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Structural selectivity provided by starburst dendrimers as pseudostationary phase in electrokinetic chromatography

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Abstract

Starburst dendrimers (SBDs) were used as a pseudostationary phase in electrokinetic chromatography (EKC) of hydrophobic compounds. The selectivity of SBD-mediated EKC (SBD-EKC) was different from those in micellar EKC (MEKC) systems, in spite of the apparent structural resemblance between micelles and SBDs. The SBDs provided similar selectivity as polymer gel packing materials in reversed-phase liquid chromatography (RPLC), showing little selectivity for alkyl groups and clear preference for aromatic compounds, especially for rigid, planar polynuclear aromatic hydrocarbons.

The alkylation of SBDs resulted in the increased retention and hydrophobic selectivity while maintaining the preference toward rigid, planar compounds. These SBDs can be used in a full range of methanol–water mixtures, showing the retention decrease with the increase in methanol content as in RPLC. The results suggest that SBDs can make a support for various pseudostationary phases for EKC.

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a highly efficient separation technique with wide applicability [1–4]. The micelles work as a separation carrier or a pseudostationary phase in electrokinetic chromatography (EKC). The solutes partition between the micelle phase and the surrounding water phase to undergo a chromatographic process based on the electroosmosis of aqueous solution and the electrophoresis of the micelle phase. Thus EKC is usually carried out in aqueous media with micelle solubilization as a retention process.

The solvents should be aqueous in order not only to be conductive, but also to allow the formation of micelles in equilibrium with the monomeric surfactant molecules. The use of organic solvents is somewhat limited by these constraints, although they have been studied intensively to broaden the applicability of this technique [5–10]. Various surfactants have also been explored for wider applications [4,11–13], including microemulsion [14] and polymeric electrolytes [15–17]. A variety of host molecules, proteins, and carbohydrate derivatives were employed for the separation of chiral species. (See

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for example Ref. [18]) The study in the structure–selectivity relationship in EKC carriers will help designing new separation carriers.

MEKC is a separation technique primarily for solutes with significant solubilities in both aqueous and organic phase. Thus MEKC of hydrophobic molecules that would mostly partition into micelle phase have been carried out by adding urea [19], cyclodextrins [20–23] or organic solvents [6,7,10] to the aqueous phase to effect the partition of such compounds into the aqueous phase. These methods, however, could be accompanied by practical difficulties including narrow migration windows, the limitation in the type and concentration of organic solvents, mechanistic complexities, or the necessity for high surfactant concentrations. The use of solvent-stable polymeric carriers [15–17] would alleviate these problems. Such systems should possess advantages experienced in RPLC where the solute retention can be manipulated simply by changing the concentration of organic solvents in mobile phase. In the case of MEKC the carrier concentration and composition are variable with the change in organic solvent content.

We have examined starburst dendrimers [poly(amidoamines), SBDs] [24,25] and compared with sodium dodecyl sulfate (SDS) micelles as carriers in EKC [26]. The SBDs possess highly symmetrical dendritic structures with overall spherical shape. Micelles and SBDs are somewhat similar in that they have ordered structures and the charged terminal groups, and the analogy has been discussed [27–29]. They also possess differences in structure. A micelle is an assembly of small molecules, while a SBD is a macromolecule. The cores of micelles are dense, fluid, and hydrophobic, whereas the core is void, rigid, and could be hydrophilic in SBDs. Alkyl chains are straight in micelles in many cases, whereas chains are highly branched in SBDs leading to the reduced mobilities.

SBDs are known to bind organic molecules in a relatively hydrophilic environment, as studied with a fluorescent probe [30]. As previously reported, SBDs functioned as a pseudostationary phase in EKC, and the selectivity of SBD carriers was different from that of micellar carriers [26,31]. As SBDs possess many functional groups, further modification of SBD structure is feasible. It will provide pseudostationary phases with different selectivity, as realized with various silica-bonded phases as well as polymer-based stationary phases in RPLC. When SBDs are to be considered as a part of a drug-delivery system [32], the SBD-EKC systems seem to be a very useful means for measuring the binding constants between various small molecules and the SBDs.

We report here the use of SBDs with ammonia and *p*-xylylenediamine cores, as well as the one with octyl modification in EKC. The results indicate the similarity between SBD carriers in EKC and polymer gel packing materials in RPLC when compared with SDS-MEKC and silica C_{18} packing material, respectively. SBDs can be a support of various pseudostationary phases to be used in a full range of water–organic solvent mixtures.

