RECEIVED
FEB 03 2000
OSTI

DOE/ER/14056-64

**DIRECT EXAMINATION OF SEPARATION PROCESSES IN
CHROMATOGRAPHY BY LASER-INDUCED FLUORESCENCE**

Final Technical Progress Report

September 1, 1989 - February 28, 1999

Victoria L. McGuffin

Department of Chemistry
Michigan State University
East Lansing, Michigan 48824

February 28, 1999

Prepared for

THE U.S. DEPARTMENT OF ENERGY
CONTRACT NO. DE-FG02-89ER14056

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

**DIRECT EXAMINATION OF SEPARATION PROCESSES IN
CHROMATOGRAPHY BY LASER-INDUCED FLUORESCENCE**

Final Technical Progress Report
September 1, 1989 - February 28, 1999

RECEIVED
FEB 03 2000
OSTI

TABLE OF CONTENTS

ABSTRACT	1
INTRODUCTION	2
PROGRESS REPORT	
I. DIRECT EXAMINATION OF SEPARATION PROCESSES IN CHROMATOGRAPHY AND ELECTROPHORESIS	3
Experimental System	3
Thermodynamic Studies of Reversed-Phase Liquid Chromatography	4
Kinetic Studies of Reversed-Phase Liquid Chromatography	15
Studies of Transition Regions	21
II. COMPUTER SIMULATION OF CHROMATOGRAPHY AND ELECTROPHORESIS	25
Development and Validation of Simulation Models	29
Applications of the Simulation	32
Correlation of Simulation and Experimental Studies	45
III. NOVEL OPTIMIZATION METHODS FOR CHROMATOGRAPHY AND ELECTROPHORESIS	45
Computer-Assisted Optimization of Liquid Chromatography by Parametric Modulation	45
Computer-Assisted Optimization of Electrophoresis	50
Photoactivation as a Means of Controlling Separations in Electrophoresis	51
IV. NOVEL DETECTION METHODS FOR CHROMATOGRAPHY AND ELECTROPHORESIS	53
Fluorescence and Fluorescence Quenching Detection	53
Photovoltaic Detection	57
SUMMARY	60
REFERENCES	61
PUBLICATIONS OF THE RESEARCH PROJECT	64
PERSONNEL OF THE RESEARCH PROJECT	67

ABSTRACT

This report summarizes the progress and accomplishments of this research project from September 1, 1989 to February 28, 1999. During this period, we have developed an experimental system for the direct examination of the chromatographic or electrophoretic column by laser-induced fluorescence. This system has been utilized to examine and to characterize the retention of model solutes on octadecylsilica, the most common stationary phase for reversed-phase liquid chromatography. Thermodynamic quantities such as the molar free energy, molar enthalpy, molar volume, enthalpy-entropy compensation temperature, and enthalpy-volume compensation pressure, as well as the kinetic rate constants for solute distribution between the mobile and stationary phases have been systematically measured as a function of bonding density (2.7 to 5.4 $\mu\text{mol m}^{-2}$), temperature (10 to 70 $^{\circ}\text{C}$), pressure (400 to 4500 psi), and mobile phase composition. In each of these studies, substantial knowledge has been gained regarding the fundamental thermodynamic and kinetic processes that are responsible for chromatographic separations.

We have also initiated the development and validation of a three-dimensional computer simulation of chromatographic and electrophoretic separations. In this simulation, the trajectories of individual molecules are monitored through the transport processes of diffusion, convection by laminar, electroosmotic, or electrophoretic flow, and surface interaction by a partition or adsorption mechanism. The molecular distribution and the corresponding zone profile may be examined and characterized by means of statistical moments at any specified time or spatial position during the simulation. This simulation has been utilized to study the partition mechanism in liquid chromatography under conditions that are representative of our experimental studies. The kinetics of solute distribution between the mobile and stationary phase have been elucidated under diffusion-limited conditions. In addition, the retention and dispersion of solute zones have been determined as a function of the distribution coefficient along the column as well as in regions of spatial and temporal transition (e.g., injection and elution).

In addition to these primary research objectives, we have made significant progress toward the development of systematic optimization methods for chromatography and electrophoresis. In these optimization methods, the theoretical knowledge gained through experimental and computer simulation studies has been applied to practical problems. Finally, we have developed novel detection methods based on laser-induced fluorescence, fluorescence quenching, and photoionization for environmental, forensic, and biomedical applications.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed or represents that its use would not infringe privately-owned rights.

INTRODUCTION

Much of our present knowledge of chromatographic and electrophoretic separation processes has been derived from experimental data and theoretical models that reflect the macroscopic behavior of solute zones. Because the separation ultimately arises through the migration and interaction of individual molecules, however, a more detailed understanding is necessary to guide future improvements in separation science. This research program is concerned with two technological advances that enable this challenge to be addressed from a unique and promising perspective.

First, an experimental system has been developed in our laboratory that allows the examination of separation processes *in situ* as the solute traverses the chromatographic or electrophoretic column. This system employs laser-induced fluorescence detectors to measure the solute zone profile at several distinct points along an optically transparent column. By effectively isolating the regions of interest, this system enables an accurate measure of thermodynamic, kinetic, and hydrodynamic processes that was not previously possible.

Second, a three-dimensional stochastic computer simulation has been developed and validated which provides a detailed understanding of mass transport processes in chromatographic and electrophoretic separations. In this simulation, the migration of individual molecules is established through diffusion and convection within a fluid phase that is in contact with a surface. Molecular interaction and, hence, retention may arise by partitioning into permeable surfaces or by adsorption at solid surfaces. The molecular distribution and the corresponding zone profile may be examined and characterized by means of statistical moments at any specified time or spatial position during the simulation. This simulation provides the opportunity to perform hypothetical experiments and to make observations that may not be possible in a real experimental system.

When appropriately and cohesively integrated in the research program, these advances in experiment and theory provide an opportunity to learn in an interactive manner about the fundamental physicochemical and hydrodynamic processes that occur in complex chromatographic and electrophoretic systems.

In addition to these primary research objectives, we have applied the knowledge gained in these studies to make important advances in several related areas. We have developed novel computer-assisted optimization methods for chromatography and electrophoresis. We have also developed novel detection techniques based on fluorescence, fluorescence quenching, and photoionization. Our progress and accomplishments in these and related areas of research supported by the Department of Energy, Office of Basic Energy Sciences, will be briefly summarized in this report.

PROGRESS REPORT

I. DIRECT EXAMINATION OF SEPARATION PROCESSES IN CHROMATOGRAPHY AND ELECTROPHORESIS

Experimental System

The experimental system, which was designed and constructed in our laboratory, is shown schematically in Figure 1. The individual components of this system are briefly described below.

Column Preparation. Several octadecylsilica materials have been evaluated in these studies. A 5.5- μm irregular silica (IMPAQ 200, PQ Corporation) with surface area of 240 $\text{m}^2 \text{g}^{-1}$ and pore size of 190 $\text{\AA}$ was reacted with monofunctional and trifunctional octadecylsilane at bonding densities of 2.7 and 5.4 $\mu\text{mol m}^{-2}$, respectively. A 3- μm spherical silica (Spherisorb, Phase Separations Ltd.) with a surface area of 200 $\text{m}^2 \text{g}^{-1}$ and pore size of 80 $\text{\AA}$ was reacted with monofunctional octadecylsilane at a bonding density of 3.5 $\mu\text{mol m}^{-2}$ and subsequently capped with monochlorotrimethylsilane.

The microcolumns were fabricated from 200- μm i.d. fused-silica capillary tubing that was terminated at the desired length with a quartz wool frit. Prior to packing, the polyimide coating was removed from the capillary at selected positions to facilitate on-column detection. A 0.25 g sample of the octadecylsilica packing material was suspended in 1.0 mL methanol, which was introduced to the column under moderate

FIGURE 1: Schematic diagram of the experimental system for on-column laser-induced fluorescence detection. I = injection valve, T = splitting tee, S = splitting capillary, R = restricting capillary, FOP = fiber-optic positioner, F = filter, PMT = photomultiplier tube, AMP = amplifier.

pressure according to the slurry packing procedure described previously (1). The resulting microcolumns were evaluated under standard test conditions to have a plate height (H) of 9.5 and 15 μm , total porosity (ϵ_T) of 0.58 and 0.84, and flow resistance parameter (ϕ') of 550 and 500 for the 3- and 5- μm silica materials, respectively. These physical properties remained relatively constant along the length of the column (2).

Chromatographic System. The mobile phase was delivered by a single-piston reciprocating pump, operated in the constant-pressure mode (0 to 6000 psi). The sample was introduced by means of a 1.0- μL injection valve and was subsequently split between the microcolumn and a capillary, resulting in a nominal injection volume of 12 nL and a nominal flow rate of 1.3 $\mu\text{L min}^{-1}$. The column, splitter, and restrictor were maintained at constant temperature in a cryogenically cooled oven (-20 and 200°C).

Detection System. Laser-induced fluorescence was utilized to probe solute zones at two to six positions along the microcolumn (3-5). A continuous-wave He-Cd laser (325 nm, 25 mW) was utilized as the excitation source and was transmitted to the column via UV-grade optical fibers. The fluorescence emission was collected in a right-angle, coplanar geometry by using optical fibers, isolated by appropriate interference filters or monochromators, and detected by a photomultiplier tube. The resulting photocurrent was amplified and converted to the digital domain. Data acquisition, storage, and display were performed simultaneously at each detector by using computer programs developed in our laboratory with the Forth-based programming language, Asyst (Keithley-McMillan).

The solute zone profiles were characterized by means of statistical moments (6) to yield an accurate measure of the mean, variance, asymmetry, etc. at each detector. In addition, the method of nonlinear regression (PeakFit, Jandel Scientific) with a Gaussian or exponentially modified Gaussian function was utilized to reconstruct the zone profile with regression coefficients (r^2) better than 0.99. The reproducibility of the mean of the zone profile was typically 0.003%, whereas that for the Gaussian and exponential contributions to the variance was typically 0.58% and 1.94%, respectively. This method provided a precise and accurate measurement of local retention and dispersion phenomena.

Thermodynamic Studies of Reversed-Phase Liquid Chromatography

In several previous studies, solute retention and selectivity in reversed-phase liquid chromatography have been shown to be substantially altered by temperature (7-13), pressure (4,5,14,15), and bonding density of the octadecyl ligand to the silica surface (7-10,13,16-20). Whereas these previous studies have elucidated the independent effects, further studies are clearly necessary to examine the simultaneous effects of these interrelated variables in a comprehensive and systematic manner. We have completed a study in which the thermodynamic and kinetic behavior of monomeric and polymeric octadecylsilica stationary phases was examined in the range of temperature from 10 to 70°C , average pressure from 400 to 4500 psi, and bonding density from 2.7 to 5.4 $\mu\text{mol m}^{-2}$ (21,22). This study was performed by measuring the retention and dispersion of homologous solutes within small isolated regions of the column, wherein temperature ($\pm 0.1^\circ\text{C}$) and pressure (± 50 psi) were maintained relatively constant. Consequently, solute retention and dispersion as well as the associated thermodynamic and kinetic parameters were measurable with very high precision and accuracy. The results of these studies for methylene homologues (C_{10} to C_{22}) are briefly described below, with more detail and additional studies of benzene homologues to be provided in the publications cited.

Effect of Temperature and Pressure on Molar Enthalpy. From classical thermodynamics, the solute retention is described by means of the well-known van't Hoff equation:

$$\ln k = \ln K - \ln \beta = \frac{-\Delta G^\circ}{R T} - \ln \beta = \frac{-\Delta H^\circ}{R T} + \frac{\Delta S^\circ}{R} - \ln \beta \quad [1]$$

where the capacity factor (k) represents the thermodynamic equilibrium constant (K) for the solute distributed between the mobile and stationary phases, compensated for the volumetric ratio of these phases (β). The capacity factor is related to thermodynamic quantities such as changes in the standard Gibbs free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) that arise from the transfer of one mole of solute between phases at isothermal temperature (T). From Equation [1], it is apparent that the logarithm of the capacity factor should be linearly related to the inverse temperature if the molar enthalpy and entropy are invariant. As shown in Figure 2 for the representative solute C_{16} , linear behavior is observed throughout the entire temperature and pressure range for the monomeric octadecylsilica phase with low bonding density ($2.7 \mu\text{mol m}^{-2}$). However, for the monomeric phase with intermediate density ($3.5 \mu\text{mol m}^{-2}$) and the polymeric phase with high density ($5.4 \mu\text{mol m}^{-2}$), two distinct regions of linear behavior are observed that are separated by a discontinuous region. These data suggest that the low-density phase exists as a single liquid-like phase, whereas the intermediate- and high-density phases undergo a phase transition at approximately 28 and 45 °C, respectively (21,22).

The molar enthalpy is usually calculated from the slope of the van't Hoff plot within the linear regions, as described in Equation [1]. However, if an equation can be obtained that accurately describes a nonlinear van't Hoff plot, then the partial derivative of $\ln k$ with respect to $1/T$ will yield the value of $-\Delta H^\circ/R$ throughout the temperature and pressure ranges (23). This method assumes that the molar enthalpy is constant within a small interval, rather than over the entire investigated temperature and pressure ranges, which provides a more accurate estimation. Figure 3 illustrates the change in molar enthalpy calculated in this manner for the representative solute C_{16} . In all cases, the change in molar enthalpy is negative, which implies that the transfer of the solute from the methanol mobile phase to the octadecylsilica stationary phases is an exothermic process. For the low-density phase, the molar enthalpy remains small and relatively constant throughout the examined temperature and pressure ranges. The values range from -1.74 to $-4.20 \text{ kcal mol}^{-1}$ for C_{10} to C_{22} , respectively, at 30 °C and 1330 psi. Moreover, the differential change in molar enthalpy per ethylene group ($\Delta\Delta H^\circ$) remains relatively constant, with an average value of $-0.41 \pm 0.02 \text{ kcal mol}^{-1}$. This suggests that each ethylene group contributes in an equal manner to the overall change in enthalpy for the homologous solutes.

For the high-density phase, the change in molar enthalpy is significantly greater with values ranging from -10.6 to $-30.5 \text{ kcal mol}^{-1}$ for C_{10} to C_{22} , respectively, at 30 °C and 1470 psi. The differential change in molar enthalpy per ethylene group remains constant for solutes C_{10} to C_{18} , within the statistical precision of the measurements. The average value of $-3.65 \pm 0.13 \text{ kcal mol}^{-1}$ is approximately ten-fold higher than that for the low-density phase under the same conditions of temperature and pressure. However, the solutes C_{20} and C_{22} have smaller enthalpy contributions of -2.91 and $-2.39 \text{ kcal mol}^{-1}$, respectively. These results are intuitively reasonable, based on the explanation proposed by Tchapla *et al.* (7,8), as the additional carbon atoms in these solutes cannot be fully embedded in the octadecylsilica stationary phase.

FIGURE 2: The logarithm of capacity factor for the methylene homologue C_{16} expressed as a function of the inverse temperature for (A) low-density monomeric, (B) intermediate-density monomeric, and (C) high-density polymeric octadecylsilica phases (21,22).

FIGURE 3: Molar enthalpy for the methylene homologue C_{16} , calculated from Figure 2, expressed as a function of the inverse temperature for (A) low-density monomeric, (B) intermediate-density monomeric, and (C) high-density polymeric octadecylsilica phases (21,22).

It is also noteworthy that temperature and pressure exhibit a significant effect upon the molar enthalpy for the high-density phase, in direct contrast to the low-density phase. The change in molar enthalpy for all solutes is linearly related to temperature in the range below 40 °C. The slope, which represents the change in heat capacity at constant pressure (ΔC_p), ranges from 0.26 to 0.86 kcal mol⁻¹ °C⁻¹ for C₁₀ to C₂₂, respectively. There is a pronounced discontinuity between 40 and 50 °C, which is consistent with a phase transition within this temperature region. Above the transition temperature, the slope ΔC_p is much smaller and ranges from 0.06 to 0.29 kcal mol⁻¹ °C⁻¹ for C₁₀ to C₂₂, respectively (21).

