A Study of Dendrimer-Solute Interactions via Electrokinetic Chromatography

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Abstract: Polyacid cascade dendrimers (generations 1−4) are used as a pseudostationary phase for electrokinetic capillary chromatography to separate mixtures of neutral species. Separation of a homologous series of molecules allow the capacity factors and distribution coefficients to be calculated, which in turn allow the standard enthalpy, entropy, and Gibbs free energy for solubilization to be evaluated by van't Hoff analysis. Comparison of these values to analogous values measured for sodium dodecyl sulfate (SDS) micelles suggests fundamental differences in the thermodynamic quantities associated with solubilization. As the size of the polyacid dendrimer increases, entropy becomes the driving force for solubilization, and enthalpy is disfavored. Gibb's free energy values are negative for all of the dendrimer/analyte interactions investigated. The capacity factor is found to increase linearly with increasing concentrations of dendrimer. Thermodynamic parameters are evaluated for several small molecules to show that the technique is not limited to molecules that are part of a homologous series.

Introduction

In recent years there has been rapid growth in the development of methods used to synthesize and characterize cascade (dendritic) polymers.[1−3] In large part, this explosion of interest in cascade polymers arises because of their unique physical properties and the ability to precisely control the molecule's chemical morphology during their synthesis. This ability to tailor the structure of dendrimers with exquisite precision has resulted in proposals for their use in a multiplicity of applications ranging from adhesive and viscosity modification to small molecule sequestration and stabilization for agricultural, fragrance, and pharmaceutical applications.[4−7] Particularly with respect to these later applications, design of appropriate dendrimer structures requires some means of characterizing structural and environmental factors which influence the sequestration of guest molecules codissolved in the solvent. Traditional techniques such as NMR spectroscopy, IR spectroscopy, mass spectrometry, low-angle light scattering, and size-exclusion chromatography have been used to establish the physical and chemical properties of dendrimers.[8] Unfortunately, the data provided by these analysis methods is increasingly difficult to interpret as the molecular size and/or structural complexity of the dendrimer increases. Furthermore, these techniques are often unable to detect subtle changes in the interaction between solutes and the dendrimer; information which can be critical if dendrimers are to be rationally designed to interact with specific solutes.

Dendrimers, or cascade polymers, have been suggested for use as a unimolecular pseudostationary phase for electrokinetic chromatography, because of their stability under widely varying separation conditions and also because of the ability to control the physiochemical properties of dendrimers. Tanaka et al. first demonstrated the utility of starburst dendrimers (SBDs) as a pseudostationary phase for the separation of polyaromatic hydrocarbons,[9,10] and, more recently, our research group[11] and Muijselaar et al.[12] have utilized dendritic macromolecules to separate mixtures of other solutes. The ability of the dendrimer to associate with or solubilize the solute determines the time required for the analyte zone to traverse the capillary. This retention time can serve as a very rapid, sensitive, and convenient means of measuring the strength of these interactions.

We herein report methods capable of determining the distribution coefficients and thermodynamic parameters associated with dendrimer-solute interactions. Whereas data generated by other analytical techniques becomes more difficult to interpret as the size and/or chemical complexity of the dendrimer increases, the data generated by the procedure reported here can be analyzed easily and efficiently. These procedures are easily implemented with commercially available equipment and allow the monitoring of interaction phenomena in a wide variety of solutions.

Experimental Section

Chemicals. The unimolecular polyacid cascades [Z-Cascade:methane[4]:(3-oxo-6-oxa-2-azaheptylidene):(3-oxo-2-azapentylidyne)G-

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1:propanoic acids] 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).[13] Methyl through butylparabens (parahydroxybenzoate esters) were obtained from Sigma Chemical Company (St. Louis, MO); the hexyl- and heptylparabens were purchased from Pflatz & Bauer (Waterbury, CT). All other chemicals were analytical reagent grade.

Instrumentation. All separations were performed on a BioFocus 3000 capillary electrophoresis instrument (Bio-Rad Laboratories, Hercules, California) in 50 μm i.d., 360 μm o.d. fused silica capillaries (Polymicro Technologies, Inc., Phoenix, Arizona). The total length of capillary was 30 cm with an inlet to detector distance of 25.4 cm. Before each analysis the separation capillary was purged with 0.3% (v:v) ammonium hydroxide for 60 s at 100 psi to provide a reproducible capillary surface and stabilize the electroosmotic flow. The anode and cathode were placed at the inlet and outlet reservoirs, respectively, and a voltage sufficient to provide a current of 20 μA was applied (corresponding voltage was approximately 11 kV). Samples were introduced into the capillary by pressure injection (2.0 psi s), and the absorbance signal for the parabens was monitored at 254 nm.

Separations for the van't Hoff analyses were performed at temperatures of 20, 25, 30, 35, and 40 °C. All other studies were performed at a constant temperature of 25 °C.

