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Polymer-supported pseudo-stationary phase for electrokinetic chromatography
Electrokinetic chromatography in a full range of methanol–water mixtures with alkylated starburst dendrimers

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Abstract

Alkylated polyamidoamine starburst dendrimers (PAMAM-SBDs) are used as high-performance carriers in electrokinetic chromatography (EKC) in a full range of water–methanol (up to 90%) mixtures. The sodium dodecyl sulfate–micellar EKC (SDS–MEKC) system showed a greater migration time window at 20% methanol, but its applicability is limited above 40% methanol. The SBDs modified with dodecyl groups provided high efficiency and much wider migration time windows than SDS micelle carriers at high methanol content, especially for hydrophobic compounds, based on their more hydrophobic properties and reduced electroosmotic mobility. Different selectivities were observed between octyl and dodecyl derivatives of SBDs, the latter showing some similarity with the SDS micelle carrier. The results, showing a nearly linear relation between log k' and methanol content, indicate the possibility for the optimization of EKC separation with polymeric carriers by simply changing the organic solvent content to manipulate the solute retention as in reversed-phase liquid chromatography.

1. Introduction

Micellar electrokinetic chromatography (MEKC) utilizing micelles of ionic surfactants such as sodium dodecyl sulfate (SDS) as a pseudo-stationary phase, or a separation carrier, provides high performance separations and is applicable to a wide range of uncharged compounds [1–4]. This separation method makes use of the solute's partitioning between an aqueous phase, which undergoes electroosmosis, and a micelle phase, which undergoes electrophoretic migration. In principle, this technique is applicable for compounds with some water solubility. All the solutes are detected in a migration time window between the migration time of an unretained solute and that of a micelle.

Narrow migration time windows were observed in SDS–MEKC for hydrophobic compounds mostly partitioned in the micelle phase. Wider separation time windows can be obtained for these solutes by increasing their water solubility (partitioning into the aqueous phase) and by reducing the electroosmotic flow of the system. This can be effected by the addition of organic solvents [5–10], cyclodextrins [11–14], or urea [15] to the system.

The addition of organic solvents to the EKC

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systems [5–10] seems to be the most straightforward approach for the separation of hydrophobic compounds, as is commonly practised in reversed-phase liquid chromatography (RPLC). The addition of organic solvents, however, is accompanied by some complexities in MEKC due to the simultaneous changes in osmotic velocity of the aqueous solution, in the partition coefficients of solutes between the micelle and aqueous phase, in micelle concentration due to the change in solubility of the surfactants, and in micelle composition due to the incorporation of an organic solvent into the micelle phase. The changes in these factors could result in complex selectivity changes, making improvement in separation unpredictable. In SDS–MEKC systems, acetonitrile, dimethylsulfoxide (DMSO), or acetone was added rather than methanol [5–10].

This approach, often studied with SDS–MEKC systems, would be more effective with polymeric carriers which are stable against organic solvents. Since we can avoid the problem of the variation in carrier concentration and composition, the changes in osmotic flow and the solute partition coefficients are the only factors to be taken into account. The variation in the separation profile is expected to be straightforward, and we can fully utilize the information from RPLC, where the solute retention can be manipulated by simply changing the concentration of an organic solvent. The use of polymeric carriers has been reported in EKC [16–18], and their possibilities should be fully examined.

Polyamidoamine-type SBDs (PAMAM-SBDs) [19,20] are used as a pseudo-stationary phase [21] and showed carbon skeleton selectivity for aromatic compounds [22]. The retentivity of SBDs, however, is relatively small due to their hydrophilic structures. The derivatization of PAMAM-SBDs is feasible, and this makes it possible to study the structural effect of a pseudo-stationary phase. The idea is similar to the modification of the silica surface with alkylsilyl groups to prepare a stationary phase for RPLC. We report here that alkylated half-generation SBDs showed increased retentivity for hydrophobic compounds, differentiating the alkyl as well as functional groups based on their hydrophobic properties. The alkyl-SBDs, particularly the dodecyl derivative of PAMAM-SBDs at generation 3.5, provided high performance and permitted the separation of hydrophobic compounds in a full range, 0–90%, of methanol content in EKC.

2. Experimental

2.1. Equipment

High-performance capillary electrophoresis (HPCE)

A high-voltage power supply, HepLL-30P0.08 (Matsusada Precision Devices, Kusatsu, Japan), a variable-wavelength UV detector, UV-8 Model II (Tosoh, Tokyo, Japan), and a data processor, C-R6A (Shimadzu, Kyoto, Japan), were used. Detection was carried out at 210 nm for SDS–MEKC systems and at 254 nm for SBD systems due to the considerable UV absorption of SBDs at the lower wavelength. The scale bars attached to all the chromatograms indicate 0.005 AU. A fused-silica capillary of 50 μm I.D. and 0.375 mm O.D. (Polymicro Technologies, Phoenix, AZ, USA) was used at ambient temperatures.

