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USE OF A NEW DIAMINOBUTANE DENDRIMER IN ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

A modified polyamidoamine-starburst dendrimer (PAMAM-SBD), 16-cascade:(1,4-diaminobutane)[4-1,4]:(1,6-diaza-7-oxo-nonylidene)^2:propanoic acid methyl ester (DABD), was synthesized and used as a carrier in electrokinetic capillary chromatography. The utility of DABD is demonstrated with the separation of a mixture of naphthalene derivatives and five neutral aromatic molecules without the need for an organic solvent as a modifier. Separation of a mixture of five neutral aromatic molecules were optimized through dendrimer concentration, pH, and organic modifier. The modified polyamidoamine-starburst dendrimer offered selectivity for polynuclear aromatic hydrocarbons as well as naphthalene based derivatives. However, DABD did not provide as much selectivity for neutral aromatic compounds in the presence of organic modifier.

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INTRODUCTION

Dendrimers are well-defined, highly branched macromolecules that emanate from a central core.[1] The first successful dendrimer syntheses were reported by Tomalia[2] and Newkome.[3] Both syntheses offered a wide range of synthetic options for controlling size, shape, and surface chemistry.

Tomalia demonstrated that the starburst dendrimer (SBD) has the same topological (hollowness) distinction of a micelle inside and outside[4] as discussed by Wennerstrom and Lindman.[5] The fundamental difference between a dendrimer and micelle is that the structure of a dendrimer is static, with all end groups covalently bonded to a central core, whereas the structure of a micelle is dynamic. In addition, the hydrophobic character of the interior of an SBD can be altered by use of relatively hydrophobic alkyl diamines to more closely mimic an aqueous micelle.[1] Aggregation numbers for micelles range from 62 to 100 depending on the surfactant.[6] Moreover, active functional groups on the surface of dendrimers play a more important role as opposed to aggregation number in micelles.

Dendrimers have been employed as substitutes in micellar electrokinetic capillary chromatography (MECC) for micelles. The mode of separation utilizing dendrimers in capillary electrophoresis (CE) has been termed dendrimer electrokinetic capillary chromatography (DECC).[7] SBDs have been used to separate uncharged aromatic compounds in EKC.[8]

Poly(propyleneimine) dendrimers have been employed in DECC to separate substituted benzyl alcohols.[9] Dendritic (cascade) molecules have been used to separate a homologous series of alkyl parabens via DECC.[7] To the best of our knowledge, no mention of diaminobutane as a hydrophobicity modifier has been reported for the linkers. We recently synthesized a modified poly(amidoamine) dendrimer, 16-cascade:(1,4-diaminobutane)[4-1,4]:(1,6-diaza-7-oxononylidene)^2:propanic acid methyl ester, herein noted as diaminobutane dendrimer (DABD). The chemical structure of DABD is shown in Figure 1. The external groups on DABD consist of methoxy groups and the internal groups consist of tertiary amines and carbonyl groups. At pH's below 9, DABD is positively charged.

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds that are difficult to separate in traditional MECC systems using sodium dodecyl sulfate (SDS) due to their strong interactions with the micelle. The cavities of DABD are more hydrophilic than that of normal SDS micelles due to the presence of carbonyl groups, and secondary as well as tertiary amines. Thus, the possibility of mild interaction of PAHs with this pseudo-stationary phase

