Supporting Information For:

Mimicking PAMAM Dendrimers with Ampholytic, Hybrid Triazine Dendrimers: A Comparison of Dispersity and Stability

Sanjiv Lalwani[†,*], *Abdellatif Chouai, Lisa M. Perez, Vanessa Santiago, Sunil Shaunak*[‡], *Eric E. Simanek*[†,*]

[†]*Department of Chemistry, Texas A&M University, College Station, TX 77843, USA*
[‡]*Faculty of Medicine, Imperial College London, Hammersmith Hospital, Ducane Road, London, W12 ONN, UK*

Table of Contents

Selection of BGE compositions and preparation of BGE solutions.

1. Analysis of **1e** and **2e**.

A pH 3.4 BGE made of formic acid/formate as the buffering species was chosen to provide significant cationic charge to the dendrimers **1e** and **2e**. Protonation of the amines allows for solubility of both dendrimers at detectable levels and provides sufficient charge to result in migration during electrophoresis. Lithium hydroxide was chosen as the titrant to (i) result in BGEs with low conductivities, and thus result in low currents during electrophoresis and (ii) minimize the effects of electromigration dispersion due arising from a mismatch between the effective mobilities of the analyte and the BGE co-ion.

2. Analysis of **1a** and **2a**.

A 50mM phosphate/lithium BGE had been previously determined as optimum for analysis of anionic and ampholytic dendrimers using capillary electrophoresis (CE).[1] As such, a pH 6.9 BGE made of dihydrogen phosphate/hydrogen phosphate as the buffering species was chosen for the analysis of dendrimers **1a** and **2a**, and to compare the dispersity of these materials with their commercially available PAMAM analogues. Lithium hydroxide was chosen as the titrant to result in BGEs with low conductivities, and thus result in low currents during electrophoresis. However, due to the presence of a "system peak" (a.k.a. eigen peak) in the vicinity of the detected signals, a pH 6.4 BGE prepared from Bis-tris and its corresponding conjugate acid as the buffering species and acetic acid as the titrant (chosen due to the similarity of effective mobility values of the analytes and the acetate ion) was used to obtain CE data that can be integrated with

certainty to determine purity. This BGE was chosen after iterative simulations using the Peakmaster software.

3. Determination of pH ranges that bracket the pI values of the respective dendrimers.

Four BGEs were used: pH 5.9 MES/lithium buffer, pH 4.7 acetate/lithium buffer, pH 3.4 formate/lithium buffer (same as used for the analysis of **1e** and **2e**) and pH 1.8 phosphate/lithium buffer. Buffering species were chosen to provide sufficient buffering capacity at the desired pH values (within ±0.5 of the pK_a value). Lithium hydroxide was chosen as the titrant due to its associated lower conductivity.

4. Preparation of BGE solutions.

In our previous work[1] it had been determined that 50-100mM BGEs provided sufficient buffering capacity for analysis of dendrimers using CE. As such, first a 1M solution of the buffering species was prepared by dissolving pure liquid or solid in 250 mL of deionized water. This was followed by titration with pure liquid or solid titrant as the pH of the solution was constantly monitored. Upon arrival at the desired pH, addition of titrant was stopped and the BGE solution was stored as the stock BGE. Typically, 100mM BGEs (with respect to the buffering species) were prepared for use in CE by diluting the stock BGE solution 10-fold. An exception to this preparation protocol was the pH 6.9 phosphate/lithium BGE: due to its high conductivity a 100mM BGE was prepared as stock BGE and a 50mM BGE was prepared for use in CE.

