs [88]. They also reported an original separation of two derivatized amino acids using anionic half generation commercial PAMAM dendrimers, and found that the separation selectivity and performance were superior to SDS micelles.

2.4. Electrokinetic chromatography–mass spectrometry

Several studies have appeared regarding the use of polymeric PSPs for the combination of EKC with mass spectrometric detection [79,89–91]. The zero cmc, low surface activity, low volatility, high electrophoretic mobility, suitable performance at low concentration, and lack of signal in the mass region of interest make polymeric PSPs attractive for this application. The suppression of signals by SDS in electrospray ionization is in part related to the large surface excess of the surfactant [92]. Thus, pseudo-stationary phases that are not surface active should perform better for this application.

Ozaki et al. utilized BBMA and electrospray ionization mass spectrometry for the analysis of pharmaceutical compounds [89]. They found that BBMA did not adversely affect the signal at concentrations below 0.5%, but signal was diminished by nearly 50% at a concentration of 1%. This was better than SDS micelles, but was considered severe enough that a partial filling approach was used.

The polymer of SUS has also been used for mass spectrometric detection of tricyclic antidepressants and β-adrenic blocker drugs [90]. Signal was severely diminished at a pSUS concentration of 0.5%, but better separations and sufficient signal were obtained at a concentration of 0.1%. The authors point out that to utilize this approach, a compromise between signal and resolution may have to be made.

Shamsi reported the use of a chiral polymeric PSP, pSUV in the L form, for the separation and electrospray ionization mass spectrometric detection of 1,1′-binaphthol (BOH) [91]. BOH at a concentration of 100 mM was detected by mass spectrometry with a signal to noise of 93. The polymeric surfactant could be employed at a concentration of at least 1% (w/v) without significant degradation of the signal or fouling of the electrospray interface.

Viberg et al. used MIP microparticles of average diameter 160 nm with mass spectrometric detection [79]. Using an orthogonal electrospray ionization interface, the mass spectrometer could be used continuously for several days without fouling of the interface. While the micro-particles did not have an effect on the signal for salbutamol, the signal for nortriptyline was diminished by 90% as the concentration of the micro-particles was increased from 0.11 to 0.44 mg/mL. It is not clear why nortriptyline behaved differently or was so adversely affected, but it may be due to ion pair formation in the ESI or impurities in the particle suspension.

3. Electrochromatography applications

Polymers of the same or similar chemistry to those described above have been used as stationary phases for capillary electrochromatography [93–101]. The advantage of this approach is that the soluble polymers can be easily introduced, and in some cases replaced, in the capillary. In most cases, the polymers are physically adsorbed to silica surfaces, often as a multilayer (up to twelve bilayers) with a polymer of opposite charge. The adsorbed layers are highly stable, and provide reproducible and stable electroosmotic flow. Analytes can interact with the immobilized polymer and are often separated by a combination of electrophoresis and electrochromatography.

Most often, the polymers are adsorbed to the interior wall of an open tubular capillary, and open-tubular CEC (OT-CEC) is performed. In such cases, especially when capillaries of $\geq$50 μm inside diameter are used, the phase ratio and mass transport effects are not conducive to high efficiency or resolution. None-the-less, several reports do indicate that OT-CEC is achieved in these capillaries with physically adsorbed polymer layers as stationary phases. Graul and Schlenoff used 6.5 bilayers of pDADMA and polystyrene sulfonate with a thickness of approximately 200 nm in a 50 μm capillary and observed separations of neutral analytes by OT-CEC [100]. They note that the efficiency suffers for later eluting analytes. Pesek et al. reported OT-CEC using two ionic polymers adsorbed to the walls of chemically etched capillary, but since ionic analytes are separated and no retention factors are reported, it is not possible to know the extent to which chromatography is responsible for the separations [101]. Kamande et al. used a single bilayer of pDADMA and pSUS as a stationary phase for OT-CEC in a 50 μm to effect the separation of phenols and benzodiazepines [97]. The separation of phenols is mostly due to electrophoresis of the anionic analytes, but the separation of benzodiazepines must be due to a chromatographic mechanism because it was not possible in an uncoated capillary. The coated capillary provided different selectivity from an EKC separation using pSUS. Later eluting peaks did show significant losses in efficiency and significant tailing, possibly because of mass transport band broadening. Kapnissi et al. used 10 bilayers of pDADMA and poly(sodium undecenoyl glycinate) in a 50 μm capillary for the OT-CEC separation of benzodiazepines [98]. The separations were found to be highly reproducible and robust. Chiral OT-CEC separations of several enantiomers using two to twelve bilayers of pDADMA and poly(sodium *N*-undecanoyl-L-leucylvalinate) in 50 μm capillaries have been reported [96]. Optimization of the coating procedure and analytical conditions to generate reproducible and robust separations with good chiral resolution and good efficiency is reported.