All experiments used to calculate dendrimer mobility were performed at a constant voltage of 25 kV. Experiments which utilized indirect detection methods (i.e., the measurement of dendrimer mobility) were performed in buffers containing 10 mM of benzoic acid as a UV-absorbing background species.

Retention times for sample components were estimated with the BioFocus 3000 Integrator (Version 5.0, Bio-Rad Laboratories) and the data manually transferred to a NeXT station computer (25 MHz 68040 processor, 16 MB RAM, NeXT Computers, Redwood City, California) for subsequent processing. Estimates of t_{eof} and t_{psp} were obtained with an Objective C program as previously described.[14]

Samples and Buffers. All separations were performed in a 0.020 M TRIS buffer adjusted to pH 8.5 with boric acid. The concentration of sodium dodecyl sulfate (SDS) used in the micellar electrokinetic chromatography experiments was 0.10 M. The paraben samples were prepared in the appropriate buffer to have a final concentration of 50 μg mL^{-1}.

Separations using dendrimers as the pseudostationary phase were performed in a 0.02 M TRIS, 0.01 M dendrimer buffer adjusted to pH 8.5. The presence of the polyacid dendrimer can significantly lower the buffer pH, so 0.1 M potassium hydroxide was added to return the pH to the desired level.

Results and Discussion

Electrokinetic chromatography (EKC) is a highly efficient separation technique with many demonstrated applications.[15,16] Previous reports have shown that it is possible to substitute polymers for the more traditional charged micelle pseudostationary phase to alter selectivity and/or eliminate environmental constraints (i.e., solution pH, temperature, etc.) on these separations.[17,18] Several researchers have also noted that when dendrimers are used as the pseudostationary phase, separation selectivities differ from those observed with sodium dodecyl sulfate (SDS) micellar phases.[9,10,12] This suggests that the mechanism of solute interaction with the pseudostationary phase is different and thus can potentially be exploited to enhance separation selectivity.

Determination of Distribution Coefficients. In EKC, the relationship between capacity factor, k', and retention time is given by[16]

$$k' = \frac{t_r - t_{eof}}{t_{eof}\left(1 - \frac{t_r}{t_{psp}}\right)} \tag{1}$$

where t_r is the solute retention time, t_{eof} is the retention time of a solute that has no interaction with the pseudostationary phase, and t_{psp} is the retention time of a solute that is completely solubilized by the pseudostationary phase. Molecular probes which reside exclusively in one phase or the other can be used to measure t_{eof} and t_{psp} under some conditions. Unfortunately, it has been observed that determination of t_{psp} can be difficult when dendrimers are utilized as the molecular carrier in EKC.[12] Recently, we have reported a procedure which uses the theory of Martin[19] to determine both t_{eof} and t_{psp} from the retention characteristics of a homologous series of molecules. This technique uses an automated search procedure to determine the t_{eof} and t_{psp} values which provide the best linear regression for the plot of log k' *vs.* the carbon number of the solutes.[14] Once t_{eof} and t_{psp} are established, it is relatively simple to estimate the capacity factor for each of the solutes with eq 1.

The capacity factor can be directly related to the distribution coefficient, K, through eq 2.

$$K = \frac{[X]_{psp}}{[X]_{mp}} \tag{2}$$

In this expression $[X]_{psp}$ is the concentration of species X associated with the pseudostationary phase, and $[X]_{mp}$ is the concentration of X in the mobile phase. The capacity factor is related to the distribution coefficient and the phase ratio

$$\frac{V_{psp}}{V_{mp}}$$

through the following equation

$$k' = K\left(\frac{V_{psp}}{V_{mp}}\right) \tag{3}$$

where V_{psp} is the volume of the pseudostationary phase, and V_{mp} is the volume of the mobile phase. With micellar phases, the phase ratio can be estimated with the following relationship:

$$\frac{V_{psp}}{V_{mp}} = \frac{\bar{v}(C_{Surf} - cmc)}{1 - \bar{v}(C_{Surf} - cmc)} \tag{4}$$

where $\bar{v}$ is the partial specific volume of the micelle, C_{Surf} is the concentration of the surfactant, and cmc is the critical micelle concentration. The use of this equation with micellar electrokinetic chromatography assumes that the concentration of monomers is constant above the cmc. Although this approximation is not strictly correct, it simplifies the associated calculations. The need for such an approximation is removed when dendrimers are the pseudostationary phase for the separation. Under these conditions the polymeric phase concentration

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is easily determined, and, unlike micellar phases, the concentration and composition do not vary with change in organic content of the solvent.

