Dendrimer Electrokinetic Capillary Chromatography: Unimolecular Micellar Behaviour of Carboxylic Acid Terminated Cascade Macromolecules

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Dendritic (cascade) macromolecules with carboxylic acid terminal moieties are examined as micellar substitutes for the separation of a homologous series of alkyl parabens *via* electrokinetic capillary chromatography employing aqueous mobile phase conditions; separations using the dendrimers yield excellent efficiency and resolution, while higher generation dendrimers demonstrate enhanced affinity for the parabens relative to lower generation dendrimers and so provide superior separations under identical conditions; the cascades are also shown to be sensitive to solution pH and the technique is extended to the separation of components found in a common cough medicine.

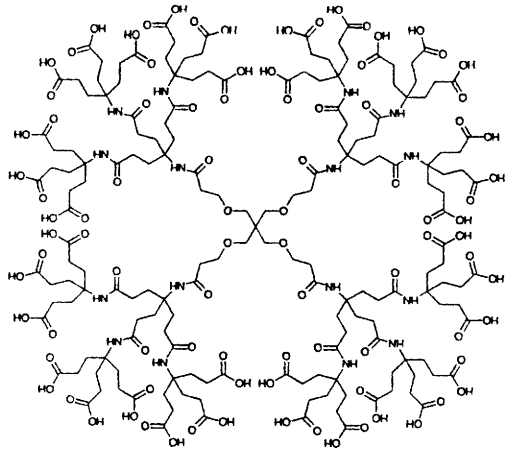
The application of micellar electrokinetic capillary chromatography (MECC)[1] to the separation of neutral organic species is increasingly of interest. Typical ionic surfactants used have included sodium dodecyl sulfate[2] (SDS), sodium cholate,[3] and cetyltrimethylammonium bromide[2] (CTAB). To alter the elution range and thereby influence the speed and range of molecules that can be separated by MECC, additives such as cyclodextrins,[4] non-ionic surfactants,[5] urea[6] and organic solvents[7] are often added to the analysis buffer.

Recently, monomolecular species that attempt to simulate globular micelle topology have been substituted for surfactant aggregates to eliminate or effectively reduce micellar concentration, solvent strength, pH and temperature constraints on MECC separations. Polymerized surfactant aggregates were used to separate alkyl phthalates and polynuclear aromatic hydrocarbons (PAHs).[8] Poly(amidoamine) dendrimers were also examined as carriers in MECC.[9] Both employed relatively high concentrations of organic cosolvents in the mobile phase (20–45% acetonitrile or 50% methanol;[8] 50% methanol[9]), hence separations based on micellar inclusion of the analytes are inhibited owing to unfavourable partition coefficients relative to the mobile phase and the surfactant substitutes.

We herein report the use of carboxylic acid terminated cascade macromolecules for the separation of a series of alkyl parabens in an aqueous environment. The paraben homologues employed included methyl, ethyl, propyl and butyl parabens[10] (4-hydroxybenzoate alkyl esters). Use of a substantially aqueous mobile phase should significantly enhance the ability of surfactant substitutes to effect separations.

The polyacid cascades[11] (or dendrimers; Fig. 1) were prepared divergently *via* a repetitive peptide-type coupling (dicyclohexylcarbodiimide–1-hydroxybenzotriazole) and deprotection (formic acid) scheme using an aminotris(*tert*-butyl ester) building block beginning with a four-directional core (synthesized by a Michael-type addition of 4 equiv. of acrylonitrile to pentaerythritol followed by acidic hydrolysis of the nitrile groups to give the corresponding tetraacid).

Fig. 2 shows the analysis of the parabens by (*a*) capillary zone electrophoresis (CZE) and (*b*) dendrimer electrokinetic chromatography employing the 36-carboxylic acid terminated cascade (Fig. 1; generation 2). All separations were performed on a commercial capillary electrophoresis instrument (BioFocus 3000, BioRad Laboratories). The separations were performed in a fused silica capillary (50 μm i.d., 360 μm o.d., 20.4 cm inlet–detector distance, Polymicro Inc.) under constant voltage conditions. Samples were introduced into the capillary with a 1 psi s pressure injection (139 pmol of each paraben introduced into the capillary). Detection was by UV



Generation	Number of terminal moieties	Nominal formula mass
0	4	424
1	12	1,341
2	36	4,092
3	108	12,345
4	324	37,102
5	972	111,373
6	2,916	334,187

Fig. 1 General structure, number of termini and molecular weights of the various generations of the amide-based cascades employed as micellar substitutes

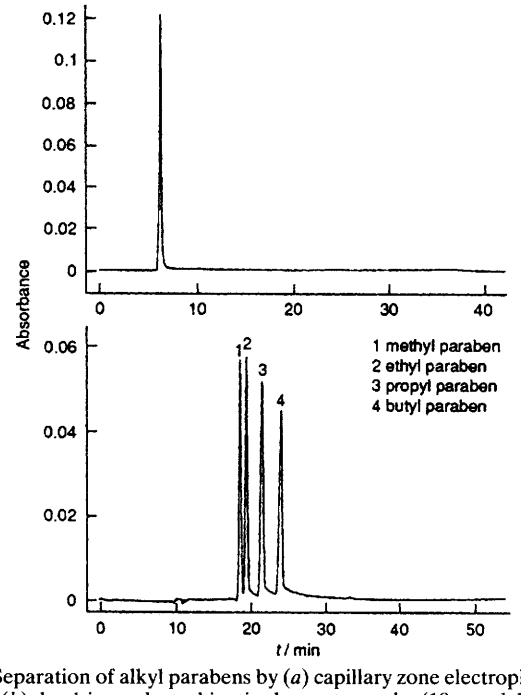


Fig. 2 Separation of alkyl parabens by (*a*) capillary zone electrophoresis and (*b*) dendrimer electrokinetic chromatography (10 mmol dm^{-3}, 2nd generation). Separations were performed in 100 mmol dm^{-3} borate buffer (pH 10.0) with a driving potential of 280 V cm^{-1}. Detection was by UV absorbance at 254 nm.