Pirogov and Buchberger used the cationic 2,10-ionene polymer to coat a silica-based cation exchanger [94]. The particles were packed into 75 μm capillaries and CEC was used for the separation of benzoic acids by an ion exchange mechanism. The approach provides much greater surface area, a more appropriate phase ratio, and fewer problems with mass transport band broadening than the open tubular approach. It was necessary to maintain a concentration of 0.1% ionene in the run buffer to maintain the coated surface. No evidence of OT-CEC was observed when the separations were run in ionene coated capillaries without packing material.

Schure et al. reported the use of a soluble entangled polymer solution for capillary gel electrochromatography [99]. A copolymer of ethyl acrylate, methacrylic acid and dodecyl methacrylate was introduced into the capillary at a concentration of 1–4% (w/w). The polymer forms an entangled gel, the ionic sites provide electroosmotic flow, and the hydrophobic sites provide retentive interactions. The gels were used for fast and efficient separation of a variety of neutral substituted benzenes and PAHs in 40% acetonitrile-modified buffers. The authors note several limitations of the approach, including the limited range of acetonitrile concentrations that are compatible with the gels and the low sample capacity of the system which is calculated to be less than that of a wall-coated 50 μm capillary. None-the-less, this seems to be an approach with many advantages and a great deal of potential. Unfortunately, there have been no additional reports of this type of CEC system.

4. Concluding remarks

Significant work continues on the development and characterization of polymeric PSPs for electrokinetic chromatography and on the application of similar polymers in electrochromatography. Studies of the selectivity and solvation characteristics of the phases have been particularly useful in determining the effects of various factors such as

backbone chemistry and pendant group chemistry on the selectivity of the phases. With few exceptions, the polymeric materials provide high efficiency separations comparable to that obtained with micellar phases. In some cases, separations of significantly higher efficiency than those with micellar phases are observed. In spite of the development of multiple novel PSP chemistries, applications of polymeric materials remain relatively few. This is at least in part due to the fact that many of the polymeric PSPs are not commercially available.

There are a wealth of polymer chemistries and structural variations that remain to be investigated. In recent years, the number of reports of the use of dendrimers has waned significantly in spite of the fact that there are many dendrimer chemistries that might provide unique chemical selectivity. The use of entangled polymers for capillary gel electrochromatography has not been further investigated since the original report. Cationic polymers with significant hydrophobic domains have recently received more attention, and future reports should continue to demonstrate reproducible separations affording unique selectivity. The use of these materials with EKC–MS continues to show promise, but is clearly not without significant challenges.