In dendrimer EKC, the volume of the dendrimer is small in comparison to the volume of the aqueous phase. The relationship between the capacity factor and the distribution coefficient can be estimated with eq 5

$$k' = K\bar{v}_{psp} C_{psp} \tag{5}$$

where $\bar{v}_{psp}$ and C_{psp} are the partial specific volume and the concentration of the dendrimer, respectively. When the radius of the dendrimer is known, $\bar{v}_{psp}$ can be calculated by

$$\bar{v}_{psp} = (6.022 \times 10^{23})\frac{4}{3}\pi r^3 \tag{6}$$

where r is the radius of the dendrimer in cm. The number of terminal carboxylic acids, formula weight, observed hydrodynamic radius, and estimated partial molar volume for the polyacid cascades used in this study are listed in Table 1. Once the capacity factor has been determined (i.e., with eq 1), the distribution coefficient can be estimated with eq 5.

Calculation of Dendrimer Mobility. Knowledge of a dendrimer's mobility can be useful for optimization of separations. Muijselaar et al. have measured the mobility of dendrimers in an electric field by capillary zone electrophoresis with indirect UV detection.[12] This method will provide an accurate estimate of dendrimer mobility under conditions of infinite dilution, but the mobility of the dendrimer will be lower when it is a buffer component since millimolar quantities of dendrimers can significantly increase the viscosity of the buffer. A potentially more accurate measure of dendrimer mobility can be obtained from the t_{psp} estimate provided by the dendrimer-based separation of homologous molecules.[14] The electrophoretic velocity of the pseudostationary phase in the electric field (v_{ep}) can be calculated by

$$v_{ep} = \frac{l}{t_{psp}} \tag{7}$$

where l is the distance from the inlet of the capillary to the detector. The pseudostationary phase's observed velocity is a function of its electrophoretic velocity and the electroosmotic flow in the capillary, as given by

$$v_{obs} = v_{ep} + v_{eof} \tag{8}$$

where v_{eof} is the velocity of the electroosmotic flow

$$v_{eof} = \frac{l}{t_{eof}}$$

and v_{ep} is the electrophoretic velocity. Substituting eq 7 into eq 8 allows the pseudostationary phase's observed velocity to be expressed as

$$v_{obs} = \frac{l}{t_{psp}} - \frac{l}{t_{eof}} \tag{9}$$

The pseudostationary phase's observed velocity is related to its electrophoretic mobility (μ_{ep}) by

$$v_{obs} = \mu_{ep} E \tag{10}$$

where E is the applied electric field. Rearranging eq 10 and substituting eq 9 and $E = V/L$, where L is the total capillary length and V is the applied voltage, the pseudostationary phase's electrophoretic mobility is then given by

$$\mu_{ep} = \left(\frac{l}{t_{psp}} - \frac{l}{t_{eof}}\right)\left(\frac{L}{V}\right) \tag{11}$$

With this equation and t_{psp} and t_{eof} values estimated by the separation of a homologous series of molecules, electrophoretic mobilities of dendrimers were estimated for 10 mM solutions of the four generations of polyacid dendrimers. The mobility of the dendrimers decrease with an increasing generation number (Table 2).

The influence of dendrimer concentration on the pseudostationary phase mobility was also investigated. As the concentration of dendrimer is increased, a corresponding increase in viscosity is apparent. A plot of dendrimer concentration *vs.* the electrophoretic mobility (Figure 2) shows the relationship between these two quantities. The decrease in electrophoretic mobility associated with higher concentrations of dendrimer is most likely the result of changes in the viscosity of the solvent.

Table 1. Physical Properties and Calculated Partial Molar Volumes for Different Generations (G) of Polyacid Dendrimers

G	no. of terminal acids	formula wt	hydrodynamic radius[a] (Å)	partial molar volume[b] (mL mol^{-1})
1	12	1 341	12.3	4 694
2	36	4 092	17.3	13 061
3	108	12 345	23.9	34 437
4	324	37 102	33.1	91 477

[a] From ref 13. [b] Estimated from the hydrodynamic radius measured at neutral pH.

Table 2. Estimated Dendrimer Migration Time[a,b] (t_{psp}), Electroosmotic Flow Time[a,b] (t_{eof}), and Dendrimer Mobility[b] (μ_{ep}) for Different Generation (G) Dendrimers

G	t_{eof} (min)	t_{psp} (min)	μ_{ep} (10^{-5} cm^2 V^{-1} s^{-1})
1	2.44 (0.01)	86.92 (0.44)	−20.2 (0.06)
2	5.41 (0.03)	99.79 (0.56)	−8.89 (0.05)
3	9.49 (0.04)	115.53 (0.65)	−4.91 (0.02)
4	31.78 (0.09)	189.50 (0.91)	−1.33 (0.01)

[a] Calculated from retention characteristics of a homologous series of parabens. Experimental conditions are described in the text. [b] Standard deviations are listed in parentheses ($n = 5$).

