

Separation of Aromatic Acids, DOPA, and Methyl-DOPA by Capillary Electrophoresis with Dendrimers as Buffer Additives

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Abstract

Polyamidoamine starburst dendrimers with terminal carboxylate groups are used as a pseudo-stationary phase in electrokinetic chromatography for separating the selected aromatic amino acids phenylalanine, phenylglycine, homophenylalanine, and tyrosine and the catecholamines 3-(3,4-dihydroxyphenyl)alanine and 3-(3,4-dihydroxyphenyl)-2-methylalanine at different pH levels. A significant difference in analyte selectivity is observed between dendrimers of different generations and at different pH levels. At pH 7.0, the utility of the dendrimers for separating these analytes is limited by relatively low selectivity and a noisy baseline. However, good separation and selectivity are obtained with low generation dendrimers (G0.5 and G1.5) at low pH. Strong association of the solute with dendrimers is observed for high generations (G2.5, G3.5, and G5.5).

Introduction

Micellar electrokinetic chromatography (MEKC) has been applied to separate neutral species, including drugs and organic compounds. Micelles formed by surfactant aggregation are employed as pseudo-stationary phases to modify the migration of solutes in EKC. When anionic surfactants (e.g., sodium dodecyl sulphate [SDS]) are added to the electrophoretic solution at a concentration greater than their critical micellar concentration (1,2), the negatively charged micelles migrate counterflow toward the anode. However, the electroosmotic flow usually transports the bulk solution, including negatively charged species, toward the cathode. The solute partitions between the micellar phase and the aqueous phase. Although MEKC is a highly efficient separation method for drugs and organic compounds, dispersion in this system is unavoidable. Micelles are dynamic systems in which surfactants are aggregated by hydrophobic or hydrophilic affinity. The number of surfactant molecules forming a micelle is usually in the range of 50–100, whereas the size of the micelle is usually in the range of

3–6 nm (3,4). The heterogeneity of micellar systems may result in peak broadening, although this is expected to be minimal. A more serious concern is the fact that hydrophobic analyte peaks tend to cluster near the migration time of the micelle. Although addition of organic solvents, cyclodextrin, or urea may improve the selectivity of MEKC either by electroosmotic flow (EOF) modification or through the presence of additional phases, the separation performance can be difficult to predict and optimize due to changes in the viscosity of the solution, solute partition coefficients, EOF, and instability of the micelle in high-organic-content buffers.

Other buffer additives (e.g., microemulsion [5], cyclodextrin derivatives [6], and ionic polymers [7]) have been explored as pseudo-stationary phases in EKC. Dendrimers, regarded as covalently fixed micelles (8), may offer an attractive alternative in EKC (9,10). These synthetic polymers are macromolecules with well-defined three-dimensional structures. The diameters of spherical dendrimers are typically in the range of 5–10 nm. The properties and features of dendrimers with well-defined generation numbers are determined by the initiator core, interior layers, and terminal groups. The highly branched interior layer is composed of repeating building blocks with special functionality. The functions of the dendrimer can be carefully designed and amplified by selecting building blocks according to the research needs. They can be made with multiple functionalities that may offer specific selectivity through well-controlled synthesis and modification (11). Dendrimers have also been reported capturing small organic compounds within their internal cavities (12). Drug delivery has been an important application in this field (13). EKC has been used to characterize dendrimer–solute interactions by the separation of paraben homologue mixtures. According to Dr. Monnig et al., Gibb's free energy values are negative for all the dendrimer–analyte interactions measured in their work (14). For high generations of polyacid dendrimer, the driving force for the partition of neutral species into the dendrimer is attributed to entropy in this work. In contrast to micelles, dendrimers maintain their three-dimensional structures in a full range of methanol–water mixtures (9,10). The uniform surface charge density and the

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structural homogeneity of dendrimers may provide an improvement in the separation performance compared to MEKC with surfactants (15).

Although dendrimers are static systems and may offer an alternative to the dynamic micelles, it should be noted that dendrimers are composed of mixtures. For instance, the dendrimer polyamidoamine (PAMAM) generation G4.5 (ammonia core) was found by electron microscopy to be 87% monodispersed (8). Mass spectrometry (MS) with electrospray ionization (ESI), which generates multiple charged ions ($z > 1$) for which the charge (z) is dependent on the size and ionization efficiency of the molecule, has been employed to study the polydispersity of PAMAM starburst dendrimers terminated with amine groups (16,17). A commercially available quadrupole mass analyzer has been used to analyze PAMAM dendrimer G0–4, and a low-frequency extended-mass-range quadrupole MS was built for use with higher molecular weight compounds. A significant mass shift was observed as the size of the dendrimer increased. Matrix-assisted laser desorption time-of-flight (MALDI–TOF) MS, which normally produces ions with an m/z ratio where z equals 1, has been used extensively for biopolymer analysis because of its mass range (400 kDa) and sensitivity (detection limit as low as fmol) (18).

Table I. Characteristics of PAMAM Dendrimers for Increasing Generations

Generation	Molecular weight	Surface charge (z)	$z/MW^{1/3}$	μ_{ep} (measured) $\times 10^3$ *
0.5	1,269	8	0.7	-10
1.5	2,935	16	1.1	-8.9
2.5	6,011	32	1.8	-9.8
3.5	12,419	64	2.8	-11
5.5	50,865	256	6.9	-14

* Average value in 100mM phosphate buffer (pH 7.0).

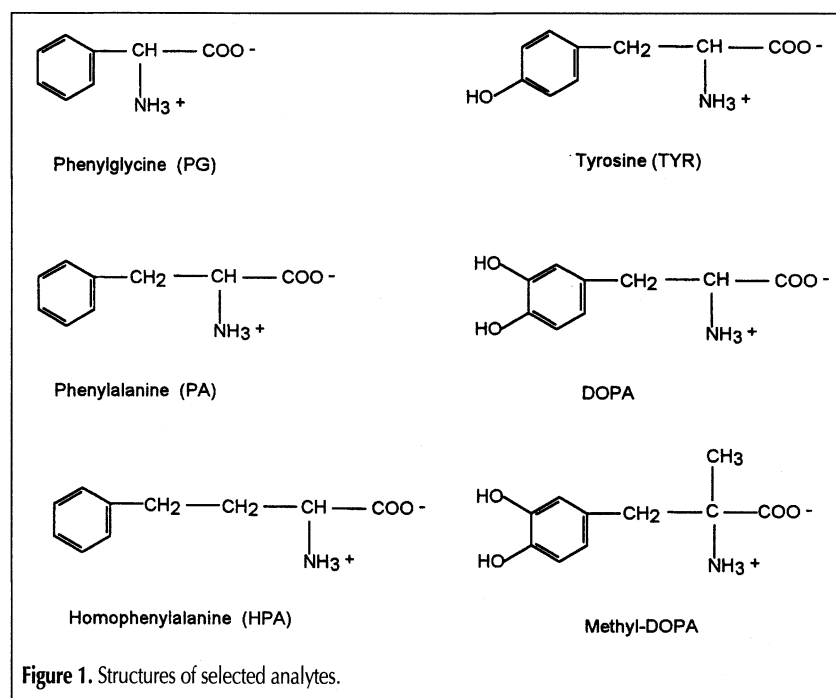


Figure 1. Structures of selected analytes.

