ELSEVIER

Journal of Chromatography A, 824 (1998) 119–124

JOURNAL OF CHROMATOGRAPHY A

Short communication

Novel sulfonic acid-modified Starburst dendrimer used as a pseudostationary phase in electrokinetic chromatography

Ann L. Gray[a], James T. Hsu[b,*]

[a]*Department of Chemistry, Lehigh University, Bethlehem, PA 18015, USA*
[b]*Department of Chemical Engineering, Lehigh University, Bethlehem, PA 18015, USA*

Received 30 December 1997; received in revised form 21 July 1998; accepted 29 July 1998

Abstract

Starburst dendrimer macromolecules modified with sulfonic acid terminal moieties were used as a pseudostationary phase in electrokinetic chromatography to separate positional isomers of neutral phenol molecules in an aqueous mobile phase by capillary electrophoresis. Using these dendrimers optimal separation was achieved at a pH below the pK_a values of the phenols and with better performance than with micellar electrokinetic chromatography using sodium dodecyl sulfate micelles.

Keywords: Pesudostationary phase; Phenols; Dendrimers; Starburst dendrimers

1. Introduction

Electrokinetic chromatography (EKC) is a powerful technique that separates both charged and neutral analytes not only on the basis of differences in electrophoretic mobility but also on differences in partitioning between an aqueous buffer and a second phase. Terabe et al. used micelles of the surfactant sodium dodecyl sulfate (SDS) as the secondary phase and thus developed micellar electrokinetic chromatography (MEKC) [1]. However, since micelles are merely aggregates of single surfactant molecules, their size and structure are variable and change with the composition (e.g., ionic strength, pH, organic solvent content) of the electrolyte solution used [2,3].

In the search for a covalently-bound molecule of defined structure and size that could serve as an alternative to SDS micelles, dendrimers have emerged as promising candidates and have been applied to EKC by several groups [4–11]. The branching structure of higher generation dendrimers make for a topology similar to that of a micelle. So far, investigations of dendrimers as pseudomicelles in EKC have focused on those with terminal carboxylate groups [8–11]. To more closely mimic the structure of an SDS micelle, we have attached terminal sulfonic acid moieties via a short alkyl chain to a poly(amidoamine) Starburst dendrimer and used it to separate positional isomers of dimethyl phenol (DMP) by capillary electrophoresis (CE).

2. Experimental

2.1. Instrumentation

Separations were performed on a 90 cm (53.4 cm

*Corresponding author.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00623-2

Table 1
Buffer composition at various pH for experiments with and without SBD-SO_3^-

pH	[Borate], m*M*	[Phosphate], m*M*	[NaOH], m*M*
Buffer alone and with SBD-SO_3^-			
7.5	11.0	27.0	–
8.0	23.8	12.6	–
8.5	18.0	16.0	–
9.0	20.0	10.0	–
9.5	30.0	–	–
10.0	10.0	5.0	24.4
10.5	6.0	10.0	23.0
11.0	5.0	10.0	24.0~
Buffer with 100 m*M* SDS			
8.0	4.5	9.0	–
9.0	15.0	–	–

from inlet to detector)×60 μm I.D.×320 μm O.D. fused bare silica capillary (SGE, Austin, TX, USA). A SpectroVision DA-30 high-voltage power supply (GTI-SpectroVision, Acton, MA, USA) was used at a constant voltage of 15 kV (current approximately 35–40 μA). On-column detection was monitored at 254 nm with a SpectroVision AD-200 variable-wavelength UV absorbance detector and electropherog-