2. Experimental

2.1. Equipment

HPCE

A high-voltage power supply, HepLL-30PO.08 (Matsusada Precision Devices, Kusatsu, Japan), and a variable-wavelength UV detector, UV-8 Model II (Tosoh, Tokyo, Japan) were used. Detection was at 210 nm for SDS-MEKC systems and at 254 nm for SBD systems. Detection at a wavelength below 240 nm is not practical with SBDs. Fused-silica capillary of 50 μm I.D. × 0.375 mm O.D. (Polymicro Technologies, Phoenix, AZ, USA) was used at ambient temperatures.

HPLC

The HPLC system consisted of a 880PU solvent-delivery system (JASCO, Tokyo, Japan) and a 440 UV detector (Waters Assoc., Milford, MA, USA) operated at 254 nm. A data processor, C-R6A (Shimadzu, Kyoto, Japan) was used with both EKC and HPLC systems. The columns were kept at 30°C with a water bath.

NMR

^{1}H NMR measurements were carried out on an XL-200 NMR instrument (Varian, Sunnyvale, CA, USA).

2.2. Materials

Preparation of SBDs

The SBD(X) and SBD(A) were prepared starting from *p*-xylylenediamine (X) and ammonia (A) as a core, respectively, according to the method reported by Tomalia et al. [27], as shown in Fig. 1. Michael addition of N–H group of the core molecule to the double bond of methyl acrylate produced a SBD of generation 0.5 (G = 0.5) having terminal ester groups, and the subsequent aminolysis with ethylenediamine produced a full-generation SBD (G = 1.0) having terminal amino groups. The higher generations were obtained by repeating the Michael addition and aminolysis. The size of the SBDs is thus described according to the original generation system [27], the generation corresponding to the number of reaction cycles starting from the core.

Hydrolysis of half-generation SBDs

All the 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 [28]. The product was added to diethyl ether to precipitate the carboxylate-form SBD.

Alkylation of SBDs

SBD(X, G = 3.5) (4.0 g) was dissolved in methanol (50 ml), to which octylamine (3.3 g, 1.2 mol equivalent to the ester groups) was added. The solution was stirred for five days. After examining the extent of alkylation by NMR, sodium hydroxide (0.65 g, 1 mol 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.

Characterization of SBDs

Monodispersity of SBDs were examined by size-exclusion chromatography by using TSK-G3000PW, 60 cm × 7.6 mm I.D. (Tosoh) in 50 m*M* phosphate buffer at pH 11. Standard SBDs (G = 2.0, 4.0, and 6.0) commercially obtained (Polysciences, Warrington, PA, USA) were used, and they showed bimodal molecular mass distributions. The reaction cycle in SBD preparation was followed by NMR and elemental analysis. The degree of alkylation was examined by NMR.

RPLC stationary phases

Silica C_{18} stationary phase prepared by a reported procedure [33] and TSK-Octadecyl 4PW (Tosoh) were used as a column of 15 cm × 4.6 mm I.D. The latter is a polymer gel packing consisting of octadecylated poly(hydroxyalkyl acrylate or methacrylate) [34].

2.3. Measurement

EKC

All separation solutions were filtered with a membrane filter. Apparent pH values were measured by a pH meter. Injection was carried out by a siphoning method. The k' values were calculated by using an equation, $k' = (t_R - t_0)/t_0(1 - t_R/t_c)$ [2–4], where t_R, t_0 and t_c stand for the elution time of a solute, an unretained solute and a carrier, respectively. Methanol or formamide was used to measure t_0 values, and Oil Yellow OB for t_{mc} in SDS-MEKC in aqueous buffer [35,36]. The t_{mc} in the presence of methanol and all t_{SBD} values were calculated based on the iterative method [37,38] assuming the linear relation between log k' and carbon numbers of alkylbenzenes and alkyl phenyl ketones. The t_{SBD} values were also estimated from the electrophoretic mobility of SBDs in aqueous buffer and electroosmotic mobility in EKC systems.

HPLC

Liquid chromatography was carried out at 30°C, with a flow-rate of 1.0 ml/min. The EKC and HPLC measurements were carried out in duplicate.

