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Starburst Dendrimers as Carriers in Electrokinetic Chromatography

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Starburst dendrimers (SBDs) were shown to be useful as carriers in electrokinetic chromatography in aqueous and aqueous-organic systems. Uncharged aromatic compounds were separated under electrophoretic conditions based on the differential binding to the SBDs. The separation was influenced by the size and ionization state of SBDs as well as the organic solvent content of the aqueous phase.

Starburst dendrimers (poly(amidoamines): SBDs)[1] were successfully used as carriers in the separation of uncharged solutes in electrokinetic chromatography (EKC).[2] While the application of micellar EKC (MEKC) in aqueous-organic solvent mixtures is limited due to the instability of micelles,[3] the SBD-mediated EKC (SBD-EKC) can separate hydrophobic compounds in a full range of water-methanol mixtures without using additives such as cyclodextrins[4] or urea[5] in the aqueous phase. The SBD-EKC gave selectivity which is different from those in MEKC systems, in spite of the apparent structural resemblance between micelles and SBDs.[6] SBDs have been attracting considerable interests in the synthesis[7] as well as their interaction with small molecules.[6c,d] The latter can be conveniently studied by the present method.

SBDs were prepared from ammonia by reacting with methyl acrylate and ethylene diamine alternately, as reported by Tomalia and coworkers previously.[1] The size and monodispersity of full generation SBDs were examined by size exclusion chromatography.[8] The composition was also confirmed by elemental analysis at half generations as an ester form. Half generation SBDs were used in EKC as a carboxylate form following hydrolysis.[6c] A fused silica capillary (375 μmOD, 50 μmID. Total length: 50 cm, effective length, from the inlet to the detector cell: 35 cm) was used with an electrophoresis system[10] at ambient temperatures. The half-generation SBDs gave an electroosmotic flow in the direction to the negative electrode above pH 7 as anionic micellar systems, while the full-generation SBDs resulted in the electroosmotic flow in the direction to the positive electrode below pH 11 as cationic micellar systems.

The SBDs worked well as carriers which migrate electrophoretically against the

electroosmotic flow of the bulk aqueous phase in EKC systems. The increase in SBD concentration resulted in a linear increase in capacity factors[11] of solutes as well as the improved separation. As shown in Fig. 1, SBD-EKC resulted in different selectivity from those in the MEKC systems with cetyltrimethylammonium chloride (CTAC) or sodium dodecyl sulfate (SDS), while similar elution orders were obtained in the two MEKC systems. The elution order in SBD-EKC was further influenced by pH.

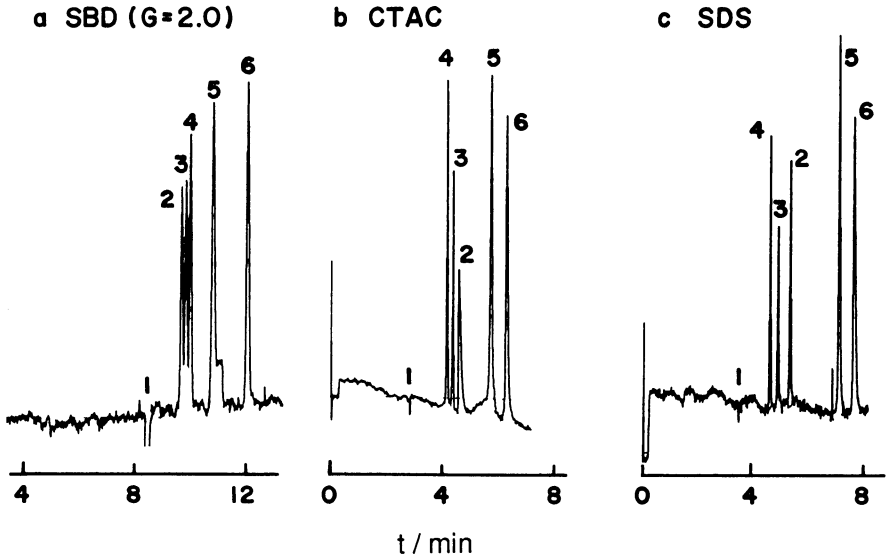

Fig. 1. Separation of some aromatic compounds by EKC. Solutes, (1) solvent front (methanol), (2) 3-phenylpropanol, (3) anisole, (4) nitrobenzene, (5) 1-naphthalenemethanol, (6) 2-naphthol. (a) 15 mM (1 M=1 mol·dm^{-3}) SBD (G=2.0, G; generation of SBD),[9] 50 mM acetate buffer, pH 5.0. 200 V/cm. (b) 30 mM CTAC, 30 mM phosphate buffer, pH 7.0. 400 V/cm. (c) 30 mM SDS, 30 mM phosphate buffer, pH 7.0. 400 V/cm.