2.2. Materials

Preparation of SBDs

The SBDs were prepared starting from *p*-xylylenediamine (X) as a core, following the method reported by Tomalia et al. [23]. The size of the SBDs is described according to the original generation system [23]. One reaction cycle, Michael addition to methyl acrylate to form an ester terminal SBD of $G = 0.5$ starting from the amine core, followed by the amide formation with ethylenediamine to regenerate an amine structure, provides a full generation SBD of $G = 1.0$. The reaction cycle was repeated to produce higher-generation SBDs. The SBDs used as carriers are described by the combination of the core (X) and the generation, that is the number of reaction cycles starting from the core. The SBD(X) of $G = 3.5$, supposedly possessing 32

terminal groups, was used as a support of alkyl groups in this study.

Hydrolysis of half-generation SBDs

Half-generation SBDs were used as carriers in EKC in a carboxylate form after hydrolyzing the terminal ester groups with equimolar sodium hydroxide in methanol [24].

Alkylation of SBDs [22]

SBD(X, $G = 3.5$) (4.0 g) was dissolved in methanol (50 ml), to which octylamine (3.3 g, 1.2 mole equivalent to the ester groups) was added to prepare SBD(X)-C_8. The solution was stirred for five days at room temperature. [SBD(X)-C_{12} was prepared at 40°C.] After examining the extent of alkylation by NMR, sodium hydroxide (0.65 g, one mole equivalent to the remaining ester groups) was added. The resulting mixture was stirred for 9 h. After concentrating the solution to 20 ml by evaporation under reduced pressure, the solution was added to acetone to precipitate the partially alkylated SBD in a carboxylate form, SBD(X)-C_8. SBD(X)-C_{12} was prepared similarly.

Characterization of SBDs

^{1}H NMR measurement was carried out on an XL-200 NMR instrument (Varian, Sunnyvale, CA, USA). The reaction cycle in SBD preparation and alkylation reaction was followed by NMR and elemental analysis.

Size exclusion chromatography (SEC)

Monodispersity of SBDs in molecular mass was examined by SEC by using TSK-G3000PW, 60 cm × 7.6 mm I.D. (Tosoh, Tokyo, Japan), in 50 m*M* phosphate buffer at pH 11. PAMAM-SBDs ($G = 2.0$, 4.0, and 6.0), commercially obtained (Polysciences, Warrington, PA, USA), were used as standards.

Electrophoresis measurement

The same instrument as described above was used in the absence of an EKC carrier. SBDs were injected as samples to the electrophoresis system to measure the electrophoretic mobility and monodispersity of alkyl SBDs. The capillary was washed with 1 *M* sodium hydroxide solution prior to every injection.

Electrokinetic chromatography (EKC)

Methanol was added to 20 m*M* borate buffer solution containing a carrier to prepare a mixed separation solution. All separation solutions were filtered with a membrane filter (0.2 μm). Apparent pH values were measured by a pH meter. Injection was carried out by a siphoning method. The k' values were calculated by using the equation $k' = (t_R - t_0)/t_0(1 - t_R/t_c)$ [2–4], where t_R, t_0, and t_c are the elution times of the solute, the unretained solute, and the carrier, respectively. Methanol or formamide was used to measure t_0 values, and Oil Yellow OB for t_{mc} in aqueous buffer for SDS–MEKC systems [25,26]. The t_{mc} in methanol–water mixtures at 40% or higher methanol content and t_{SBD} were calculated by the iterative method [27,28] assuming a linear relation between log k' and carbon numbers of alkyl phenyl ketones and alkylbenzenes.

3. Results and discussion

3.1. Characterization of alkylated SBDs

The PAMAM-SBDs prepared in this study showed better monodispersity in SEC than the standard SBDs obtained commercially that contained low-molecular-mass species. The alkylation of SBDs is expected to give a variety of products with a varying extent of alkylation. The NMR measurements indicated that SBD(X)-C_8 and SBD(X)-C_{12} possess 6 and 14 alkyl groups per molecule on average, respectively, out of 32 terminal groups on the SBD(X, $G = 3.5$). The difference in the extent of alkylation is presumably caused by the difference in reaction temperature, room temperature (ca. 25°C) for SBD(X)-C_8 and 40°C for SBD(X)-C_{12}.