Enthalpy-Entropy Compensation Behavior. The enthalpy-entropy compensation behavior for the octadecylsilica stationary phases has been evaluated by using the method of Melander *et al.* (24):

$$\ln k_T = \frac{-\Delta H^\circ}{R} (1/T - 1/T_{com}) - \frac{\Delta G^\circ_{Tcom}}{R T_{com}} + \ln \beta \quad [2]$$

where k_T is the capacity factor measured at temperature T , ΔG°_{Tcom} is the Gibbs free energy at the compensation temperature (T_{com}), and all other variables are as previously defined. A graph of $\ln k_T$ versus $-\Delta H^\circ$ can be used to characterize the retention mechanism for homologous solutes that obey a linear free energy relationship.

As shown in Figure 4, a linear graph of $\ln k_T$ versus $-\Delta H^\circ$ is observed for the low-density phase under all examined conditions of temperature and pressure. This behavior suggests that the balance of enthalpic and entropic forces remains the same for all methylene homologues under all experimental conditions; i.e., the retention mechanism is invariant. Similar behavior is observed for the intermediate-density phase above the transition temperature (28 °C) and also for the high-density phase below the transition temperature (45 °C). It is a common feature within these regions that the capacity factor at a constant $-\Delta H^\circ$ value decreases with increasing temperature and increases with increasing pressure. This is expected from the van't Hoff relationship in Equation [1], if ΔS° and β are constant with temperature. These results suggest that temperature and pressure exhibit the thermodynamically expected effect on solute retention within the liquid-like and solid-like regions.

For the intermediate-density phase below the transition temperature and for the high-density phase above the transition temperature, a nonlinear graph of $\ln k_T$ versus $-\Delta H^\circ$ is observed. These curves consist of two approximately linear segments, which are separated by a discontinuity. At the highest temperature within this transition region, the discontinuity occurs between solutes C₁₆ and C₁₈. The discontinuity occurs at progressively lower carbon number with decreasing temperature until, at the lowest temperature within this region, the discontinuity occurs between solutes C₁₀ and C₁₂. This discontinuity indicates that the homologous solutes have a different balance of enthalpic and entropic forces and, hence, are not retained by the same retention mechanism. The solutes with carbon number below the discontinuity have a high slope (which reflects the change in retention arising from a given change in molar enthalpy, $\Delta(\ln k) / \Delta(-\Delta H^\circ)$), comparable to that in the liquid-like phase. In contrast, solutes with carbon number above the discontinuity have a lower slope approaching that in the solid-like phase. These results support the conclusion that the phase transition is discontinuous and progressive with respect to carbon number. Throughout the entire transition region, the capacity factor at a constant $-\Delta H^\circ$ value increases with increasing temperature and decreases with increasing pressure. These results, which are directly

FIGURE 4: The logarithm of capacity factor for the methylene homologues expressed as a function of the molar enthalpy for (A) low-density monomeric, (B) intermediate-density monomeric, and (C) high-density polymeric octadecylsilica phases.

contrary to thermodynamic expectations according to Equation [1], suggest that ΔS° is no longer constant with temperature and pressure and its variation becomes significant compared to the variation of ΔH° .

According to Equation [2], the compensation temperature ($T_{\text{com}} = \Delta\Delta H^\circ / \Delta\Delta S^\circ$) can be determined from the slope of the graph of $\ln k_T$ versus $-\Delta H^\circ$ (24). This represents the hypothetical temperature at which enthalpic and entropic forces would be exactly balanced if thermodynamic conditions at the specified temperature T prevailed throughout the entire range. For the low-density phase, the compensation temperature ranges from approximately 446 to 687 °C. This range of values is comparable to that reported by Tchapla *et al.* (25) for another series of homologous solutes (497 to 641 °C). For the intermediate-density phase in the liquid-like region, the compensation temperature ranges from 448 to 462 °C, which is at the lower boundary but still within the range of values for the low-density phase. For the high-density phase in the solid-like region, the compensation temperature ranges from 50 to 77 °C. Because this range of values is substantially lower than the liquid-like region, it is indicative of an entropically dominated retention process.

Within the transition region between the liquid-like and solid-like regions, a relatively broad range of compensation temperatures is observed: 45 to 139 °C for the intermediate-density phase and 91 to 238 °C for the high-density phase. The compensation temperature decreases as the temperature decreases, which is consistent with the progressive transition from liquid-like to solid-like state.

Effect of Temperature and Pressure on Molar Volume. To elucidate further the thermodynamic behavior of the octadecylsilica stationary phases, it is desirable to examine the change in molar volume for the methylene homologues. The partial derivative of $\ln k$ with respect to P will yield the value of $-\Delta V^\circ / RT$, provided that the phase ratio remains constant. Figure 5 illustrates the change in molar volume calculated in this manner for the methylene homologues. In most cases, the change in molar volume is negative which implies that the solute occupies a smaller volume in the octadecylsilica stationary phases than in the methanol mobile phase. For the low-density phase, the molar volume remains small and relatively constant throughout the examined temperature and pressure ranges. The values range from +1.92 to -4.31 cm³ mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C. The differential change in molar volume per ethylene group ($\Delta\Delta V^\circ$) remains relatively constant, with an average value of -1.0 ± 0.4 cm³ mol⁻¹. This suggests that each ethylene group contributes in an equal manner to the overall change in volume for the homologous solutes (21,26).

For the high-density phase, the change in molar volume is significantly greater with values ranging from -27.1 to -104 cm³ mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C. The differential change in molar volume per ethylene group remains constant for solutes C₁₀ to C₁₈, within the statistical precision of the measurements. The average value of -14.1 ± 2.8 cm³ mol⁻¹ is approximately ten-fold higher than that for the low-density phase under the same conditions of temperature and pressure. In order to gain an appreciation for the magnitude of this change, it is helpful to compare with the molar volume of the ethylene group in liquid octadecane. From the density of octadecane (0.77 g cm⁻³ at 30 °C), the molar volume of the ethylene group (28 g mol⁻¹) can be estimated as 36 cm³ mol⁻¹. Thus, the compression of the ethylene group in the high-density octadecylsilica phase is approximately 39% of its total volume in the bulk liquid. The solutes C₂₀ and C₂₂ have notably smaller volume contributions of -9.55 and -8.89 cm³ mol⁻¹, respectively. Again, these results are intuitively reasonable because the additional carbon atoms cannot be fully embedded in the octadecylsilica phase (21).

FIGURE 5: Molar volume for the methylene homologues for (A) low-density monomeric, (B) intermediate-density monomeric, and (C) high-density polymeric octadecylsilica phases (5,21).

Enthalpy-Volume Compensation Behavior. As noted previously, the changes in molar enthalpy and molar volume are linearly related to the carbon number for the methylene homologues. It is, therefore, not too surprising that the molar enthalpy and volume show a linear correlation. For the low-density phase at 30 °C, the slope is 2.37 cm³ kcal⁻¹, the intercept is 5.64 cm³ mol⁻¹, and the linear correlation coefficient (r^2) is 0.987. For the high-density phase at 30 °C, the slope is 3.93 cm³ kcal⁻¹, the intercept is 16.6 cm³ mol⁻¹, and the linear correlation coefficient (r^2) is 0.997. These results suggest that there may be an enthalpy-volume correlation similar to the enthalpy-entropy correlation discussed above. Since enthalpy-entropy compensation arises through the Gibbs free energy as

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \quad [3]$$

it follows that enthalpy-volume compensation may arise in an analogous manner through the internal energy (ΔU°) as

$$\Delta U^\circ = \Delta H^\circ - P \Delta V^\circ \quad [4]$$

Thus, we may define a compensation pressure ($P_{\text{com}} = \Delta\Delta H^\circ / \Delta\Delta V^\circ$) which represents the hypothetical pressure at which enthalpic and volume effects would be exactly balanced. From the slopes above, the compensation pressures for the low-density and high-density phases are determined to be 13,300 and 10,400 psi, respectively, at 30 °C. Further investigations of the enthalpy-volume compensation phenomenon are presently underway in our laboratory (21,27).

Effect of Wetting Solvents on Thermodynamic Behavior. We have extended these thermodynamic studies to include the effect of mobile-phase composition. Methanol, which was used as the mobile phase for all studies described above, is not miscible with the bulk solvent octadecane; however, ethanol is slightly miscible and propanol is miscible in all proportions. It has been suggested that the surface excess of solvent and the surface tension may influence solute retention on octadecylsilica (11,28-31). If these interfacial phenomena are a significant contribution to the thermodynamic behavior, then solvents with good wetting and solvation properties should exhibit distinctly different temperature and pressure dependence. In order to examine this effect, retention of the methylene homologues was measured on the octadecylsilica stationary phases with different bonding density (2.7 to 5.4 $\mu\text{mol m}^{-2}$) using mobile phases of methanol, 10% ethanol in methanol, and 10% propanol in methanol.

From classical thermodynamics, the effect of pressure on solute capacity factor at constant temperature can be expressed by Equation [1]. For the low-density phase (Table I), an increase in pressure has a slight effect on retention ranging from a +2.0% to +9.4% increase for C_{10} to C_{22} , respectively, for the methanol mobile phase. The effect is of comparable magnitude for the ethanol and propanol mobile phases. For the high-density phase, the effect of pressure ranges from +5.4% to +21.1% above the transition temperature (Table II), and from +22.3% to +124.1% below the transition temperature (Table III) for C_{10} to C_{22} , respectively. Again, the effect is of comparable magnitude for the ethanol and propanol mobile phases. Thus, the wetting properties of the mobile phase have little or no consequences upon the thermodynamic effect of pressure.

Similarly, from classical thermodynamics, the effect of temperature on solute capacity factor at constant pressure can be expressed by Equation [1]. For the high-density phase (Table IV), an increase in temperature causes a significant decrease in retention

TABLE I: Effect of pressure at constant temperature (30 °C) on solute capacity factor for the low-density monomeric octadecylsilica stationary phase using various mobile phases.

SOLUTE CARBON NUMBER	METHANOL		10% ETHANOL		10% i-PROPANOL	
	k 370 psi	$\Delta k / k$ 4600 psi	k 370 psi	$\Delta k / k$ 4600 psi	k 340 psi	$\Delta k / k$ 4630 psi
10	0.28	+2.0 %	0.25	+2.0 %	0.24	+1.2 %
12	0.42	+2.9 %	0.37	+2.8 %	0.34	+2.5 %
14	0.61	+4.3 %	0.53	+4.1 %	0.49	+3.1 %
16	0.88	+5.2 %	0.75	+5.2 %	0.69	+4.5 %
18	1.25	+6.8 %	1.05	+6.1 %	0.95	+5.8 %
20	1.75	+7.8 %	1.45	+7.3 %	1.30	+7.0 %
22	2.43	+9.4 %	1.99	+8.3 %	1.75	+7.8 %

TABLE II: Effect of pressure at constant temperature (60 °C) on solute capacity factor for the high-density polymeric octadecylsilica stationary phase using various mobile phases.

SOLUTE CARBON NUMBER	METHANOL		10% ETHANOL		10% i-PROPANOL	
	k 560 psi	$\Delta k / k$ 3560 psi	k 560 psi	$\Delta k / k$ 3560 psi	k 560 psi	$\Delta k / k$ 3560 psi
10	0.13	+5.4 %	0.12	+8.0 %	0.11	+7.2 %
12	0.17	+9.4 %	0.16	+9.5 %	0.15	+10.3 %
14	0.24	+11.2 %	0.21	+11.6 %	0.20	+11.9 %
16	0.32	+13.8 %	0.28	+13.5 %	0.26	+13.7 %
18	0.42	+16.4 %	0.36	+16.0 %	0.33	+15.7 %
20	0.53	+18.2 %	0.46	+17.9 %	0.42	+17.8 %
22	0.67	+20.1 %	0.57	+19.4 %	0.52	+19.5 %

TABLE III: Effect of pressure at constant temperature (30 °C) on solute capacity factor for the high-density polymeric octadecylsilica stationary phase using various mobile phases.

SOLUTE CARBON NUMBER	METHANOL		10% ETHANOL		10% i-PROPANOL	
	k 560 psi	$\Delta k / k$ 3560 psi	k 560 psi	$\Delta k / k$ 3560 psi	k 560 psi	$\Delta k / k$ 3560 psi
10	0.30	+22.3 %	0.27	+22.2 %	0.26	+22.5 %
12	0.53	+33.7 %	0.47	+33.4 %	0.45	+34.5 %
14	0.94	+50.6 %	0.83	+49.3 %	0.78	+51.1 %
16	1.74	+72.1 %	1.49	+71.0 %	1.40	+73.2 %
18	3.17	+95.2 %	2.67	+91.8 %	2.47	+95.2 %
20	5.37	+112.7 %	4.45	+109.4 %	4.07	+113.9 %
22	8.33	+124.1 %	6.82	+120.7 %	6.14	+124.7 %

TABLE IV: Effect of temperature at constant pressure (560 psi) on solute capacity factor for the high-density polymeric octadecylsilica stationary phase using various mobile phases.

SOLUTE CARBON NUMBER	METHANOL		10% ETHANOL		10% i-PROPANOL	
	k 30 °C	$\Delta k / k$ 60 °C	k 30 °C	$\Delta k / k$ 60 °C	k 30 °C	$\Delta k / k$ 60 °C
10	0.30	-57.2 %	0.27	-57.2 %	0.26	-56.9 %
12	0.53	-67.2 %	0.47	-66.6 %	0.45	-67.4 %
14	0.94	-74.7 %	0.83	-74.3 %	0.78	-74.5 %
16	1.74	-81.7 %	1.49	-81.3 %	1.40	-81.4 %
18	3.17	-86.8 %	2.67	-86.5 %	2.47	-86.4 %
20	5.37	-90.0 %	4.45	-89.7 %	4.07	-89.6 %
22	8.33	-91.9 %	6.82	-91.6 %	6.14	-91.5 %

ranging from -57.2% to -91.9% for C₁₀ to C₂₂, respectively. The effect is of similar magnitude for the ethanol and propanol mobile phases. Thus, the wetting properties of the mobile phase have little or no consequences upon the thermodynamic effect of temperature. These results suggest that the structure and properties of the octadecylsilica stationary phase in aggregate, not merely the interfacial region, control the thermodynamics of solute distribution between the mobile and stationary phase.

Kinetic Studies of Reversed-Phase Liquid Chromatography

Effect of Temperature and Pressure on Kinetic Rate Constants. It is also desirable to examine the extent to which temperature, pressure, and wetting solvents influence the kinetic rate of solute distribution between the mobile and stationary phases. This understanding is essential to identify and to control the rate-limiting processes so that separation speed may be increased without sacrificing efficiency.

The overall rate of mass transport within the chromatographic column is reflected in the dispersion of the solute zones. If all sources of dispersion are independent, then the total variance of the solute zone may be expressed as the summation of the contributions from diffusion, laminar flow, multiple paths between and within particles, stationary phase mass transfer, etc. (6,32-34). Among these contributions, those that are rapid compared with the linear velocity will generally lead to symmetric (e.g., Gaussian) profiles, whereas those that are slow will lead to asymmetric (e.g., exponential) profiles. These latter contributions are the most detrimental to resolution and, therefore, are of greatest concern in increasing the speed of separations. It must be noted that other processes can lead to exponential or otherwise asymmetric profiles, among which nonlinear isotherms (35) and extracolumn contributions from injection, detection, and connections (6) are prevalent.