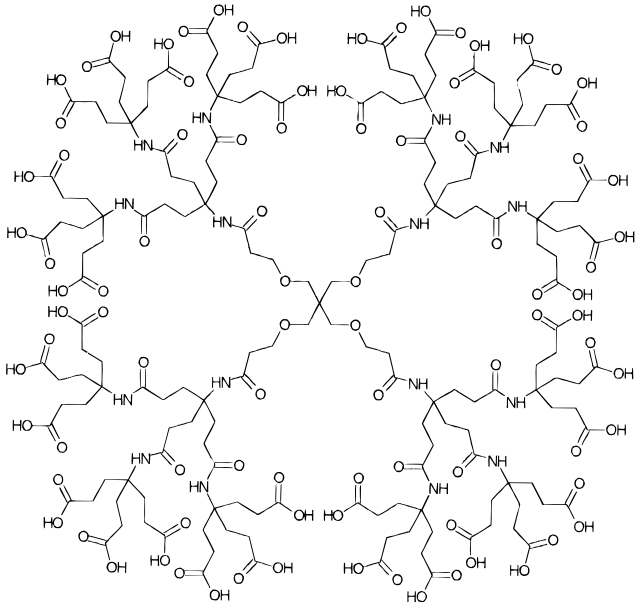

Figure 1. Structure of polyacid dendrimer (second generation, 36 carboxylic acid terminal groups).

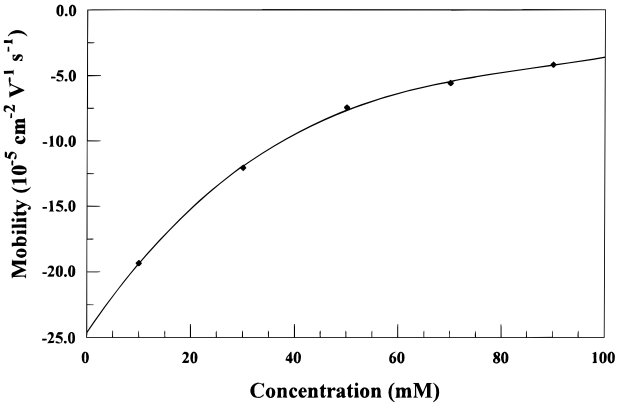

Figure 2. Polyacid dendrimer electrophoretic mobility, μ_{ep}, dependence on dendrimer concentration ($G = 1$).

Capillary electrophoresis experiments using indirect detection (as proposed by Muijselaar et al.[12]) were performed to compare the dendrimer mobility at "infinite dilution" and the mobility obtained from the analysis of retention characteristics of homologous series of molecules.[14] A sample of first generation ($G = 1$) polyacid dendrimer was subjected to electrophoretic analysis in a buffer which contained benzoic acid to allow indirect detection but did not contain any dendrimer. Peaks for t_{eof} and t_{psp} were observed at 2.02 ($\pm$ 0.01) min and 84.1 ($\pm$ 0.4) min, respectively. Substituting these times in eq 11 indicates an average dendrimer mobility of -24.7 ($\pm$ 0.1) $\times$ 10^{-5} cm^2 V^{-1} s^{-1}. This value is very close to the *y*-intercept (-24.6×10^{-5} cm^2 V^{-1} s^{-1}) of the curve used to model the data in Figure 2. Thus, determination of the mobility by the procedure described here provides a more versatile and potentially more accurate means of determining a dendrimer's mobility.

Influence of Dendrimer Concentration on Capacity Factor. To determine the influence of dendrimer concentration on the capacity factor, k', a mixture of parabens were separated in solutions containing different concentrations of first generation dendrimer. These dendrimer solutions were all carefully adjusted to the same pH using the procedure described in the Experimental Section. Solutions containing the larger polyacid dendrimers required addition of potassium hydroxide to counteract the reduction of pH caused by the increased number of carboxylic acid terminating groups. Potassium hydroxide was used because it generated a negligible effect on electrophoretic voltage during constant current analysis, whereas sodium hydroxide produced very noticeable effects.

A plot of capacity factor, k', as a function of the concentration of dendrimer for five parabens is shown in Figure 3. As the concentration of dendrimer increases, the capacity factor for each of the parabens also increases. This is similar to what has been reported for micellar systems, although the slope of the lines in equivalent plots for SDS micellar separations[20] are larger than those shown in Figure 3. Increasing the dendrimer concentration has a less pronounced influence on the capacity factor when compared with micellar systems.

Comparison of Distribution Coefficients. Calculated partial molar volumes from Table 1 and capacity factors estimated from the retention of a homologous series of parabens were substituted into eq 5 to determine distribution coefficients for solutes in the dendrimer solution. Separations were performed at different temperatures to determine the temperature dependence of the distribution coefficient, *K*. Representative separations of parabens at different temperatures are shown in Figure 4. In general, the dendrimers used in this study do not solubilize the hydrophobic compounds as efficiently as SDS micelles, but higher concentrations of organic modifier can be used to help solubilize these compounds. Organic modifiers were not used in this study so that similar conditions could be used for both SDS and dendrimer separations. As illustrated in Table 3, distribution coefficients increase as the parabens become more hydrophobic, and the distribution coefficients decrease with increasing temperature.