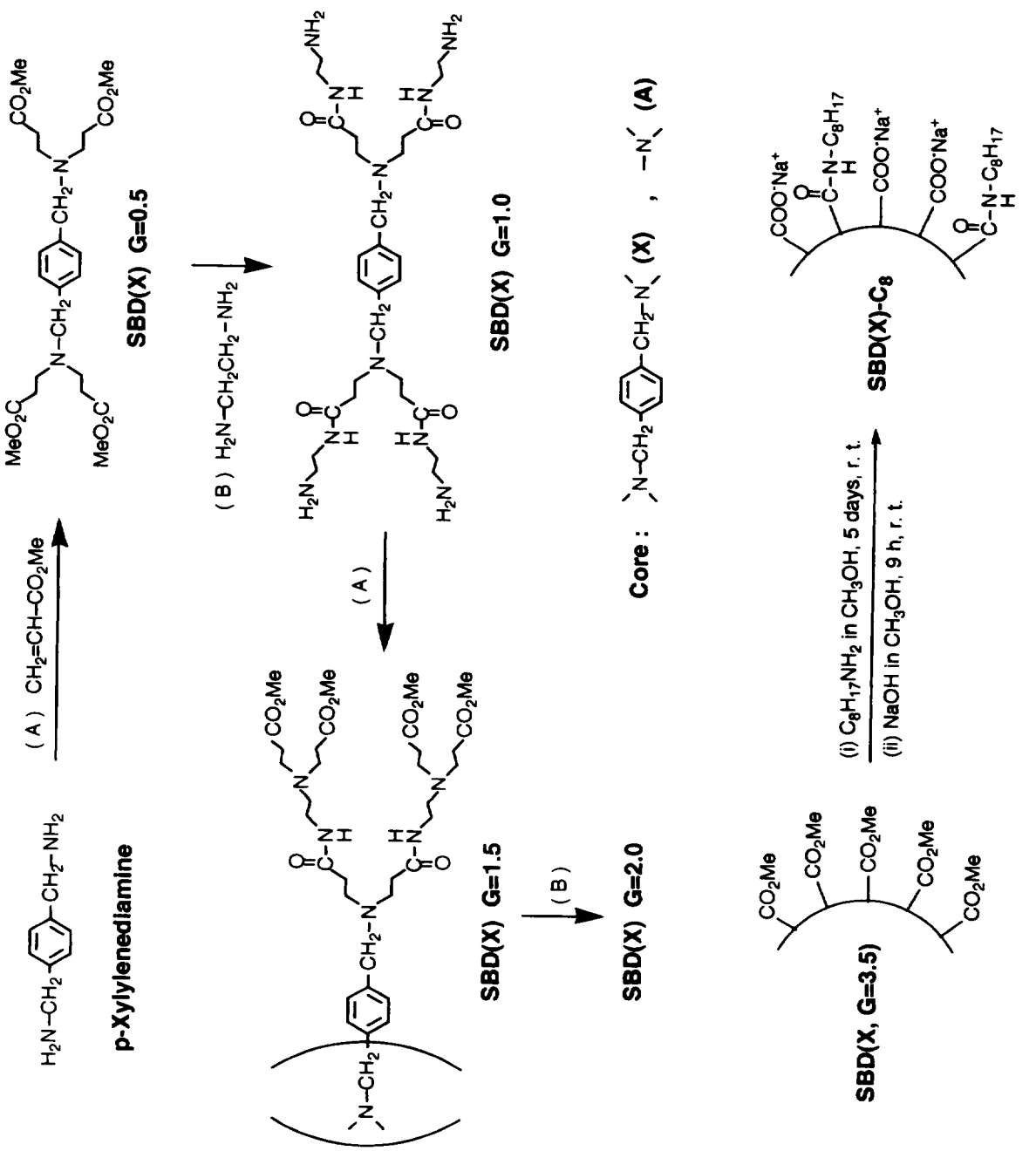

Fig. 1. Preparation of starburst dendrimers [24] and their alkyl derivatives.

3. Results and discussion

3.1. Preparation of SBDs and the alkylated derivatives

The SBDs used in this study, poly(amidoamines), possess alternating amide and *tert.*-amine functionalities, as shown in Fig. 1. One can use either an ester form, called a half generation, or an amine form, called a full generation [24,25], for EKC. By hydrolyzing the ester group of half-generation SBDs, one can get a carboxylate form. Then, the structures of half-generation and full-generation SBDs are somewhat similar to anionic and cationic micelles, respectively. The presence of tertiary amino groups in all poly(amidoamine)-type SBDs should be noted.

The molecular mass of a SBD is almost doubled with one full reaction cycle, attaining the size of ordinary micelles at a relatively low molecular mass (G = 3 or 4) due to the sparse internal structures. The preparation of SBDs becomes increasingly hard as the molecular size increases [24,25]. The SBD(X, G = 3.5) corresponds to about 5 nm size with a molecular mass of about 6000. Alkylation of SBDs were carried out by reacting alkylamines with half-generation SBDs to form amide bonds with the terminal ester groups. The resulting partially alkylated SBDs maintain the solubility in water as long as the SBDs possess prevailing numbers of external ionic groups. NMR measurement indicated that the degree of alkylation to be about 20% in the case of SBD(X)-C_8 prepared from SBD(X, G = 3.5) by the reaction with octylamine. This indicates that about six octyl groups were introduced in 32 terminal groups in SBD(X, G = 3.5) on an average.