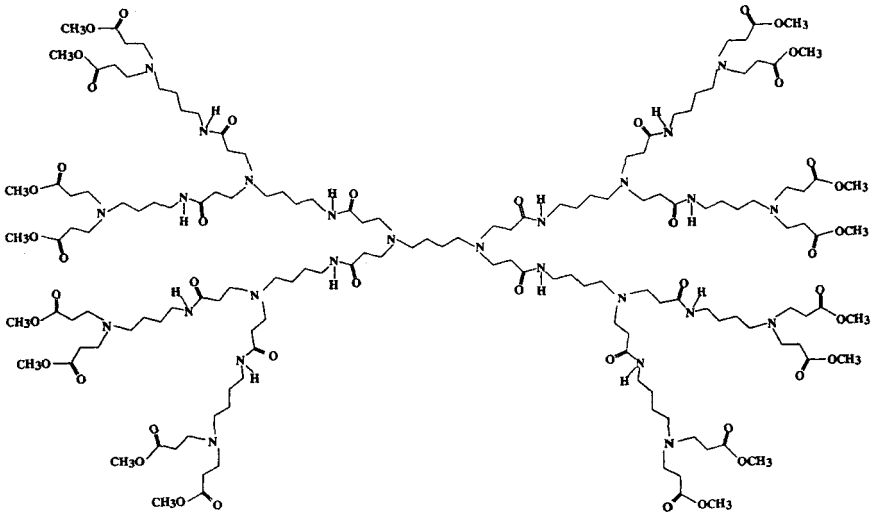

Figure 1. Chemical structure of DABD.

could improve separation. In order to determine the utility of DABD, a systematic optimization of the separartion of five analytes [nitrobenzene (NB), naphthalene (NA), acenaphthene (ANA), fluorene (FLU), and phenanthrene (PTN)] was first studied.

Separation factors such as pH, organic modifier, and concentration of DABD are optimized to achieve the separation of a mixture of those molecules.

EXPERIMENTAL

Apparatus

The DECC separations were performed using a BioFocus 3000 CE (Bio-Rad, Hercules, CA, USA) equipped with a UV lamp detector operated at 254 nm. The run voltage ranged from -10 to -30 kV. All DECC separations were carried out at ambient temperature (~25°C).

Materials

Analytical grade sodium acetate, acetic acid, sodium hydroxide, nitrobenzene (NB), naphthalene (NA), phenanthrene (PTN), fluorene (FLU), acenaphthene (ANA), 2-naphthalene methanol (NAM), naphthol (NAL), phenol (PNL), benzylamine (BA), naphthylamine (NAA), nitronaphthalene (NNA), and benzyl alcohol (BAL) were purchased from Aldrich (Milwaukee, WI, USA). Binaphthol (BNL) was purchased from Fisher (Fair Lawn, NJ, USA). The 6-Bromo-2-naphthol (BNAL) was purchased from Sigma (St. Louis, MO, USA). Sodium dodecyl sulfate was purchased from Mallinckrodt (Paris, Kentucky, USA). Methanol used was of spectroscopic grade and was purchased from EM Science (Darmstadt, Germany). Deionized distilled water (18.3 MΩ/cm) was obtained from a PURELAB™ Polishing System (Lowell, MA, USA). All reagents were used as received.

Synthesis of DABD

The DABDs were synthesized starting from 1,4-diaminobutane and methylacrylate, following the method reported by Tomalia, et al.[10] An exhaustive Michael addition to 1,4-diaminobutane with methyl acrylate at room temperature, followed by addition of a large excess of diaminobutane at room temperature, was performed to yield a first generation (G = 1) DABD. Repeating steps 1, 2, and 1, DABD of G = 2.5 was synthesized. Half and whole generations were distinctly different and characterized by ^{1}H NMR. The half number generations displayed the disappearance of the methoxy singlet at d 3.65 ppm. The ^{1}H NMR and ^{13}C NMR measurements were performed on the AC 250 Bruker NMR (Germany). ^{1}H NMR (250 MHz, C^2H_3OH): δ = 1.46 ppm (m, 52 H;NCH_2-$\boldsymbol{CH_2CH_2}CH_2N$), 2.34 (t, 28 H; $N\boldsymbol{CH_2}CH_2CH_2\boldsymbol{CH_2}N$), 2.45 (t, 56 H; $CH_2N(\mathbf{CH_2}CH_2)_2$), 2.74 (t, 56 H; $CH_2N(CH_2\boldsymbol{CH_2})_2$), 3.17 (s, 24 H; $CH_2CONH\mathbf{CH_2}$), 3.65 (s, 48 H: $COCH_3$): Fast atom bombardment mass spectrometry (FAB-MS) measurements were performed on a Finnigan MAT (Germany), (M+H 3188.6). The size of the DABD is described according to the generation system[10] and named according to standard nomenclature.[11] Further characterization of this system is underway.