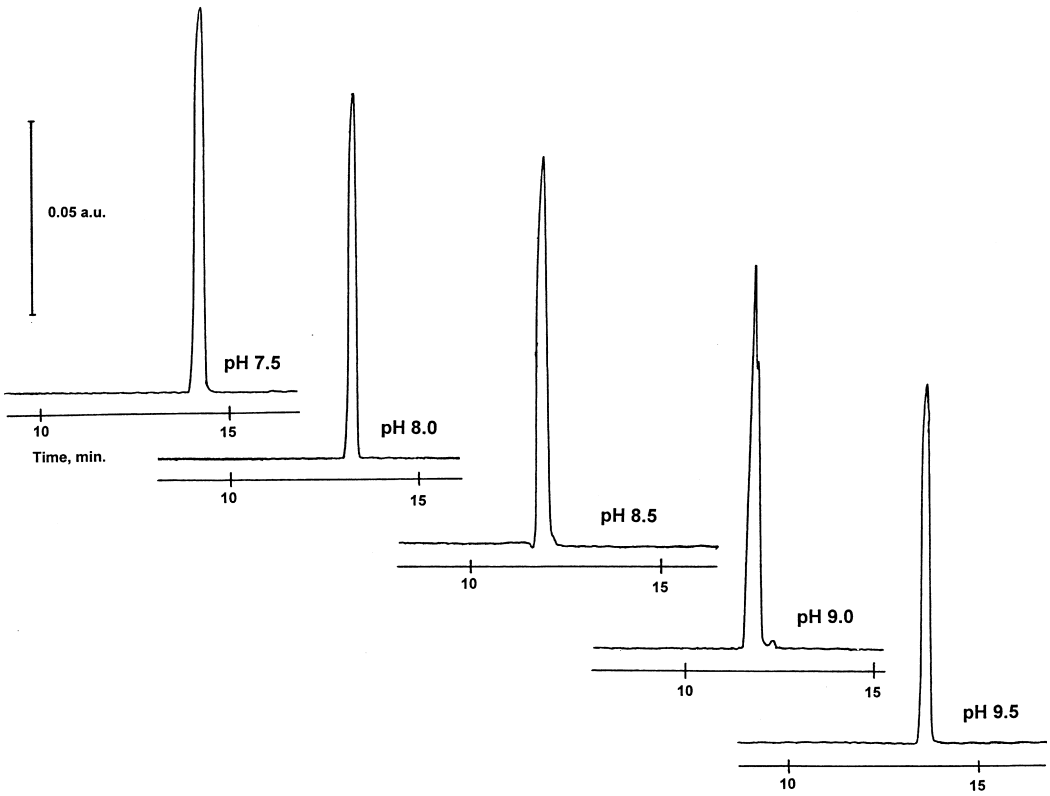

Fig. 1. Separation of DMPs by CZE at various pH. Buffer, see Table 1; separation potential, 15 kV; current, 35–40 μA; detection, 254 nm. Peaks: 1=2,6-DMP (50 m*M*); 2=2,5-DMP (50 m*M*).

rams were recorded on a strip-chart recorder (Kipp&Zonen, Netherlands).

2.2. Materials

Sulfonic acid-modified Starburst dendrimer (SBD-SO_3^-) was prepared by reacting the terminal amino groups of a Generation 2.0 Starburst dendrimer (SBD) with 1,4-butane sultone, extending each arm by four methylene groups and adding a terminal SO_3^- moiety [12–15].

Generation 2.0 Starburst (PAMAM) dendrimer having 16 surface amino groups was obtained as a 20% (w/w) solution in methanol from Aldrich (Milwaukee, WI, USA). After removing the methanol in vacuo, the dendrimer was suspended in tetrahydrofuran (THF) and 1.1 equivalents per amino group of 1,4-butane sultone (Aldrich) were added. The mixture was stirred in a closed vessel at 80–100°C for three days after which the THF was evaporated. The remaining oil was extracted twice with a hot mixture of ethyl acetate–cyclohexane (50:50), washed with warm cyclohexane and evaporated at 90°C and 10 mbar to produce a white hygroscopic foam. The pH of a 1.0 m*M* solution of the product in Ultrapure water was 7.0. Physical characteristics were in agreement with the proposed modified structure: ^{13}C NMR (150 MHz, D_2O, 50 mg/ml) δ 23.9, 24.0, 25.3, 27.2, 35.0, 37.6, 38.6, 39.2, 41.8, 49.7, 49.8, 51.5, 51.7, 52.7, 52.8, 53.9, 55.6, 177.0, 177.1, 178.1. Elemental analysis indicated that all 16 dendrimer arms possess a sulfonic acid group (9.39% sulfur found vs. 9.41% calculated).