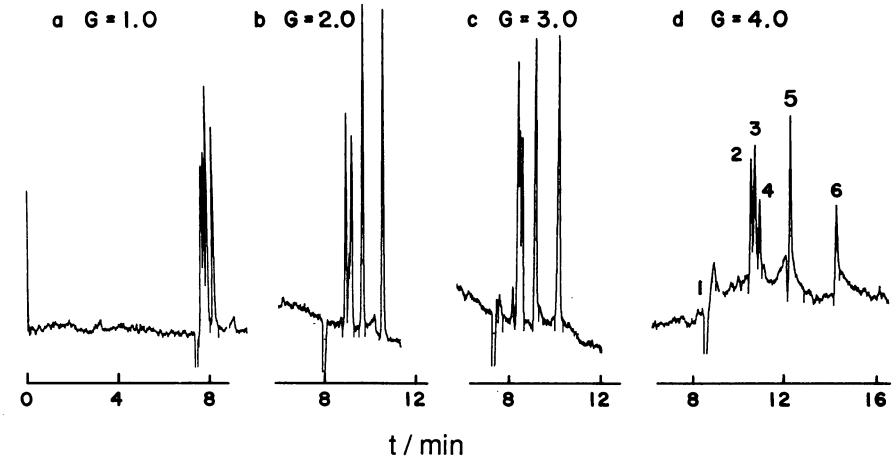

Fig. 2. Effect of SBD size on EKC separation. (a, b) 10 mM SBD (G=1.0 and 2.0), (c, d) 5 mM SBD (G=3.0 and 4.0), 50 mM acetate buffer, pH 5.0. 200 V/cm. Solutes as in Fig. 1.

Figure 2 indicates that the binding of solutes increased with the size of the SBDs, leading to the improved separation with the SBDs of higher generation. Increased binding of hydrophobic molecules to high-generation SBDs has been reported.[6c] The capacity factors for the aromatic compounds with the SBDs, however, were much smaller than those with micelles of a similar

size, presumably due to much smaller hydrophobic properties of the SBDs. The k' value[11] for 2-naphthol was 0.61 for SBD (G=4.0),[9] compared with 3.1 in MEKC with SDS under the conditions for Figs. 1 and 2.

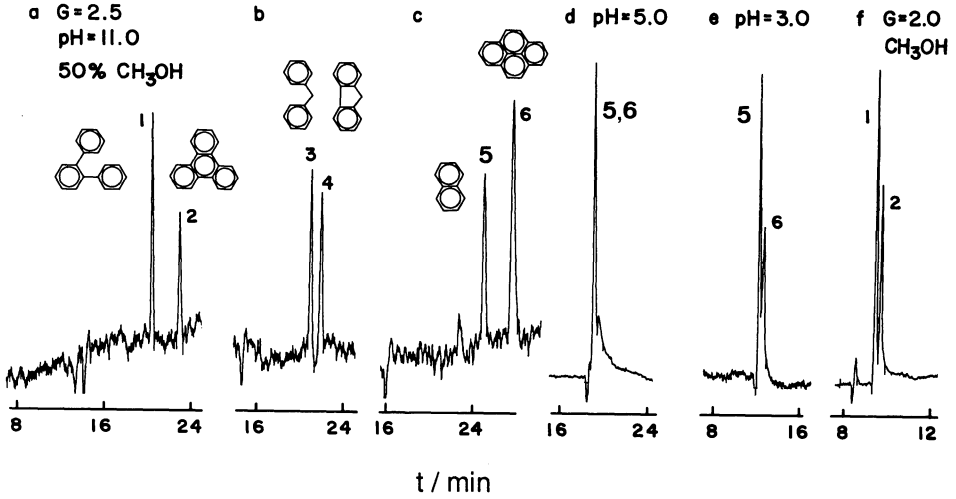

Fig. 3. SBD-EKC separation of aromatic hydrocarbons. Solutes: (1) o-terphenyl, (2) triphenylene, (3) diphenylmethane, (4) fluorene, (5) naphthalene, (6) pyrene. (a-e) 5 mM SBD (G=2.5) in methanol/water=50/50, (a-c) pH 11.0,[12] (d) pH 5.0,[12] (e) pH 3.0,[12] (f) 10 mM SBD (G=2.0) in methanol in the presence of 0.05% acetic acid.