absorbance measurement at 254 nm. As expected, the CZE analysis produced no separation as the paraben molecules are uncharged. Addition of the dendritic micellar substitutes to the analysis buffer separated the parabens as a function of their affinity for the hydrophobic environment of the dendrimer. Electrophoretic processes cause the dendrimers to migrate towards the more positive electrode at the capillary inlet at a rate dependent upon their size and charge. Electroosmotic flow within the capillary causes the neutral analytes to be swept towards the capillary outlet. Thus, the retention times are proportional to the time the analyte spends interacting with the dendrimers. More lipophilic analytes are retained longer and elute at longer retention times.

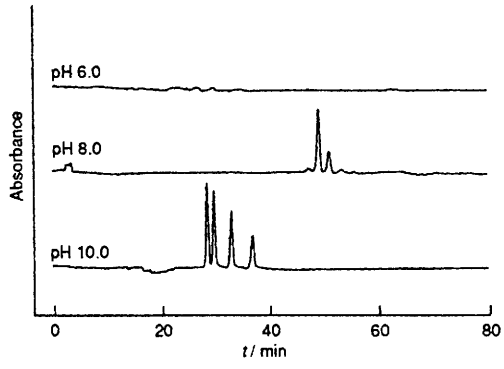


Fig. 3 Separation of parabens as a function of pH. Conditions are as in Fig. 2 except the driving potential was 200 V cm^{-1}. The buffers were as follows: pH 10 100 mmol dm^{-3} borate; pH 8.0 100 mmol dm^{-3} phosphate; pH 6.0 100 mmol dm^{-3} carbonate.

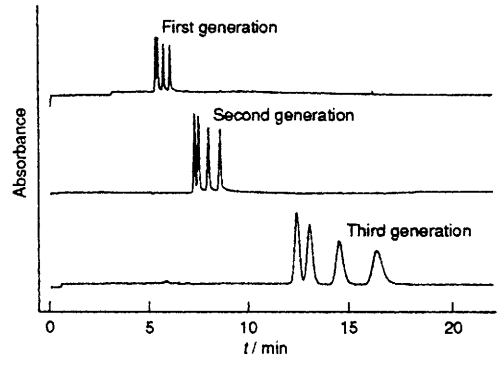


Fig. 4 Separation of alkyl parabens with dendrimers of different generation. Conditions are as in Fig. 2 except that the driving potential was 440 V cm^{-1}.

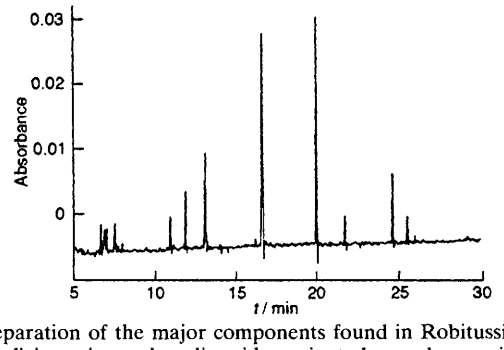


Fig. 5 Separation of the major components found in Robitussin CF cough medicine using carboxylic acid terminated cascades as micellar substitutes. Separation was in 100 mmol dm^{-3} borate buffer (pH 10.0) with detection at 340 nm.

Separations with amide-based cascade polymers were strongly influenced by the pH of the analysis buffer. In general, the optimum conditions for analysis were established at high pH values (Fig. 3). Dendrimer mobility was noticeably reduced at neutral pHs. This may result from increasing cationic behaviour at these lower pHs, and may ultimately cause the dendrimer to interact with the negatively charged walls of the fused silica capillary.

The influence of dendrimer size on separation was also investigated (Fig. 4). Paraben separation with 10 mmol dm^{-3} first, second and third generation cascades revealed baseline resolution could easily be achieved using second or higher tier polyacids. Comparison of the separations using the third tier dendrimer to that obtained with the second tier macromolecule reveals significant peak broadening and an approximately doubled run time. Interestingly, a 100 mmol dm^{-3} solution of the first generation dendrimer behaves similarly to a 10 mmol dm^{-3} solution of the second generation dendrimer, which in turn behaves similarly to a 4 mmol dm^{-3} solution of the third generation. Thus retention appears to increase proportionally with the phase ratio (*i.e.* mass of dendrimer/mass of buffer).

The use of dendrimer electrokinetic chromatography was further examined for the separation of more complex mixtures. Fig. 5 illustrates the analysis of Robitussin CF cough medicine. Previous analysis of Robitussin by SDS-MECC has shown that it is possible to separate acetaminophen, caffeine, naproxen, gauifenesin, phenacetin and noscapine in this mixture.[12] The equivalent separation by dendrimer electrokinetic capillary chromatography is shown in Fig. 5. At least eight major components are observed in this analysis.

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12 Examination of Robitussin CF cough medicine was the topic of a poster presented at HPCE '93 (Orlando, Florida, January 1993) by D. N. Heiger.