The detection system developed in our laboratory (Figure 1) is particularly well suited for such measurements because the solute zone profile is examined at several positions *in situ* along the column. The zone profiles are then analyzed by nonlinear regression to determine the Gaussian (σ^2) and exponential (τ^2) contributions to variance. By graphing the variance as a function of distance travelled, the column contributions may be determined from the slope (σ^2/L , τ^2/L) to effectively eliminate extracolumn contributions (which are invariant with distance). Moreover, column contributions due to a nonlinear isotherm (which decrease with distance and increase with solute concentration) can be readily distinguished from contributions due to kinetic processes (which increase linearly with distance and are independent of solute concentration).

Once the exponential contribution to the variance has been reliably determined, the kinetic rate constants for solute transfer between the mobile and stationary phases (k_{ms} and k_{sm}) can be calculated. By modification of the approach by Giddings (36,37)

$$\frac{\tau^2}{L} = \frac{(1+k)^2}{u^2} \left(\frac{2k_{ms} u}{(k_{ms}+k_{sm})^2} \right) \quad [5]$$

where u is the linear velocity of the mobile phase. These rate constants are a colligative measure of all mass transport processes cited above that are slow on the time-scale of the separation. They also represent a time-weighted average of all local environments (e.g., different surface sites, pore diameters, particle sizes, etc.) to which the solute is exposed between the on-column detectors.

The rate constants for the high-density octadecylsilica phase are shown as a function of temperature in Figure 6 (38). Below the transition temperature (45 °C), the rate constants decrease logarithmically with carbon number. The magnitude of both rate constants changes significantly; k_{ms} ranges from 5.1 to 0.83 s⁻¹ and k_{sm} from 9.1 to 0.013 s⁻¹ for C₁₀ to C₂₂, respectively, at 25 °C and 4750 psi. Whereas both rate constants are important to the overall rate of mass transfer, k_{ms} is rate-limiting for solutes with capacity factors less than unity and k_{sm} is limiting for those greater than unity. An increase in temperature causes a marked increase in the rate constants that is greater for k_{sm} than for k_{ms} . Above the transition temperature, the rate constants increase with carbon number. The magnitude of k_{ms} ranges from 7.9 to 90 s⁻¹ and k_{sm} from 56 to 104 s⁻¹ for C₁₀ to C₂₂, respectively, at 60 °C and 4750 psi. The rate constants measured in this region are comparable in magnitude to the reciprocal relaxation time for sorption/desorption of a trimethylhexadecylammonium ion pair on octadecylsilica materials measured using the pressure-jump kinetic method by Marshall *et al.* (39,40).

Using a simple Arrhenius model, the rate constants can be expressed as

$$k_{ms} = A_{m\ddagger} \exp\left(\frac{-\Delta E_{m\ddagger}}{R T}\right) \quad [6a]$$

$$k_{sm} = A_{s\ddagger} \exp\left(\frac{-\Delta E_{s\ddagger}}{R T}\right) \quad [6b]$$

where $A_{m\ddagger}$ and $A_{s\ddagger}$ are the pre-exponential factors and $\Delta E_{m\ddagger}$ and $\Delta E_{s\ddagger}$ are the activation energies from the mobile and stationary phases, respectively, to the transition state ($\ddagger$). Thus, the activation energies can be calculated from the slope of a graph of $\ln k_{ms}$ or $\ln k_{sm}$ versus T . Below the transition temperature (45 °C), the activation energy $\Delta E_{m\ddagger}$ decreases with carbon number whereas $\Delta E_{s\ddagger}$ increases with carbon number. Typical values for $\Delta E_{m\ddagger}$ range from +30.2 to +19.3 kcal mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C and 4750 psi. Typical values for $\Delta E_{s\ddagger}$ range from +41.6 to +55.9 kcal mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C and 4750 psi. Above the transition temperature, both $\Delta E_{m\ddagger}$ and $\Delta E_{s\ddagger}$ increase with carbon number. Typical values for $\Delta E_{m\ddagger}$ range from -4.9 to +15.6 kcal mol⁻¹ and $\Delta E_{s\ddagger}$ range from -0.2 to +28.1 kcal mol⁻¹ for C₁₀ to C₂₂, respectively, at 60 °C and 4750 psi. In all cases both above and below the transition temperature, the barrier for the stationary phase transition is greater than that for the mobile phase (38).

The rate constants for the high-density octadecylsilica phase are shown as a function of pressure in Figure 7 (41). An increase in pressure causes a decrease in the rate constants that is greater for k_{sm} than for k_{ms} . The activation volumes $\Delta V_{m\ddagger}$ and $\Delta V_{s\ddagger}$ can be calculated from the slope of a graph of $\ln k_{ms}$ or $\ln k_{sm}$ versus P . Typical values for $\Delta V_{m\ddagger}$ range from +55.3 to +110 cm³ mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C. Typical values for $\Delta E_{s\ddagger}$ range from +91.4 to +211 cm³ mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C. These changes in activation volume are positive, which implies that the solute molar volume in the transition state is larger than that in the mobile or stationary phases. Moreover, the changes are quite large with respect to the overall change in molar volume ΔV° , which ranges from -27.1 to -104 cm³ mol⁻¹ for C₁₀ to C₂₂, respectively, at 30 °C (38).

These results suggest that the kinetics of solute distribution in octadecylsilica stationary phases is strongly influenced by temperature and, to a lesser extent, by pressure. From these results, it is apparent that kinetic processes are a significant contribution to zone broadening that must be understood and controlled in order to improve chromatographic performance.

FIGURE 6: Kinetic rate constants k_{ms} (A) and k_{sm} (B) for the methylene homologues for the high-density polymeric octadecylsilica phase as a function of temperature at constant pressure (4750 psi) (38).

FIGURE 7: Kinetic rate constants k_{ms} (A) and k_{sm} (B) for the methylene homologues for the high-density polymeric octadecylsilica phase as a function of pressure at constant temperature (30 °C) (38).

FIGURE 8: Kinetic rate constants k_{ms} (A) and k_{sm} (B) for the methylene homologues for the high-density polymeric octadecylsilica phase as a function of mobile-phase composition (38).

Effect of Wetting Solvents on Kinetic Rate Constants. We have extended these kinetic studies to include the effect of mobile-phase composition. As noted previously, methanol is immiscible, ethanol is slightly miscible, and propanol is miscible in all proportions with octadecane. If surface tension and the resulting interfacial resistance to mass transfer are a significant contribution (28-31), then the solvents with good wetting properties should exhibit distinctly different kinetic behavior.

The rate constants for the high-density polymeric phase are shown as a function of mobile-phase composition in Figure 8. The use of wetting solvents causes a small but statistically significant increase in the rate constants. The effect is comparable for solutes C_{10} to C_{22} , but is greater for k_{sm} than for k_{ms} . These results are consistent with stationary phase mass transfer as the predominant contribution to kinetic behavior, with interfacial phenomena due to solvent wetting having a small and comparable effect for all solutes (38). It is noteworthy that the wetting solvents have far less effect on the kinetic rate of transfer than either temperature or pressure.

Summary of Thermodynamic and Kinetic Studies

Based on the results summarized above, we can identify and characterize three distinctly different regions of behavior for the octadecylsilica stationary phases:

In the liquid-like region, the octadecyl chains can be envisioned as rotationally and translationally mobile with little short-range or long-range order. The changes in molar enthalpy (Figure 3) and molar volume (Figure 5) are linearly related to solute carbon number, which suggests that each ethylene group contributes equivalently to solute retention. These thermodynamic quantities are influenced to a small extent by temperature and pressure in the theoretically expected manner (Equation [1]). Although the molar enthalpy is relatively small, entropic effects are also small; thus, the solute capacity factor or equilibrium constant increases rapidly (slope $\Delta(\ln k)/\Delta(-\Delta H^\circ)$ in Figure 4). The kinetics of solute distribution are rapid at all temperatures and pressures.

In the solid-like region, the octadecyl chains are relatively immobile because of the more compact and rigid organization. The changes in molar enthalpy (Figure 3) and molar volume (Figure 5) are smoothly but nonlinearly related to carbon number, and are significantly influenced by temperature and pressure. The molar enthalpy is much larger than that in the liquid-like region, but the solute capacity factor does not increase commensurately because of unfavorable entropic effects (slope $\Delta(\ln k)/\Delta(-\Delta H^\circ)$ in Figure 4). The kinetics of solute distribution are slow and are significantly influenced by temperature and pressure. The kinetic rate constants decrease logarithmically with carbon number.

The transition from liquid-like to solid-like regions appears to be similar to the order-disorder transitions in Langmuir monolayers and Langmuir-Blodgett films (9,41-43). This transition involves the progressive organization of the alkyl chain, beginning at the proximal (bound) end and advancing to the distal (interfacial) end. This transition appears to be progressive and discontinuous with respect to the variables of temperature, pressure, and carbon number. Solutes with low carbon number can access only the interfacial region, which has the greatest mobility and least organization. These solutes show behavior similar to that in the liquid-like region. Solutes with higher carbon number can access the highly organized regions near the surface and, thus, show behavior that approaches that in the solid-like region (Figure 4). Within this transition region, the effects of temperature and pressure on solute capacity factor are directly contrary to thermodynamic expectations according to Equation [1].

These results suggest that both molar enthalpy and molar entropy are not constant, but changing significantly with temperature and pressure.

Among the interrelated variables of state, the bonding density appears to have the greatest influence on the thermodynamic and kinetic behavior. Moreover, the bonding density circumscribes the magnitude of the effects of temperature and pressure. For example, stationary phases with sufficiently low bonding density may exhibit only liquid-like behavior within the normal operating range of temperature and pressure. As bonding density increases, different ranges within the liquid-like, transition, and solid-like regions become attainable. Temperature and pressure serve to modify, to a gradually increasing extent, the local density and organization of the stationary phase. Finally, a stationary phase with sufficiently high bonding density may be expected to show only solid-like behavior within the normal operating range of temperature and pressure.

Most of the commercially available octadecylsilica materials are in the range of 2.0 to 4.0 $\mu\text{mol m}^{-2}$ and, thus, will exhibit behavior in the liquid-like and transition regions. Because the transition region shows the greatest anomalies in thermodynamic and kinetic behavior, deviations from ideality will be problematic for the phases with higher bonding density. Therefore, octadecylsilica materials with low bonding density may be preferred for practical applications because of the greater predictability as well as precision and accuracy of solute retention. Alternatively, a material with shorter alkyl chain length may be beneficial because of the higher transition temperature (7).

Studies of Transition Regions

After any rapid change in the physical or chemical environment, some time is necessary before equilibrium can be reestablished. In chromatographic applications, such nonequilibrium conditions exist to some extent continually as the solute zone travels along the column (34). However, a further departure from equilibrium occurs specifically on injection of a solute zone onto the chromatographic column and on elution of the zone from the column. The unique ability of the on-column fluorescence detectors to isolate specific regions of the column affords a direct view of both injection and elution processes that was not previously possible. A brief description of our research progress in each area follows, with more depth and detail to be provided in the publications cited.

Injection Nonequilibrium. In most theoretical models of chromatographic separations, the solute is initially presumed to be present on the column with a narrow rectangular profile, fully equilibrated between the mobile and stationary phases. In practice, however, the solute zone must pass from a nonretentive injection valve or open tube onto the retentive packed bed. This spatial discontinuity in the physical and chemical environment inherent in the injection process may influence both the retention and dispersion of the solute zone. Previous investigations of the injection process have been limited by the inability to probe solute zones directly in the inlet region of the column.

In this study, the solute zones are monitored *in situ* by positioning one detector along an open tube immediately prior to the packed bed and five detectors sequentially along the column at 5-cm intervals. Upon injection of the model solutes, a decrease in length variance and a concomitant increase in concentration are measured as a function of the solute capacity factor. This decrease in variance may be predicted theoretically using a simple steady-state model of the injection process developed in our laboratory (44):

$$(\sigma_L^2)_{ON} = (\sigma_L^2)_{OFF} \frac{R_{OFF}^4}{R_{ON}^4} \frac{1}{\epsilon_T^2} \frac{1}{(1 + k_{INJ})^2} \quad [7]$$

where $(\sigma_L^2)_{OFF}$ and $(\sigma_L^2)_{ON}$ are the length variances measured immediately prior to and directly on the packed bed, respectively. In this equation, the physical or structural transition within this region is reflected in the terms that contain the radius (R) and total porosity (ϵ_T), which are the same for all solutes; the transition in chemical environment is reflected in the term involving the capacity factor (k), which is clearly solute dependent. Excellent agreement is observed between experimental measurements and theoretical predictions based on this model, as shown in Figure 9.

Equation [7] suggests that the solute zone variance is influenced by the composition of the injection solvent; a solvent that is weaker than the mobile phase ($k_{INJ} > k_{MP}$) will cause zone compression, whereas one that is stronger ($k_{INJ} < k_{MP}$) will cause expansion. This hypothesis has been tested by using 90% and 95% methanol-water mixtures (weaker) as well as 90% and 95% methanol-acetone mixtures (stronger) as injection solvents with a pure methanol mobile phase. Although the overall retention does not change because the solute zone is rapidly separated from the injection solvent, the zone variance is altered dramatically with injection solvent composition and the experimental measurements agree well with theoretical predictions using Equation [7] (44). This effect may be exploited to preconcentrate the solute at the column inlet and to reduce extracolumn dispersion from the injection process. Contrary to common practice, however, only small changes in the injection solvent composition are necessary to produce a marked improvement in the zone profile. When the injection solvent differs substantially from the mobile phase (i.e., pure water or acetone), deleterious changes in the zone profile are observed that are not presently predictable (44).

Elution Nonequilibrium. Nonequilibrium effects are also significant at the exit of the chromatographic column, where the solute zone passes from the retentive packed bed to a nonretentive open tube or detector flow cell. Upon elution of the solute zone, an increase in length variance and a concomitant decrease in concentration are observed as a function of the capacity factor. This increase in variance may be predicted theoretically using a simple steady-state model of the elution process developed in our laboratory (45,46):

$$(\sigma_L^2)_{OFF} = (\sigma_L^2)_{ON} \frac{R_{ON}^4}{R_{OFF}^4} \epsilon_T^2 (1 + k_{MP})^2 \quad [8]$$

where $(\sigma_L^2)_{ON}$ and $(\sigma_L^2)_{OFF}$ are the length variances measured directly on and immediately after the packed bed, respectively, and all other variables are as previously defined. Excellent agreement is observed between experimental measurements and theoretical predictions based on this model, as shown in Figure 10.

Because there is an abrupt change in retention that is propagated spatially and temporally along the solute zone profile, the steady-state model described above may not adequately represent this source of dispersion. Consequently, it is desirable to test this model under a variety of practical operating conditions where departure from equilibrium may be expected. In these studies, we have attempted to disrupt the local equilibrium by altering the linear velocity, temperature, and mobile-phase composition.

FIGURE 9: Measured ratio of the length variance measured off- and on-column after injection as a function of the capacity factor. (—) Theoretical prediction according to Equation [7]; (O) Experimental measurement using methylene homologues as model solutes (44).

FIGURE 10: Measured ratio of the length variance measured on- and off-column after elution as a function of the capacity factor. Theoretical prediction according to Equation [8]. Experimental measurement with varying mobile-phase linear velocity using methylene homologues as model solutes (45,46).

FIGURE 11: Measured ratio of the length variance measured on- and off-column after elution as a function of the capacity factor. Theoretical prediction according to Equation [8]. Experimental measurement with varying temperature using methylene homologues as model solutes (46).

FIGURE 12: Measured ratio of the length variance measured on- and off-column after elution as a function of the capacity factor. Theoretical prediction according to Equation [8]. Experimental measurement with varying mobile-phase composition using methylene homologues as model solutes (46).

Although the linear velocity of the mobile phase is known to influence solute zone dispersion along the column, it is not implicated directly or indirectly in Equation [8]. If the steady-state model is correct, then the length variance ratio should be independent of linear velocity. This expectation is verified in Figure 10 for velocities near the optimum value ranging from 0.060 to 0.129 cm/s.