Table 1 lists the electrophoretic mobilities of the SBD carriers in buffer solution without SBDs as a component of the separation solution, and the electroosmotic mobilities under EKC conditions. The electroosmotic flow is suppressed by

Table 1
Electrophoretic and electroosmotic mobilities of carriers

Methanol %	SDS		SBD(X)		SBD(X)-C_8		SBD(X)-C_{12}	
	μ_{eo}	μ_{ep}	μ_{eo}	μ_{ep}	μ_{eo}	μ_{ep}	μ_{eo}	μ_{ep}
0%	7.30	−4.37	5.12	−4.18	5.16	−3.79	6.11	−3.84
20%	4.14	−3.29	–	−2.42	3.34	−2.47	3.54	−2.38
40%	2.45	−2.31	2.43	−1.79	2.00	−1.81	2.62	−1.80
60%	1.47	−0.71	1.54	−1.71	1.46	−1.58	1.54	−1.47
80%	1.29	−0.22	1.77	−2.07	1.49	−2.21	1.00	−1.61

Electroosmotic mobilities were obtained from EKC experiments.
Electrophoretic mobilities of SBDs were measured in the absence SBDs in the separation solution.
Electrophoretic mobilities of SDS micelle were calculated from t_{mc} obtained by the iteration method from EKC experiments.

the presence of SBDs under EKC conditions, presumably due to the contribution of amino functionality of the SBDs [22]. The results agreed fairly well with those calculated from the t_{SBD} values by the iterative method. The mobilities of the SDS carriers were calculated from t_0 and t_{mc} in EKC. SBDs migrated as a band under electrophoretic conditions in the presence of electroosmotic flow, as shown in Fig. 1. The electrophoresis under the reduced electroosmotic flow in the presence of organic solvents showed broader peaks, presumably due to polydispersity in the alkylated SBDs which have different numbers of alkyl and terminal carboxylate groups.

An increase in the number of alkyl groups should lead to a decrease in the number of carboxylate groups, which in turn may lead to lower electrophoretic mobility. The contribution of this factor to EKC, however, can be counteracted, because the partition of a solute into such heavily alkylated SBDs would be greater

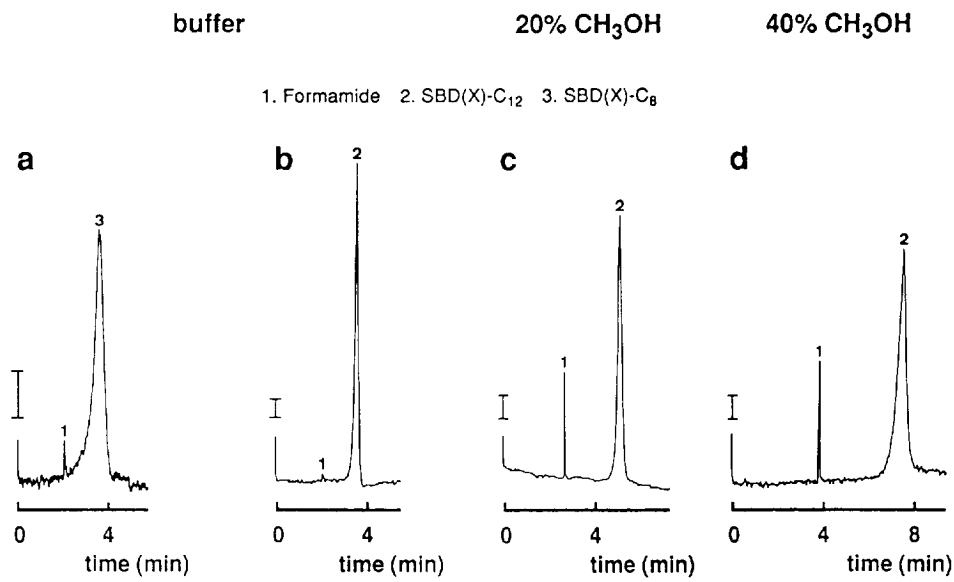


Fig. 1. Electrophoresis of SBD(X)-C_8 and SBD(X)-C_{12} in 0–40% methanol–buffer. Buffer: 20 m*M* borate, pH 8.9. $L = 48$ cm, $l = 33$ cm. Detection: 210 nm. Sample: 1 = formamide, 2 = SBD(X)-C_{12}, 3 = SBD(X)-C_8. (a and b) Field strength 300 V/cm, in aqueous buffer, and (c and d) 400 V/cm, in 20 and 40% methanol.

than in less alkylated SBDs, which are supposed to show higher electrophoretic mobility. Therefore, the broad band seen with the SBD carriers will not directly mean greater broadening of a solute band under EKC conditions.

Although SDS–MEKC provided a wider separation time window at 20% methanol, the SBD carriers showed greater $-\mu_{ep}/\mu_{eo}$ ratios, resulting in wider separation time windows than SDS–MEKC systems at higher methanol content. An infinite time window is expected when the ratio becomes unity. Some SBDs may have this property in the presence of 60–80% methanol.

3.2. SBD–EKC in aqueous solution

Although SDS–MEKC provided good separation for the relatively hydrophilic compounds, relatively narrow band spacing was observed between peaks 4 and 5, as shown in Fig. 2. This effect is generally seen with hydrophobic compounds that migrate near the elution time of the micelle, and is caused by the nearly complete partitioning of the hydrophobic solutes into the micelle phase [2–4]. SBD(X)-C_8 and SBD(X)-C_{12} showed a large increase in retention and separation based on the size of the alkyl group of a solute compared with the parent SBD(X). SBD(X)-C_{12} showed a similar migration profile to SDS micelles, with good efficiency. The high efficiency of the SBD(X)-C_{12} system is possibly provided by the narrow band of the carrier under electrophoretic conditions (Fig. 1).