Preparation of Electrolyte and Analytes

Aqueous buffer solutions were prepared by adding an appropriate volume of 1.0 mM sodium acetate/acetic acid buffer. Separation buffers for DECC were prepared by weighing the appropriate amount of dendrimer into a volummetric flask, adding the aqueous acetate buffer, adjusting the pH, and

then sonicating the mixture to promote complete dissolution of the dendrimer and to simultaneously degas the run buffer. Stock solutions of each analyte were prepared at a concentration of 1.5 mg/mL in methanol. A mixture of analytes (1-3 mM) were prepared in methanol from the respective stock solutions.

The uncoated capillary purchased from Polymicro Technologies (Phoenix, AZ, USA), with an internal diameter of 50 μm and a total length of 47 cm (39.4 cm to detector window) was used.

A new capillary was conditioned by flushing successively with 1.0 M NaOH (60 min), 0.1 M NaOH (30 min), and deionized distilled water (10 min) before use. Between each injection, the capillary was rinsed with 0.1 M NaOH (1 min), deionized distilled water (1.5 min), and with the respective buffer (2 min). All samples were dissolved in methanol, filtered with 0.45 μm Nalgene nylon filters (Rochester, NY, USA), and introduced into the capillary with a 14 kPa*sec pressure injection.

RESULTS AND DISCUSSION

Optimization Study of DABD

Influence of DABD concentration

Figure 2A illustrates the attempted separation of the mixture of the five analytes, NB, NA, ANA, FLU, and PTN using SDS. Under the given electrophoretic conditions, SDS showed no selectivity toward these analytes. Figure 2B illustrates that the separation of the same five analytes (using SDS) was possible only when acetonitrile was added as a co-modifier. Acetonitrile reduces the strong interaction between SDS and the neutral compounds, thus aiding in separation. These analytes were separated with DABD without the need for any organic co-modifiers.

Figure 3 shows a plot of retention time as a function of concentration of DABD for the separation of the five analytes. Using negative polarity CE, the observed elution order was NB<NA<ANA<FLU<PTN, which is consistent with the trends in which retention is directly proportional to the hydrophobicity of the analytes. Lower retention times were observed at 2.2 and 4.4 mM. However, ANA and FLU co-elute at this concentration. An increase in DABD concentration up to 6.6 mM resulted in a linear increase in retention time of the