The thermodynamic partition coefficient and, hence, the capacity factor (k) is inversely related to temperature according to the Van't Hoff equation (Equation [1]). If the steady-state model is correct, this should be the only influence of temperature on elution nonequilibrium processes. Under steady-state conditions, Equation [8] predicts that a graph of the length variance ratio *versus* the function of capacity factor $(1 + k)^2$ should be independent of temperature. This expectation is verified in Figure 11 for temperatures ranging from 25 to 90 °C, where capacity factors decrease three- to ten-fold for solutes C_{10} to C_{20} , respectively.

Similarly, the mobile-phase composition is known to influence the solute capacity factor. According to regular solution theory, the logarithm of the capacity factor is quadratically related to the volume fraction of organic solvent in the mobile phase (47,48). If this is the only influence of mobile-phase composition, Equation [8] predicts that a graph of the length variance ratio *versus* the function of capacity factor $(1 + k)^2$ should be independent of composition. This expectation is verified in Figure 12 for mobile-phase compositions ranging from 90% to 100% methanol-water mixtures, where capacity factors decrease five- to fifteen-fold for solutes C_{10} to C_{20} , respectively.

As shown in Figures 10 – 12, the extent of solute zone broadening upon elution from the chromatographic column is substantial, and increases with the capacity factor as $(1 + k)^2$. The excellent agreement with Equation [8] indicates that, while nonequilibrium exists across the entire solute zone profile, at any spatial location the solute is in local thermodynamic equilibrium between the mobile and stationary phases. This observation suggests that a temporal gradient in temperature or mobile-phase composition would be expected to minimize this source of dispersion by reducing the capacity factors for late-eluting solutes, whereas a temporal gradient in linear velocity would not be effective in this manner. Furthermore, a spatial gradient in any of these variables would be ineffective to reduce this source of dispersion (46). Because this appears to be a substantial contribution to the general elution problem in chromatography, a more thorough understanding of this dispersion process is required.

II. COMPUTER SIMULATION OF CHROMATOGRAPHY AND ELECTROPHORESIS

A three-dimensional stochastic computer simulation has been developed in order to provide a unified and detailed treatment of chromatographic and electrophoretic separations. In this simulation, the migration of individual molecules or ions is established through diffusion and convection by means of a pressure or electric-field gradient within a fluid phase that is in contact with a surface. Molecular interaction and, hence, retention may arise by absorption (partition) into permeable surfaces or by adsorption at solid surfaces. The molecular distribution and the corresponding zone profile may be examined and characterized at any specified time or spatial position during the simulation. This simulation provides a powerful and versatile model with which to characterize transport phenomena in complex chromatographic and electrophoretic separation systems (49-52).

The simulation program was written in the FORTRAN 90 programming language and optimized for execution on an IBM RS/6000 Model 580 computer. A flowchart for the simulation program is shown schematically in Figure 13. This program incorporates algorithms for the processes of diffusion, convection, and retention, which are repeated for each molecule at each time increment (t) until the total simulation time (T) is reached. The simulation may be performed in Cartesian global coordinates, which is most appropriate for separations in planar media, or alternatively in cylindrical global coordinates for separations in capillary tubes or fibers. Because of its mathematical simplicity, the latter case will be briefly described herein with more depth and detail to be provided in the publications cited.

Simulation Input. The input parameters required for the simulation may be divided into three general categories, as shown in Table V. The system parameters describe properties of the fluid and the surface, as well as the spatial dimensions of the separation system to be simulated. The molecular parameters describe attributes of the solute molecules, and the computational parameters describe certain constraints that are required for the simulation. On the basis of these input parameters, an array is created that contains the properties and coordinates of each molecule. To initialize the simulation, the molecules are distributed randomly with a delta, rectangular, or Gaussian profile of specified variance at a specified mean distance in the global coordinate frame.

Simulation Output. The simulation program allows the molecular zone profile to be examined as the distance distribution at specified times or, correspondingly, as the time distribution at specified distances. The statistical moments of the molecular distribution are then calculated in either the distance or time domain (49-52). For example, the first statistical moment $M1$ or mean distance $\bar{Z}$ is determined from

$$M1 = \bar{Z} = N^{-1} \sum_{i=1}^N Z_i \quad [9]$$

where Z_i is the axial coordinate of an individual molecule and N is the total number of molecules. The second statistical moment $M2$ or variance σ^2 is determined from

$$M2 = \sigma^2 = N^{-1} \sum_{i=1}^N (Z_i - \bar{Z})^2 \quad [10]$$

and the higher-order statistical moments are determined in a similar manner. These statistical moments, as well as the chromatographic or electrophoretic figures of merit derived therefrom, are stored in a standard data file at each specified time (or distance). For example, the capacity factor, effective mobility, velocity, plate height, etc. can be calculated since the beginning of the simulation (net average) or since the most recent data file output (local average). Other information such as the number of molecules in the fluid and surface phases, the time spent by each molecule in each phase, and the number of transitions between phases are also recorded in the standard data file.

In addition to these numerical output parameters, the molecular population is summed in discrete segments and then smoothed by Fourier transform methods (53) to provide a continuous zone profile for graphical display. Because the molecular distribution may be examined at any time (or distance), these output routines provide an extensive visual and numerical record of transport processes throughout the simulation.

FIGURE 13: Flowchart of the computer program for stochastic simulation of chromatography and electrophoresis (49-52).

TABLE V: Variable parameters of the computer program for stochastic simulation of chromatography and electrophoresis (49-52).

SYSTEM PARAMETERS	SYMBOL
Radius of fluid phase	R_f
Radius of surface phase	R_s
Length of fluid phase	L
Position of injection zone	L_{inj}
Length, variance of injection zone	l_{inj}, σ^2_{inj}
Position of detection zone	L_{det}
Length, variance of detection zone	l_{det}, σ^2_{det}
Zeta potential of surface phase	ζ
Velocity of fluid phase	v_0
pH of fluid phase	pH
pC of complexing agent in fluid phase	pC
Ionic strength of fluid phase	I
Viscosity of fluid phase	η
Dielectric constant of fluid phase	ϵ
Temperature	T_0
Pressure	P
Voltage	V
MOLECULAR PARAMETERS	SYMBOL
Diffusion coefficient in fluid phase	D_f
Diffusion coefficient in surface phase	D_s
Equilibrium constant for acid/base reaction	K_a
Equilibrium constant for complexation reaction	K_c
Absorption coefficient	K_{abs}
Adsorption energy	E_{ads}
Electrophoretic mobility	μ
Charge	
COMPUTATIONAL PARAMETERS	SYMBOL
Number of molecules	N
Time increment	t
Total simulation time	T
Molecular-frame coordinates	
Spherical	ρ, ϕ, θ
Cartesian	x, y, z
Global-frame coordinates	
Cylindrical	R, Θ, Z
Cartesian	X, Y, Z

Development and Validation of Simulation Models

Diffusion. Molecular diffusion is simulated by using a three-dimensional extension of the Einstein-Smoluchowski equation (54-56). The radial distance ρ travelled during the time increment t is selected randomly from the following probability distribution (51)

$$P_{\rho} = \frac{\rho^2}{(4\pi D_{f,s} t)^{1/2}} \exp\left(\frac{-\rho^2}{4D_{f,s} t}\right) \quad [11]$$

where $D_{f,s}$ represents the binary diffusion coefficient of the molecule in the fluid or surface phase, as appropriate. This approach provides a variable step size derived from a normal (Gaussian) distribution, where the direction of travel is subsequently randomized through the spherical coordinate angles (ϕ, θ). The coordinate increments in the molecular frame are used to calculate the new molecular position in the global coordinate frame.

To verify the accuracy of the diffusion algorithm, the zone distance and variance for an ensemble of 750 molecules were monitored as a function of the simulation time. These results were compared with classical models based on the Einstein equation (54). Excellent agreement was observed for the range of diffusion coefficients from 10^{-1} to 10^{-10} $\text{cm}^2 \text{s}^{-1}$, with average relative errors for the zone distance and variance of 0.81% and 3.67%, respectively (50,51,57).

Convection. Molecular convection in the fluid phase may be induced by means of a pressure or electrical field gradient applied tangential to the surface. Under these conditions, the axial distance z travelled by the molecule in time increment t is given by

$$z = v t \quad [12]$$

For pressure-induced flow under fully developed laminar conditions, the radial velocity profile in the cylindrical global frame is given by the Taylor-Aris equation (58)

$$v = 2v_0 \left(1 - \frac{R^2}{R_f^2}\right) \quad v_0 = \frac{R_f^2 P}{8\eta L} \quad [13a,b]$$

The mean velocity v_0 may be specified as an input parameter or may be calculated from the Hagen-Poiseuille equation (32,33,59), where P is the applied pressure, η is the viscosity of the fluid phase, and R_f and L are the radius and length. The coordinate increment in the molecular frame determined from Equations [12] and [13a] is used to calculate the new molecular position in the global coordinate frame.

To verify the accuracy of the laminar convection algorithm, the zone distance and variance for an ensemble of 750 molecules were monitored as a function of the simulation time. These results were compared with theoretical predictions based on the Taylor-Aris equation (58) including both diffusion and resistance to mass transfer in the fluid phase. Excellent agreement was observed for the range of linear velocities from 0.001 to 100 cm s^{-1} , with average relative errors for the zone distance and variance of 0.49% and 2.24%, respectively (50).

For electric field-induced flow due to electroosmosis, the radial velocity profile in the cylindrical global frame is given by the Rice-Whitehead equation (60,61)

$$v = v_0 \left(1 - \frac{I_0(\kappa R)}{I_0(\kappa R_f)} \right) \quad v_0 = \frac{\epsilon \zeta V}{4\pi\eta L} \quad [14a,b]$$

where κ^{-1} is the Debye length, and I_0 is the zero-order modified Bessel function of the first kind. The maximum velocity v_0 may be specified as an input parameter or may be calculated from the Helmholtz-Smoluchowski equation (62,63), where ϵ is the permittivity of the fluid phase, ζ is the zeta potential of the fluid-surface interface, and V is the applied voltage. The coordinate increment in the molecular frame determined from Equations [12] and [14a] is used to calculate the new molecular position in the global coordinate frame.

To verify the accuracy of the electroosmotic convection algorithm, the zone distance and variance for an ensemble of 500 molecules were monitored as a function of the simulation time. These results were compared with classical models based on the analytical solution of the Rice-Whitehead equation by McEldoon and Datta (64) including both diffusion and resistance to mass transfer in the fluid phase. Excellent agreement was observed for the range of linear velocities from 0.01 to 1.0 cm s⁻¹, with average relative errors for the zone distance and variance of 0.12% and 4.42%, respectively (51).

These convection algorithms may be used individually or in combination to simulate a wide variety of hydrodynamic conditions for chromatography, electrophoresis, or electrochromatography (52).

Electrophoretic Migration. For charged molecules under the influence of an applied electric field (65), the velocity of electrophoretic migration is given by

$$v = \mu \frac{V}{L} \quad [15]$$

The electrophoretic mobility μ is corrected by means of the modified Onsager equation (66) to the specified ionic strength of the fluid phase.

If the molecule exists as a single species, the mobility is constant. This convection algorithm provides equal axial displacement of all molecules during each time increment according to Equations [12] and [15]. The axial coordinate increment in the molecular frame is used to calculate the new position of each molecule in the global frame.

If the molecule exists as n multiple species in dynamic equilibrium (e.g., phosphate may exist as H_3PO_4 , $H_2PO_4^-$, HPO_4^{2-} , or PO_4^{3-}), the mobility of an individual molecule is determined from statistical probability at each time increment. The fraction α_i of each species i is calculated from the appropriate equilibrium constants for acid/base or complexation reactions, which are corrected for ionic strength by means of the Davies equation (67,68). The identity of a molecule is determined by selecting a random number, ξ , between zero and one to establish the value of i that satisfies the relationship

$$1 - \sum_{j=1}^{n-i} \alpha_{n-j} < \xi \leq \sum_{j=1}^{i+1} \alpha_{j-1} \quad [16]$$

The molecule is then assigned the mobility μ_i corresponding to species i during that time increment and its electrophoretic migration is subsequently calculated via Equations [12]

and [15]. The resulting migration of the zone is similar, but not identical, to that for a single species whose average mobility is given by

$$\mu = \sum_{i=0}^n \alpha_i \mu_i \quad [17]$$

To verify the performance of the electrophoretic migration algorithms corresponding to a single species and n multiple species, the zone distance and variance for an ensemble of 500 molecules were monitored as a function of the simulation time. These results were compared with classical models. Excellent agreement was observed for single species with positive and negative electrophoretic mobilities in the range from $+10^{-3}$ to $-10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, with average relative errors for the zone distance and variance of 0.04% and 2.67%, respectively (51). The agreement was similarly good for multiple species, with average relative errors for the zone distance and variance of 0.01% and 3.38%, respectively, for phosphate at pH values from 3.0 to 9.0 (51).

Surface Interaction. Molecular interaction is simulated as an absorption process if the surface is permeable (e.g., thin polymer film or chemically bonded organic ligands) or as an adsorption process if the surface is solid (e.g., silica or alumina).

For the absorption process, the probability of transport between the fluid and surface phases is given by

$$\begin{cases} P_{fs} = aK_{abs}(D_s/D_f)^{1/2} \\ P_{sf} = a \end{cases} \quad \text{or} \quad \begin{cases} P_{fs} = a \\ P_{sf} = aK_{abs}^{-1}(D_f/D_s)^{1/2} \end{cases} \quad [18a,b]$$

where K_{abs} is the absorption coefficient and the constant a represents the fraction of effective collisions with the interface, which is equal to unity when there is no barrier to transport (diffusion-limited case). When a molecule in the fluid phase encounters the fluid-surface interface during the simulation, a random number between zero and one is selected. If the selected number is less than or equal to the probability P_{fs} given by Equation [18a,b], the molecule will be transferred to the surface phase. Otherwise, the molecule will remain in the fluid phase and will undergo an elastic collision at the interface. A similar routine is performed when a molecule in the surface phase encounters the interface, except that the random number is compared with the probability P_{sf} given in Equation [18a,b]. Finally, when a molecule in the fluid or surface phase encounters a spatial boundary of the system, an elastic collision is performed.

To verify the accuracy of the absorption algorithm, the zone distance and variance for an ensemble of 750 molecules were monitored as a function of the simulation time. These results were compared with classical models based on the extended Golay equation (69) including both diffusion and resistance to mass transfer in the fluid and surface phases. Excellent agreement was observed for the range of distribution coefficients from 0.01 to 100.0, with average relative errors for the zone distance and variance of 0.55% and 4.02%, respectively (50).

For the adsorption process, the surface is considered to be a uniform distribution of localized lattice sites, each of equal area and interaction energy with the molecule. The molar adsorption energy E_{ads} is related to the mean time for desorption τ_{ads} in the following manner

$$\tau_{\text{ads}} = \tau_0 \exp\left(\frac{E_{\text{ads}}}{k_A k_B T_0}\right) \quad [19]$$

where τ_0 is the vibrational period, typically $10^{-12} - 10^{-13}$ s, k_B is the Boltzmann constant and k_A is the Avogadro number. If a molecule encounters the surface, the probability for adsorption P_{fs} is equal to unity if the site is vacant and zero if it is occupied. The desorption time of the molecule is then randomly selected from an exponential distribution based on the mean desorption time τ_{ads} in Equation [19].

The absorption and adsorption algorithms may be used for homogeneous surfaces, as described above, or for heterogeneous surfaces with a fractional coverage of two or more types of surface sites. In this case, a random number is selected to establish the identity of the individual surface site by comparison with the fractional coverage. A second random number is then selected to determine whether the molecule is transferred to the surface phase for the absorption mechanism or to determine the desorption time for the adsorption mechanism. These algorithms may be used individually or in combination to describe a wide variety of retention mechanisms for chromatography, electrophoresis, and electrochromatography (52).