References

[1] S. Terabe, K. Otsuka, A. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] S.C. Beale, Anal. Chem. 70 (1998) R279.
[3] J.P. Quirino, S. Terabe, J. Chromatogr. A 856 (1999) 465.
[4] M.G. Khaledi, High Performance Capillary Electrophoresis: Theory, Techniques, and Applications, Wiley, New York, 1998.
[5] C.P. Palmer, N. Tanaka, J. Chromatogr. A 792 (1997) 105.
[6] C.P. Palmer, J. Chromatogr. A 780 (1997) 75.
[7] C.P. Palmer, Electrophoresis 21 (2000) 4054.
[8] J.S. Fritz, M.C. Breadmore, E.F. Hilder, P.R. Haddad, J. Chromatogr. A 942 (2002) 11.
[9] H.H. Yarabe, E. Billiot, I.M. Warner, J. Chromatogr. A 875 (2000) 179.
[10] B. Maichel, E. Kenndler, Electrophoresis 21 (2000) 3160.
[11] C.P. Palmer, Electrophoresis 23 (2002) 3993.
[12] S.A. Shamsi, C.P. Palmer, I.M. Warner, Anal. Chem. 73 (2001) 140A.
[13] I. Peric, E. Kenndler, Electrophoresis 24 (2003) 2924.
[14] A.V. Pirogov, A.V. Shpak, O.A. Shpigun, Anal. Bioanal. Chem. 375 (2003) 1199.
[15] C.P. Palmer, K.T. Tellman, J. Microcol. Sep. 11 (1999) 185.
[16] T.W. Moy, P.L. Ferguson, A.H. Grange, W.H. Matchett, V.A. Kelliher, W.C. Brumley, J. Glassman, J.W. Farley, Electrophoresis 19 (1998) 2090.
[17] K.T. Tellman, C.P. Palmer, Electrophoresis 20 (1999) 152.
[18] S.A. Shamsi, C. Akbay, I.M. Warner, Anal. Chem. 70 (1998) 3078.
[19] D. Norton, S.A. Shamsi, Anal. Chim. Acta 496 (2003) 165.
[20] C. Akbay, I.M. Warner, S.A. Shamsi, Electrophoresis 20 (1999) 145.
[21] C.P. Palmer, S. Terabe, Anal. Chem. 69 (1997) 1852.
[22] S.H. Edwards, S.A. Shamsi, J. Chromatogr. A 903 (2000) 227.
[23] C.W.I. Henry, S.A. Shamsi, I.M. Warner, J. Chromatogr. A 863 (1999) 89.
[24] S.M. Mwongela, A. Numan, N.L. Gill, R.A. Agbaria, I.M. Warner, Anal. Chem. 75 (2003) 6089.
[25] C. Fujimoto, Electrophoresis 22 (2001) 1322.
[26] C. Fujimoto, Y. Fujise, S. Kawaguchi, J. Chromatogr. A 871 (2000) 415.
[27] D.S. Peterson, C.P. Palmer, J. Chromatogr. A 924 (2001) 103.
[28] D.S. Peterson, C.P. Palmer, J. Chromatogr. A 959 (2002) 255.
[29] D.S. Peterson, C.P. Palmer, Electrophoresis 22 (2001) 3562.
[30] S. Schulte, C.P. Palmer, Electrophoresis 24 (2003) 978.
[31] M. Aguilar, A. Farran, C. Serra, M.J. Sepaniak, K.W. Whitaker, J. Chromatogr. A 778 (1997) 201.
[32] S.K. Wiedmer, H. Tenhu, P. Vastamaki, M.L. Riekkola, J. Microcolumn Sep. 10 (1998) 557.
[33] M.S. Leonard, M.G. Khaledi, J. Sep. Sci. 25 (2002) 1019.
[34] C.P. Palmer, Presented at the 9th International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques, Anaheim, CA, 26–30 January 1997.