The influence of dendrimer generation number on the distribution coefficient was also examined. Distribution coefficients for parabens separated with polyacid dendrimers are listed in Table 4. Interestingly, the *K* values are inversely proportional to generation number. One possible explanation for the reduction in *K* is the decrease in the porosity near the surface of the larger dendrimers. As the generation size increases, the amount of branching also increases, and the

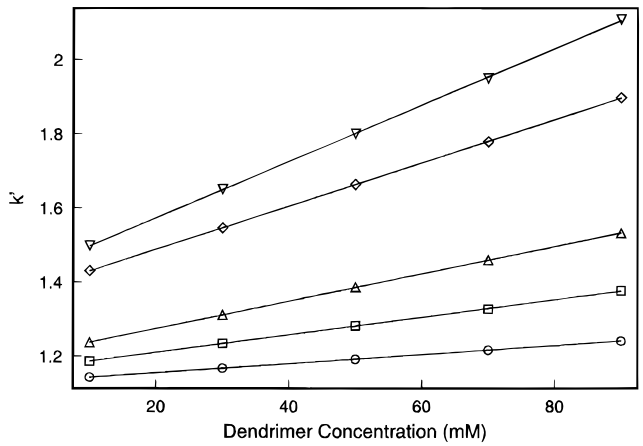

Figure 3. Capacity factor, k', dependence on dendrimer concentration ($G = 1$) for parabens (ethylparaben O, propylparaben □, butylparaben △, hexylparaben ◇, heptylparaben ▽).

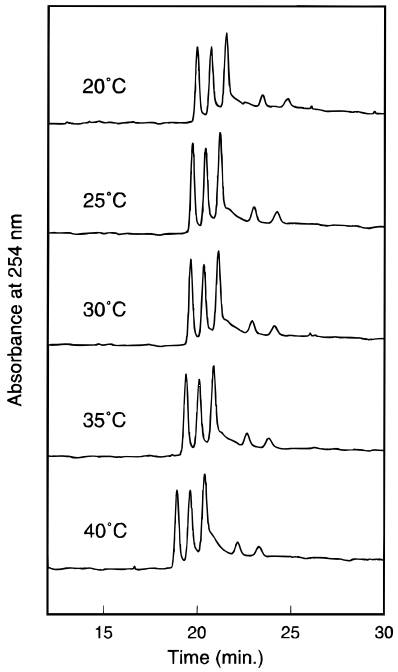

Figure 4. Parabens separations in solutions containing polyacid by dendrimers ($G = 3$) at different temperatures. Peaks correspond to ethyl-, propyl-, butyl-, hexyl-, and heptylparaben, respectively. The peaks for hexylparaben and heptylparaben are smaller due to their lower solubility in the aqueous solution.

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Table 3. Average Distribution Coefficients[a] at Different Temperatures for Paraben Homologues Solubilized by Second Generation Polyacid Dendrimers

compound	20 °C	25 °C	30 °C	35 °C	40 °C
ethylparaben	10.12 (0.03)	9.95 (0.02)	9.90 (0.03)	9.77 (0.02)	9.62 (0.02)
propylparaben	11.28 (0.04)	10.96 (0.03)	10.85 (0.03)	10.70 (0.04)	10.46 (0.03)
butylparaben	12.64 (0.04)	12.13 (0.04)	11.97 (0.03)	11.73 (0.04)	11.44 (0.04)
hexylparaben	17.01 (0.04)	16.06 (0.05)	15.77 (0.04)	15.26 (0.04)	14.60 (0.04)
heptylparaben	19.15 (0.05)	17.89 (0.05)	17.50 (0.05)	16.86 (0.04)	16.04 (0.04)

[a] Standard deviations are listed in parentheses ($n = 5$).

Table 4. Average Distribution Coefficients[a] for Paraben Homologues Solubilized by Polyacid Dendrimers of Different Generations (G)

compound	$G = 1$	$G = 2$	$G = 3$	$G = 4$
ethylparaben	26.04 (0.01)	9.62 (0.02)	3.43 (0.03)	2.07 (0.03)
propylparaben	27.57 (0.01)	10.46 (0.03)	3.72 (0.04)	2.63 (0.05)
butylparaben	29.31 (0.02)	11.44 (0.04)	4.03 (0.04)	3.45 (0.05)
hexylparaben	36.52 (0.02)	14.60 (0.04)	4.77 (0.05)	6.77 (0.06)
heptylparaben	39.22 (0.03)	16.04 (0.04)	5.18 (0.05)	8.87 (0.06)

[a] Standard deviations are listed in parentheses ($n = 5$).

unoccupied area near the surface of the dendrimer decreases.[8] If this is indeed the case, then a reduction in the degree of branching in the larger generation dendrimers may actually enhance dendrimer-solute interactions. It is also possible that higher generations possess higher surface charge-density barriers, thereby inhibiting dendrimer/analyte interaction.