Applications of the Stochastic Simulation

Based on the validation studies described above, the stochastic simulation is able to model accurately and precisely the processes of diffusion, convection, and surface interaction. Consequently, it may now be applied with confidence to examine and characterize the combined transport processes in greater detail. Because this simulation monitors the migration of individual molecules, it provides the opportunity to perform simulated experiments and to make observations that may not be possible in a real system. For example, the kinetic rate constants for solute transfer between the fluid and surface phases have been directly determined, and their dependence upon the simulation variables has been established. In addition, the effect of these rate constants upon the properties of the solute zone profile along the chromatographic column have also been examined under both steady-state and nonequilibrium conditions. Finally, the properties of the solute zone profile arising in regions of discrete spatial and temporal transition have been examined. The studies described herein will focus on characterization of the absorption (partition) mechanism, with later studies to be focused on the adsorption mechanism.

Determination of Kinetic Rate Constants. In the absorption process, the solute X is distributed between the fluid and surface phases by a reversible mechanism

where k_{fs} and k_{sf} are the pseudo-first-order rate constants for mass transport. Under these conditions, the distribution of solute molecules can be described by a simple kinetic model (70,71). The net rate of change in the number of molecules in the fluid and surface phases (N_f and N_s , respectively) is governed by the following system of ordinary differential equations

$$\frac{dN_f}{dT} = -k_{fs} N_f + k_{sf} N_s \quad [21a]$$

$$\frac{dN_s}{dT} = k_{fs} N_f - k_{sf} N_s \quad [21b]$$

If all molecules initially reside in the fluid phase, the solution of Equation [21a,b] is given by

$$\frac{N_f}{N} = \frac{k_{sf} + k_{fs} \exp(-(k_{fs} + k_{sf}) T)}{k_{fs} + k_{sf}} \quad [22a]$$

$$\frac{N_s}{N} = \frac{k_{fs} - k_{fs} \exp(-(k_{fs} + k_{sf}) T)}{k_{fs} + k_{sf}} \quad [22b]$$

from which it follows directly that

$$\frac{\tilde{N}_f}{N} = \frac{k_{sf}}{k_{fs} + k_{sf}} \quad [23a]$$

$$\frac{\tilde{N}_s}{N} = \frac{k_{fs}}{k_{fs} + k_{sf}} \quad [23b]$$

where $\tilde{N}_f$ and $\tilde{N}_s$ represent the number of molecules in the fluid and surface phases at equilibrium ($T \rightarrow \infty$). Hence, the ratio of the number of molecules $\tilde{N}_s/\tilde{N}_f$ under the equilibrium definition is equal to the ratio of the rate constants k_{fs}/k_{sf} under the kinetic definition:

$$\frac{\tilde{N}_s}{\tilde{N}_f} = \frac{k_{fs}}{k_{sf}} = \frac{K_{abs} V_s}{V_f} = k \quad [24]$$

Furthermore, this ratio defines the capacity factor (k), which represents the equilibrium constant (K_{abs}) adjusted for the relative volumes of the fluid and surface phases (V_f and V_s , respectively). For the cylindrical model system, these volumes are given by

$$V_f = \pi R_f^2 L \quad [25a]$$

$$V_s = \pi (R_s^2 + 2R_f R_s) L \quad [25b]$$

To examine the kinetic and equilibrium behavior of the model system, the number of molecules in the fluid phase is monitored as a function of time during the stochastic simulation. Three to five repetitive simulations with 10,000 molecules are averaged to obtain the simulation data ($T, N_f/N$). The kinetic rate constants are then determined by nonlinear regression of the simulation data, as illustrated in Figure 14. In addition, the ratio of the number of molecules $\tilde{N}_s/\tilde{N}_f$ is calculated after equilibrium has been attained. This regression approach has been evaluated by theoretical and numerical means to elucidate the conditions for the most reliable determination of the rate constants (72). The two-parameter exponential relationship given in Equation [22a,b] as well as a four-parameter biexponential relationship that provides for intrinsic error compensation have been assessed. In addition, the effects of the initial conditions ($(N_f)_0, (N_s)_0$), total number of molecules (N), and time increment (t) on the relative standard deviation and relative error have been examined over a wide range of simulation conditions. In general, the individual rate constants k_{fs} and k_{sf} can be determined with 0.49% relative standard deviation, and the ratio of the rate constants k_{fs}/k_{sf} with 0.70% relative standard

deviation and 2.25% relative error. The ratio of the number of molecules $\bar{N}_s/\bar{N}_f$ can be determined with 0.29% relative standard deviation and 0.39% relative error (50,72,73).

This approach has been applied to characterize the kinetic behavior of the absorption mechanism under diffusion-limited conditions. Although the ratio of the rate constants k_{fs}/k_{sf} can be readily predicted by means of Equation [24], no theoretical model is available to determine the magnitude of the individual rate constants k_{fs} and k_{sf} . For this reason, the simulation was used to elucidate the relationships between the diffusion-limited rate constants and the fundamental parameters of the system (73). The equilibrium constant K_{abs} was varied from 0.1 to 10.0 ($n = 7$). The diffusion coefficient in the fluid phase D_f was varied from 1.0×10^{-4} to $1.0 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ and the diffusion coefficient in the surface phase D_s was varied from 1.0×10^{-4} to $1.0 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ ($n = 17$). The radius of the fluid phase R_f was varied from 1.13×10^{-3} to $8.33 \times 10^{-2} \text{ cm}$ and the radius of the surface phase R_s was varied from 9.76×10^{-5} to $4.63 \times 10^{-3} \text{ cm}$ ($n = 13$). These radii correspond to a volume ratio of the fluid and surface phases (V_f/V_s) ranging from 0.1 to 50.0 according to Equation [25a,b]. On the basis of these simulations, the following expressions for the rate constants were established (73):

FIGURE 14: Kinetic evolution of the absorption process by monitoring the relative number of molecules in the fluid phase (N_f/N) as a function of simulation time (T). Simulation conditions: $N = 10,000$; $t = 5.0 \times 10^{-4} \text{ s}$; $K_{abs} = 1.0$; $D_f = 1.0 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$; $D_s = 1.0 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$; $R_f = 2.00 \times 10^{-3} \text{ cm}$; $R_s = 8.28 \times 10^{-4} \text{ cm}$; $v_0 = 0.0 \text{ cm s}^{-1}$. (—) Nonlinear regression analysis according to Equation [22a], yielding rate constants $k_{fs} = 0.353 \pm 0.001$, $k_{sf} = 0.357 \pm 0.001$ ($r^2 = 0.974$).

$$k_{fs} = \left(\frac{K_{abs}(2 R_f R_s + R_s^2)}{R_f^2 + K_{abs}(2 R_f R_s + R_s^2)} \right) \left(\frac{1}{D_f} + \frac{1}{D_s} \right)^{-0.5} \\ \times \left(\frac{\pi R_f}{D_f} + \frac{\pi R_s}{D_s} \right)^{-0.5} \left(\frac{(R_f + \pi R_s)^2}{R_f^{1.5} R_s^2} \right) \quad [26a]$$

$$k_{sf} = \left(\frac{R_f^2}{R_f^2 + K_{abs}(2 R_f R_s + R_s^2)} \right) \left(\frac{1}{D_f} + \frac{1}{D_s} \right)^{-0.5} \\ \times \left(\frac{\pi R_f}{D_f} + \frac{\pi R_s}{D_s} \right)^{-0.5} \left(\frac{(R_f + \pi R_s)^2}{R_f^{1.5} R_s^2} \right) \quad [26b]$$

Figure 15 demonstrates the excellent agreement obtained between the rate constants determined by the stochastic simulation and those predicted by Equation [26a,b]. Because the slopes of these graphs appear to be nearly unity and the intercepts appear to be nearly zero, this suggests that all important parameters have been considered. In addition, the ratio of the rate constants k_{fs}/k_{sf} evaluated by using Equation [26a,b] is equivalent to $K_{abs} (V_s/V_f)$, as expected from Equation [24]. Thus, we may conclude that the relationships for the rate constants in a homogeneous fluid and surface phase under diffusion-limited conditions have been fully resolved. The individual rate constants k_{fs} and k_{sf} can be predicted by using Equation [26a,b] with relative errors of 4.36% and 6.59%, respectively, in the range from 10^{-3} to 10^3 s^{-1} (73).

The overall kinetic behavior of the system can be described in terms of the characteristic time τ . This represents the time required for the number of molecules in the fluid phase N_f/N to reach $1 - (1/e)$ of the number at equilibrium $\bar{N}_f/N$. For the reversible pseudo-first-order system in Equation [20], the characteristic time is given by

$$\tau = \frac{1}{k_{fs} + k_{sf}} \quad [27]$$

which can be readily evaluated by substitution of k_{fs} and k_{sf} from Equation [26a,b].

Next, it is desirable to evaluate the sensitivity of the rate constants k_{fs} and k_{sf} as well as the characteristic time τ to changes in the various parameters. This can be easily accomplished by calculating the partial derivatives of Equations [26a,b] and [27]; e.g. $\delta k_{fs}/\delta K_{abs}$, $\delta k_{sf}/\delta K_{abs}$, $\delta \tau/\delta K_{abs}$. It is noteworthy that the derivatives of the rate constants k_{fs} and k_{sf} with respect to the equilibrium constant K_{abs} are opposite in sign. These contributions balance such that the characteristic time τ is independent of K_{abs} . The derivatives of both rate constants with respect to the diffusion coefficients D_f and D_s are positive, leading to a strong negative dependence of the characteristic time τ . Finally, the derivatives of both rate constants with respect to the radii R_f and R_s are negative, leading to a strong positive dependence of the characteristic time τ . These conclusions are in accord with the general trends observed from the stochastic simulations (73). By evaluation of the sensitivity of the rate constants in this manner, we can identify which of the various parameters is the most significant limitation to the transport rate. Under many conditions representative of gas, supercritical fluid, and liquid chromatography, the limiting factor appears to be the diffusion coefficient in the surface phase. In order to provide the most effective transport, the diffusion coefficient in the surface phase must be maintained within two orders of magnitude of that in the fluid phase (52).

FIGURE 15: Relationship between the individual rate constants k_{fs} (A) and k_{sf} (B) determined by stochastic simulation and predicted by Equation [26a,b]. (—) Linear regression analysis with slopes of 1.01 and 1.11, intercepts of -0.02 and -0.60 , and linear correlation coefficients (r^2) of 0.989 and 0.999, respectively.

Finally, this simulation approach can be extended to consider the situation where there is some resistance to mass transport at the interface and all collisions are not sufficiently energetic to overcome this barrier. To represent this situation, the constant a in the probability expressions of Equation [18a,b] was varied from 1.0 to 0.001. The effect of the constant a on the rate constants k_{fs} and k_{sf} is illustrated in Figure 16. The rate constants appear to increase linearly with the constant a up to approximately 0.5, whereafter the system becomes diffusion limited for these simulation conditions. From the rate constants, we can estimate the barrier to interfacial transport relative to the diffusion-limited case ($a = 1.0$). These barriers correspond to $1.70 k_B T_0$ for $a = 0.1$, $3.92 k_B T_0$ for $a = 0.01$, and $6.20 k_B T_0$ for $a = 0.001$. From these calculations, it is evident that relatively small barriers can have a significant effect upon the kinetic behavior of the system. Consequently, we must seek to minimize sources of interfacial resistance to mass transport in order to develop chromatographic systems with optimal kinetic performance. This may involve minimizing surface tension effects, minimizing configurational or orientational effects, and choosing fluid phase solvents and modifiers that can be easily and rapidly disassociated from solute molecules at the interface (52).

FIGURE 16: Effect of interfacial resistance to mass transport on rate constants k_{fs} and k_{sf} . Simulation conditions: $N = 10,000$; $t = 1.0 \times 10^{-5}$ s; $K_{abs} = 1.0$; $D_f = 1.0 \times 10^{-5}$ cm² s⁻¹; $D_s = 1.0 \times 10^{-5}$ cm² s⁻¹; $R_f = 2.00 \times 10^{-3}$ cm; $R_s = 8.28 \times 10^{-4}$ cm; $v_0 = 0.0$ cm s⁻¹.

From these types of simulations, a greatly improved understanding is derived of the kinetic processes involved in the absorption mechanism. The diffusion-limited rate constants can now be predicted via Equation [26a,b] for any simple system consisting of a homogeneous fluid phase in contact with a homogeneous surface phase. These rate constants provide a direct indication of the relative magnitude and importance of the fluid- and surface-phase mass transfer as well as the interfacial contributions to the solute zone profile. This understanding is essential if the retention and dispersion processes inherent in complex chromatography, electrophoresis, and electrochromatography systems are to be understood and controlled. The rate constants are also necessary to evaluate the effect of a discrete spatial or temporal transition on the solute zone profile. These effects are discussed in the following sections.

Characterization of Solute Zone Profiles in Chromatography and Electrochromatography. In the presence of convective flow, the characteristic time τ will influence the appearance of the solute zone profiles. If τ is sufficiently small, the system will be nearly at equilibrium and the zone profile will be a symmetric Gaussian distribution. Under these conditions, the profile will be well described by the classical equations of mass balance using the equilibrium-dispersive model (32,33,69). We have shown excellent correspondence between these classical theoretical models and the stochastic simulation under equilibrium conditions (50).

As τ increases, however, the system may depart from equilibrium conditions and the zone profile may become highly asymmetric. As a measure of the degree of departure from equilibrium for convective systems, we may define a unitless kinetic parameter P as

$$P = \frac{\tau}{T} = \frac{\tau v_0}{\bar{Z}} \quad [28]$$

where T is time, $\bar{Z}$ is the mean distance, and v_0 is the mean linear velocity. This parameter directly reflects the sources of kinetic stress that are placed on the system and will approach a limiting value of zero for a system that is at equilibrium. It is apparent from this definition that kinetic stress arises from the characteristic time τ as well as the linear velocity and the distance travelled. The stochastic simulation is uniquely well suited to examine and characterize systems that are far from equilibrium, where the classical theoretical models are not appropriate.

In order to examine the influence of each source of kinetic stress, a standard system has been selected for simulation. For a solute with an equilibrium constant $K_{abs} = 1.0$, diffusion coefficients $D_f = 1.0 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $D_s = 1.0 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, and radii $R_f = 2.00 \times 10^{-3} \text{ cm}$ and $R_s = 8.28 \times 10^{-4} \text{ cm}$, the characteristic time τ shown graphically in Figure 14 is 1.408 s. The evolution of the solute zone profile has been examined as a function of the distance travelled for laminar and electroosmotic flow at a fixed linear velocity of 0.1 cm s^{-1} . The degree of asymmetry is high at a distance of 0.1 cm ($P = \tau = 1.408$) and gradually decreases until it is only slightly apparent at a distance of 5.0 cm ($P = \tau/50 = 0.028$). The first statistical moment ($M1$, Equation [9]), which represents the mean retention time, increases in the theoretically expected linear manner with distance. The second and third statistical moments ($M2$ and $M3$, Equation [10]), which represent the variance and asymmetry, also increase linearly with distance. The skew of the zone profile, represented by the Gram-Charlier series as $M3/(M2)^{3/2}$, therefore decreases with the square root of the distance. In other words, the solute zone profile becomes broader but more symmetric as it traverses the chromatography or

electrochromatography system. It is noteworthy that the profiles with laminar flow are not visibly broader or less symmetric than those with electroosmotic flow (73).

Next, the effect of the linear velocity has been examined at a fixed distance of 5.0 cm and a fixed characteristic time τ for the standard system described above. The solute zone profile becomes increasingly asymmetric as the linear velocity is increased from 0.1 cm s⁻¹ ($P = \tau/50 = 0.028$) to 1.0 cm s⁻¹ ($P = \tau/5 = 0.282$) for both laminar and electroosmotic flow. The first statistical moment varies in the theoretically expected inverse manner with velocity. The second and third moments also vary inversely with velocity. Consequently, the skew $M3/(M2)^{3/2}$ increases with the square root of the velocity. In other words, the solute zone becomes less broad but more skewed as the linear velocity increases.