[35] W. Shi, D.S. Peterson, C.P. Palmer, J. Chromatogr. A 924 (2001) 123.
[36] W. Shi, C.P. Palmer, J. Sep. Sci. 25 (2002) 215.
[37] W. Shi, C.J. Watson, C.P. Palmer, J. Chromatogr. A 905 (2001) 281.
[38] W. Shi, C.P. Palmer, J. Sep. Sci. 25 (2002) 543.
[39] W. Shi, C.P. Palmer, Electrophoresis 23 (2002) 1285.
[40] J.P. Quirino, S. Terabe, Science 282 (1998) 465.
[41] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023.
[42] N. Tanaka, K. Nakagawa, H. Nagayama, K. Hosoya, T. Ikegami, A. Itaya, M. Shibayama, J. Chromatogr. A 836 (1999) 295.
[43] N. Tanaka, K. Nakagawa, H. Iwasaki, K. Hosoya, K. Kimata, T. Araki, D.G. Patterson, J. Chromatogr. A 781 (1997) 139.
[44] N. Tanaka, K. Nakagawa, K. Hosoya, C.P. Palmer, S. Kunugi, J. Chromatogr. A 802 (1998) 23.
[45] S. Kolb, J.P. Kutter, T. Welsch, J. Chromatogr. A 792 (1997) 151.
[46] B. Maichel, K. Gogova, B. Gas, E. Kenndler, J. Chromatogr. A 894 (2000) 25.
[47] F.B. Erim, J. Chromatogr. A 768 (1997) 161.
[48] B. Maichel, B. Potocek, B. Gas, M. Chiari, E. Kenndler, Electrophoresis 19 (1998) 2124.
[49] B. Maichel, B. Potocek, B. Gas, E. Kenndler, J. Chromatogr. A 853 (1999) 121.
[50] B. Potocek, E. Chmela, B. Maichel, E. Tesarova, E. Kenndler, B. Gas, Anal. Chem. 72 (2000) 74.
[51] G. Izzo, M.A. Raggi, B. Maichel, E. Kenndler, J. Chromatogr. B 752 (2001) 47.
[52] K. Gogova, B. Maichel, B. Gas, E. Kenndler, J. Chromatogr. A 916 (2001) 79.
[53] K. Kopecka, E. Tesarova, A. Pirogov, B. Gas, J. Sep. Sci. 25 (2002) 1027.
[54] A.V. Pirogov, K.V. Stepanov, O.A. Shpigun, J. Anal. Chem. 58 (2003) 478.
[55] F.H. Billiot, E.J. Billiot, I.M. Warner, J. Chromatogr. A 922 (2001) 329.
[56] S.A. Shamsi, B.C. Valle, F. Billiot, I.M. Warner, Anal. Chem. 75 (2003) 379.
[57] F. Haddadian, E.J. Billiot, I.M. Warner, J. Chromatogr. A 950 (2002) 233.
[58] S.J. Thibodeaux, E. Billiot, E. Torres, B.C. Valle, I.M. Warner, Electrophoresis 24 (2003) 1077.
[59] S.J. Thibodeaux, E. Billiot, I.M. Warner, J. Chromatogr. A 966 (2002) 179.
[60] J. Tarus, R.A. Agbaria, K. Morris, F.H. Billiot, A.A. Williams, T. Chatman, I.M. Warner, Electrophoresis 24 (2003) 2499.
[61] J. Tarus, S.A. Shamsi, K. Morris, R. Agbaria, I.M. Warner, Langmuir 19 (2003) 7173.
[62] F.H. Billiot, M. McCarroll, E.J. Billiot, J.K. Rugutt, K. Morris, I.M. Warner, Langmuir 18 (2002) 2993.
[63] J.K. Rugutt, E. Billiot, I.M. Warner, Langmuir 16 (2000) 3022.
[64] M.E. McCarroll, F.H. Billiot, I.M. Warner, J. Am. Chem. Soc. 123 (2001) 3173.