Whereas a consistent reduction in distribution coefficients is found as dendrimer size increases for ethyl-, propyl-, and butylparaben, distribution coefficients for the larger parabens (hexyl- and heptylparaben) deviate from this trend. Distribution coefficients for more hydrophobic molecules separated with fourth generation ($G = 4$) dendrimers are larger than separations utilizing third generation ($G = 3$) dendrimers. The cause of these variations have not been determined; however, increasingly unfavorable analyte/aqueous phase interactions are suspected.

van't Hoff Analyses. The temperature dependence of the distribution coefficient, K, is is modeled by the van't Hoff equation

$$\ln K = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \qquad (12)$$

where, R is the ideal gas constant, T is the absolute temperature, and ΔH° and ΔS° are the change in enthalpy and entropy corresponding to micellar[16] or dendrimer solubilization, respectively.

Thermodynamic parameters for dendrimer-solute interactions are calculated with the following procedure. Distribution coefficients at five different temperatures are determined as previously described. A van't Hoff plot is constructed by plotting the natural logarithm of the distribution coefficient against the reciprocal temperature (ln K *vs.* 1/T). An example of a typical van't Hoff plot is shown in Figure 5. The slope of the resulting line is equal to

$$\frac{-\Delta H^\circ}{R}$$

and the intercept is

$$\frac{\Delta S^\circ}{R}$$

Once ΔH° and ΔS° are established, the standard Gibbs free energy (ΔG°) can be estimated with the following expression:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad (13)$$

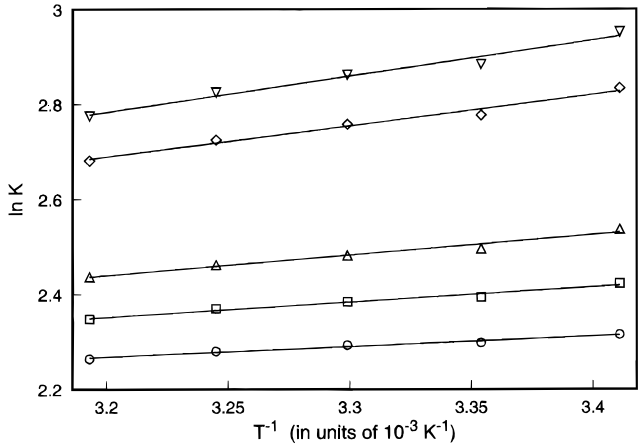

Figure 5. van't Hoff plots for paraben homologues (ethylparaben ○, propylparaben □, butylparaben △, hexylparaben ◇, heptylparaben ▽) separated by polyacid dendrimers ($G = 2$).

Changes in enthalpy, entropy, and Gibb's free energy for solubilization of parabens in the dendrimer are presented in Table 5. Solubilization with the first and second generation dendrimers is favorable in terms of enthalpy and less favorable in entropy. For separations with the first generation dendrimer, enthalpy values increase with increasing carbon number for ethyl-, propyl-, and butylparaben and decrease for the more hydrophobic hexyl- and heptylparaben. A smaller increase in entropy for more hydrophobic solutes ($G = 1$, 2) would be expected, because of the limited freedom of motion of the parabens within the dendrimer interior. When the paraben molecule interacts with the dendrimer's cavities, it becomes severely limited in the orientations it can assume. The constraints imposed by the branches of the molecules restrict the motions of sequestered analytes.[21] A reduced increase of entropy accompanies this constrained molecular motion.

For the higher generation dendrimers ($G = 3$, 4), enthalpy changes become unfavorable (i.e., increase) for all solutes, whereas entropy changes appear more favorable (i.e., increase). The rationale for these observations is similar to that used to explain the reduction in K with increasing dendrimer size.

As the generation number increases, the increase in branching lessens porosity near the dendrimer surface.[8,22] Surface charge-density increases as well. Increased branching associated with larger dendrimers would inhibit surface penetration of the dendrimer and lessen its interaction with the dendrimer skeleton. Once inside, the solute would partially disrupt the ordered structure of the dendrimer. It may also be possible that the solute prevents the dendritic branches from interacting with each other in the most efficient manner and so exacts a corresponding enthalpy penalty.

(21) (a) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders, M. J.; Grossman, S. H. *Angew. Chem., Int. Ed. Engl.,* **1991**, *30*, 1178. (b) *Angew. Chem.* **1991**, *103*, 1205.

(22) This also relates to dendritic fractality; for a comprehensive treatment, see: (a) Chapter 2 of ref 1 and (b) Avnir, D.; Farin, D. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1408.