Finally, the effect of the characteristic time τ has been examined at a fixed linear velocity of 0.1 cm s⁻¹ and a fixed distance of 5.0 cm. Although any of the parameters in Equation [26a,b] may be used, we have chosen to vary the parameter that has the greatest influence upon τ . By evaluation of the partial derivatives under the conditions of the standard system, $\delta\tau/\delta K_{abs} = 0$, $\delta\tau/\delta D_f = -2.5 \times 10^3$, $\delta\tau/\delta D_s = -1.5 \times 10^7$, $\delta\tau/\delta R_f = 4.8 \times 10^2$, and $\delta\tau/\delta R_s = 6.0 \times 10^3$. From this analysis, it is apparent that the diffusion coefficient in the surface phase has the most significant effect. The solute zone profile is symmetric for diffusion coefficients of 1.0×10^{-5} cm² s⁻¹ ($P = 0.0007$) and 1.0×10^{-6} cm² s⁻¹ ($P = 0.003$), becomes slightly asymmetric for 1.0×10^{-7} cm² s⁻¹ ($P = 0.028$), and highly asymmetric for 1.0×10^{-8} cm² s⁻¹ ($P = 0.261$). The first statistical moment is independent of the characteristic time τ , as theoretically expected. The second moment increases linearly with τ and the third moment increases with the square of τ . Consequently, the skew $M3/(M2)^{3/2}$ increases with the square root of τ . Thus, the solute zone becomes more broad and more skewed as the characteristic time τ increases, and has the relationships shown in Equation [26a,b] to the parameters of the system.

Through these stochastic simulation studies, we have demonstrated for the first time how the properties of the solute zone profile such as retention time, variance, and asymmetry develop under nonequilibrium conditions. We have also clarified how the zone profile is influenced by the rate constants, linear velocity, and distance travelled and have identified the factors that are most severely limiting the rate of mass transport. To date, we have examined a broad range of chromatographic conditions including those representative of gas, dense gas, supercritical fluid, enhanced fluidity liquid, and liquid chromatography (52) as well as electrochromatography (73). This understanding is necessary in order to significantly increase the speed of chromatographic separations without sacrificing efficiency and resolution.

Characterization of Solute Zone Profiles During Spatial and Temporal Transitions. As discussed in the previous section, nonequilibrium conditions can exist continuously as the solute zone is transported along the chromatographic column. However, a further departure from equilibrium occurs whenever there is an abrupt change in the local conditions of the solute zone. Specifically, such changes occur upon injection to and elution from the column or upon a stepwise or linear gradient in velocity, temperature, or solvent composition. During these processes, the solute zone encounters a spatial and/or temporal transition in which its retention is abruptly and permanently altered. Depending upon the specific conditions, the effect of these transitions may be beneficial, detrimental, or inconsequential to the separation. For example, the resolution and detection sensitivity will be improved if the resultant solute zone is compressed, and will be degraded if the solute zone is expanded. The stochastic simulation permits a more rigorous and detailed examination of these injection and elution effects than classical theoretical models (44,45).

In order to study the injection and elution processes, it is necessary to modify the simulation to incorporate spatial transitions (i.e., discontinuous or continuous functions of a simulation variable, such as distribution coefficient, that arise at a specified distance). In this simulation, time has been chosen as the independent and discrete variable, whereas distance is the dependent and continuous variable. Therefore at each time increment, the position of each molecule must be determined before invoking a distance-dependent function for the distribution coefficient or other variable. This approach has been successfully employed for some preliminary simulations of the injection and elution processes (50).

Upon injection to the system, the solute zone encounters an abrupt spatial transition from the nonretained state ($K_{abs,i} = 0.0$) to the retained state ($K_{abs,f} = 1.0, 2.0, \text{ and } 10.0$). The solute zone profiles, together with their corresponding variances, are evaluated at five simulation times during this transition (Figure 17). The initial profile is evaluated in the nonretentive region and, hence, is identical for all solute zones. The second through fourth profiles are assessed during the transition, whereas the final profile is evaluated when the solute zone is completely within the retentive region. Because the average linear velocity of molecules is reduced after the transition, the resultant solute zone is compressed by the nonequilibrium process. The extent of compression clearly increases with the equilibrium constant, as indicated by the decrease in variance and increase in amplitude between the initial and final zone profiles. The results of these simulations are summarized and compared with classical models in Table VI. It is clear that the simple steady-state model developed previously in our laboratory (44) exaggerates the compression effect of injection nonequilibrium and significantly underestimates the final variance. When dispersion processes from the chromatographic column are included, the Taylor-Aris equation (58) for a nonretained solute underestimates the variance whereas the extended Golay equation (69) for a retained solute overestimates the variance (50).

The complementary elution effect is shown in Figure 18, where the solute zone encounters an abrupt spatial transition from the retained state ($K_{abs,i} = 1.0, 2.0, \text{ and } 10.0$) to the nonretained state ($K_{abs,f} = 0.0$). The initial profile is evaluated in the retentive region, the second through fourth profiles during the transition, and the final profile in the nonretentive region. Because the average linear velocity of molecules increases after the transition, the resultant solute zone is expanded by the nonequilibrium process. The extent of expansion increases dramatically with the distribution coefficient, as revealed by the change in variance and amplitude between the initial and final profiles. The results of these simulations are summarized and compared with the classical models in Table VI. It is evident that the simple steady-state model (45) underestimates the expansion effect of elution nonequilibrium, and neither the Taylor-Aris (58) nor the Golay equation (69) adequately addresses the contributions from system processes (50).

FIGURE 17: Solute zone profiles, together with their corresponding variances (●), during the injection process. Column inlet is at 0.0 cm, as shown in schematic diagram at top. Simulation conditions: $N = 500$; $t = 5.0 \times 10^{-4}$ s; $R_f = 2.00 \times 10^{-3}$ cm; $R_s = 4.49 \times 10^{-4}$ cm; $D_f = 1.0 \times 10^{-5}$ cm² s⁻¹; $D_s = 1.0 \times 10^{-6}$ cm² s⁻¹; $v_0 = 0.1$ cm s⁻¹; $K_{abs,i} \rightarrow K_{abs,f} = 0.0 \rightarrow 1.0$ (A), $0.0 \rightarrow 2.0$ (B), $0.0 \rightarrow 10.0$ (C).

FIGURE 18: Solute zone profiles, together with their corresponding variances (●), during the elution process. Column outlet is at 0.0 cm, as shown in schematic diagram at top. Simulation conditions: $N = 500$; $t = 5.0 \times 10^{-4}$ s; $R_f = 2.00 \times 10^{-3}$ cm; $R_s = 4.49 \times 10^{-4}$ cm; $D_f = 1.0 \times 10^{-5}$ cm² s⁻¹; $D_s = 1.0 \times 10^{-6}$ cm² s⁻¹; $v_0 = 0.1$ cm s⁻¹; $K_{abs,i} \rightarrow K_{abs,f} = 1.0 \rightarrow 0.0$ (A), $2.0 \rightarrow 0.0$ (B), $10.0 \rightarrow 0.0$ (C).

TABLE VI: Initial and final zone variance arising from nonequilibrium processes during injection (Figure 17), elution (Figure 18), and stepwise gradients (50).

$K_{\text{abs},i}$	$K_{\text{abs},f}$	V_f/V_s	Simulation Model				Classical Models		
			$\bar{Z}_i$ (cm)	$\bar{Z}_f$ (cm)	σ_i^2 (cm ²)	σ_f^2 (cm ²)	σ_f^2 (cm ²)*	σ_f^2 (cm ²)†	σ_f^2 (cm ²)‡
0.0	1.0	2.0	-0.30	0.24	9.93×10^{-3}	6.64×10^{-3}	4.41×10^{-3}	5.42×10^{-3}	7.12×10^{-3}
0.0	2.0	2.0	-0.30	0.18	9.76×10^{-3}	4.66×10^{-3}	2.44×10^{-3}	3.34×10^{-3}	5.16×10^{-3}
0.0	10.0	2.0	-0.30	0.07	1.03×10^{-2}	1.31×10^{-3}	2.86×10^{-4}	9.77×10^{-4}	2.08×10^{-3}
1.0	0.0	2.0	-0.30	0.47	9.84×10^{-3}	2.88×10^{-2}	2.21×10^{-2}	2.36×10^{-2}	2.57×10^{-2}
2.0	0.0	2.0	-0.30	0.61	9.61×10^{-3}	5.58×10^{-2}	3.84×10^{-2}	4.01×10^{-2}	4.32×10^{-2}
10.0	0.0	2.0	-0.30	1.83	9.69×10^{-3}	5.04×10^{-1}	3.49×10^{-1}	3.53×10^{-1}	3.58×10^{-1}
10.0	1.0	2.0	0.00	0.32	9.72×10^{-3}	3.91×10^{-3}	1.56×10^{-3}	2.14×10^{-3}	—
10.0	2.0	2.0	0.00	0.25	9.53×10^{-3}	6.04×10^{-3}	3.43×10^{-3}	3.90×10^{-3}	—
2.0	1.0	2.0	0.00	0.73	1.01×10^{-2}	9.95×10^{-3}	4.49×10^{-3}	5.85×10^{-3}	—
1.0	10.0	2.0	0.00	0.78	9.41×10^{-3}	9.18×10^{-2}	5.88×10^{-2}	6.03×10^{-2}	—
2.0	10.0	2.0	0.00	0.43	1.01×10^{-2}	3.99×10^{-2}	2.81×10^{-2}	2.88×10^{-2}	—
1.0	2.0	2.0	0.00	1.08	9.26×10^{-3}	3.78×10^{-2}	2.08×10^{-2}	2.29×10^{-2}	—

* Calculated by using steady-state model of nonequilibrium dispersion (44,45).

† Calculated by using steady-state model of nonequilibrium dispersion (44,45) and the Taylor-Aris equation (58) for column dispersion of a nonretained solute.

‡ Calculated by using steady-state model of nonequilibrium dispersion (44,45) and the extended Golay equation (69) for column dispersion of a retained solute.

It is also desirable to examine nonequilibrium during more complex transitions, such as a stepwise or linear gradient in velocity, temperature, or solvent composition. In order to characterize these gradients by means of the stochastic simulation, it is necessary to incorporate two additional types of transitions: temporal transitions (i.e., discontinuous or continuous functions of a simulation variable, such as distribution coefficient, that arise at a specified time) and nonstationary spatiotemporal transitions (i.e., discontinuous or continuous functions of the variable that are not fixed in either distance or time, but migrate at a linear velocity different from that of the solute zone). Because time has been chosen as the independent variable for this simulation, temporal transitions may be achieved directly by invoking a time-dependent function for the variable. In contrast, nonstationary transitions present a more complicated and time-consuming computational problem because, at each time increment, the position of each molecule as well as that of the transition itself must be determined before invoking a distance-dependent function for the variable. Some preliminary results are described below in which discontinuous functions of the distribution coefficient are considered as nonstationary transitions (50). These simulations are representative of practical conditions such as injection of a solute in a solvent other than the fluid phase, as well as stepwise gradients in composition of the fluid phase.

The first simulation is representative of a stepwise increase in solvent strength. During this transition, the distribution coefficient of the solute decreases ($K_{\text{abs},i} \rightarrow K_{\text{abs},f} = 10.0 \rightarrow 1.0$, $10.0 \rightarrow 2.0$, and $2.0 \rightarrow 1.0$), whereas that of the solvent remains constant (0.0). The solute zone profiles, together with their corresponding variances, are evaluated periodically throughout the transition. The average linear velocity of molecules increases after the transition to higher solvent strength, hence the resultant solute zone is compressed by the nonequilibrium process. Several important conclusions are evident from these simulations: First, the greater the ratio of the equilibrium constants in the initial and final solvents, the greater the extent of solute zone compression. If the equilibrium constant changes too significantly, however, the solute zone profile may exhibit severe distortion and asymmetry during the transition. Finally, the greater the magnitude of the initial and final equilibrium constants, the more rapidly the solute zone is surpassed by the solvent zone. The results of these simulations are summarized and compared with the classical models in Table VI (50).

The complementary case is representative of a stepwise decrease in solvent strength. During this transition, the distribution coefficient of the solute increases ($K_{\text{abs},i} \rightarrow K_{\text{abs},f} = 1.0 \rightarrow 10.0$, $2.0 \rightarrow 10.0$, and $1.0 \rightarrow 2.0$), whereas that of the solvent remains constant (0.0). Because the average linear velocity of molecules decreases after the transition, the resultant solute zone is expanded by the nonequilibrium process. The smaller the ratio of equilibrium constants in the initial and final solvents, the greater the extent of solute zone expansion. Again, if the equilibrium constant changes too significantly, the solute zone profile may exhibit severe distortion and asymmetry during the transition. The results of these simulations are summarized and compared with the classical models in Table VI (50).

These simulations provide additional insight into the injection process when a solvent other than the fluid phase is employed. If the solute is injected in a solvent that is weaker than the fluid phase ($K_{\text{abs},i} > K_{\text{abs},f}$), the solute zone is compressed to a greater extent during the spatial transition from the nonretentive to retentive regions at the column inlet. Then, as the injection solvent surpasses the solute zone and is replaced by the fluid phase, the zone is further compressed. The opposite effect is observed when the solute is injected in a solvent that is stronger than the fluid phase ($K_{\text{abs},i} < K_{\text{abs},f}$). During the spatial transition, the solute zone is compressed to a lesser extent; then, as the injection solvent is replaced by the fluid phase, the solute zone is further

expanded. Thus, these two independent transition processes act in a cooperative manner to decrease or increase the solute zone variance during the injection process.

Correlation of Simulation and Experimental Studies

The correlation between the stochastic simulation and the experimental measurements has been a fundamental goal of this research program. To date, correlations have been successfully attempted in four general areas: 1) agreement of zone profiles (retention time, variance, etc.) for electrophoretic separations with nucleotide monophosphates as well-characterized model solutes, 2) agreement of zone profiles for liquid chromatographic separations with fatty acids as well-characterized model solutes, 3) agreement of kinetic rate constants under diffusion-limited conditions in liquid chromatography, and 4) agreement of zone profiles during the processes of injection and elution in liquid chromatography.

Because the stochastic simulation monitors the migration of individual molecules, it provides the opportunity to perform hypothetical experiments and to make observations that may not be practical in an experimental chromatographic or electrophoretic system. Thus, these simulation studies enhance and expand upon the knowledge gained in our previous experimental studies.

III. NOVEL OPTIMIZATION METHODS FOR CHROMATOGRAPHY AND ELECTROPHORESIS

Computer-Assisted Optimization of Liquid Chromatography by Parametric Modulation

A variety of methods has been developed for univariate optimization of chromatographic separations. Among these methods, the regression approach has proven to be the most widely used and commercially successful (74). In this approach, a few preliminary experiments are performed and the resulting chromatographic data are analyzed by numerical regression to a predefined mathematical equation. While this approach can lead rapidly to the identification of the most promising experimental conditions, it requires that the mathematical model used to express the relationship between the parameter and the chromatographic properties such as capacity factor, plate number, etc. be well defined. Unfortunately, deviations from ideal behavior frequently lead to nonlinear relationships for the parameters of interest in liquid chromatography, including mobile phase composition (75,76), stationary phase composition (77), temperature (23,78,79), and flow rate. Such deviations cannot be predicted *a priori* and, hence, serve to limit the accuracy with which the optimum conditions can be identified. This problem, which is detrimental in univariate optimization, becomes prohibitive when two or more parameters are to be optimized simultaneously.