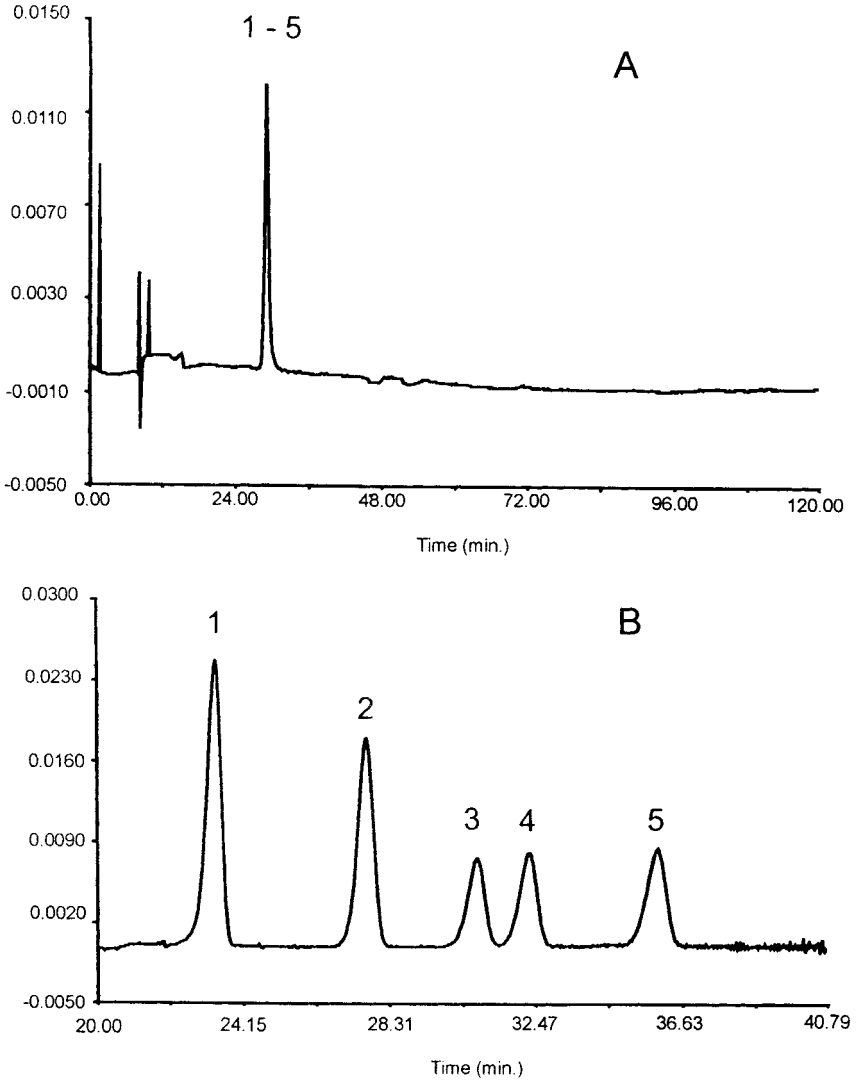

Figure 2. Electrokinetic chromatogram: Separation of a mixture of five neutral molecules using SDS. CE conditions: (A) 50 mM borate buffer, 30 mM sodium dodecyl sulfate, pH 9, (B) 50 mM borate buffer (40 % acetonitrile, 60 % borate buffer) 30 mM sodium dodecyl sulfate, Peaks: 1 -5 NTB, NAP, ACE, FLU, PHEN. 15 kV applied for separation, current ~57 μA for (A) and ~35 μA for (B). UV detection 214 nm.

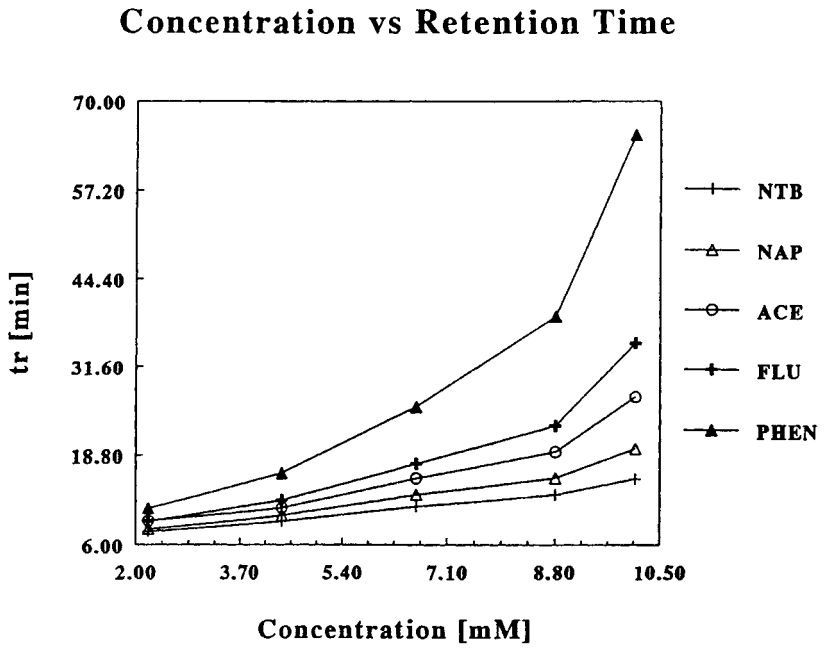

Figure 3. Effect of concentration of DABD on the retention time of the mixture of five neutral molecules. Separation voltage was -10 kV, current 15-18 μA.