Table 5. Standard Enthalpy ($\Delta H°$), Standard Entropy ($\Delta S°$), and Standard Gibbs Free Energy ($\Delta G°$) of Parabens for Solubilization by Dendrimers of Different Generation (G)

	$G = 1$				$G = 2$				$G = 3$				$G = 4$			
compound	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r
ethylparaben	−2.5 (0.1)	19.2 (0.3)	−8.2 (0.2)	0.993	−1.8 (0.2)	13.0 (0.4)	−5.7 (0.3)	0.987	0.2 (0.2)	11.0 (0.5)	−3.0 (0.3)	0.970	13.3 (0.4)	48.4 (0.6)	−1.1 (0.3)	0.964
propylparaben	−2.4 (0.1)	19.9 (0.3)	−8.3 (0.2)	0.992	−2.7 (0.2)	11.0 (0.5)	−6.0 (0.3)	0.987	0.5 (0.2)	12.7 (0.5)	−3.2 (0.3)	0.959	17.3 (0.4)	62.9 (0.6)	−1.5 (0.4)	0.960
butylparaben	−2.3 (0.1)	20.7 (0.3)	−8.5 (0.2)	0.972	−3.6 (0.2)	8.8 (0.4)	−6.2 (0.3)	0.987	0.8 (0.2)	14.0 (0.6)	−3.4 (0.3)	0.988	22.2 (0.4)	80.8 (0.7)	−1.9 (0.3)	0.956
hexylparaben	−5.2 (0.2)	13.5 (0.4)	−9.2 (0.2)	0.988	−5.4 (0.2)	4.9 (0.4)	−6.9 (0.3)	0.988	0.9 (0.3)	15.9 (0.6)	−3.8 (0.4)	0.971	36.9 (0.6)	132.9 (1.8)	−2.8 (0.4	0.961
heptylparaben	−5.5 (0.2)	12.9 (0.4)	−9.4 (0.2)	0.986	−6.3 (0.3)	2.9 (0.4)	−7.2 (0.3)	0.988	11.1 (0.3)	17.3 (0.6)	−4.0 (0.3)	0.975	41.8 (0.7)	150.9 (2.4)	−3.2 (0.5)	0.960

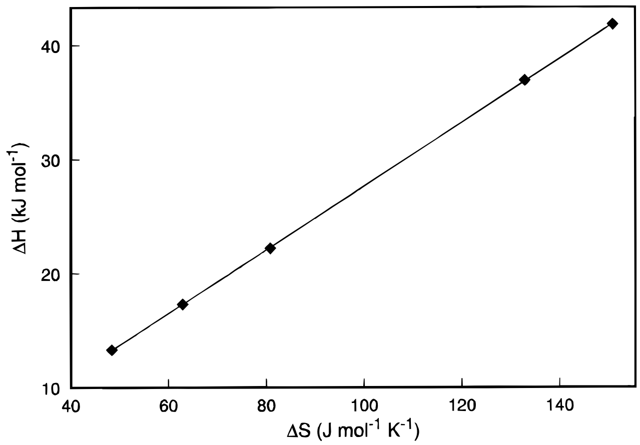

Figure 6. Linear free energy relationship for paraben separation (G = 4). (r^2 = 0.9999).

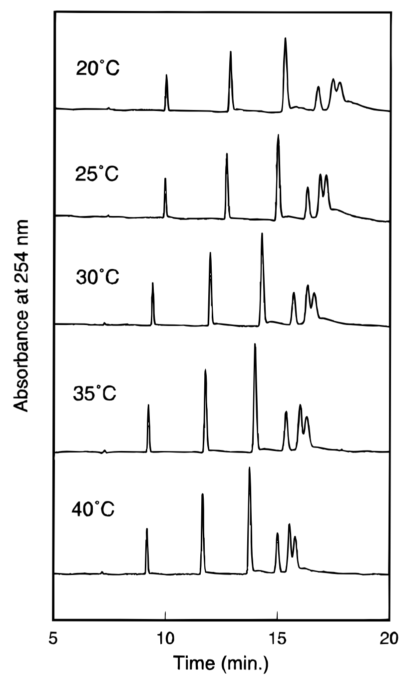

Figure 7. Parabens separated by SDS at different temperatures for thermodynamic analysis. Peaks correspond to ethyl-, propyl-, butyl-, amyl-, and hexylparaben, respectively.

The linear free energy relationship for the largest generation dendrimer (G = 4) is shown in Figure 7. The profound influence of entropy is apparent for separations with the higher generation cascades. Entropy influences solute interactions with porous phases.[23] Overall, all four generations of dendrimers showed decreasing $\Delta G°$ values for solutes with increasing carbon number, indicating an enhanced ability to solubilize the more hydrophobic parabens.

Comparison of Solute Interactions with Dendrimers and Micellar Structures. To compare the dendrimer-solute interactions with micellar-solute interactions, the paraben series was separated using SDS micelles as the pseudostationary phase. Under the stated experimental conditions, hexylparaben and heptylparaben coelute so heptylparaben was replaced with amylparaben. The mobility of the SDS micelles was found to be -6.1×10^{-5} cm^2 V^{-1} s^{-1}, which is between the mobilities of the second and third generation polyacid dendrimers.