PAMAM, terminated with carboxylate groups, was the starburst dendrimer used in this investigation. Evaluation of the effect of the generation number on the quality of separation was a major focus. As an ancillary part of this study, capillary electrophoresis (CE) and MALDI–TOF MS were employed to characterize the various half-generations of PAMAM dendrimer (ethylenediamine core) used in this study.

This study was aimed at exploring the potential application of dendrimers as buffer additives in CE for separating some small organic molecules and drugs, including phenylalanine (PA), phenylglycine (PG), homophenylalanine (HPA), tyrosine (TYR), 3-(3,4-dihydroxyphenyl)alanine (DOPA), and 3-(3,4-dihydroxyphenyl)-2-methylalanine (methyl-DOPA). Figure 1 shows the selected analytes. DOPA is one of the natural catecholamines, and tyrosine is its precursor. Clinical determination of DOPA and TYR is critical because of their important functions in the central nervous system and the cardiovascular system. The concentration of catecholamines and their metabolites can be useful for the diagnosis of some diseases. Methyl-DOPA, an antihypertension drug commonly used to treat women with hypertension during pregnancy (19), unfortunately interferes with the determination of catecholamines because of its structural similarity to DOPA.

Experimental

Chemicals

Starburst dendrimers (PAMAM, G0.5–3.5) in methanol were purchased from Aldrich Chemical (Milwaukee, WI). G5.5 was purchased from Dendritech (Midland, MI). Analytes were obtained from Aldrich or Sigma Chemical (St. Louis, MO) or Fluka Chemie AG (Buchs, Switzerland). Phosphoric acid, potassium phosphate, potassium hydroxide, and nitromethane were obtained from Fisher Scientific (Fair Lawn, NJ). The water was obtained from a Barnstead water purification system.

All buffers were made by blending equimolar solutions of two different phosphate salts in water to yield the appropriate pH. PAMAM was recovered from a methanol solution under a nitrogen stream, then dissolved in monobasic phosphate or phosphoric acid solution to the appropriate pH, and diluted with phosphate buffer at the same pH to the appropriate concentration. All buffers were filtered with a 0.45- μ m membrane filter before use.

Stock solutions of 30mM DOPA, methyl-DOPA, and PA were made by dissolving the solid compound in 1M acetic acid. Stock solutions of 30mM PG, TYR, and HPA were made by dissolving the solid compound in 0.4M KOH. The 0.5mM or 3mM solutions of each analyte were made by diluting the stock solution with run buffer. All sample solutions were filtered with a 0.45- μ m

membrane filter before injection.

Dendrimer solutions of 1mM PAMAM (G0.5, G1.5, G2.5, G3.5, and G5.5) were prepared in methanol for the MALDI-TOF sample analysis. The matrix solution of 10 mg/mL sinapinic acid (SIN) was made by dissolving the solid in a 60:40 (v/v) acetonitrile-water mixture. The matrix solution of 12 mg/mL α -cyano-

4-hydroxycinnamic acid (CHCA) was prepared by dissolving the solid in 60:40:0.1% acetonitrile-water-trifluoroacetic acid mixtures.

Instrumentation and methods

All CE experiments were performed using a P/ACE system 5050 (Beckman, Palo Alto, CA) interfaced to an IBM-compatible personal computer equipped with a Beckman System Gold for data collection and operation. Fused-silica capillary tubing (75- μ m i.d.) from Polymicro Technologies (Phoenix, AZ) was used for all experiments. A 57-cm-long capillary was used for the experiments at pH 7.0, and a 27-cm-long capillary was used for experiments from pH 2.5 to 2.10. All experiments were carried out at 20°C. The capillary was pre rinsed with 0.4M KOH for 2 min, with water for 2 min, and then with the separation buffer for 3 min before each injection. Samples were injected hydrodynamically by applying high pressure. The detection wavelength was 214 nm. The diode array was set to scan from 198 to 442 nm. Nitromethane was employed as the neutral marker to measure the EOF.

A VG ToFSpec-SE (Manchester, UK) MALDI-TOF MS was used for the mass analysis of the dendrimers. The dendrimers were analyzed in the linear mode with positive ion detection. A nitrogen laser was used to ionize the samples in a matrix solution. The dendrimer solution of 1 μ L PAMAM G1.5 (1mM in methanol) was mixed with 1 μ L CHCA matrix solution; all the other dendrimers (PAMAM G0.5, G2.5, G3.5, and G5.5) were mixed with a 1- μ L SIN matrix solution. The mixtures were allowed to dry on the probe spot. The spots were rinsed with 1 μ L of water before analysis to remove excess salts. The measured molecular weights were based on internal mass calibration using mixtures of substance P ($MH^+ = 1349$ da), adrenocorticotrophic hormone fragment (18-39) ($MH^+ = 2466$ da), cytochrome c ($MH^+ = 12360$ da), myoglobin (horse) ($MH^+ = 16953$ da), and bovine serum albumin ($MH^+ = 66431$ da).

Results and Discussion

Characterization of PAMAM

According to the vendor, the commercially available PAMAM dendrimer was synthesized from an initial core of ethylenediamine. The branches were built up by the repetition of a two-step process involving an initial Michael addition to the core molecule with methyl acrylate followed by amidation of the terminal ester with ethylenediamine. Repetition of the two-step pro-