Different selectivity was observed when the pseudo-stationary phase was composed with shorter alkyl groups. SBD(X)-C_8 showed a greater migration time window than SDS–MEKC, especially for alkyl phenyl ketones with C_3–C_5. The less hydrophobic structure of SBD(X)-C_8 than SDS micelles seems to contribute to the smaller partition coefficients for hydrophobic compounds. This effect and the greater t_{SBD} of this carrier make the separation profile similar to that in RPLC. SDS–MEKC and SBD(X)-C_{12}–EKC gave a greater peak capacity for relatively hydrophilic solutes, and a smaller peak capacity for the hydrophobic solutes than SBD(X)-C_8 due to the more hydrophobic structure. In a sense, the SBD(X)-C_{12} system showed some similarity with SDS–MEKC. The peak capacity for the hydrophobic compounds can be improved by additives, such as urea, cyclodextrins, or organic solvents, that should increase the solute partition into the aqueous phase.

Fig. 3 shows the separation of some benzene and naphthalene derivatives. SBD(X) without alkyl substituents separated the compounds with

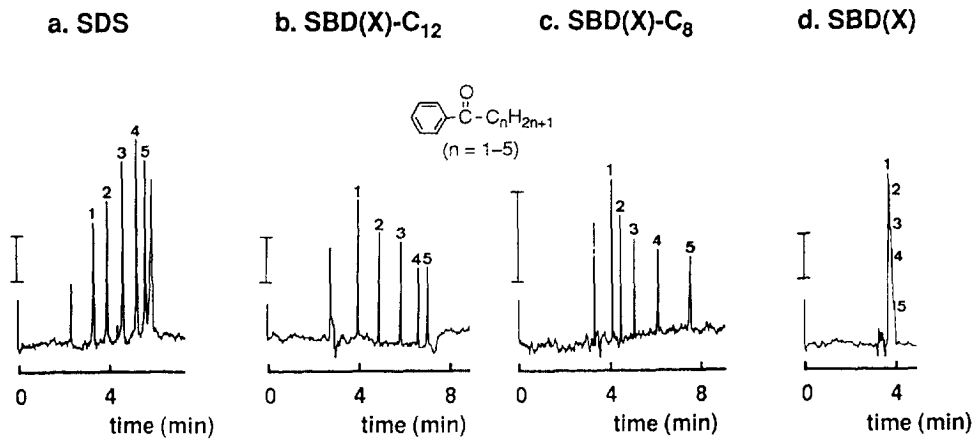
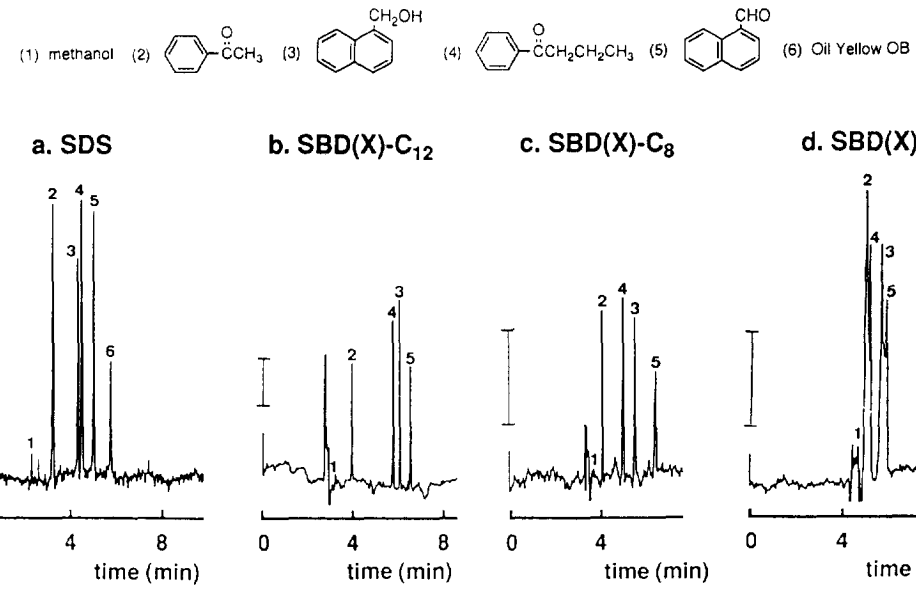


Fig. 2. Comparison of carriers for the separation of alkyl phenyl ketones. (a) SDS 30 m*M*, pH 9.4, (b) SBD(X)-C_{12} 5 m*M*, pH 10.4, (c) SBD(X)-C_8 5 m*M*, pH 10.1, and (d) SBD(X) 5 m*M*, pH 10.3. Buffer solution: 20 m*M* borate. Solute: alkyl phenyl ketones (C_6H_5–CO–C_nH_{2n-1}, n = 1–5). Numbers indicate the numbers of carbon atoms in the alkyl group. Field strength 300 V/cm. The last peak in (a) is Oil Yellow OB.