2.2.1. Buffers

All water used was ultrapure from a Milli-Q System (Millipore, Bedford, MA, USA). Buffers were prepared from 50 m*M* stock solutions of sodium tetraborate decahydrate (Fisher Scientific,

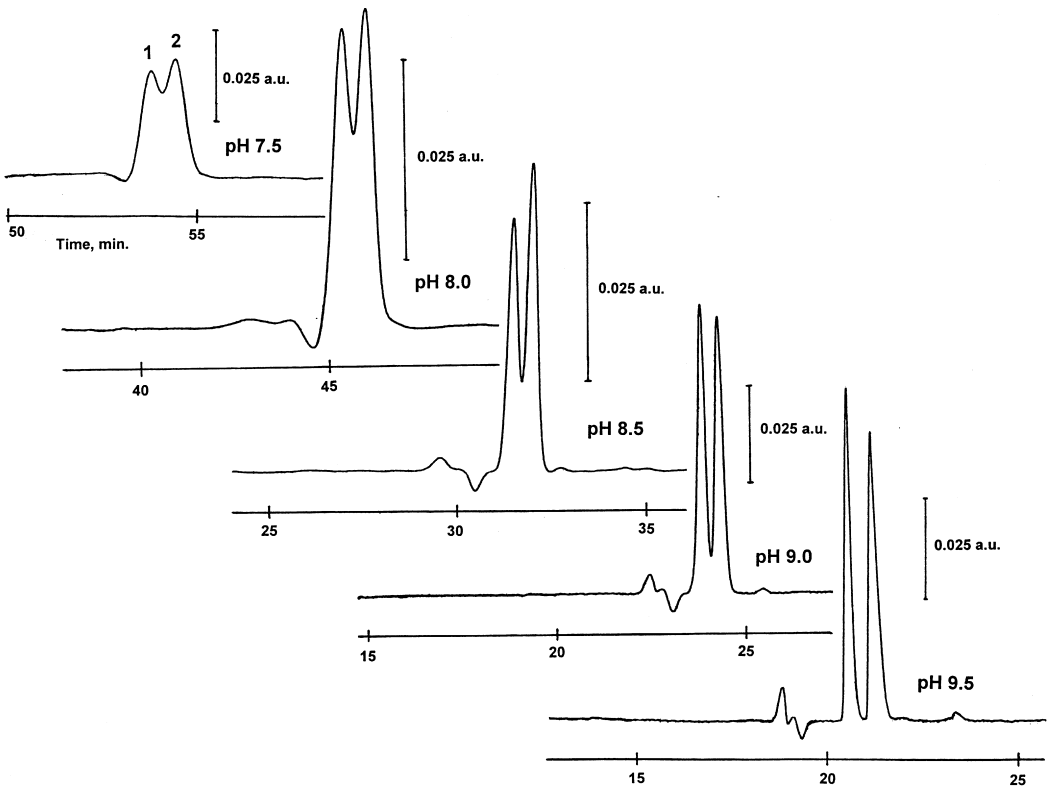

Fig. 2. Separation of DMPs by EKC using 1.0 m*M* SBD-SO_3^- at various pH. Conditions as in Fig. 1. Peaks: 1=2,6-DMP (50 m*M*); 2=2,5-DMP (50 m*M*).

Fair Lawn, NJ, USA) and potassium dihydrogen phosphate (Sigma, St. Louis, MO, USA), both reagent grade. Buffers were mixed until the correct pH was achieved; when necessary, the pH was adjusted with 0.5 *M* NaOH. Ionic strength varied slightly at each pH but remained in the 30–40 m*M* range for all experiments except those containing SDS (Table 1). The ionic strength of buffers used for SDS micelle experiments were lower in order to prevent precipitation of SDS. All electrolyte solutions were degassed and filtered before use. Modified Starburst dendrimer solutions were 1.0 m*M* in the appropriate buffer. The SDS concentration was 100 m*M* [16].