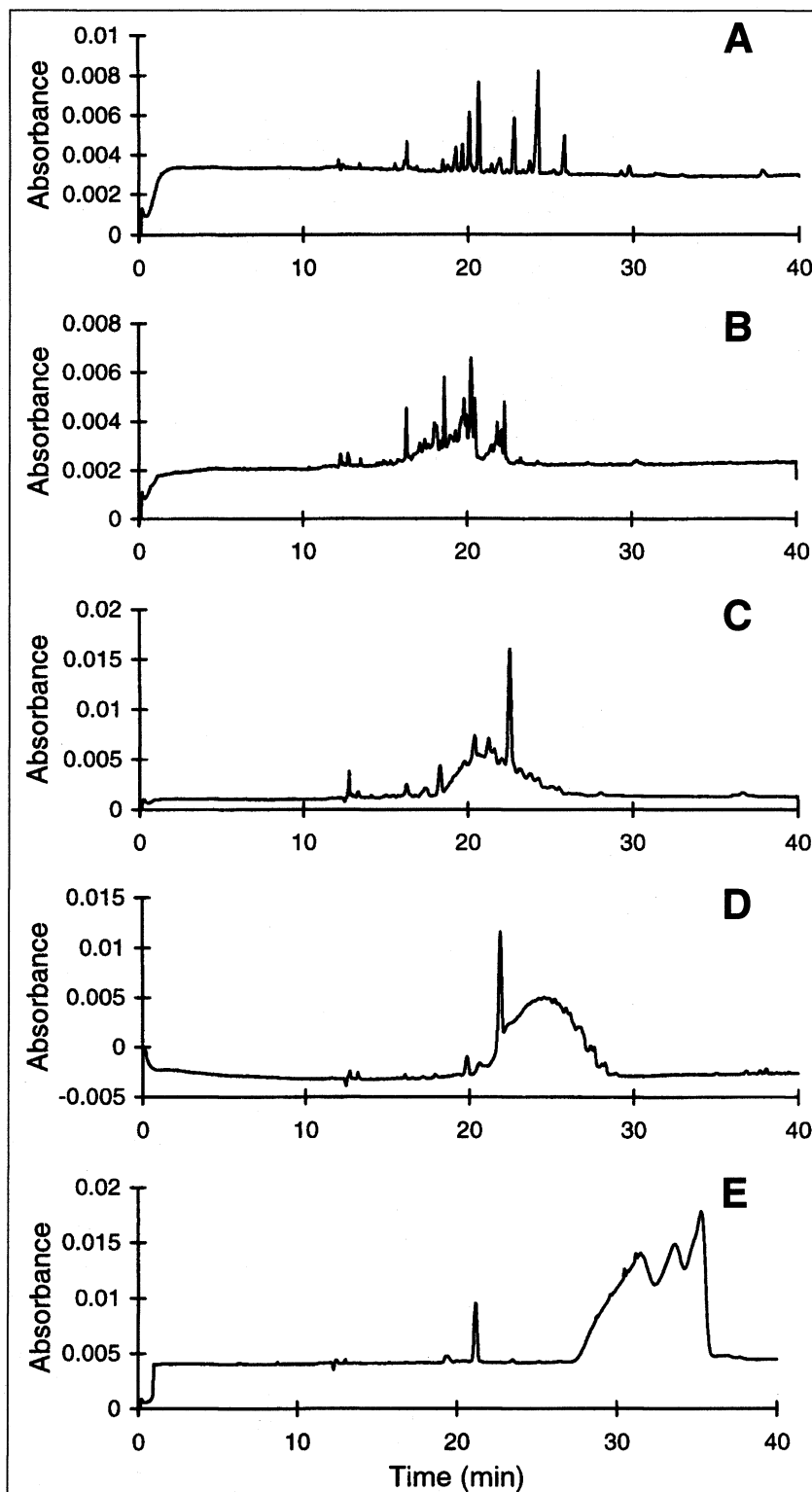


Figure 2. Migration of PAMAM. (A) G0.5, (B) G1.5, (C) G2.5, (D) G3.5, and (E) G5.5 in 100mM phosphate buffer (pH 7.0). Silica capillary, 57 cm (50 cm to detector); applied voltage, 10 kV (175 V/cm).

cess formed whole generations terminated with amine groups. Cessation with the Michael-addition step constituted half-generations terminated with carboxylate groups. The dimensions of dendrimers were estimated by the degree of polymerization, diameter, molecular weight, and number of surface groups. The molecular weight was estimated by applying the molecular weight of the core molecule, repeating units, and terminal units into Tomalia's equation (8). Assuming total reactive conservation, Table I lists the calculated surface charge, molecular weight, and an average measured mobility of PAMAM for each of the generations used in this study.

CE was used to separate the components of each dendrimer to study the polydispersity of each of the dendrimers used in this study. The electrophoretic mobilities of several generations of PAMAM were measured by performing CE analysis on each dendrimer in 100mM, pH 7.0 phosphate buffer. The electropherogram for each generation showed multiple migration peaks, as in Figure 2. The diode-array detection was set to scan from 198 to 442 nm for each peak. Each component had the same spectrum; absorbance was from 200 to 320 nm, which was consistent with the individual components having similar structures. The electropherograms indicate increased complexity for higher generations of dendrimer. Not surprisingly, each additional step in the

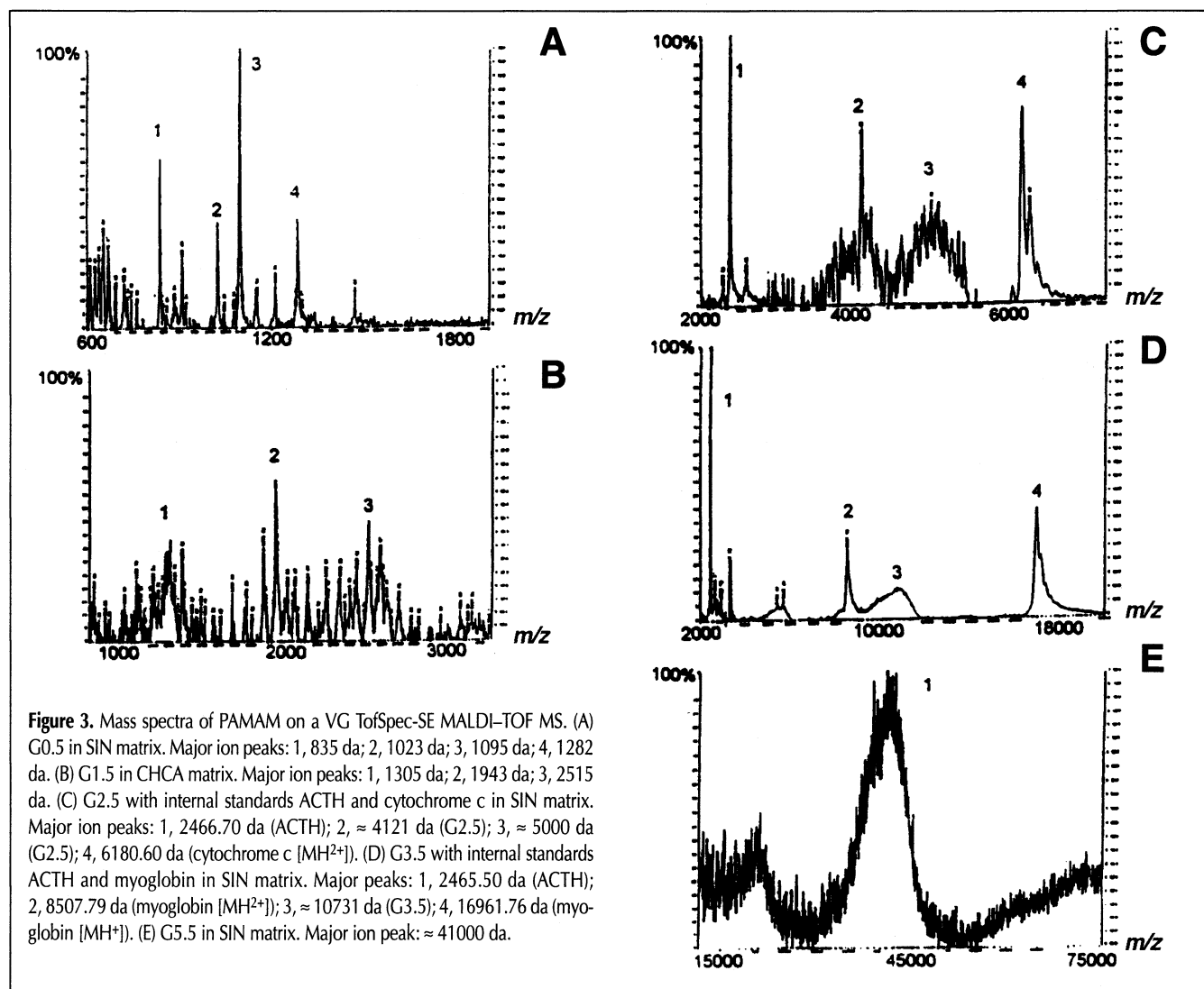
synthesis seemingly increases the likelihood of the presence of incompletely formed reaction products.