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different skeletons, benzene and naphthalene derivatives, but did not separate well the compounds with a difference in alkyl or functional groups on one skeleton. The hydrophobic recognition is very poor with this carrier [22]. This is in contrast to SDS–MEKC, where benzene derivatives with hydrophobic substituents are retained longer than naphthalene derivatives with hydrophilic substituents. The alkylated SBDs showed selectivities which lay in-between, with greater differentiation for the benzene and naphthalene derivatives than in SDS–MEKC, and greater differentiation for alkyl and polar substituents on one skeleton than the parent SBD(X). SBD(X)-C_8 showed selectivities somewhat similar to the parent SBD(X), and SBD(X)-C_{12} to SDS micelle. The contribution of SBD skeleton to the solute binding seems to be more significant with SBD(X)-C_8.

3.3. SBD–EKC in methanol–water mixtures

Fig. 4 shows the separation of alkyl phenyl ketones in 20–80% methanol with SBD(X)-C_{12} and SDS micelle as a carrier. While SDS–MEKC showed narrow band spacing for hydrophobic alkyl phenyl ketones in the absence of methanol, it showed much greater peak capacity in 20% methanol due to the increased partition of the solutes into the aqueous phase and the increased migration time window because of the reduced electroosmosis. The SDS system, however, showed an abnormal elution profile in 40% methanol, and very narrow migration time windows above 40% methanol. SBD(X)-C_{12} showed consistent separation in the presence of 40–80% methanol. The comparison clearly shows the advantage of a polymer carrier in EKC that is stable against organic solvents.

Figs. 5 and 6 show the separation of aromatic hydrocarbons in 40–80% methanol. Good chromatograms were not obtained for aromatic hydrocarbons in 40% methanol with SDS carrier, as was the case with alkyl phenyl ketones. SDS–MEKC and SBD(X)-C_8 gave similar performance at 60% methanol for aromatic hydrocarbons. At 80%, however, the SDS carrier gave poorer resolution. SBD(X)-C_{12} showed much better performance with a methanol content of 60% or greater for highly hydrophobic com-