Stock solutions of DMPs (Aldrich) were prepared by dissolving them in HPLC grade methanol (Fisher) for a final concentration of 1.0 *M*. Samples contained 20–50 m*M* of each phenol in the appropriate pH buffer (final methanol concentration 10–12%, v/v).

2.3. Procedures

At the start of each day and between changes in electrolyte, the capillary was conditioned first for 3 min with 0.1 *M* NaOH, followed by 5 min of rinsing with ultrapure water and 3 min of rinsing with the electrolyte to be used in the experiment. The capillary was rinsed and filled by suction from the cathode end. Between runs using the same electrolyte, the capillary was flushed for 3 min with electrolyte solution.

Samples were introduced by electrokinetic injection at 15 kV for 3–8 s. All runs were performed at ambient temperature (25°C).

Table 2
Measured migration times of 2,6-DMP and 2,5-DMP, respectively

pH	Buffer only (min)	100 m*M* SDS (min)	SBD-SO_3^- (min)	
			1 m*M*	5 m*M*
7.5	14.2		53.1, 53.7	
8.0	13.2	31.5, 33.2	47.2, 47.8	
8.5	10.2		30.9, 31.4	
9.0	12.0	29.2, 30.6	24.6, 25.0	
9.5	13.6		20.4, 21.0	32.9, 34.1
10.0	11.3		18.5, 19.0	
10.5	10.7		16.0, 16.3	
11.0	13.7, 14.9		16.9, 17.5	

3. Results and discussion

In free-solution capillary zone electrophoresis (CZE), ionic analytes separate on the basis of electrophoretic mobility. However, neutral analytes have no electrophoretic mobility and are swept through the capillary by electroosmotic flow (EOF). Neutral molecules of similar size are therefore difficult to separate.

Fig. 1 shows the separation of two test analytes, 2,5-DMP (pK_a=10.22) and 2,6-DMP (pK_a=10.59) by CZE at various pH [17–21]. At pH 9.5 and below, the neutral phenol form predominates and the two isomers essentially coelute.

In contrast, with a 1.0 m*M* solution of the sulfonic

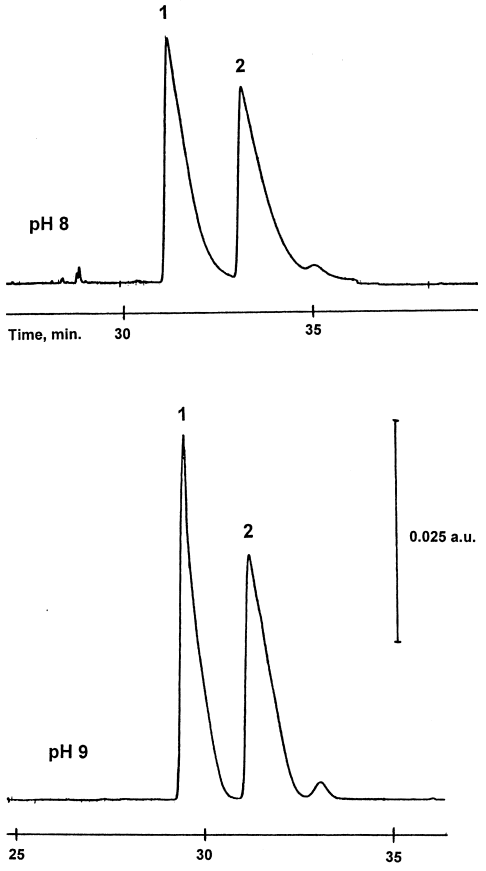

Fig. 3. Separation of DMPs by MEKC using SDS micelles. Buffer, see Table 1; SDS, 100 m*M*; separation potential, 15 kV; current, 35–40 μA; detection, 254 nm. Peaks: 1=2,6-DMP (50 m*M*); 2=2,5-DMP (50 m*M*).

acid-modified Starburst dendrimer (SBD-SO_3^-) in the same buffer, the presence of two distinct components can be seen already at pH 7.5 (Fig. 2). Separation improved with increasing pH and the two isomers were completely resolved at pH 9.5. Partitioning of the analytes into the negatively-charged SBD-SO_3^-, which are attracted to the anode in the direction opposite to EOF, should result in longer migration times for the phenols in the dendrimer system, and this is indeed observed (Table 2).