Thermodynamic data for the SDS separations are listed in Table 6. Partial molar volumes and critical micelle concentra-

(23) Giddings, J. C. *Unified Separation Science;* Wiley-Interscience: New York, 1991, Chapter 2.

Table 6. Standard Enthalpy ($\Delta H°$), Standard Entropy ($\Delta S°$), and Standard Gibbs Free Energy ($\Delta G°$) of Parabens for Solubilization by SDS Micelles[a]

compound	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r
ethylparaben	−3.3 (0.3)	32.6 (0.8)	−13.0 (0.4)	0.978
propylparaben	−3.8 (0.3)	38.3 (0.9)	−15.2 (0.4)	0.990
butylparaben	−4.2 (0.3)	44.2 (0.9)	−17.4 (0.4)	0.980
amylparaben	−4.9 (0.3)	47.8 (1.1)	−19.2 (0.5)	0.988
hexylparaben	−5.6 (0.4)	52.5 (1.4)	−21.2 (0.5)	0.987

[a] Standard deviation are listed in parentheses ($n = 5$).

tions used to calculate these values were obtained from refs 24 and ref 25, respectively. Micellar solubilization of parabens of increasing hydrophobicity is found to be increasingly favorable with respect to both enthalpy and entropy. These data are consistent with trends previously observed for a homologous series of phenols.[26]

In comparing polyacid dendrimers with SDS micelles, the trends indicate that solubilization by the dynamic micelle structure is favored relative to the more highly ordered and less lipophilic polyacid dendrimer. The change in enthalpy for parabens separated with second generation dendrimers are very similar to those measured in solutions containing SDS micelles. However, the changes in entropy are less favorable when these same molecules are solubilized by dendrimers, most likely because of reduced porosity in the dendrimer structure.

To illustrate the utility of this technique for samples that are not part of a homologous series, acetaminophen, dimethoxynitrobenzyl alcohol, caffeine, theophylline, and xanthine were added to the paraben mixture and separated in a 0.010 M second generation polyacid dendrimer and 0.02 M TRIS solution adjusted to pH 8.5. Capacity factors, partition coefficients, and thermodynamic parameters were measured using the previously described procedures. Thermodynamic data for these compounds are summarized in Table 7. The ionic xanthines (theophylline and xanthine) are retained longer than the nonionic caffeine. This increase in retention time may be correlated with a decrease in the pK_a of the ionic xanthines: theophylline (pK_a = 8.8) and xanthine (pK_a = 7.5). Interestingly, changes in Gibb's free energy for all three xanthines were equivalent within the error of the measurement, but the changes in entropy were greater for the more ionic xanthines.

Conclusions

A procedure capable of monitoring changes in dendrimer-solute solution equilibrium has been described. This technique utilizes a previously published procedure to determine the size of the elution window based on the retention characteristics of a homologous series of molecules and allows very rapid measurements of distribution coefficients and thermodynamic parameters. These parameters were determined for several solutes, illustrating that these measurements can be made for a wide variety of analytes. The mobility of the dendrimer, a quantity useful for optimization, was also obtained from electrokinetic chromatographic experiments.

Using the procedure described in this paper, rapid and sensitive measurements of very small changes in the solution equilibrium can be made for a wide variety of dendrimer/solute associations. These measurements do not interfere with the dendrimer/solute interaction and can be performed in many different buffer systems. Furthermore, these measurements can be fully automated, and estimates of interaction phenomena and thermodynamic parameters can be made in several hours. These measurements are not limited to dendritic macromolecules but could be easily extended to allow monitoring of solute interactions with novel polymeric molecules.[17,18]

Dendrimers have been proposed for a multiplicity of uses including the stabilization and sequestration of small molecules for pharmaceutical and agricultural purposes. Dendrimer electrokinetic chromatography is capable of providing the requisite sensitivity and speed to characterize dendrimer/solute interactions for these applications. As data are accumulated and interactions for several dendrimers are characterized, a greater understanding of dendrimer solubilization will be obtained. Eventually this information could be exploited to enhance the selectivity of electrokinetic chromatography.

Table 7. Standard Enthalpy ($\Delta H°$), Standard Entropy ($\Delta S°$), and Standard Gibbs Free Energy ($\Delta G°$) for Solubilization by Polyacid Dendrimers ($G = 2$)[a]

compound	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r
acetaminophen	−0.8 (0.1)	15.0 (0.5)	−5.3 (0.3)	0.978
dimethoxynitrobenzyl alcohol	−1.8 (0.1)	12.9 (0.4)	−5.7 (0.4)	0.990
caffeine	−3.8 (0.2)	4.2 (0.4)	−5.1 (0.3)	0.980
theophylline	−3.7 (0.2)	4.8 (0.4)	−5.1 (0.3)	0.988
xanthine	−3.1 (0.2)	6.6 (0.4)	−5.1 (0.3)	0.987

[a] Standard deviation are listed in parentheses ($n = 5$).

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