3.2. Selectivity of SBD-EKC

Increased retention and separation were observed with the increase in the generation of SBDs. The benzene derivatives were well resolved with SBD(A, G = 4.0) or greater by using full-generation dendrimers in an amine form, as shown in Fig. 2. Very similar results were obtained with the half-generation SBDs. Note that the concentration of G = 4.0 is half that of G = 3.0 in Fig. 2. We observed differences in selectivity between the full-generation and half-generation SBDs [26]. The SBD system also showed different selectivity from MEKC with either SDS or cetyltrimethylammonium chloride micelles [26].

As expected from the presence of both the external carboxyl groups and the internal *tert.*-

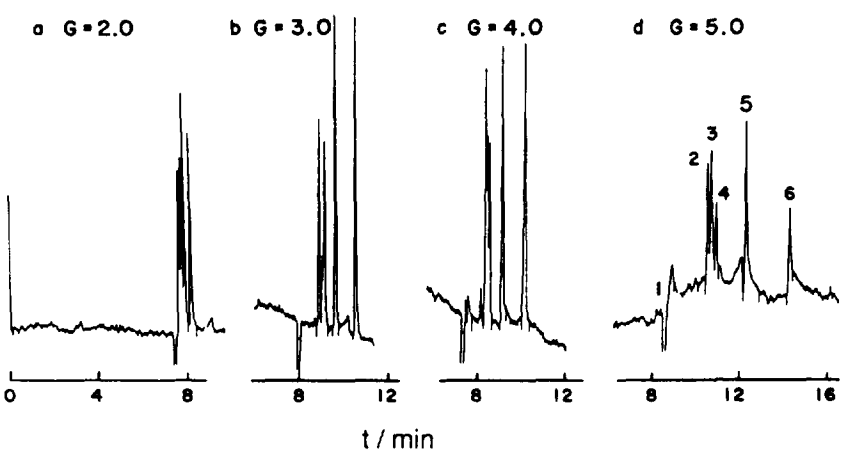

Fig. 2. Effect of SBD size on EKC separation. (a, b) 10 m*M* SBD(A, G = 2.0 and 3.0), (c, d) 5 m*M* SBD(A, G = 4.0 and 5.0), 50 m*M* acetate buffer, pH 5.0. 200 V/cm. Peaks: 1 = methanol; 2 = 3-phenyl-1-propanol; 3 = anisole; 4 = nitrobenzene; 5 = 1-naphthalenemethanol; 6 = 2-naphthol.

amino groups in the carboxylate form SBDs, the direction and the magnitude of the electroosmotic flow depend on the pH of the system, as shown in Fig. 3. At low pH, the reversed osmotic flow was observed, whereas at high pH a regular osmotic flow was observed. This effect should be related to the suppression of the ionization of the terminal carboxyl groups and the internal tertiary amino groups in SBDs at either pH extremes. Full-generation dendrimers always caused a reversed osmotic flow, presumably due to the adsorption of SBDs of polyamine structures onto the capillary wall. For the later part of this study, we mainly worked at pH > 9 where carboxyl groups are fully ionized and the ionization of alkylamino groups suppressed to compare the carboxylate form SBDs with the SDS micelles as carriers in EKC.

Fig. 4 shows the comparison of selectivity between SDS-MEKC and SBD-EKC. The difference in selectivity is obvious. The hydrophobic benzene derivatives eluted in-between the naphthalene derivatives with hydrophilic substituents in SDS-MEKC, whereas clear separation was seen between the two types of compounds with different carbon skeletons with the SBD system. It is interesting to note that the SBDs did not differentiate the functional groups on one type of carbon skeleton, but they differentiated the skeleton. It would be of much interest to compare the selectivity with those obtained with dimeric or polymeric surfactants [11,15–17].