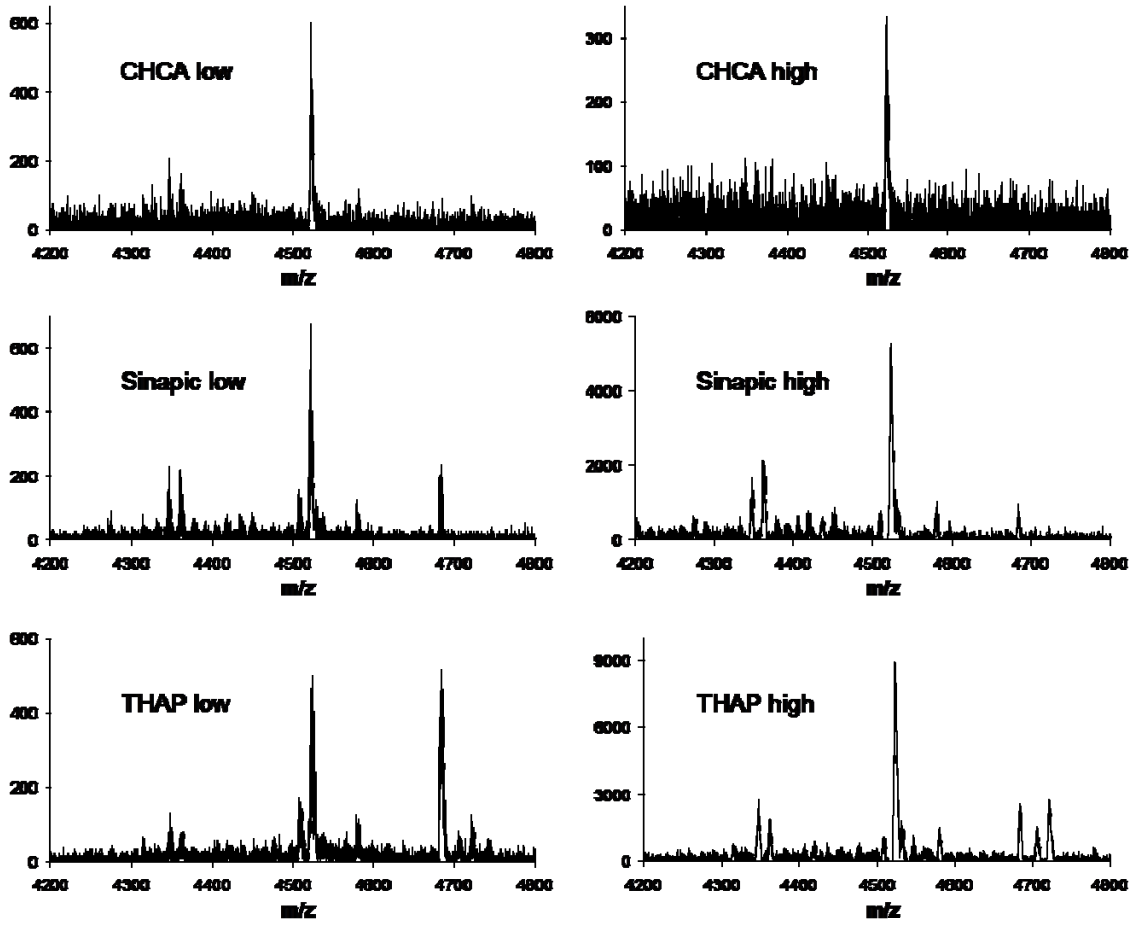
Optimization of MS analysis.

Figure 1. MALDI-TOF-MS analysis of the methanolic reaction mixture for the synthesis of **1e** using different matrices. Analysis was performed using "low" and "high" laser power settings.

CHCA : (E)-2-cyano-3-(4-hydroxyphenyl)prop-2-enoate or α-Cyano-4-hydroxycinnamic acid.

Sinapic : 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid or 3,5-Dimethoxy-4-hydroxycinnamic acid or Sinapinic acid.

THAP : 2,4,6-trihydroxyacetophenone



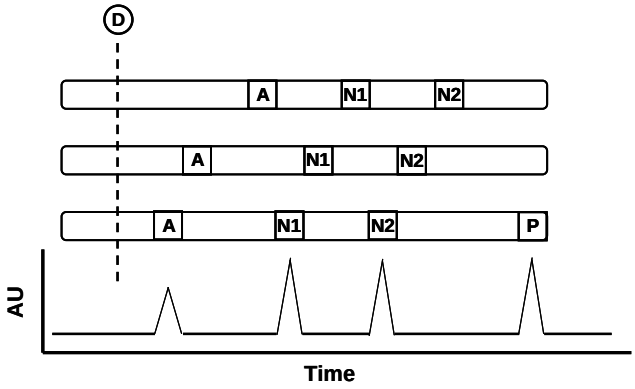
Detailed description of the 3-band PreMCE method.

The pH ranges that bracket the respective pI values of the dendrimers included in this study were obtained by determining their electrophoretic mobilities as a function of the pH of the BGEs in the $3 < pH < 7$ range, adapted from the work of Glukhovskiy and Vigh.[2] A 3-band PreMCE method was used, which may be divided into two steps: separation and mobilization. The sequence of events in the separation step proceeds as follows: a band of the analyte (A) is injected for 3 seconds, t_{inj}, into the capillary at a pressure setting of 0.5 psi; a band of the pure BGE is injected into the capillary for either 30 seconds or 1 minute, t_{transf}, using the same pressure setting. This step (first transfer step) moves the analyte band (A) into the capillary by a fixed distance, l_{transf}. Next, a band of the neutral marker is injected for t_{inj} (first neutral marker band, N1) and transferred into the capillary by another band of the pure BGE injected for time t_{transf}. This step is followed by the injection of another band of the neutral marker (second neutral marker band, N2) for time t_{inj}, and its transfer into the capillary by yet another band of the pure BGE, injected for time t_{transf}. Next, 5 kV potential, U_{appl}, is applied across the capillary for 1 or 2 minutes, (migration time, t_{migr}) under positive-to-negative polarity (the anode at the injection end and the cathode at the detector end of the capillary). U_{appl} and t_{migr} were selected to maximize the observed electrophoretic migration distance for all three bands, however, electrophoresis was terminated before any of the bands migrated past the detector. Top two panels in Figure 2 illustrate the position of the bands in the capillary, before and after electrophoresis, respectively.