In order to overcome this problem, a new approach has been developed for univariate and multivariate optimization called parametric modulation (74,80-83). The fundamental strategy of this approach is that chromatographic properties may be accurately predicted if the solute is constrained to undergo interactions independently within each mobile phase, stationary phase, and temperature environment. This strategy is implemented by maintaining each of these variables in discrete and separate zones along the chromatographic column, as illustrated schematically in Figure 19. The overall retention time (t_r) of the solute is calculated by summation of its retention within each individual environment

$$t_i = \sum_{p=1}^q \sum_{j=0}^n \frac{\pi r_p^2 \varepsilon_p x_j}{F} \left(\frac{1+k_{ijp}}{k_{ijp}} \right) \quad [29]$$

where k_{ijp} is the capacity factor of solute i in solvent j on column p , which has radius r_p , length l_p , particle size d_p , total porosity ε_p , and reduced plate height h_p (80). The limit of the summation index (n), which represents the total number of solvent zones required to elute the solute from column p , is determined by evaluating the expression

$$\sum_{j=0}^n \frac{x_j}{k_{ijp}} = l_p \quad [30]$$

In a similar manner, the variance (σ_i^2) of the solute zone in temporal units is calculated by independent summation (80)

$$(\sigma_i)^2 = \sum_{p=1}^q \sum_{j=0}^n (\sigma_{ijp})^2 = \sum_{p=1}^q \frac{h_p d_p}{l_p} \left(\sum_{j=0}^n \frac{\pi r_p^2 \varepsilon_p x_j}{F} \left(\frac{1+k_{ijp}}{k_{ijp}} \right) \right)^2 \quad [31]$$

The resolution between adjacent solute zones ($R_{i,i+1}$) is then calculated by substitution of Equations [29] and [31] in the following expression

$$R_{i,i+1} = \frac{(t_{i+1} - t_i)}{2[(\sigma_{i+1})_t + (\sigma_i)_t]} \quad [32]$$

FIGURE 19: Schematic illustration of the parametric modulation concept for n solvent zones in q serially coupled columns (74).

Finally, a criterion such as the chromatographic resolution statistic (CRS) is used to evaluate the overall quality of the separation (84)

$$\text{CRS} = \left(\sum_{i=1}^{m-1} \left(\frac{R_{i,i+1} - R_{\text{opt}}}{R_{i,i+1} - R_{\text{min}}} \right)^2 \frac{1}{R_{i,i+1}} + \sum_{i=1}^{m-1} \frac{(R_{i,i+1})^2}{(m-1) R_{\text{avg}}^2} \right) \frac{t_f}{m} \quad [33]$$

where m is the total number of solutes, t_f is the elution time of the final solute, R_{opt} is the optimum or desired resolution, R_{min} is the minimum acceptable resolution, and R_{avg} is the average resolution for all solute pairs. The optimum separation is achieved by the experimental conditions that yield the minimum value of the CRS function. The optimum conditions for parametric modulation may be determined in two ways: 1) by varying the sequence and length of the mobile phase, stationary phase, and temperature zones in a systematic manner to construct a complete response surface, from which the optimum is identified by visual inspection, or 2) by using a sequential method such as the simplex search routine (85).

The parametric modulation approach has several advantages over traditional methods of chromatographic optimization. First, the predicted retention is inherently more accurate because it is calculated by summation of the known and measured behavior in discrete environments rather than by numerical regression in unknown or poorly characterized mixed environments. Second, the number of preliminary experiments necessary to fully characterize the chromatographic system is dramatically reduced in comparison with traditional methods. For a system composed of n mobile phases, q stationary phases, and t temperature zones, only $n \cdot q \cdot t$ retention measurements are necessary for each solute. From these preliminary measurements, the solute retention time and variance for any combination, sequence, and length of the zones may be calculated. Other physical parameters of interest given in Equations [29] and [31], such as particle size, column dimensions, and flow rate, may be optimized simultaneously with no addition experimental measurements. Thus, a complete multivariate response surface may be systematically generated and the optimum may be identified by visual inspection or by computer-assisted search methods.

The potential limitations of this approach arise from the assumptions inherent in development of the theoretical equations. First, it is assumed that solute retention is controlled independently within each individual environment. This requires that the zones for each mobile phase, stationary phase, and temperature exhibit minimal mixing at the boundaries. A statistical treatment may be used to estimate the minimum zone length required to maintain an acceptable level of zone purity (80,83,86). This approach also requires that the solute be able to achieve steady-state conditions rapidly after each change in environment. This may limit the selection of mobile and stationary phases to those exhibiting rapid kinetics and linear isotherms for the solutes of interest. The validity of these assumptions has been examined for both univariate (81,85-87) and multivariate optimization (80,83).

Experimental verification of this technique for univariate optimization of the mobile phase has been demonstrated in several studies (81,82,85,87). Using seven model solutes with varying chemical interactions, the accuracy of predicting retention by using solvent modulation was compared with that using traditional mixed mobile phases. For binary and ternary solvent systems with varying strength and selectivity, the relative error in the predicted capacity factor was typically less than $\pm 10\%$ for solvent modulation but as great as $\pm 60\%$ for mixed solvents due to deviation from ideal behavior. The accuracy and precision of the solvent modulation technique were also examined as a function of the number and length of the solvent zones (86,87). In a later study, the

solvent modulation technique was applied to optimize the separation of fourteen saturated and unsaturated fatty acids on an octadecylsilica column (85). The mobile phases of 90 and 100% methanol as well as 85 and 100% acetonitrile were selected to optimize both solvent strength and selectivity (4 total experiments). By applying the method outlined in Equations [29] to [33] above, all possible permutations of these solvents were examined. The optimum solvent sequence was determined to be 100% methanol and 90% methanol in zones of 116 and 1810 cm length, respectively. Under these conditions, the least-resolved solute pairs were myristic and arachidonic acids as well as arachidonic and palmitoleic acids, both of which had limiting resolution of 1.24. The experimental chromatogram agreed well with theoretical predictions, having an average relative error in capacity factor of $\pm 1.5\%$. The separation of fatty acids achieved by using the solvent modulation technique was similar to that obtained by traditional optimization methods (88), but achieved with significantly fewer preliminary experiments.

Experimental verification of the parametric modulation technique for multivariate optimization has been demonstrated in two independent studies. In the first study, isomeric four- and five-ring PAHs were separated on octadecyl- and β -cyclodextrin-modified silica stationary phases (80). Although both stationary phases interact with the solutes by van der Waals forces, octadecylsilica is dominated by enthalpic interactions whereas β -cyclodextrinsilica has a considerable entropic contribution. Thus, the combination of these phases should provide good selectivity for the isomeric PAHs. The capacity factors for ten PAH standards were measured on these stationary phases using mobile phases of 80% and 90% aqueous methanol as well as 50% and 70% aqueous acetonitrile (8 total experiments). Although there are 64 possible permutations of these mobile and stationary phases, the computer-assisted search routines developed in this work provided a rapid and effective means to identify the most promising permutation. By systematic examination of all variables, this optimization routine was also able to construct a response surface which allowed facile identification of the most promising sequence and length of the mobile and stationary phase zones in the selected permutation. The predicted optimum conditions consisted of a 75 cm octadecylsilica column (no β -cyclodextrinsilica) using a solvent modulation sequence of 70% aqueous acetonitrile and 80% aqueous methanol in zones of 203 and 634 cm length, respectively. These conditions provided an excellent separation of the isomeric PAH standards, with baseline resolution of many solute pairs and a limiting resolution of 0.88. The experimental chromatogram agreed well with theoretical predictions having average relative errors in retention time and variance of $\pm 3.5\%$ and $\pm 46\%$, respectively (80).

In the second study, temperature was investigated as the means to vary selectivity on an octadecylsilica stationary phase with high bonding density (83). At temperatures above the melting point of the stationary phase, enthalpic interactions with the solute are predominant whereas, below the melting point, entropic contributions become significant (12,13). The capacity factors for nine PAH standards were measured at 23, 35, 40, and 45 °C using mobile phases of methanol and acetonitrile (8 total experiments). The computer-assisted optimization routine was used to select from among the 64 possible permutations, and to identify the most promising sequence and length of the temperature and mobile phase zones. The optimum conditions consisted of a 90 cm octadecylsilica column, with 85 cm maintained at 23 °C and 5 cm maintained at 40 °C, using a methanol mobile phase. A good separation of the isomeric PAH was achieved under these conditions, with limiting resolution of 1.31. The experimental chromatogram agreed well with theoretical predictions, as shown in Figure 20, having average relative errors in retention time and variance of $\pm 0.9\%$ and $\pm 16\%$, respectively (83).

FIGURE 20: Comparison of predicted (A) and experimental (B) chromatograms obtained by parametric modulation of mobile phase and temperature (83). Column: 200 μm I.D. x 90 cm fused-silica capillary, packed with 5 μm octadecylsilica. Mobile phase: 100% methanol, 0.95 $\mu\text{L}/\text{min}$. Temperature: 85 cm of column maintained at 23 $^{\circ}\text{C}$ and 5 cm maintained at 40 $^{\circ}\text{C}$. Detector: laser-induced fluorescence with excitation at 325 nm and emission at 420 nm. Solutes: (1) benzo[c]phenanthrene, (2) pyrene, (3) phenanthrophenanthrene, (4) benz[a]anthracene, (5) tetrabenzonaphthacene, (6) chrysene, (7) benzo[e]pyrene, (8) perylene, (9) benzo[a]pyrene.

Based on these results, parametric modulation appears to be a powerful and versatile strategy for the univariate and multivariate optimization of chromatographic separations. This optimization can be achieved with greater accuracy and with fewer preliminary experiments than traditional optimization methods. Although this discussion has focussed on liquid chromatography, the parametric modulation strategy should also be applicable in gas and supercritical fluid chromatography as well as capillary electrophoresis.

Computer-Assisted Optimization of Electrophoresis

A computer program has been developed to model separations by capillary electrophoresis that is based on simple but reliable models for zone migration and dispersion. The migration time of each zone is determined by the net rate of zone migration, which is a vectorial summation of the electrophoretic (v_{ep}) and electroosmotic (v_{osm}) velocities:

$$t_i = \frac{L_{det}}{v_{ep} + v_{osm}} = \frac{L_{det} L_{tot}}{(\mu_{ep} + \mu_{osm}) V} \quad [34]$$

where L_{tot} and L_{det} are the total capillary length and the length between the injector and detector, respectively.

In the electroosmotic migration subroutine, the response of the fused-silica capillary surface to changes in buffer composition is modelled in analogy to an ion-selective electrode (89). A mathematical function based on the Nernst equation is used to relate the zeta potential to the pH and sodium concentration of the buffer solution. The electroosmotic velocity is then calculated from the zeta potential by means of the Helmholtz-Smoluchowski equation (62). The electrophoretic migration subroutine is based on classical equilibrium calculations, which require knowledge of the solute dissociation constant(s) and mobility(s) with activity corrections (90).

The temporal variance of each zone is calculated by summation of the individual contributions that result from longitudinal diffusion and a finite injection and detection volume (90). The resolution between adjacent solute zones is estimated by using Equation [32] and the overall quality of the separation is assessed by means of the CRS function in Equation [33].

As input to the computer program, variables related to the buffer composition (pH, concentration, and ionic strength), capillary dimensions (diameter and length), and instrumental parameters (applied voltage or current, injection and detection volumes) are considered. By methodically varying the input parameters and evaluating the overall quality of the separation, this computer program can be used to predict the experimental conditions required for optimal separation of the solutes. The computer optimization routine was experimentally validated with a mixture of nucleotide mono- and di-phosphates in phosphate buffer solutions, with average errors in the electroosmotic mobility, the effective electrophoretic mobility, and the zone variance of 2.3%, 2.9%, and 9.4%, respectively (90).

This computer program has been applied to the separation of the antibiotics tetracycline, chlortetracycline, demeclocycline, oxytetracycline, doxycycline, methacycline, and minocycline (91). A complete set of dissociation constants and electrophoretic mobilities was determined and, subsequently, was used to assess the

separation. Under the predicted optimum condition (pH 7.5, 4.3 mM buffer concentration, 18.2 mM ionic strength, and constant current of 20 μ A), the separation was performed satisfactorily and all tetracyclines were readily identified. Moreover, the common impurities in tetracycline resulting from dehydration and epimerization reactions were discriminated under the same conditions. The determination of tetracycline, doxycycline, and minocycline was performed in commercially available pharmaceutical formulations, with a detection limit of 1×10^{-5} M and a linear range of two orders of magnitude using UV-absorbance detection at 260 nm (91).

Photoactivation as a Means of Controlling Separations in Electrophoresis

Many parameters may be used to manipulate the mobility and enhance electrophoretic separations, including the buffer type, pH, and ionic strength as well as the type and concentration of an organic solvent, complexing agent, or other modifier. In addition, a secondary phase with sieving properties such as a gel or entangled polymer matrix may be added. While these traditional parameters are effective in altering the solute mobility, the strength and selectivity cannot be easily or rapidly changed during the electrophoretic separation. This limitation is especially important for high-speed applications and for two-dimensional electrophoresis–electrophoresis and chromatography–electrophoresis applications.

In this work, photoactivation is explored as an alternative approach to mediate electrophoretic separations. In this approach, the solute molecules or ions are exposed to radiant energy (photons) rather than a chemical potential or other source of energy. The molecules that absorb this radiation will selectively undergo a transition to the excited state. If this transition induces a change in either the mass or the charge of the solute molecule, its electrophoretic mobility will be concomitantly altered. There are two simple mechanisms by which a change in mass and/or charge may occur: photoionization and photodissociation. In the former case, an electron will be ejected from the molecule if the energy of the absorbed photon exceeds the ionization energy. The resulting positively charged ion will have a higher mobility than the unperturbed molecule. In the latter case, the equilibrium constant for an acid–base, complexation, or other reversible reaction may differ significantly between the ground and excited states. As the molecules associate or dissociate in order to reach equilibrium, their charge and mass will be altered, thereby influencing their mobility. In this study, the conceptual and theoretical basis of this technique are developed together with preliminary experimental studies that support its feasibility (92).

The experimental system for these studies is shown schematically in Figure 21. The electrophoretic system is similar to that used for the studies described above, except that the polyimide coating is removed from the fused-silica capillary so that it is optically transparent. Photoactivation is achieved by means of an excimer laser at 248 nm with typical pulse energy of 70 – 250 mJ, pulse width of 2.3×10^{-8} s, and repetition rate of 10 s^{-1} . A series of fused-silica plates are situated to reflect and transmit this light in order to provide uniform irradiance along a 10- to 25-cm length of the capillary tube. Detection is accomplished by using either UV-visible absorbance or fluorescence immediately after the photoactivated region.

In preliminary studies, it was necessary to establish that any apparent changes in the electrophoretic separation are due to selective photoactivation of the solute, rather than other nonselective processes that may arise from laser irradiation. To this end, we demonstrated that photothermal heating from irradiation ($0 - 1.4 \times 10^5$ W cm^{-2}) may be considered negligible in comparison with Joule heating under both constant voltage and

constant current conditions. In addition, we demonstrated that irradiation causes no significant change in the electroosmotic velocity, which implies that the buffer and capillary wall are not significantly affected. Finally, we demonstrated the feasibility of solute photoactivation by using chemical reactions that are irreversible. Although such reactions do not have analytical utility, they conclusively verify the ability to generate ions in good yield by photoactivation and to separate those ions from the parent neutral solutes. The first validation study is representative of the photoionization mechanism: N,N,N',N'-tetramethyl-1,4-phenylenediamine was photoactivated to form the cation and the two species were readily separated by electrophoresis (92). The second validation study is representative of the photodissociation mechanism: phenol has an acid-base equilibrium constant of 10.0 in the ground state and 3.62 in the excited state. After photoactivation, a proton is dissociated and the resulting phenolate ion may be separated from the neutral phenol (92). Other applications of this method are discussed, including the analysis of benzo[a]pyrene and its monohydroxyl and dihydroxyl positional isomers. The acid-base equilibrium constants of these isomers differ substantially between the ground and excited states, thereby facilitating their selective photodissociation.