The average mobility of the G0.5 PAMAM was estimated by the following equation:

$$\mu = \sum_i^n \mu_i \times \frac{t_i}{t_1} \times S_i$$

where t_i is the migration time of the peak, t_1 is the migration time of the first peak, S_i is the percentage of peak area, and μ_i is the mobility of the peak.

Because the dendrimers of higher generation migrated as a broad band instead of discrete peaks under electrophoretic conditions, the migration times for generations from 1.5 to 3.5 and 5.5 were averaged with the starting point and the ending point of the band peak in their electropherogram. Although the apparent mobility of G1.5 seemed to be slightly decreased relative to the other generations, the intrinsic electrophoretic mobility of the starburst dendrimer indicated by the migration time seemed to increase generally as a function of the dendrimer generation because the electrophoretic mobility of the dendrimers opposed the EOF under these conditions. This increased migration time



was consistent with the increased charge-to-mass ratio with increasing generation reported in Table I.

Preliminary results by MALDI-TOF MS on the dendrimers (Figure 3) not only confirmed that the mass of PAMAM increased as a function of generation but also confirmed the polydispersity of the polymer observed in the CE experiments (Figure 2). The G0.5 spectrum (Figure 3A) showed collections of distinct peaks with major ions present at 835, 1023, 1095, and 1282 da. The G1.5 spectrum (Figure 3B) displayed a number of low-intensity peaks with major ions present at 1305, 1943, and 2515 da. The G2.5 (Figure 3C) spectrum showed two broad peaks with estimated centroids of approximately 4121 and 5000 da, whereas G3.5 (Figure 3D) and G5.5 (Figure 3E) appeared as large broad

peaks with estimated centroids of approximately 10,731 (G3.5) and 41,000 da (G5.5). The internal standard peaks were well-resolved with G2.5 (Figure 3C) and G3.5 (Figure 3D); for example, discrete peaks were obtained in the spectrum with G2.5 (Figure 3C) at 2466.70 (ACTH) and 6180.60 da (cytochrome c [MH²⁺]) as well as with G3.5 (Figure 3D) at 2465.60 (ACTH), 8507.79 (myoglobin [MH²⁺]), and 16961.76 da (myoglobin [MH⁺]). This suggests that the broad peaks for G2.5, G3.5, and G5.5 resulted from the sample heterogeneity compounded by the decreasing resolution of the MS as the measured mass increased.

Effect of buffer concentration

Phosphate buffer was selected as the electrolyte carrier because of its wide buffering pH range. The initial buffer concentration was 25mM because the EOF decreases in proportion to the square root of the buffer concentration, and Joule heating can be a problem at high buffer concentrations. It was found that buffer capacity must be a primary concern with the addition of the dendrimer. The addition of 2mM dendrimer G0.5 into 25mM pH 7.0 phosphate buffer changed the pH from 7.0 to 8.0. The resulting electropherogram is shown in Figure 4A. Adjustment of the pH back to 7.0 with acid reduced the EOF and delayed the analytes, as shown in Figure 4B. Increasing the buffer concentration 10-fold provided sufficient buffer capacity to maintain the pH at 7.0 upon addition of the dendrimer. Increasing the buffer concentration also lengthened the retention time by decreasing the EOF and improved the separation, as can be seen in Figure 4C.

Effect of pH

The separation mechanism can vary with pH because of changes in the ionization state of the PAMAM and the charges on the analytes. At pH 7.0, the dendrimers were negatively charged and migrated against the EOF. The analytes were neutral zwitterions carrying zero overall charge. Hence, the migration of these analytes was close to that of neutral species. The electrokinetic separation under this condition should be mainly determined by the partition of solute between dendrimer and aqueous phase and should be manifested as a delay in the migration in the presence of the dendrimer relative to their migration in the absence of the dendrimer.

pH 7.0

Overall, increased retention was observed with the increase in starburst dendrimer generation, as shown in Figure 5. In Figure 5, the retention of the analytes increased with the addition of 2mM PAMAM to G0.5 (Figure 5A), G1.5 (Figure 5B), and G2.5 (Figure 5C) in the phosphate buffer. The selectivity of G2.5 (Figure 5C) may have been