analytes. The optimum concentration for baseline separation of all five analytes was found to be 6.6 mM. At concentrations above 6.6 mM, retention times increased significantly. For this series of analytes, concentrations higher than 6.6 mM are not advantageous for separations unless enhanced selectivity and resolution are desired at the expense of longer retention times.

Influence of buffer pH

The effect of pH on elution time of a mixture of the five analytes was examined with 190 mM acetate buffer containing 6.6 mM DABD, over the pH range of 4.5 - 7 (Figure 4). Initially, at pH 4.5, retention times were longer and peaks were broad.

Upon increased pH, retention times decreased and peak shapes improved. This can be attributed to a close mobility match between background electrolyte and analyte at lower pH.

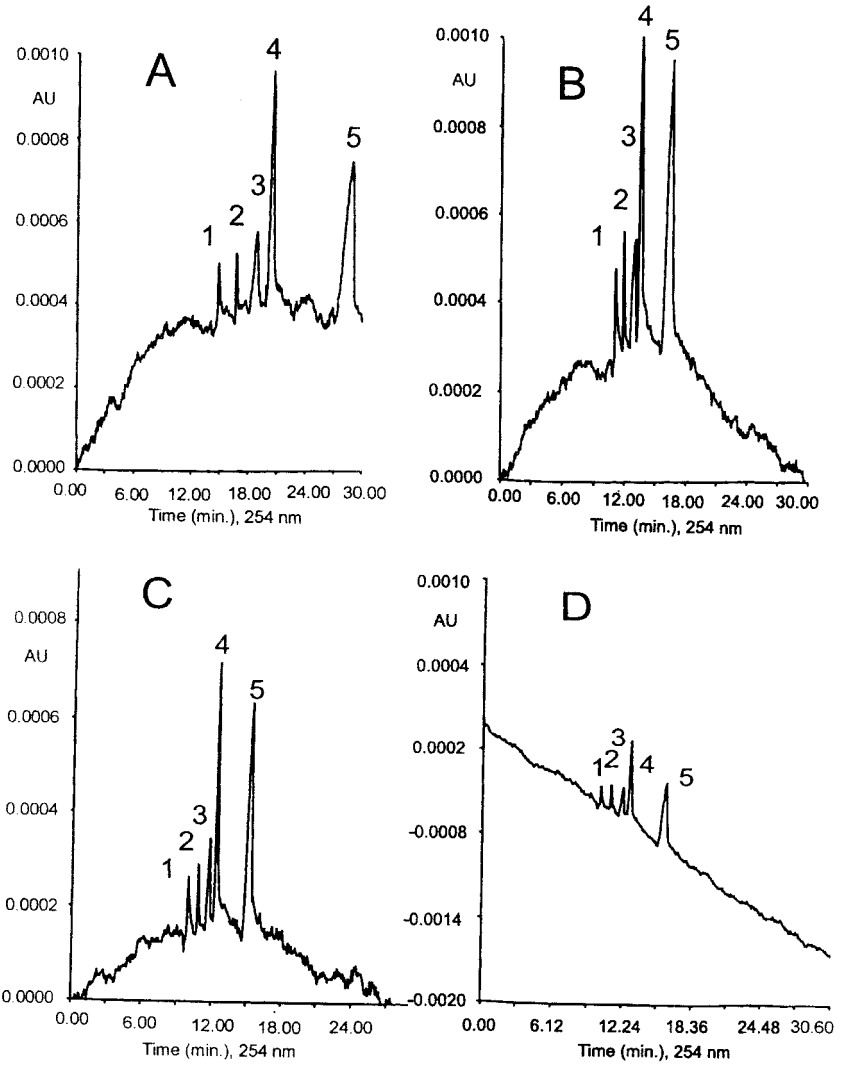