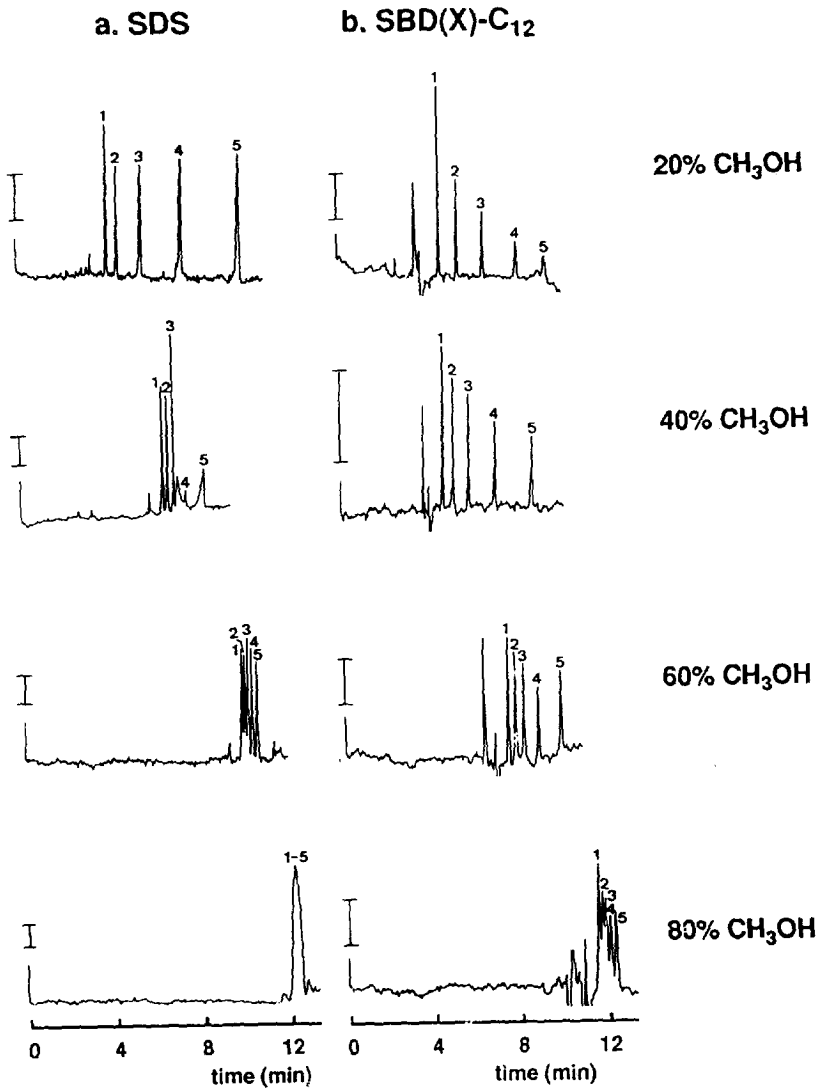

Fig. 4. Effect of methanol addition on the separation of alkyl phenyl ketones at 20–80% methanol. Solutes: alkyl phenyl ketones, C_6H_5–CO–C_nH_{2n+1} (n = 1–5), as in Fig. 2. (a) 30 m*M* SDS, 20 m*M* borate, 400 V/cm, pH 9.3 in 20% methanol, pH 9.3 in 40% methanol, pH 9.0 in 60% methanol, and pH 9.5 in 80% methanol; (b) 5 m*M* SBD(X)-C_{12}, 20 m*M* borate; pH 10.2, 400 V/cm in 20% methanol; pH 10.8, 500 V/cm in 40% methanol; pH 11.0, 500 V/cm in 60% methanol; and pH 11.2, 500 V/cm in 80% methanol.

pounds. The peak capacity for these solutes with this system is maximum at 60% methanol.

The improvement of separation with SBD(X)-C_{12} was mainly due to the following two factors: (i) the increase in migration time of the solutes was provided by the increase in the migration time of the carrier at higher methanol content, and (ii) the optimum k' range was attained by the decrease in k' values at higher methanol content. Relatively small k' values, 1–3, are required for optimum resolution [2–4].

It is interesting to note the elution-order change of the aromatic hydrocarbons with a difference in planarity with methanol content. The relative migration time of the bulky molecules, peaks No. 1 and 2 in Fig. 5, clearly shows

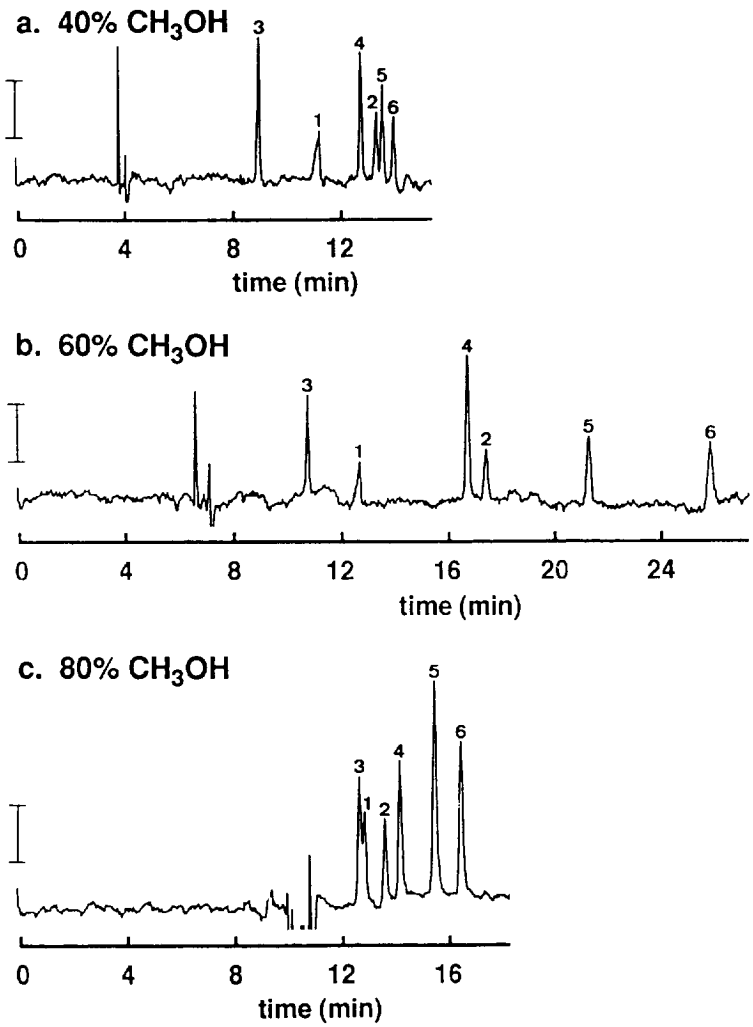

Fig. 5. Effect of methanol addition on the separation of aromatic hydrocarbons with SBD(X)-C_{12}. SBD(X)-C_{12} 5 m*M*, 20 m*M* borate, 500 V/cm. (a) pH 10.8 in 40% methanol, (b) pH 11.0 in 60% methanol, (c) pH 11.2 in 80% methanol. Solutes: 1 = diphenylmethane, 2 = *ortho*-terphenyl, 3 = naphthalene, 4 = anthracene, 5 = pyrene, 6 = triphenylene.

the tendency of preferential retention of planar polynuclear aromatic hydrocarbons relative to non-planar, bulky polyphenylalkanes with SBD(X)-C_{12} at higher methanol content. The results with SBD(X)-C_{12} in EKC are similar to those with C_{18} stationary phases in RPLC, where planar compounds are preferentially retained at higher methanol contents [29]. The effect was not observed with short alkyl bonded phases, and seems to be related to the ordering of long alkyl chains at a higher methanol content [29,30]. The effect of organic solvent content on the solute–carrier interaction will be an interesting subject to study.