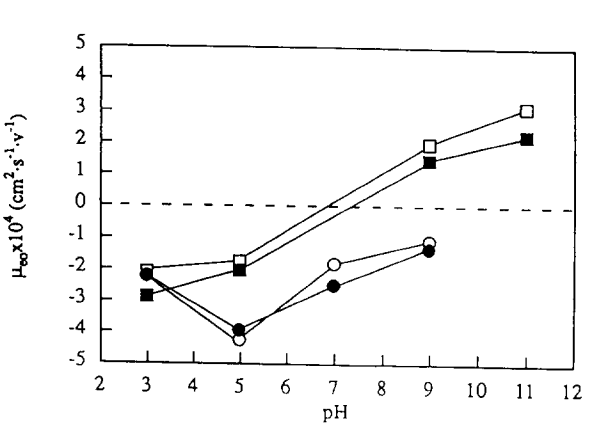

Fig. 3. Effect of pH on electroosmotic flow in SBD-EKC systems. ○: G = 2.0; ●: G = 3.0; ■: G = 2.5; □: G = 3.5; 200 V/cm. SBD 10 m*M*.

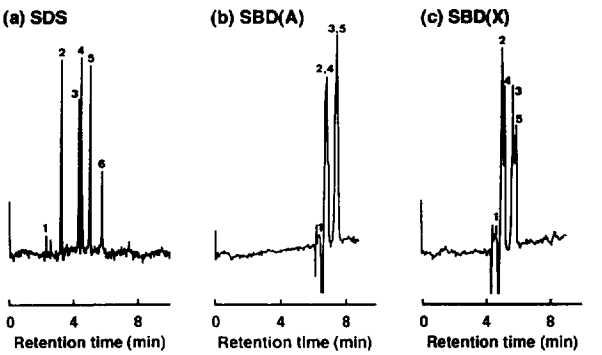

Fig. 4. Difference in selectivity between SDS-MEKC and SBD-EKC. $L = 48$ cm, $l = 33$ cm. Conditions: (a) 210 nm detection, 30 m*M* SDS, 20 m*M* borate buffer pH 8.9, (b) 254 nm detection, 5 m*M* SBD(A, G = 3.5), 20 m*M* borate buffer pH 8.5, (c) 254 nm detection, 5 m*M* SBD(X, G = 3.5), 20 m*M* borate buffer pH 10.0. Peaks: 1 = methanol; 2 = acetophenone; 3 = 1-naphthalenemethanol; 4 = phenyl propyl ketone; 5 = 1-naphthaldehyde; 6 = Oil Yellow OB.

3.3. Comparison with RPLC systems

The difference in selectivity provided by stationary phase is well known in RPLC [39]. Fig. 5 shows the plots of log k' values on polymer gel against log k' values on silica C_{18} for benzene and naphthalene derivatives in RPLC. These stationary phases show a clear difference in selectivity. Silica C_{18} phase consists of a monolayer of hydrophobic alkyl chains having flexible liquid-like structure. In contrast, polymer gels possess rigid cross-linked network structures with many branches that are not so hydrophobic as alkyl chains on silica C_{18}. Polymer gels showed preferential retention for naphthalene derivatives over benzene derivatives relative to the silica C_{18}. The SBD-EKC showed similar tendency to a much greater extent, relative to SDS-MEKC.

The reason why the naphthalene skeleton is favored so much by SBDs in comparison with a benzene ring is not clear at present. One possible

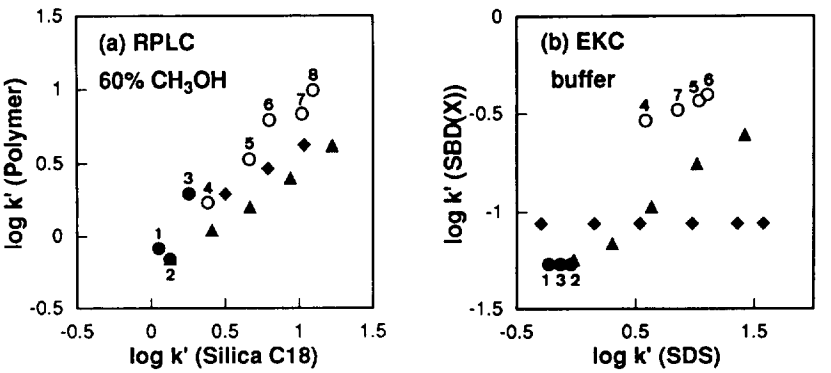

Fig. 5. Comparison of selectivity between RPLC and EKC. EKC conditions as in Fig. 4. See Experimental for RPLC conditions. Solutes: 1 = benzaldehyde; 2 = acetophenone; 3 = nitrobenzene; 4 = 1-naphthalenemethanol; 5 = 1-naphthaldehyde; 6 = 1-nitronaphthalene; 7 = naphthalene; 8 = 1-chloromethylnaphthalene. ▲: $PhCOC_nH_{2n+1}$ ($n = 1$–5); ◆: PhC_nH_{2n+1} ($n = 0$–5), $n = 0$–2 for RPLC.

explanation is based on the contribution of the presence of stiff chains due to the presence of many amide bonds and branching that favor rigid molecules compared with the solute with alkyl groups of similar hydrophobic property. Another possible explanation is based on the difference in the size of the aromatic ring that undergoes preferential association with the SBD backbones. The branches in SBDs become increasingly dense and crowded at the external surface to attain spherical shape [30]. The results showed preferential retention of large molecules that are to be retained near the core, compared to the small molecules to be retained near the hydrophilic terminals.