For comparison, the analytes were separated with SDS micelles (100 m*M* SDS) using otherwise nearly identical conditions (Fig. 3). Ionic strength for the SDS solutions however, was lower than for the buffer and dendrimer systems in order to keep the SDS from salting out of solution. Separation of the phenols at both pH 8.0 and 9.0 was comparable to the best resolution achieved with SBD-SO_3^- (at pH 9.5), but with considerably more band broadening. Theoretical plate numbers with 1 m*M* dendrimer were in the range of 20 000 to 30 000 theoretical plates, while SDS micelles (100 m*M*) were significantly lower with only 12 000 to 13 000 theoretical plates. The plate numbers obtained with SDS MEKC on our system were lower than are usually reported.

Additionally, the concentration of SBD-SO_3^- could be increased, resulting in a greatly improved separation: 44 000 theoretical plates were obtained at a dendrimer concentration of 3 m*M*, and over 60 000

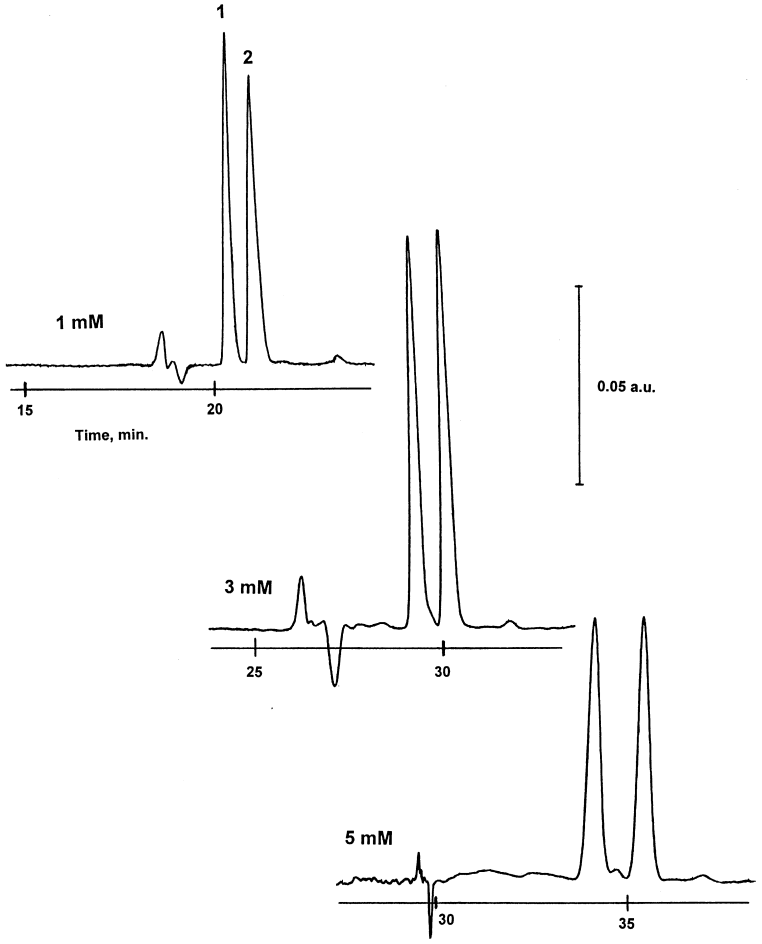

Fig. 4. Separation of DMPs by EKC using various concentrations of SBD-SO_3^- at pH 9.5. Conditions as in Fig. 1. Peaks: 1=2,6-DMP (50 m*M*); 2=2,5-DMP (50 m*M*).

theoretical plates were achieved with a dendrimer concentration of 5 m*M* (Fig. 4).