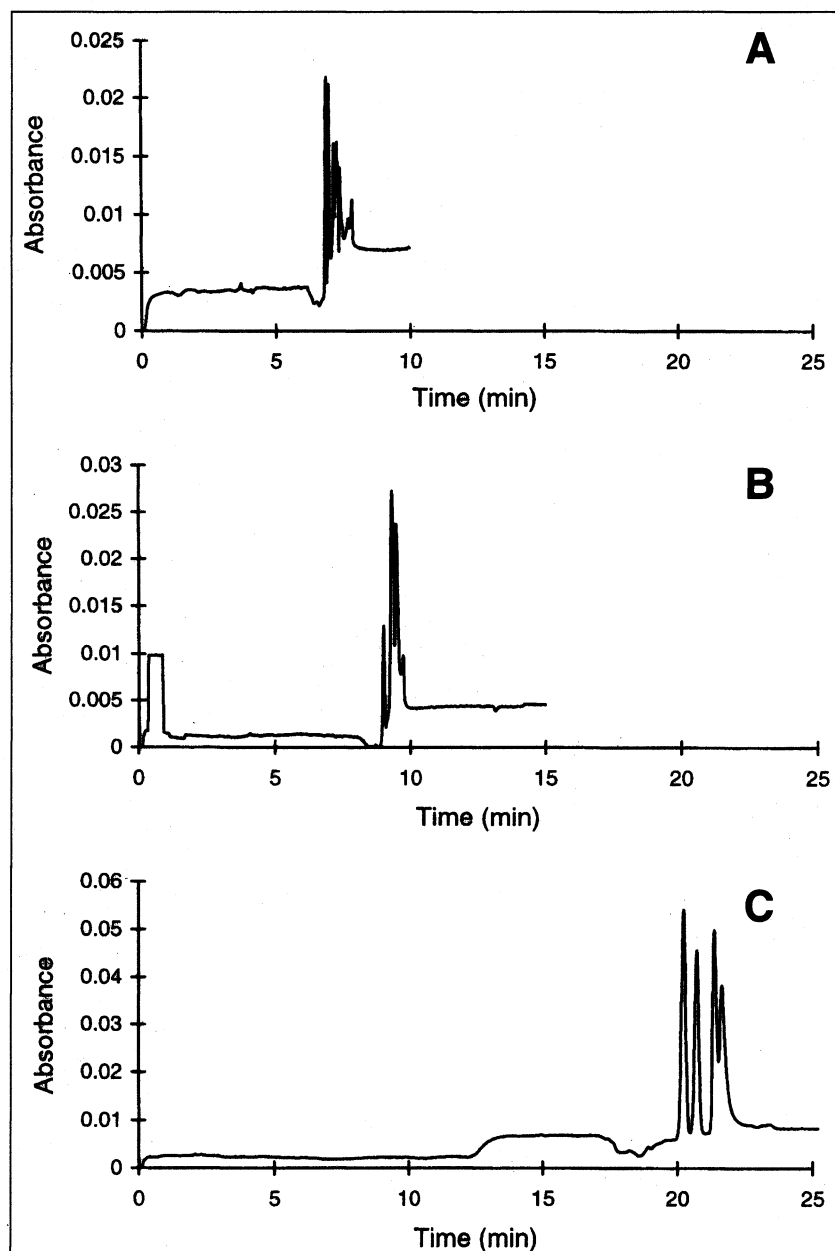


Figure 4. Effect of phosphate buffer concentration on separation of analytes with addition of 2mM PAMAM G0.5. (A) 25mM phosphate buffer with an initial pH of 7.0 and a pH of 8.0 after the addition of dendrimer; applied voltage, 15 kV (263 V/cm). (B) 25mM phosphate (pH 7.0); applied voltage, 15 kV (263 V/cm). (C) 100mM phosphate (pH 7.0); applied voltage, 10 kV (175 V/cm); silica capillary, 57 cm (50 cm to detector). Peaks: 1, HPA; 2, PA; 3, TYR; 4, methyl-DOPA; 5, DOPA; 6, PG.

slightly better than those of G0.5 (Figure 5A) and G1.5 (Figure 5B), though the overall separation of the aromatic acids was not complete. In general, analyte mobilities decreased as the concentration of G1.5 increased, as shown in Figure 6. The EOF, as measured by nitromethane, decreased as the dendrimer concentration increased, resulting from the increased ionic strength in the buffer with the addition of the polyelectrolytic dendrimer. Thus, the slowed migration may be attributed to not only increased association with dendrimer, but also the reduction of EOF.

Some practical difficulties in the dendrimer system were compounded by the relatively poor separation at pH 7.0. The baseline in the electropherogram was usually high because of the absorbance of the dendrimer over a wide range of wavelengths; the baseline fluctuated, possibly as a result of the polydisperse dendrimer eluting from the column. A fairly high concentration of analytes dissolved in separation buffer was required to obtain a good signal-to-noise ratio. These problems became more severe with higher generations of dendrimer as buffer additives.

pH 2.4

At a low pH, the analytes carry a net positive charge. However, the ionization states of the dendrimers are complicated because

of their polyelectrolyte nature. G0.5 and G1.5 dendrimers may carry little charge and should consequently move slowly in the capillary with the EOF, which should be minimal at a low pH. Thus the dendrimer may function as a pseudo-stationary phase in the column. The positively charged analytes moved cathodically with only weak assistance from the EOF. Association with the dendrimer impeded their migration. However, higher generations of the dendrimer (G2.5, G3.5, and G5.5) may carry significant negative charges, even at pH 2.4. The polarity of the applied

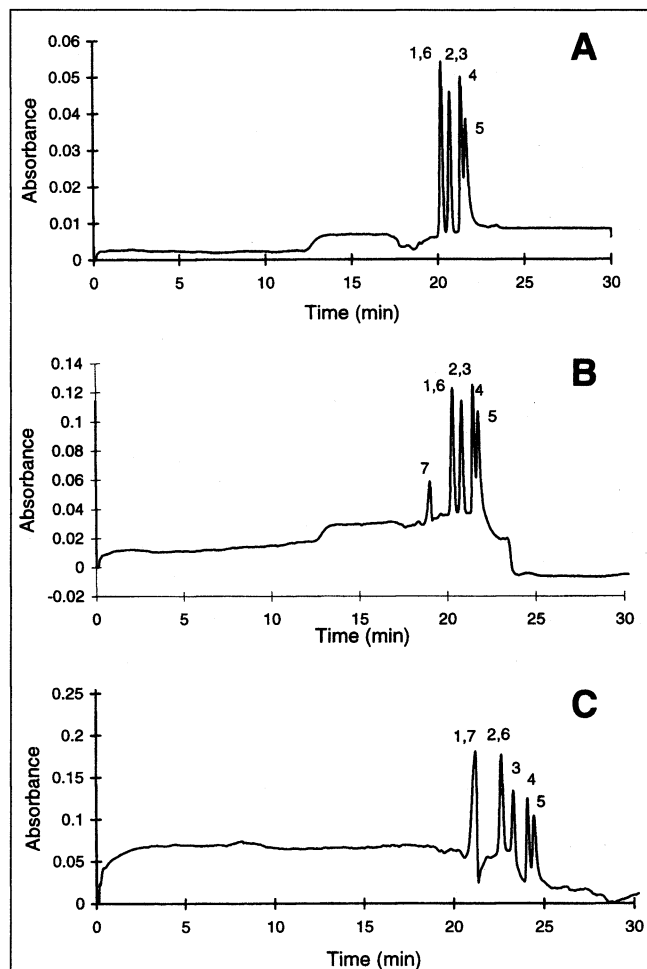


Figure 5. Separation of analytes in 100mM phosphate (pH 7.0) with 2mM PAMAM (A) G0.5, (B) G1.5, and (C) G2.5. Silica capillary, 57 cm (50 cm to detector); applied voltage, 10 kV (175 V/cm). Peaks: 1, HPA; 2, PA; 3, TYR; 4, methyl-DOPA; 5, DOPA; 6, PG; 7, nitromethane.