Figure 4. Electrokinetic chromatogram showing the effect of pH on the separation of a mixture of PAH's by use of DABD (G = 2.5). CE conditions: 190 mM acetate buffer (a) pH 4.5, (b) pH 5.5, (c) pH 6.0, (d) pH 7.0. Peaks: 1 = NTB, 2 = NAP, 3 = ACE, 4 = FLU, 5 = PHEN. -10 kV applied for separation, current 15 -18 μA.

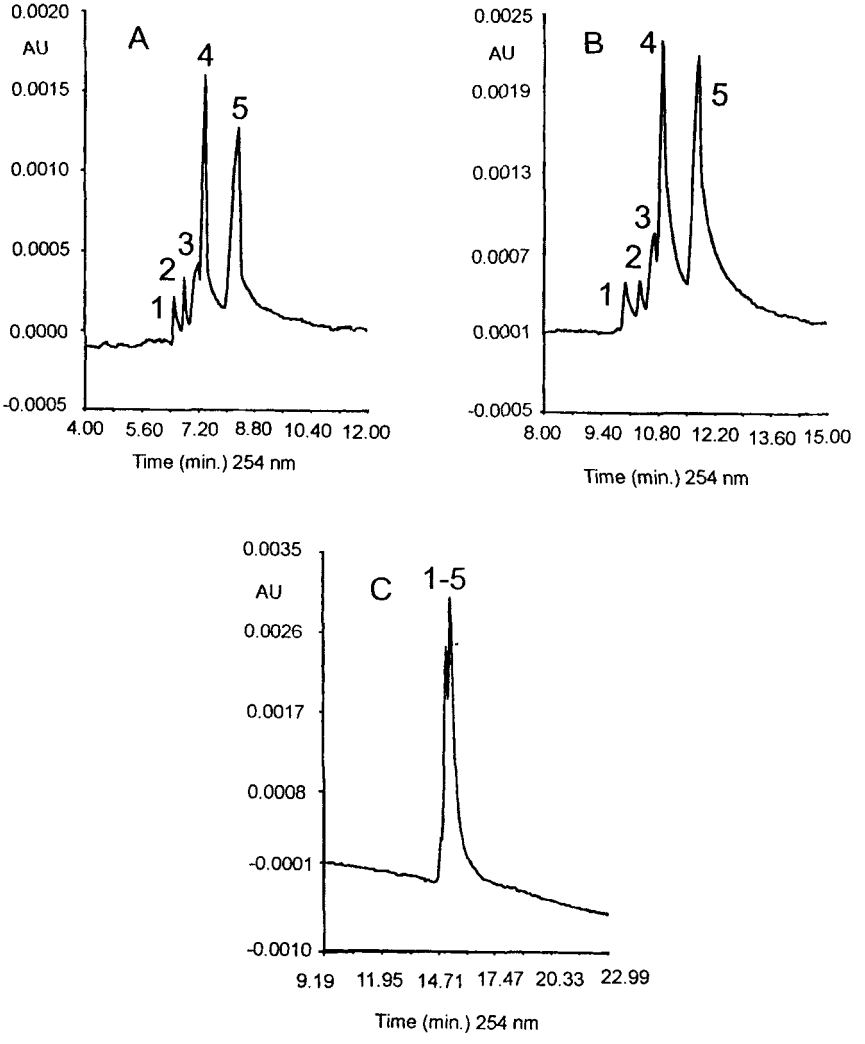

Figure 5. Effect of organic modifier on the separation of a mixture of five neutral molecules. CE conditions: -25 kV applied for separation, pH 5.5* (a) 190 mM acetate buffer-methanol (1:10), (b) 190 mM acetate buffer-methanol (1:5), (c) 190 mM acetate buffer-methanol (1:2), current: 17.5 μA, 14.8 μA, 11.5 μ A for (a), (b), and (c) respectively. UV detection: 254 nm, Peaks: 1 = NTB, 2 = NAP, 3 = ACE, 4 = FLU, 5 = PHEN. * measured pH before addition of methanol.