Steric selectivity provided by the stationary phases in RPLC is most clearly seen with the hydrocarbons having different rigidity and bulkiness [39], shown in Fig. 6. Easy separation was seen for alkylbenzenes in SDS-MEKC in 60%

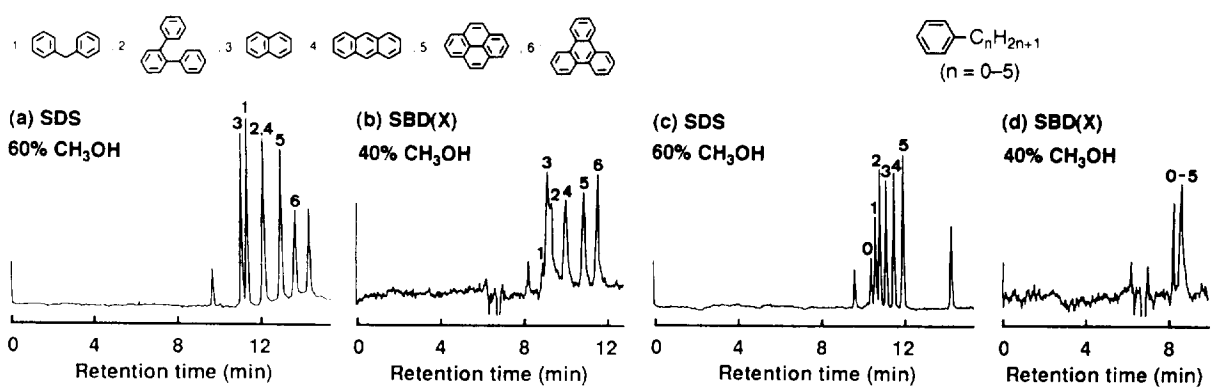

Fig. 6. Difference in selectivity between SDS-MEKC and SBD-EKC for aromatic hydrocarbons. $L = 48$ cm, $l = 33$ cm. Conditions: (a, c) 210 nm detection, 30 m*M* SDS, 20 m*M* borate buffer–methanol (4:6), pH 9.4; (b, d) 254 nm detection, 5 m*M* SBD(X, G = 3.5), 20 m*M* borate buffer–methanol (6:4), pH 10.6. Peaks: (a and b) 1 = diphenylmethane, 2 = *ortho*-terphenyl, 3 = naphthalene, 4 = anthracene, 5 = pyrene, 6 = triphenylene; (c and d) PhC_nH_{2n+1} ($n = 0$–5).

methanol according to the size of the alkyl group. The most hydrophobic alkylbenzene tested, amylbenzene, eluted very close to *ortho*-terphenyl and anthracene, having three aromatic ring systems (Fig. 6a and c).

The free energy change associated with the transfer of one methylene group from the aqueous phase to the organic phase, $G^0(CH_2)$, can be calculated from the separation factor, $\alpha(CH_2)$, which corresponds to the difference in retention or partition coefficient by one methylene unit. The $G^0(CH_2)$ in 60% methanol is −187 cal/mol for SDS-MEKC (1 cal = 4.184 J), much smaller than with the stationary phases in RPLC systems, −284 cal/mol for the polymer gel, and −395 cal/mol for C_{18} phase with the same mobile phase. Small free energy changes have been reported with micelle partition [40]. No separation of alkylbenzenes was observed with SBD(X, G = 3.5) indicating very low hydrophobic property of these carriers for alkyl groups. This is presumably due to the presence of hydrophilic amide and amino groups in the SBD structure. The alkylbenzenes eluted before the naphthalene or other aromatic hydrocarbons with two or more aromatic rings in SBD-EKC.

A difference in elution order was also seen with the hydrocarbon molecules having the difference in molecular planarity, as shown in Fig. 6. While triphenylene and fluorene are rigid, planar molecules, *ortho*-terphenyl and diphenylmethane, having the same numbers of carbon atoms and double bonds, are bulky molecules. Diphenylmethane (peak 1) and *ortho*-terphenyl (peak 2) eluted very early in the SBD system, whereas the planar aromatic hydrocarbons were retained much longer. Fluorene eluted between naphthalene and anthracene.