The plots of t_R/t_0 against methanol content in Fig. 7 indicate that the migration time window first increases by the addition of methanol to SDS–MEKC and SBD(X)-C_{12} systems, as shown in Figs. 4 and 5. The separation with the SDS system, however, quickly diminishes upon further addition of methanol. The SDS system failed to provide separation even for the highly

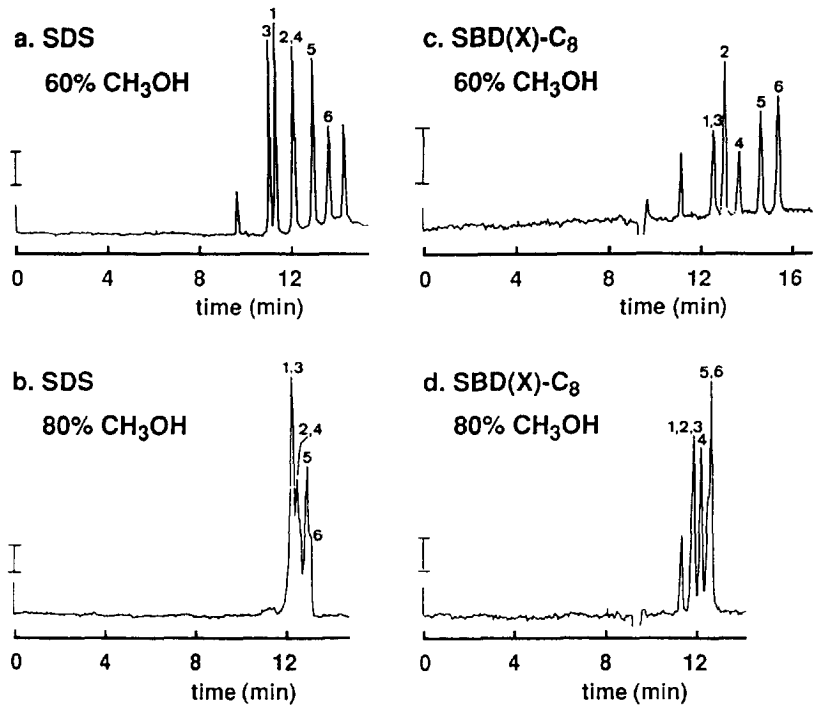

Fig. 6. Effect of methanol addition on the separation of aromatic hydrocarbons with SBD(X)-C_8 and SDS. (a, b) SDS: 30 m*M*, 20 m*M* borate, 400 V/cm. pH 9.4 in 60% and pH 11.4 in 80% methanol. (c, d) SBD(X)-C_8: 5 m*M*, 20 m*M* borate, 400 V/cm. pH 9.9 in 60% and pH 11.4 in 80% methanol. Solutes as in Fig. 5.

hydrophobic compounds in 80% methanol. In contrast, SBD(X)-C_{12} provided a much greater migration time window at higher methanol contents, with a maximum at 20% for the ketones and at 60% for the aromatic hydrocarbons.

It is generally seen that the migration times and resolution of hydrophobic solutes which migrated near the carrier at low methanol concentration first increase with methanol content, followed by a decrease with further addition of methanol. The composition providing maximum migration time and peak capacity shifts toward the higher methanol content as the solutes become more hydrophobic. Once the maximum is reached, the migration time and peak capacity decrease with an increase in methanol content, as in RPLC.

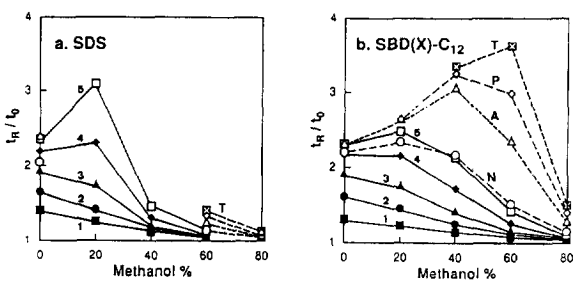

Fig. 7. Plots of t_R/t_0 values for alkyl phenyl ketones and aromatic hydrocarbons against methanol content of the separation solution. Chromatograms are shown in Figs. 2 and 4–6. (a) SDS, (b) SBD(X)-C_{12}. Solutes: alkyl phenyl ketones (n = 1–5) as in Fig. 2, N = naphthalene, A = anthracene, P = pyrene, T = triphenylene.

The results suggest that the SBD(X)-C_{12} keeps its hydrophobic property even at high methanol content, as the stationary phases for RPLC. This was not the case with the SDS micelle, which shows hydrophobic properties similar to or greater than that of SBD(X)-C_{12} in an aqueous system, but much lower in the presence of methanol. The results suggest a change in the composition of the micelle with the surrounding solvent.