[65] H.H. Yarabe, J.K. Rugutt, M.E. McCarroll, I.M. Warner, Electrophoresis 21 (2000) 2025.
[66] K.A. Agnew-Heard, S.A. Shamsi, I.M. Warner, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 1301.
[67] S.H. Edwards, S.A. Shamsi, Electrophoresis 23 (2002) 1320.
[68] C. Akbay, N.L. Gill, R.A. Agbaria, I.M. Warner, Electrophoresis 24 (2003) 4209.
[69] S. Mwongela, C. Akbay, X. Zhu, S. Collins, I.M. Warner, Electrophoresis 24 (2003) 2940.
[70] J.R. Mazzeo, E.R. Grover, M.E. Swartz, J.S. Petersen, J. Chromatogr. A 680 (1994) 125.
[71] S.A.A. Rizvi, S.A. Shamsi, Electrophoresis 24 (2003) 2514.
[72] A. Dobashi, M. Hamada, J. Yamaguchi, Electrophoresis 22 (2001) 88.
[73] M. Hamada, A. Dobashi, Anal. Sci. 18 (2002) 83.
[74] K. Zhang, R. Krishnaswami, L. Sun, Anal. Chem. Acta 496 (2003) 185.
[75] T. de Boer, R. Mol, R.A. de Zeeuw, G.J. de Jong, D.C. Sherrington, P.A.G. Cormack, K. Ensing, Electrophoresis 23 (2002) 1296.
[76] P. Spegel, L. Schweitz, S. Nilsson, Electrophoresis 24 (2003) 3892.
[77] P. Spegel, L. Schweitz, S. Nilsson, Anal. Chem. 75 (2003) 6608.
[78] P. Spegel, S. Nilsson, Am. Lab. 34 (2002) 29 and 32.
[79] P. Viberg, M. Jornten-Karlsson, P. Petersson, P. Spegel, S. Nilsson, Anal. Chem. 74 (2002) 4595.
[80] L. Schweitz, L.I. Andersson, S. Nilsson, Analyst 127 (2002) 22.
[81] L. Schweitz, P. Spegel, S. Nilsson, Electrophoresis 22 (2001) 4053.
[82] P. Spegel, L. Schweitz, S. Nilsson, Electrophoresis 22 (2001) 3833.
[83] L. Schweitz, P. Spegel, S. Nilsson, Analyst 125 (2000) 1899.
[84] A. Amini, U. Paulsen-Sorman, D. Westerlund, Chromatographia 50 (1999) 497.
[85] C. Stathakis, E.A. Arriaga, N.J. Dovichi, J. Chromatogr. A 817 (1998) 233.
[86] A.L. Gray, J.T. Hsu, J. Chromatogr. A 824 (1998) 119.
[87] H. Gao, J. Carlson, A.M. Stalcup, W.R. Heineman, J. Chromatogr. Sci. 36 (1998) 146.
[88] M. Castagnola, C. Zuppi, D.V. Rossetti, F. Vincenzoni, A. Lupi, A. Vitali, E. Meucci, I. Messana, Electrophoresis 23 (2002) 1769.
[89] H. Ozaki, S. Terabe, J. Chromatogr. A 794 (1998) 317.
[90] W.Z. Lu, S.A. Shamsi, T.D. McCarley, I.M. Warner, Electrophoresis 19 (1998) 2193.
[91] S.A. Shamsi, Anal. Chem. 73 (2001) 5103.
[92] K.L. Rundlett, D.W. Armstrong, Anal. Chem. 68 (1996) 3493.
[93] A.V. Pirogov, A.V. Yur'ev, O.A. Shpigun, J. Anal. Chem. 58 (2003) 781.
[94] A.V. Pirogov, W. Buchberger, J. Chromatogr. A 916 (2001) 51.
[95] C.P. Kapnissi-Christodoulou, X. Zhu, I.M. Warner, Electrophoresis 24 (2003) 3917.
[96] C.P. Kapnissi, B.C. Valle, I.M. Warner, Anal. Chem. 75 (2003) 6097.
[97] M.W. Kamande, C.P. Kapnissi, X. Zhu, C. Akbay, I.M. Warner, Electrophoresis 24 (2003) 945.
[98] C.P. Kapnissi, C. Akbay, J.B. Schlenoff, I.M. Warner, Anal. Chem. 74 (2002) 2328.
[99] M.R. Schure, R.E. Murphy, W.L. Klotz, W. Lau, Anal. Chem. 70 (1998) 4985.
[100] T.W. Graul, J.B. Schlenoff, Anal. Chem. 71 (1999) 4007.
[101] J.J. Pesek, M.T. Matyska, S. Sentellas, M.T. Galceran, M. Chiari, G. Pirri, Electrophoresis 23 (2002) 2982.