The plots of log k' on polymer gel against those on silica C_{18} (Fig. 7a) clearly shows the preferential retention of rigid, compact solutes by the polymer gels compared with the C_{18} in RPLC. The retention on polymer gel increases in the order, alkanes, cycloalkanes, alkylbenzenes, polyphenylalkanes, and rigid planar polynuclear aromatic hydrocarbons; that is the order of increasing rigidity in structure. Selectivity difference between polymer gel and silica C_{18} is also seen with aliphatic hydrocarbons that are free from strong electronic effects indicating the contribution of steric effects [34].

Fig. 7b shows similar plots between the log k' values in SBD-EKC and those in SDS-MEKC. There is an obvious similarity between Fig. 7a and b. The SBD preferentially retained the rigid, compact hydrocarbons relative to SDS micelles, just like the polymer gels in RPLC relative to silica C_{18}. The retention characteristics of SBDs that is similar to polymer gels in RPLC can be understood by taking into account the contribu-

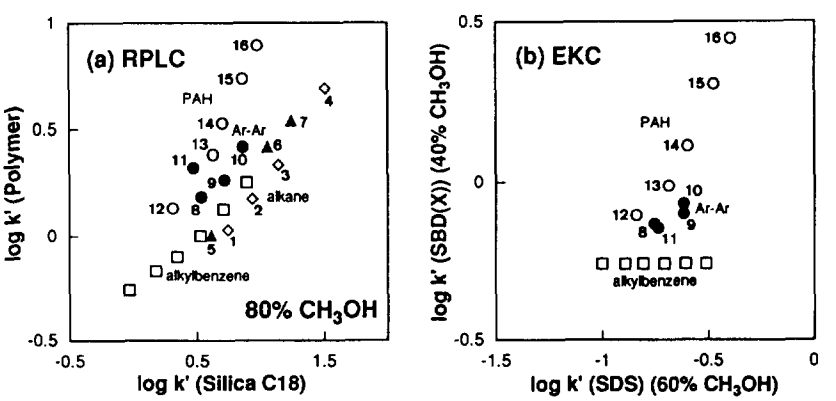

Fig. 7. Comparison of selectivity between RPLC and EKC. EKC conditions as in Fig. 6. See Experimental for RPLC conditions. Solutes: □ = PhC_nH_{2n+1} (n = 0–5); 1 = hexane; 2 = heptane; 3 = octane; 4 = decane; 5 = cyclohexane; 6 = adamantane; 7 = *trans*-decalin; 8 = diphenylmethane; 9 = *ortho*-terphenyl; 10 = triphenylmethane; 11 = triptycene; 12 = naphthalene; 13 = fluorene; 14 = anthracene; 15 = pyrene; 16 = triphenylene.

tion of the hydrophilic, rigid structure of highly branched polymers, as mentioned above. Similar selectivity was observed with poly(methyl methacrylate)-coated silica packing in RPLC where the polymer was not cross-linked [41].

3.4. Alkylated SBDs as a carrier

In spite of the interesting steric selectivity, the use of SBDs as carriers in EKC as they are may be accompanied by some difficulty. Due to the hydrophilic nature, SBD provided relatively short retention. Increase in the concentration resulted in high current leading to noisy baseline. High-generation SBDs can give longer retention, but their preparation is not easy. The efficiency of the SBD-EKC system with SBD(A) or SBD(X) was slightly lower than that of the SDS system. Size monodispersity of carriers and slow equilibration associated with polymeric carriers can work against each other affecting the efficiency in the EKC system.

EKC with an alkylated SBD, SBD(X)-C_8, provided better efficiency with better peak shape, more stable baseline, and much longer retention for the aromatic compounds than the parent SBD(X, G = 3.5), as shown in Fig. 8. The efficiency was slightly better than in SDS-MEKC. The polymer carrier SBD(X)-C_8 can be used in a wide range of methanol–water mixtures (0–80% methanol) just like the stationary phase in RPLC by simply changing the organic solvent content in the electrolyte solutions. Retention decrease was observed with the increase in methanol concentration in the SBD-EKC system, as commonly seen in RPLC. The structural selectivity, or the preference toward rigid planar aromatic hydrocarbons, was maintained.