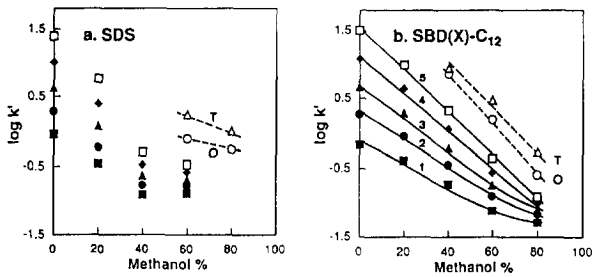

Fig. 8. Plots of log k' values of alkyl phenyl ketones and aromatic hydrocarbons against methanol content. Chromatograms are shown in Figs. 2 and 4–6. Solutes as in Fig. 7.

Fig. 8 shows further similarity of SBD–EKC with SBD(X)-C_{12} to the RPLC system. The nearly linear relation between log k' and methanol content observed with this carrier, but not with SDS–MEKC, is very common in RPLC [31]. It is also noted that the selectivity in SBD–EKC can be altered by methanol content, as shown in Fig. 5. Such selectivity change with methanol content would appear as a cross-over in the plot. This suggests the possibility of optimization of separation conditions by changing the methanol content with these polymeric carriers, as in RPLC. Simulation of the migration profile from k' values requires electroosmotic and electrophoretic mobilities of the carrier.

Fig. 9 shows the separation of some aromatic hydrocarbons with SBD(X)-C_{12} in 90% methanol. This carrier provided separation of hydrophobic compounds at high methanol content. The application of this type of carrier to the separation of more hydrophobic aromatic compounds would be of much interest in environmental analysis [11].

Further study is needed on the effect of the type of organic solvents and the structure of alkyl groups on SBDs on the selectivity in SBD–EKC. SBDs were employed as pseudo-stationary phase for EKC of neutral as well as ionic analytes [32,33]. The present results show that SBDs can be used as a support to construct a wide range of pseudo-stationary phases that may not be possible with a micellar carrier in terms of the density and chain length of the alkyl group. Various pseudo-stationary phases can also be developed by varying interacting groups as well

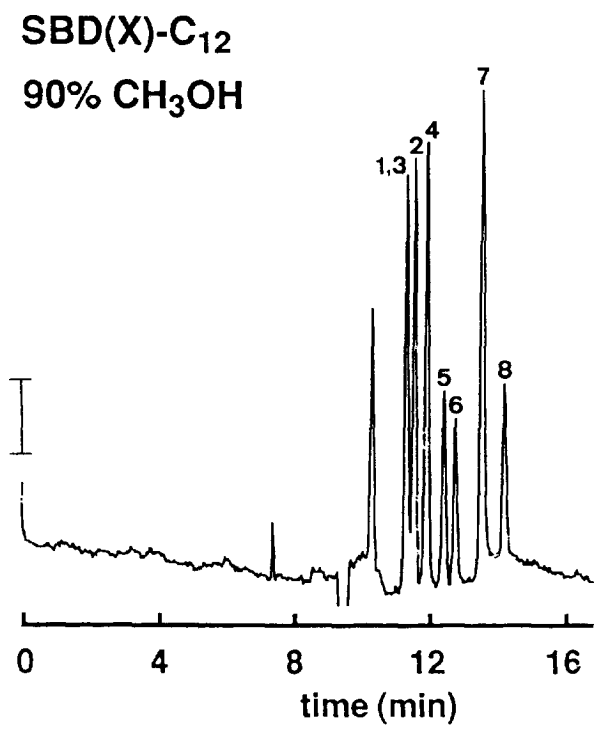

Fig. 9. Separation of aromatic hydrocarbons with SBD(X)-C_{12} in 90% methanol–10% 20 mM borate buffer, 5 mM SBD(X)-C_{12}, pH 10.1, 500 V/cm. Solutes: 1–6 = aromatic hydrocarbons as in Fig. 5, 7 = 3,4-benzpyrene, 8 = indeno[1,2,3-*cd*]pyrene.

as support structures. Suitable hydrophilic polymers that can be used as a support will further increase the usefulness of EKC. In the case of RPLC, stationary phase modification is mostly limited to silica surfaces.

4. Conclusion

SDS–MEKC showed excellent performance at low methanol content, but is not suitable for the separation of hydrophobic compounds at high methanol content. The alkylated SBDs showed increased retention and hydrophobic selectivity compared with the parent PAMAM-SBD(X). Selectivity can be altered with the chain length of the alkyl group. SBD(X)-C_{12} can be used in 0–90% methanol and provided high efficiency as well as a much wider migration time window, especially for hydrophobic compounds, than

SDS–MEKC. A nearly linear relation was observed between log k' values and methanol content. Optimum separation conditions can be attained for a wider range of solutes in SBD–EKC by adjusting the organic solvent content.

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