After the separation step is complete, the sequence of events in the mobilization step are initiated: a third band of the neutral marker is injected (called the push peak, P) for time t_{inj}; then, the contents of the entire capillary are mobilized through the detector window by applying 0.7 psi of pressure from the injection end. The third panel in Figure 2 illustrates the position of the bands as a result of the injection of the push peak, and the corresponding detector trace that is recorded is shown in the bottom panel of Figure 2.

Although the effective mobility of the analyte and the mobility due to the electroosmotic flow can be calculated from the traces obtained using PreMCE[1], since the objective was to determine the pH range which brackets the pI of the dendrimer, simple determination of whether the analyte band was moving as an anion or as a cation sufficed. This could be inferred from the direction in which A migrates with respect to N1 as a result of the applied potential: if the distance between A and N1 increases, it can be inferred that the analyte band moved further away from N1, and migrated as a cation; if the opposite is true and the analyte band comes closer to N1, then the analyte must have migrated as an anion.

Figure 2. Illustration showing the position of the bands injected into the capillary before electrophoresis (top illustration), after electrophoresis (second illustration from the top), and after injection of the last band of neutral marker (third illustration from the top). The corresponding trace that is recorded is shown as the bottom illustration.

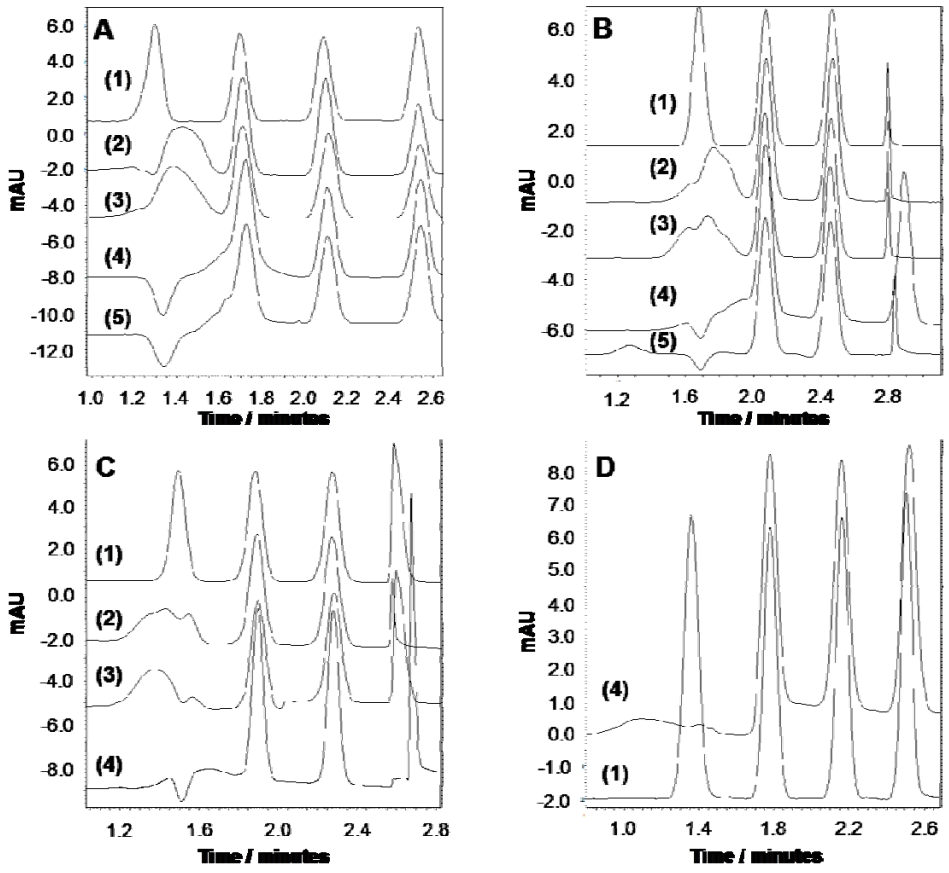


Complete PreMCE traces recorded.

Figure 3. Complete PreMCE traces recorded for the different dendrimers in BGEs of varying pH values. Legend is listed below:

A : traces obtained using pH 5.9 BGE
B : traces obtained using pH 4.7 BGE
C : traces obtained using pH 3.4 BGE
D : traces obtained using pH 1.8 BGE

1 : all peaks correspond to neutral marker (N)
2 : PAMAM G 1.5, N, N, N
3 : PAMAM G 2.5, N, N, N
4 : **1a**, N, N, N
5 : **2a**, N, N, N



References

(1) Lalwani, S.; Venditto, V. J.; Rivera, G. E.; Chouai, A.; Shaunak, S.; Simanek, E. E. *Macromolecules* **2009,** *42,* 3152-3161.
(2) Glukhovskiy, P.; Vigh, G.; *Electrophoresis* **1998,** *19,* 3166-3170.