Partition into SDS micelles is based on the hydrophobic environment created by the long hydrocarbon chains of the SDS molecule. However, the SBD-SO_3^- has a much shorter four-carbon chain connected to an amine, so any hydrophobic interactions are presumably augmented by hydrogen bonding of the phenol analytes with the connecting amine group. This would also explain the improving separation observed as the pH increased, approaching the pK_a of the amine. As the pH increases, the connecting amine group will be increasingly deprotonated, making the lone pair of the nitrogen available to hydrogen bond with the analyte molecules.

4. Conclusions

In summary, we have demonstrated that SBD-SO_3^- molecules can be used successfully in the separation of neutral phenols by EKC. While the resolution is comparable, SBD-SO_3^- gives higher theoretical plate numbers and better peak shape. The covalent nature of sulfonic acid-modified Starburst dendrimers makes them less sensitive to buffer concentration, pH or added organic solvents than surfactants, which may make them attractive as additives in EKC. Additionally, it may be possible to modify dendrimers or synthesize them de novo for specific EKC applications, something that is not possible with surfactants.

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] D.F. Evans, H. Wennerström, The Colloidal Domain – Where Physics, Chemistry, Biology and Technology Meet, VCH, New York, 1994.
[3] A.T. Balchunas, M.J. Sepaniak, Anal. Chem. 59 (1987) 1466.
[4] D.A. Tomalia, V. Berry, M. Hall, D. Hedstrand, Macromolecules 20 (1987) 1164.
[5] G.R. Newkome, C.N. Moorefield, G.R. Baker, M.J. Saunders, S.H. Grossman, Angew. Chem. 103 (1991) 1207.
[6] K.L. Wooley, G.J. Hawker, J.M.J. Fréchet, J. Am. Chem. Soc. 115 (1993) 11496.
[7] C.J. Hawker, K.L. Wooley, J.M.J. Fréchet, J. Chem. Soc., Perkin Trans. 1 (1993) 1287.
[8] N. Tanaka, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, S. Terabe, Chem. Lett. 6 (1992) 959.
[9] S.A. Kuzdzal, C.A. Monnig, G.R. Newkome, C.N. Moorefield, J. Chem. Soc., Chem. Commun. 23 (1994) 2139.
[10] P.G.H.M. Muijselaar, H.A. Claessens, C.A. Cramers, J.F.G.A. Jansen, E.W. Meijer, E.M.M. de Brabander-van den Berg, S. van der Wal, J. High Resolut. Chromatogr. 18 (1995) 121.
[11] M. Castagnola, L. Cassiano, A. Lupi, I. Messana, M. Patamia, R. Rabino, D.V. Rossetta, B. Giardina, J. Chromatogr. A 694 (1995) 463.
[12] K. Aoi, K. Itoh, M. Okada, Macromolecules 28 (1995) 5391.
[13] N. Tanaka, T. Fukutome, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, K.K. Unger, J. Chromatogr. A 699 (1995) 331.
[14] N. Tanaka, T. Fukutome, K. Hosoya, K. Kimata, T. Araki, J. Chromatogr. A 716 (1995) 57.
[15] S. Stevelmans, J.C.M. van Hest, J.F.G.A. Jansen, D.A.F.J. van Boxtel, E.M.M. de Brabander-van den Berg, E.W. Meijer, J. Am. Chem. Soc. 118 (1996) 7398.
[16] S.A. Kuzdzal, C.A. Monnig, G.R. Newkome, C.N. Moorefield, J. Am. Chem. Soc. 119 (1997) 2255.
[17] C.D. Gaitonde, P.V. Pathak, J. Chromatogr. 514 (1990) 389.
[18] S.C. Smith, M.G. Khaledi, Anal. Chem. 65 (1993) 193.
[19] A.J. Zemann, J. Cap. Electrophoresis 2 (1995) 131.
[20] N.J. Benz, J.S. Fritz, J. High Resolut. Chromatogr. 18 (1995) 175.
[21] S.M. Masselter, A.J. Zemann, J. Chromatogr. A 693 (1995) 359.