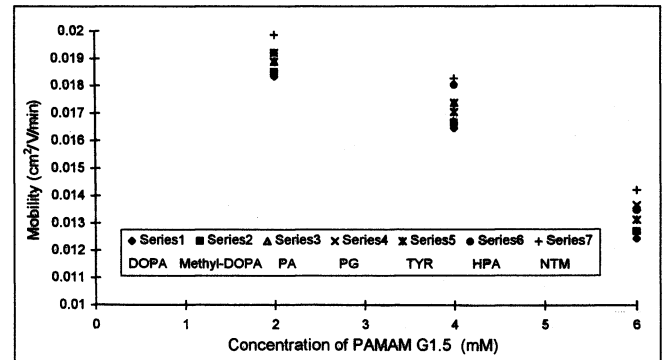


Figure 6. Effect of PAMAM G1.5 concentration on the mobility of EOF and analytes. Buffer was made with PAMAM G1.5 in 100mM phosphate (pH 7.0); all other conditions were the same as in Figure 5.

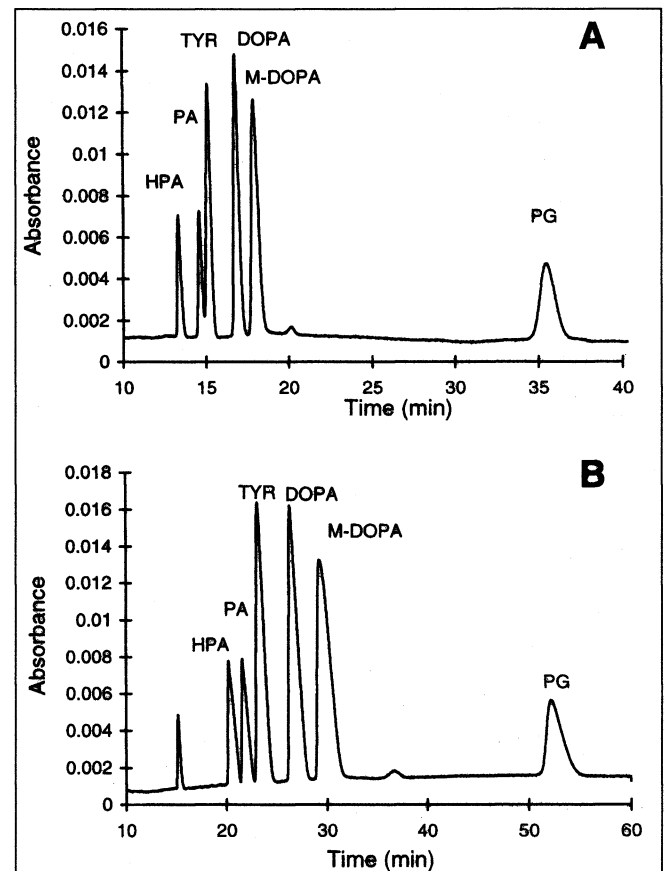


Figure 7. Separation of analytes with low generation numbers at low pH. Silica capillary, 27 cm (20 cm to detector); (A) 25mM phosphate buffer (pH 2.4) with 1mM PAMAM G0.5; applied voltage, 10 kV (370 V/cm); (B) 25mM phosphate buffer (pH 2.15) with 0.1mM PAMAM G1.5; applied voltage, 7 kV (259 V/cm).

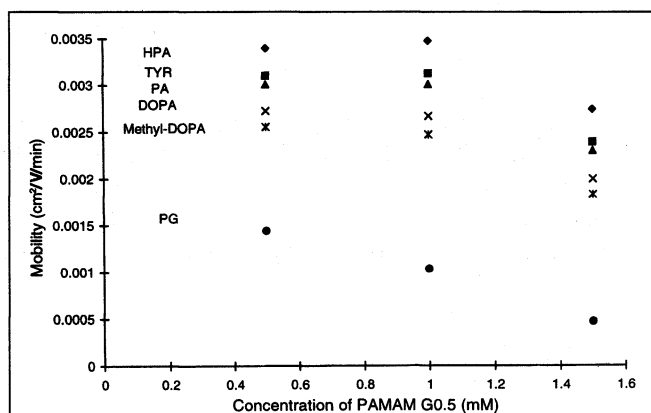


Figure 8. Effect of PAMAM G0.5 in 25mM phosphate buffer (pH 2.4). Silica capillary, 27 cm (20 cm to detector).