The separation of aromatic hydrocarbons and the effect of pH in SBD-EKC in a methanol-water mixture (50/50 v/v) are shown in Fig. 3a-e. The SBD-EKC using SBD (G=2.5) gave good performance in the presence of methanol. The increase in methanol concentration resulted in reduced binding of the aromatic compounds. It is possible to manipulate the retention and separation of the hydrophobic compounds by simply changing the methanol concentration as in reversed-phase liquid chromatography.

As shown in Figs. 3c-e, greater retention was obtained under alkaline conditions with the SBD (G=2.5). At pH 11, the dissociation of the internal amino groups of the SBD is suppressed, while the dissociation of the external carboxyl groups should be suppressed at pH 3.[1] The results indicate that the hydrophobicity of the internal region of the half-generation SBD plays a major role in binding of these aromatic hydrocarbons, whereas increased binding of hydrophobic molecules at the surface region of higher-generation SBDs has been suggested based on the high density of terminal groups.[6c] Compared with the half-generation SBDs, a full-generation SBD (G=2.0) gave less complete and invariable separation of the aromatic compounds with methanol contents 50% or higher. Figure 3f, however, still suggests the possibility of EKC separation in non-aqueous solvents by using a SBD.

The present preliminary results with relatively small SBDs with molecular weight 2000-10000 indicate that SBDs can be used as solvent-stable carriers in EKC to provide different selectivity from MEKC systems. In addition, SBDs possess other attractive features for the use in EKC including the operational variables in terms of pH and the use of organic solvents as well as the possibility of further structural modification. The SBD-EKC system will also be useful for

examining the effects of the size, symmetry, and the density of branched structures as well as the ionization state on the binding properties of SBDs.

References

1) D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, and P. Smith, *Polym. J.* (Tokyo), **17**, 117 (1985); D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, and P. Smith, *Macromolecules,* **19**, 2466 (1986).

2) S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, and T. Ando, *Anal. Chem.,* **56**, 111 (1984); S. Terabe, K. Otsuka, and T. Ando, *ibid.,* **57**, 834 (1985).

3) K. Otsuka, S. Terabe, and T. Ando, *Nippon Kagaku Kaishi,* **1986**, 950; A. T. Balchunas and M. J. Sepaniak, *Anal. Chem.,* **59**, 1466 (1987); J. Vindevogel and P. Sandra, *ibid.,* **63**, 1530 (1991); The addition of acetonitrile to electrophoresis systems has been reported, although not in an EKC mode. See Y. Walbroehl and J. W. Jorgenson, *Anal. Chem.,* **58**, 479 (1986); S. Fujiwara and S. Honda, *ibid.,* **59**, 487 (1987).

4) S. Terabe, Y. Miyashita, O. Shibata, E. R. Barnhart, L. R. Alexander, D. G. Patterson, B. L. Karger, K. Hosoya, and N. Tanaka, *J. Chromatogr.,* **516**, 23 (1990).

5) S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama, and K. Otsuka, *J. Chromatogr.,* **545**, 359 (1991).

6) a) D. A. Tomalia, M. Hall, and D. M. Hedstrand, *J. Am. Chem. Soc.,* **109**, 1601 (1987); b) D. A. Tomalia, V. Berry, M. Hall, and D. M. Hedstrand, *Macromolecules,* **20**, 1164 (1987); c) G. Caminati, N. J. Turro, and D. A. Tomalia, *J. Am. Chem. Soc.,* **112**, 8515 (1990); d) K. R. Gopidas, A. R. Leheny, G. Caminati, N. J. Turro, and D. A. Tomalia, *ibid.,* **113**, 7335 (1991).

7) A. B. Padias, H. K. Hall, D. A. Tomalia, and J. R. McConnell, *J. Org. Chem.,* **52**, 5305 (1987); K. L. Wooley, C. J. Hawker, and J. M. J. Fréchet, *J. Am. Chem. Soc.,* **113**, 4252 (1991).

8) Column: TSK gel G3000PW, 7.6 mmIDx60 cm, eluent: 50 mM phosphate buffer at pH 11, by using the standard SBDs (G=2.0, 4.0, and 6.0)[9] commercially available (Dow Chem.).

9) According to the newer generation system.[6c]

10) The system consisted of a high voltage power supply (Matsusada, HepLL-30P0.08) and a variable wave-length UV detector for HPLC (Tosoh UV-8II), operated at 254 nm.

11) Calculated by using an equation, $k'=(t_R-t_0)/t_0(1-t_R/t_c)$,[2] where t_R, t_0, and t_c stand for the elution times of a solute, an unretained solute, and a carrier, respectively. The t_c in SBD-EKC was estimated from the electrophoretic velocity measurement with SBDs.

12) Apparent pH measured by a pH meter.

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