Narrow separation time windows are seen in SDS-MEKC for hydrophobic compounds. The addition of organic solvents to SDS-MEKC systems can provide wider migration windows by reducing electroosmotic flow and increasing the solute partition into the aqueous phase containing organic solvents. The increase in organic solvent concentration, however, would also affect micelle concentration and composition in addition to the extent of solute–solvent interaction in the aqueous phase, resulting in narrow migration windows at high organic solvent content. Higher concentration of surfactants for the operation of MEKC systems at high organic

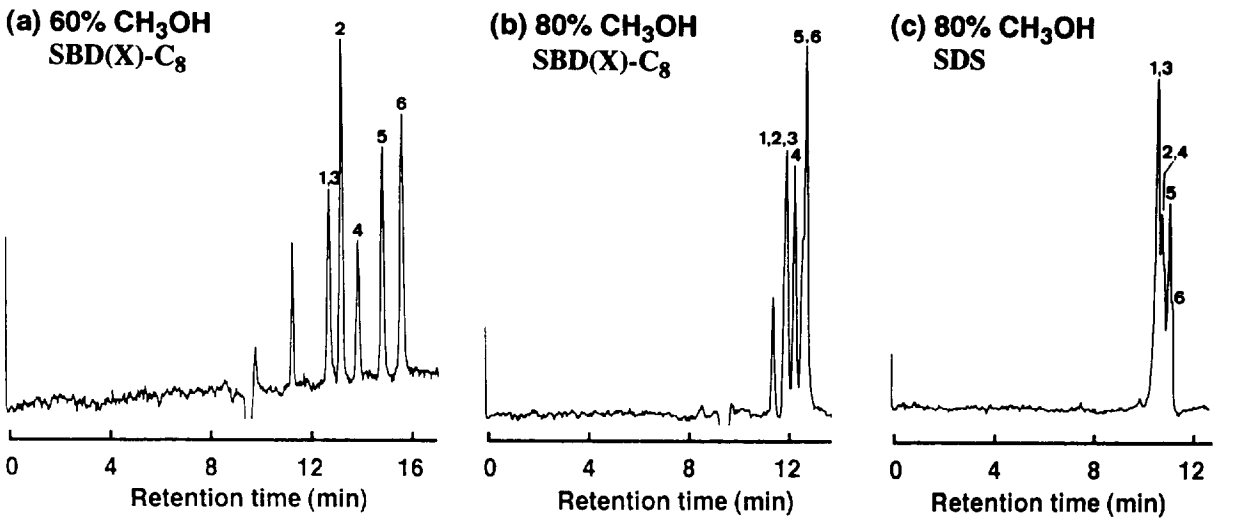

Fig. 8. SBD-EKC with an octylated SBD as a carrier as compared with SDS-MEKC in methanol–water mixtures. $L = 48$ cm, $l = 33$ cm, 254 nm detection. Conditions: (a) 5 mM SBD(X)-C_8, 20 mM borate buffer–methanol (4:6), pH 9.9. (b) 5 mM SBD(X)-C_8, 20 mM borate buffer–methanol (2:8), pH 11.4. (c) 30 mM SDS, 20 mM borate buffer–methanol (2:8), pH 11.4. Solutes as in Fig. 6a and b.

solvent contents can have practical difficulty of solid formation upon the evaporation of organic solvents. The use of cyclodextrin for the separation of hydrophobic compounds [20–23] can be accompanied by the unpredictable elution orders.

Organic solvent content does not affect the carrier concentration in SBD-EKC, although it might affect the solute retention orders as in RPLC. The alkylated SBDs can provide much wider separation windows for aromatic hydrocarbons compared with SDS-MEKC [42]. It will be of much interest to use SBDs as a support for interacting groups such as electron donors, electron acceptors as well as hydrophobic groups to prepare various pseudostationary phases for EKC. One might be able to control selectivity in EKC, as one can do by using the selective stationary phases in RPLC [39]. This approach will contribute to the further development of EKC.

4. Conclusions

SBDs functioned as pseudostationary phase in EKC. Retention and separation in SBD-EKC increased with the increase in generation of SBDs. The SBD-EKC system is different from SDS-MEKC in that SBDs are more hydrophilic than micelles, and provide separation based on the molecular rigidity, planarity, and the size of the carbon skeletons. SDS micelle showed relatively similar selectivity as silica C_{18}, whereas the SBDs are relatively similar to polymer gel packings in RPLC showing little selectivity for hydrophobic groups as well as for the functional groups. The SBDs seem to recognize the carbon skeletons rather than the substituents. The different selectivity would be useful when selectivity change is required in EKC. Alkylation of SBDs resulted in increase in retention and separation, along with the improvement in the performance. The alkylated SBDs can be used in highly organic medium, showing the SBDs to be a promising support for various interacting groups in EKC pseudostationary phase.

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