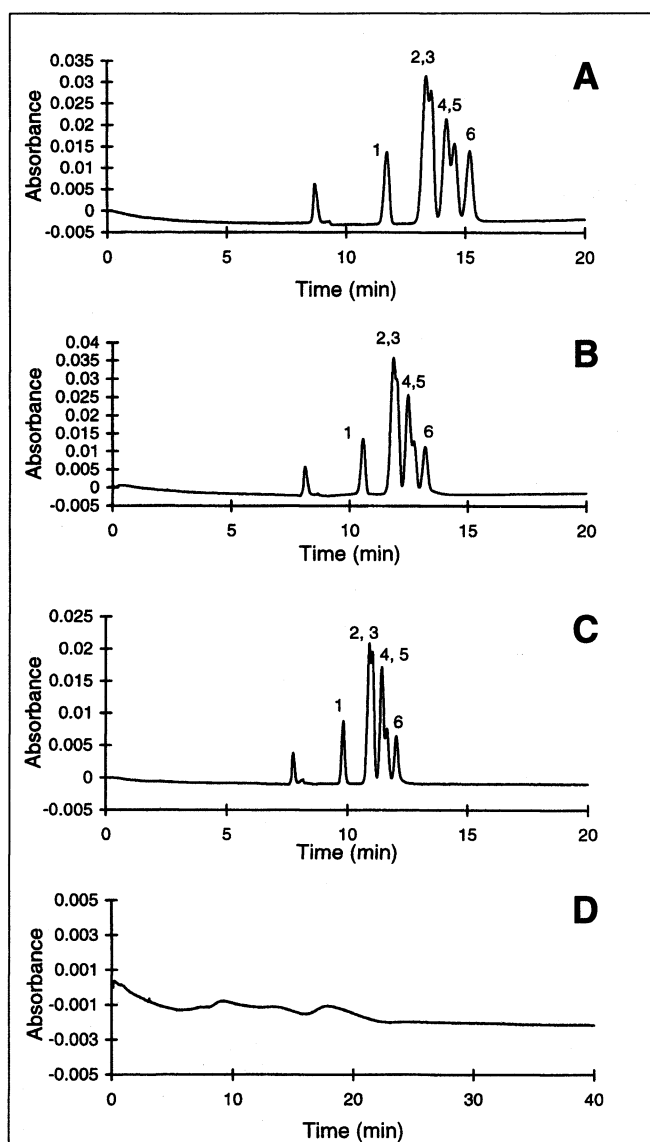


Figure 9. Separation of analytes with G2.5, G3.5, and G5.5 in 100mM phosphate buffer (pH 2.4) by reversed polarity. Silica capillary, 27 cm (20 cm to detector); (A) 10µM G2.5, (B) 5µM G3.5, (C) 1µM G5.5, (D) no dendrimers; applied voltage, 5 kV (185 V/cm). Peaks: 1, PG; 2, methyl-DOPA; 3, DOPA; 4, TYR; 5, PA; 6, HPA.

voltage had to be reversed to enable the negatively charged dendrimers to bring the positively charged analytes to the detection window. Ion-pairing formation may take place between positively charged analytes and partially negatively charged dendrimers.

Selectivity and baseline were improved by decreasing the pH to 2.4, as shown in Figure 7. Increased selectivity was observed with G1.5 (Figure 7B) as the buffer additive compared with G0.5 (Figure 7A). It should be noted that the concentration used for G1.5 in Figure 7 was only 0.1mM, whereas the concentration of G0.5 was 1mM. The extremely slow migration of PG with both dendrimers, despite having the largest charge-to-mass ratio, may have resulted in part from the low pK_a of PG as well as the strong association of PG with the three-dimensional structure of the dendrimer. Increasing the concentration of G0.5 decreased the mobility of analytes, as shown in Figure 8. However, as can also be seen in Figure 8, a significant improvement in selectivity was not observed as a function of the dendrimer concentration.

As indicated in Table I and seen in the electropherograms of the individual dendrimers, the intrinsic electrophoretic mobility of the dendrimers increased with increasing generations. Reversing the polarity was necessary to carry out the separation

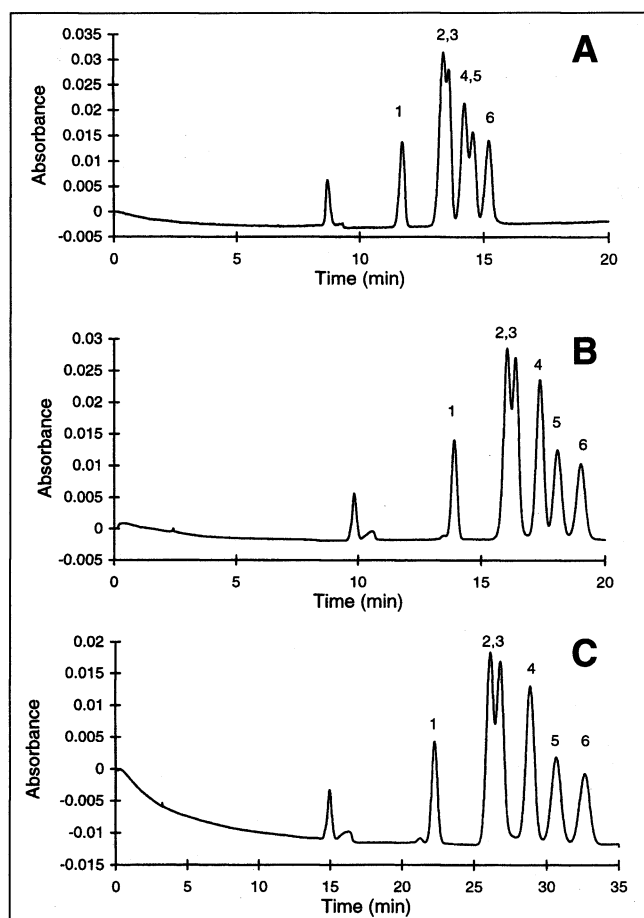


Figure 10. Effect of phosphate buffer concentration at pH 2.4 by reversed polarity. Separation was performed with 10µM PAMAM G2.5. Silica capillary, 27 cm (20 cm to detector); (A) 100mM phosphate buffer; applied voltage, 5 kV (185 V/cm); (B) 150mM phosphate buffer; applied voltage, 5 kV (185 V/cm); (C) 200mM phosphate buffer; applied voltage, 4 kV (148 V/cm). Peaks: 1, PG; 2, methyl-DOPA; 3, DOPA; 4, TYR; 5, PA; 6, HPA.

with PAMAM G2.5, G3.5, and G5.5 as carriers in electrokinetic chromatography at pH 2.4, as discussed previously. Because the negatively charged dendrimers brought the positively charged analytes to the detection window, the elution order was opposite to the one obtained with the cathodic polarity used for G0.5 and G1.5.

The strong association of solutes with dendrimers was indicated by the short retention time, as shown in Figure 9. The analytes migrated out with micromolar concentrations of PAMAM G2.5 (Figure 9A), G3.5 (Figure 9B), and G5.5 (Figure 9C) within 18 min. It should be noted that these analytes failed to elute in the absence of dendrimers (Figure 9D) under these conditions. The migration time of the analytes decreased, indicating both an increasing interaction between analytes and dendrimers, as a function of dendrimer generation and the increased mobility of the higher generation dendrimers.