A buffer pH of 5.5 can be adopted for standard application since it combines the best resolution, efficiency, and short analysis time for the mixture. Above pH 6.0, even shorter retention times can be achieved but peak fronting and baseline drifts were evident.

Influence of organic modifier

The influence of volume:volume (v:v) ratio of methanol added to DABD (6 mM and 190 mM with acetate buffer of pH 5.5) on separation of five neutral molecules is depicted in Figure 5. Normal migration behavior similar to MECC was observed. Initially, as the v:v ratio of methanol:water increased to 1:10, migration times increased with a slight increase in resolution of ANA and FLU (Fig. 5, peaks 3 & 4) at the expense of a decrease in selectivity for FLU and PHE (Fig. 5, peaks 4 & 5). However, at 1:5, selectivity between DABD and the analytes is dramatically reduced. These results contrast with the results reported by Terabe *et al.*, where selectivity of SBD-EKC for aromatic hydrocarbons was observed in the presence of high concentrations of the organic modifier methanol.[12] The cavities of DABD become more polar with the addition of organic modifier, disabling partitioning of neutral analytes between the bulk medium and dendrimer, and thus a decrease in selectivity occur.

DABD Selectivity of Naphthalene Derivatives

In order to explore further applications of DABD in the separation of neutral molecules, naphthalene derivatives were studied. Terabe, *et al.* demonstrated that SBD favored the naphthalene skeleton over benzene derivatives in the separation of neutral molecules.[13] DABD exhibited no selectivity toward benzyl derivatives. However, DABD exhibited selectivity and separation of naphthalene derivatives. A mixture of benzyl derivatives and the parent naphthalene derivatives were examined. Figure 6A shows the attempted separation of a mixture of BAL, BA, NB and PTN. No separation of this mixture was achieved. Under the same conditions, a mixture of NNA, NAA, NAL and NAM was separated (Figure 6B). However, NNA and NAM separate whereas NAA and NAL coelute. These experiments illustrate that for a mixture of the benzyl derivatives, there is no selectivity whereas selectivity is shown toward the mixture of parent naphthalene derivatives. To demonstrate further utility of DABD, separation of a mixture of four naphthalene derivatives [NA, NAM, BNAL, BNL] is shown in Figure 7. As expected, the analytes eluted in order of increasing hydrophobicity.