The migration time of the solutes and the peak width increased as the buffer concentration increased from 100mM (Figure 10A) to 150mM (Figure 10B) as in Figure 10, suggesting the importance of electrostatic interactions between the analytes and the dendrimers. With buffer concentration increasing to

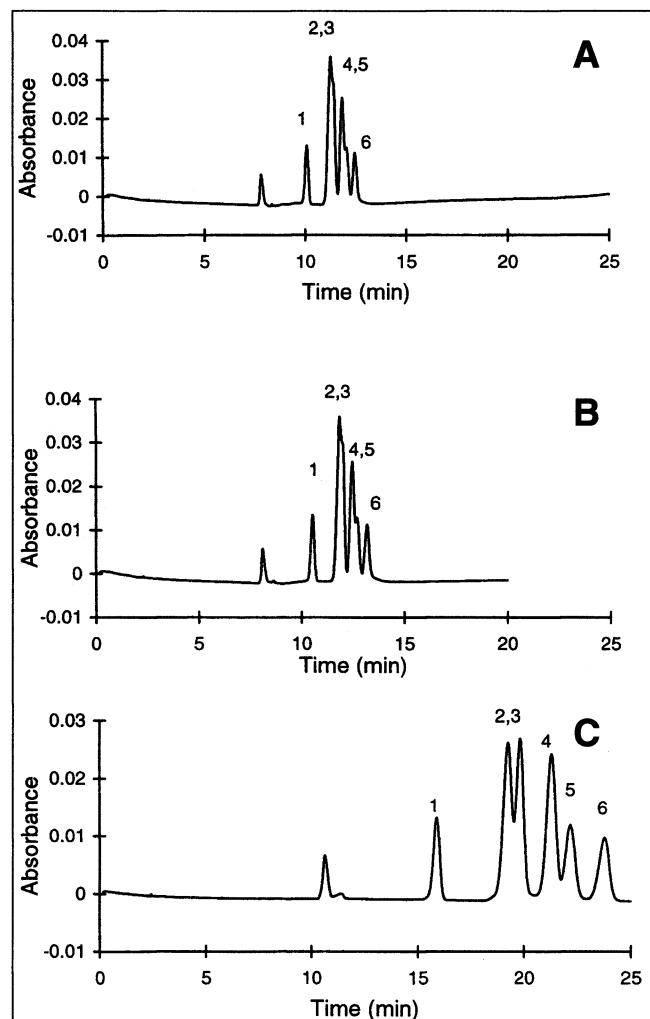


Figure 11. Effect of G3.5 concentration at pH 2.4 by reversed polarity in 100mM phosphate buffer. Silica capillary, 27 cm (20cm to detector); (A) 10 μ M; (B) 5 μ M; (C) 1 μ M PAMAM G3.5; applied voltage, 5 kV (185 V/cm). Peaks: 1, PG; 2, methyl-DOPA; 3, DOPA; 4, TYR; 5, PA; 6, HPA.

200mM (Figure 10C), Joule-heating became a significant problem, requiring a reduction in the applied voltage.

As expected, the migration times of the solutes increased with the decrease in concentration of PAMAM G3.5, as shown in Figure 11. The migration time slightly increased with a decrease in dendrimer concentration from 10 μ M (Figure 11A) to 5 μ M PAMAM G3.5 (Figure 11B) and significantly increased with a concentration of 1 μ M PAMAM G3.5 (Figure 11C).

The migration times of the analytes and the separation was fairly insensitive to the concentration of the G5.5, as shown in Figure 12. The electropherogram of the analytes barely changed with the decrease of concentration of PAMAM G5.5 from 5 μ M (Figure 12A) to 1 μ M (Figure 12B) and even down to 0.5 μ M (Figure 12C). It should be noted that the dendrimer G5.5 should have had a high negatively charged density, and the analytes were positively charged at pH 2.4. Thus, the analytes should have had a high affinity for the dendrimer. In addition, the large size of the dendrimer provided considerable surface area (as well as internal volume, although access to the interior may be sterically limited) for interaction and may have presented multiple independent interaction sites for the analytes. This may account in part for the insensitivity of migration times and separation performance of these analytes with respect to G5.5 concentration.

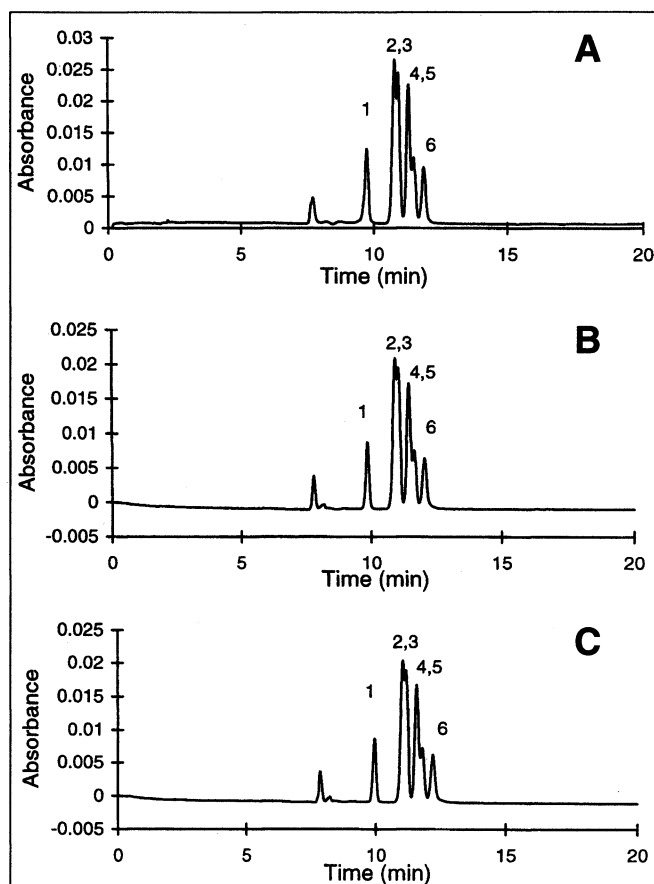


Figure 12. Effect of PAMAM G5.5 concentration at pH 2.4 by reversed polarity in 100mM phosphate buffer. (A) 5 μ M; (B) 1 μ M; (C) 0.5 μ M PAMAM G5.5. All other conditions were the same as in Figure 11. Peaks: 1, PG; 2, methyl-DOPA; 3, DOPA; 4, TYR; 5, PA; 6, HPA.

Conclusion

Dendrimers can be employed as a pseudo-stationary phase for electrokinetic chromatography. The interaction between analytes and dendrimers was different at different pH values because the ionization states of both the analytes and the dendrimers varied. The best separation of the analytes used in this study was obtained at a low pH (e.g., pH 2.4), whereas only limited selectivity of dendrimers was obtained at pH 7.0. The size of the dendrimer may offer another alternative for improved selectivity.

As polyelectrolytes, care must be taken to ensure proper pH control with the addition of the dendrimers. In these analytes, working with low pH eased detection problems. The rapid development in the dendrimer synthesis field may reduce or solve the polydispersity problem of the dendrimer in the near future. This study also demonstrated that electrokinetic chromatography is a useful method to study the interaction between macromolecules and drugs.

Acknowledgment

The authors wish to thank Dr. Ralph Spindler at Dendritech, Inc. for his helpful comments and suggestions. We also thank the National Institute of Health for the funds to purchase the CE instrument through the Small Instrumentation Grant program. The Department of Chemistry at the University of Cincinnati generously supported H. Gao through the Lange Fellowship.

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Manuscript accepted January 14, 1998.