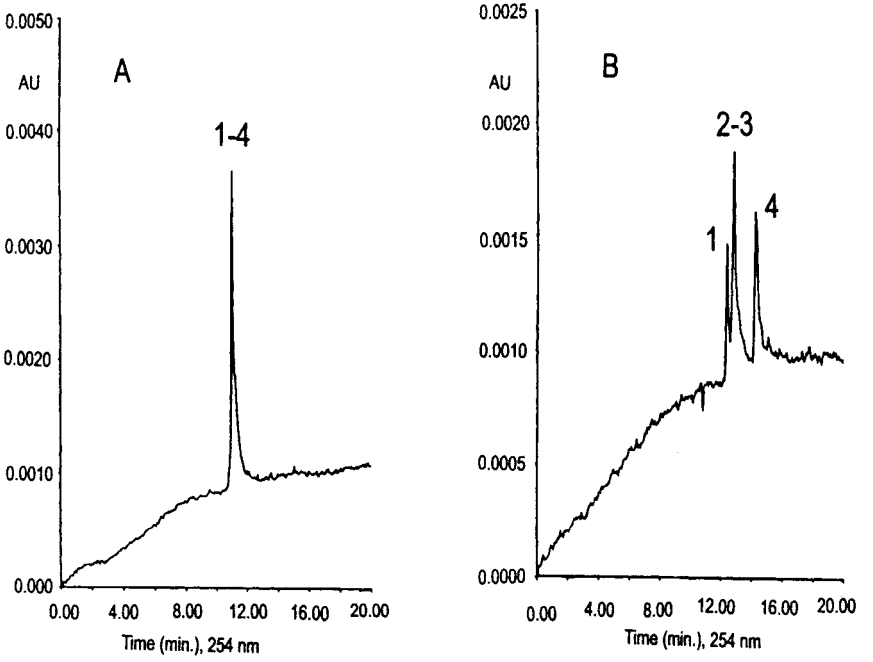

Figure 6. Electrokinetic chromatogram of benzyl and corresponding naphthalene derivatives CE conditions: 190 mM acetate buffer pH 5.5 (a) benzyl derivatives: Peaks: 1-4 NB, BA, PNL, BAL. (b) naphthalene derivatives: Peaks: 1 = NNA, 2 = NAA, 3 = NAL, 4 = NAM. -15 kV applied for separation, current 23.5 μA. UV detection: 254 nm.

CONCLUSION

DABD functioned as a pseudo-stationary phase in DECC. A range of pH's and concentra-tions of DABD were applicable in the separation of neutral aromatic molecules and naphthalene derivatives. For separation of the selected analytes, organic co-modifier was not required to show selectivity. However, DABD did not show any selectivity toward smaller benzyl derivatives.

More hydrophobic linkers such as diaminobutane did not enhance selectivity for smaller benzyl groups but offered selectivity for naphthalene derivatives and PAH's. By using a longer linker for the modified PAMAM-SBD, we are making DABD more hydrophobic than conventional SBD's. Results showed that DABD offered less selectivity in the presence of organic modifier.

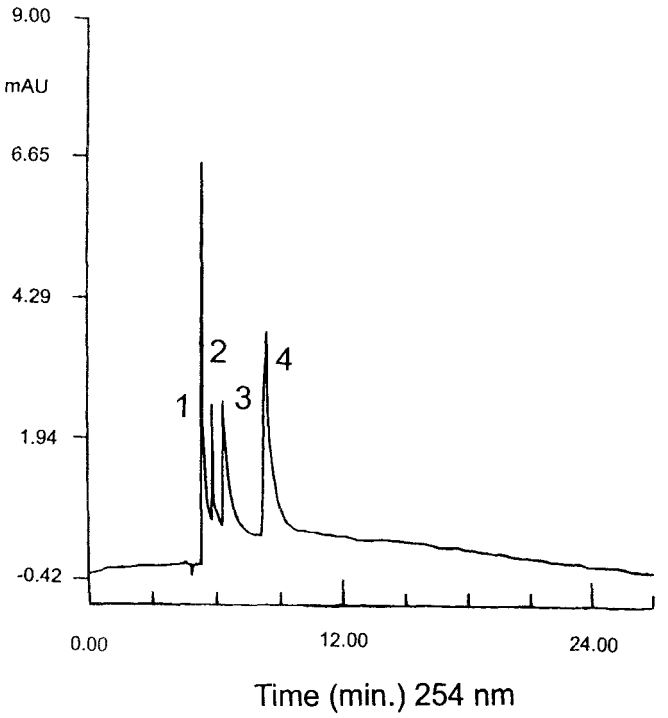

Figure 7. Separation of a mixture of four naphthalene derivatives by use of 6.6 mM DABD (G = 2.5). CE conditions: 190 mM acetate buffer (pH = 5.5), -25 kV applied for separation, current 23.5 μA. UV detection: 254 nm. Peaks: 1 = NAL, 2 = NAP, 3 = BNAL, and 4 = BNL.

The combination of even more hydrophobic diamines (i.e. octylamine) with higher branched surface groups will possibly offer better structural selectivity toward benzyl derivatives and large polycyclic compounds. Future studies will focus on improving selectivity for larger PAH's and the influence of higher branch surface groups on separation of neutral compounds.

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