FIGURE 21: Schematic diagram of the experimental system for capillary electrophoresis with photoactivation. Solute photoactivation is achieved by reflection and transmittance of laser radiation using fused-silica plates. Detection is performed by UV-visible absorbance or fluorescence immediately after the photoactivated region (92).

Based on these results, the enhancement of electrophoretic separation by photoactivation of the solute appears to be a novel and promising approach. Unlike other means to control electrophoretic mobility, this method has the advantage that it is mediated through an externally applied electromagnetic field that can be rapidly modified in both strength and selectivity. The strength of photoactivation may be controlled by the laser irradiance and pulse width, whereas the selectivity may be controlled by the wavelength and polarization. Moreover, stepwise and linear gradients in irradiance and wavelength may be implemented using standard spectroscopic instrumentation. Although this investigation has been limited to the solute as the photoactive species, it is apparent that this general principle can be extended to other species in the separation system. Thus, this approach can potentially be applied to membrane and chromatographic separation systems where a component of the mobile or stationary phase is photoactivated.

IV. NOVEL DETECTION METHODS FOR CHROMATOGRAPHY AND ELECTROPHORESIS

Fluorescence and Fluorescence Quenching Detection

Photophysical Properties of Pyrene as a Polarity Probe in Supercritical and Liquid Solutions. The use of pyrene as a fluorescence probe to classify solvent strength and to characterize surface or interfacial environments has become a well-established and well-accepted technique (93-96). The fluorescence spectrum of monomeric pyrene exhibits five major vibronic bands between 370 and 400 nm, labelled in progressive numerical order for convenience. Band 1 shows significant intensity enhancement with increasing solvent polarity compared with band 3, so that the ratio of emission intensities ($Py = I_1/I_3$) serves as a quantitative empirical measure of polarity. In this study (97), it is employed to explore the effects of temperature and pressure on the solvent strength of carbon dioxide, a common solvent for supercritical-fluid extraction and chromatography. The Py parameter scale was expected to be more representative than Kamlet-Taft and other solvatochromic scales (98-100) because the primary interaction of pyrene arises from van der Waals (dispersion) forces, much like carbon dioxide itself.

The Py value was found to increase with the square of the density in the range from 0.54 to 0.75 g/cm³. This observation is consistent with the expectation that the cohesive energy of induced dipole forces is proportional to $1/r^6$ or to ρ^2 . However, the Py value was also found to decrease with temperature at constant density, which is not consistent with the expectation that van der Waals interactions are independent of temperature. Although carbon dioxide is widely regarded as a nonpolar solvent, the temperature dependence was observed to be comparable to that for polar liquid solvents. The cause of this temperature effect is not fully understood, and has been attributed previously to local clustering of carbon dioxide around the probe molecule (101,102). However, because the temperature dependence of the Py parameter appears to be continuous throughout the supercritical fluid and liquid regions for carbon dioxide, clustering cannot be wholly responsible for this phenomenon.

We believe that the temperature effect may be attributed to the intrinsic photophysical and photochemical properties of pyrene, and have examined this possibility through computer simulation at the molecular level. First, the structure and electronic charge distribution for the ground state (1A_g) and excited states ($^1B_{2u}$ and $^1B_{1u}$) of pyrene were generated from semiempirical molecular orbital (MNDO) calculations. These structures were used in a molecular mechanics and dynamics program to simulate the interaction

of pyrene with selected solvents. The total interaction energy, as well as the contributions from van der Waals and electrostatic interactions, were then determined by force-field calculations (103). Based on these simulations (97), a strong electrostatic attraction was shown to arise between pyrene and carbon dioxide that is similar in magnitude to that with polar solvents such as acetone, acetonitrile, methanol, and tetrahydrofuran. The energy of this electrostatic interaction is directly and quantitatively correlated to the observed temperature dependence of the Py parameter ($\Delta\text{Py}/\Delta T$). This suggests that the temperature effect arises from strong local dipole orientation forces between carbon dioxide and pyrene, which are known to be inversely dependent on temperature (104). As a result of this study, we believe that the changes in the fluorescence spectrum of pyrene arise from specific solute-solvent interactions on the molecular scale, rather than from colligative properties of the bulk solvent. For this reason, we do not recommend the Py parameter scale for carbon dioxide or other solvents with strong local dipole or quadrupole moments.

Fluorescence Quenching of Polycyclic Aromatic Hydrocarbons. Quenching is usually considered to be a detrimental phenomenon in fluorescence spectroscopy. However, the deliberate quenching of fluorescence can be used to analytical advantage if applied in a judicious and carefully controlled manner. This method can provide valuable photophysical and photochemical information about an individual fluorophore that can be used for its classification or identification. In addition, when combined with a separation method, fluorescence quenching can be used to simplify the qualitative and quantitative analysis of complex samples by selective discrimination against interfering components (105).

In preliminary studies, we have systematically investigated the fluorescence quenching of polycyclic aromatic hydrocarbons (PAH) (105,106). Nitromethane was found to quench the emission intensity of alternant PAH, while the emission of nonalternant PAH with five-membered rings was essentially unaltered. A modified Stern-Volmer relationship was derived to distinguish and to compensate mathematically for primary and secondary absorption effects. Utilizing this expression, the Stern-Volmer quenching constants were determined to be 125 and 0.15 M^{-1} for pyrene and fluoranthene, respectively, using nitromethane as the quenching agent in methanol at ambient temperature. Because of the large difference in quenching constants, an analytical method was developed for the class-selective identification of PAH separated by microcolumn liquid chromatography with laser-induced fluorescence detection. This method was applied to the sixteen PAH identified as priority pollutants by the U.S. Environmental Protection Agency (NIST SRM 1647) and a complex coal-derived fluid (NIST SRM 1597). From the ratio of the peak heights in the absence and presence of nitromethane, the alternant and nonalternant PAH could be readily classified. In addition, the nonalternant PAH could be accurately quantitated with fewer interferences (106).

In subsequent studies, we examined other potentially useful quenching agents for alternant and nonalternant PAH. Nitromethane and nitrobenzene (electron acceptors) were compared with 1,2,4-trimethoxybenzene (electron donor) for their class selectivity and efficiency as fluorescence quenchers. From the Stern-Volmer constants, nitromethane was found to be the most selective quencher for alternant PAH, 1,2,4-trimethoxybenzene was more effective but somewhat less selective for nonalternant PAH, and nitrobenzene was an effective but nonselective quencher. The application of these quenchers was demonstrated for the priority pollutant PAH mixture and the coal-derived fluid (107).

Although the fluorescence quenching method appears to show great promise, only a few quenching agents have been characterized in sufficient depth and detail to permit their routine and reliable application to unknown samples. Substantially more research is necessary to identify promising new quenching agents and to evaluate their effectiveness and selectivity. In order to facilitate this process, a novel experimental system shown in Figure 22 has been developed and validated (108). In this system, capillary flow injection is used to automate the preparation and mixing of the fluorophore and quencher solutions. Because of the small diameter of the capillary, fluorescence measurements can be made without corrections for primary and secondary absorbance effects. The fluorescence spectrometer is equipped with a charge-coupled device (CCD) that has a detection limit of 3×10^{-9} M and a linear dynamic range of 10^5 for integration times of 0.01 – 10 s. This spectrometer has a 300 nm spectral range with 1 nm resolution, allowing the Stern–Volmer constants to be calculated at single wavelengths or over integrated wavelength ranges. This system was validated by comparison to traditional methods for the determination of quenching constants for alternant and nonalternant PAH with nitromethane, as shown in Figure 23 (108). In addition, we have recently completed a study in which this system is used to evaluate the quenching constants for PAH with a variety of aliphatic amines (electron donors) in methanol and acetonitrile. These results suggest that triethylamine is a highly selective quencher for nonalternant PAH, more so than 1,2,4-trimethoxybenzene. Moreover, it shows an interesting selectivity for nitrogen-containing PAH that may be useful in profiling complex petroleum-based samples (109).

FIGURE 22: Schematic diagram of the experimental system for flow injection with laser-induced fluorescence detection to determine Stern–Volmer quenching constants (108). I = injection valve, T = mixing tee, F = filter, L = lens, CCD = charge-coupled device, PMT = photomultiplier tube.

FIGURE 23: Typical results of fluorescence quenching studies using the experimental system shown in Figure 22 (108). Fluorophore: 75 μL injections of 10^{-5} M pyrene in methanol, 35 $\mu\text{L}/\text{min}$. Quencher: 1 – 0.05 M nitromethane in increments of 0.01 M per 20 min step, 35 $\mu\text{L}/\text{min}$. Lower trace shows UV absorbance at 254 nm. Middle trace shows fluorescence detected by PMT at 371 nm, 1 nm bandpass. Upper traces show fluorescence spectra detected by CCD detector at 350 – 500 nm, 1 nm bandpass.

Finally, we have begun to use *ab initio* calculations to aid in the identification of promising new quenching agents and to elucidate their mechanisms of fluorescence quenching (110). Ground- and excited-state calculations (HF/6-31G* and CIS/6-31G*) have been performed for four representative alternant and nonalternant PAH: pyrene, benzo(a)pyrene, fluoranthene, and benzo(b)fluoranthene. The ground- and excited-state geometries, vibrational frequencies, excitation energies, etc. have been calculated and show good agreement with experimental data. Upon excitation, all PAH lengthen along their axis of polarization, and the changes in electron density appear to correlate with changes in the excited-state geometry. These calculations have been used to examine characteristic differences between the alternant and nonalternant PAH. The nonalternant PAH tend to possess higher ground-state energies, lower excitation energies, and greater changes in their excited-state electron densities and geometries than the alternant isomers (110). We are presently extending these calculations to examine ground- and excited-state complexes of the representative PAH with nitromethane in various configurations. These calculations will aid in our understanding of fluorophore-quencher complexes and the mechanism of fluorescence quenching.

Photovoltaic Detection

The direct measurement of absorbance is one of the most common detection techniques. However, the sensitivity of this technique decreases rapidly with the optical pathlength, which limits its applicability in miniaturized systems such as capillary liquid chromatography and capillary electrophoresis. In addition, because the absorbance is measured as the difference in two large transmittance signals, an increase in the radiant source power will generally not improve the sensitivity. Other techniques based on the indirect measurement of absorbance, such as photothermal, photoacoustic, photoionization, and photoluminescence techniques, appear to be more suitable for this application. In these techniques, the signal is proportional to the radiant source power and is measured against little or no background. Consequently, high-power coherent laser sources can be readily focused into flow cells of capillary dimensions to achieve higher sensitivity. Among the indirect techniques, photoluminescence has been the most broadly applied and has proven to be the most successful. However, many molecules of interest have small fluorescence and phosphorescence quantum yields and cannot be readily derivatized to incorporate a luminescent label. Hence, it is desirable to develop and characterize other indirect methods of absorbance detection. Photoionization seems especially promising, as the optical system is simpler than that for photothermal or photoacoustic techniques and rejection of stray and scattered radiation is not necessary.

A novel photovoltaic detector has been developed which is shown schematically in Figure 24. In this detector, the sample is enclosed between two optically transparent electrodes and is irradiated transversely by a pulsed laser. The transient photopotential developed across the unbiased electrodes after illumination is measured relative to external ground. By eliminating the bias voltage used in traditional photoconductive measurements, several important advantages are gained. First, contributions from intrinsic solvent conduction are substantially reduced or eliminated. Hence, measurements of the photovoltaic response may be accomplished in polar solvents and in solutions of relatively high ionic strength, where a photoconductive measurement may not be possible. Finally, this approach may reduce background current from the photoelectric effect, which is caused by electron ejection from the electrodes when directly irradiated in an applied field.

In preliminary studies, we investigated the origin of the photovoltaic signal and found that it arose from photoionization, photooxidation/reduction, or photodecomposition at or near the surface of the front electrode. The photovoltaic response was then characterized as a function of the composition, thickness, illuminated area, and separation distance of the optically transparent electrodes as well as the laser pulse energy and frequency (111). The photopotential for N,N,N',N'-tetramethyl-1,4-phenylenediamine (TMPD) was measured with excitation by a nitrogen laser (337 nm, 3.7 eV) and an excimer laser (KrF, 248 nm, 5.0 eV) in a variety of polar solvents. Very good agreement was observed between the measured photopotential and the calculated liquid-phase ionization energy for TMPD (111).

In subsequent studies, the photovoltaic method was compared to the traditional photoconductive method (112). Theoretical models were developed for each method and verified by experimental measurements. Each system was characterized by means of signal and signal-to-noise ratio measurements with respect to laser pulse energy, applied voltage, electrode separation distance, solute concentration, and solvent composition. Although both techniques exhibited comparable detection limits (1×10^{-7} M) for solutes in polar solvents such as methanol and water, the photovoltaic technique had a more extensive linear dynamic range (10^3). Moreover, the photovoltaic technique allowed detection of solutes in ionic solutions with comparable sensitivity and linearity, where the photoconductive technique was not suitable. In contrast, the photoconductive technique provided excellent sensitivity in nonpolar solvents such as hexane, where the photovoltaic technique was not responsive. Thus, the photoconductive and photovoltaic methods appear to be highly complementary rather than competitive techniques (112).

FIGURE 24: Schematic diagram of the experimental system for liquid chromatography with photovoltaic detection (113). I = injection valve, PD = photodiode, M = mirror.

FIGURE 25: Separation of aromatic amines by reversed-phase liquid chromatography with photovoltaic detection (113). Mobile phase: 60% methanol, 20% acetonitrile, and 20% water adjusted to pH 8.0; 0.3 mL/min. Solutes: (1) N,N-diethylaniline, (2) N,N-dimethylaniline, (3) *m*-toluidine, and (4) aniline at 1×10^{-4} M in methanol.

The photovoltaic technique was then developed as a detection method for liquid chromatography (113). To evaluate the applicability of this technique, we examined a wide variety of aliphatic and aromatic compounds including those with amine, carbonyl, carboxyl, hydroxyl, and halogen functional groups. The sensitivity and flow rate dependence of the photovoltaic method were characterized and were compared with direct UV-visible absorbance. Finally, the photovoltaic detection was applied for the detection of a series of substituted anilines and aldehydes separated by reversed-phase liquid chromatography. In general, the photovoltaic method exhibited improved sensitivity over direct UV-visible absorbance for aliphatic aldehydes, while showing comparable response for aromatic aldehydes and amines (Figure 25). Thus, this novel technique shows promise for the sensitive and selective detection of a wide variety of compounds with nonbonding (n) and aromatic (π) electrons.

SUMMARY

In summary, we believe that significant progress has been made on all of the primary scientific objectives of this research project. We have developed a laser-induced fluorescence system that allows *in situ* detection at multiple points along a chromatographic or electrophoretic column. We have utilized this system to examine the thermodynamic and kinetic behavior of octadecylsilica stationary phases as a function of bonding density (2.7 to 5.4 $\mu\text{mol m}^{-2}$), temperature (10 to 70 $^{\circ}\text{C}$), pressure (400 to 4500 psi), and mobile-phase composition. Concurrently, we have developed and validated a computer simulation of chromatographic and electrophoretic separations that follows the trajectories of individual molecules in three-dimensional space. We have utilized this simulation to examine the kinetics of solute distribution as well as the retention and dispersion of solute zones along the column and in regions of spatial and temporal transitions. In each of these experimental and simulation studies, substantial knowledge has been gained of the fundamental processes arising in chromatographic and electrophoretic separations.

In addition to these primary research objectives, we have made notable progress in several related areas. Reliable computer-assisted methods have been developed for univariate and multivariate optimization in liquid chromatography and capillary electrophoresis. We have also developed novel detection strategies based on laser-induced fluorescence, fluorescence quenching, and photoionization. All of these methods extend the advanced separation techniques developed in our laboratory to practical applications of environmental and forensic significance (polycyclic aromatic hydrocarbons, nitrated explosives) as well as biomedical significance (fatty acids, corticosteroids, nucleotides, tetracyclines).

REFERENCES

1. J.C. Gluckman, A. Hirose, V.L. McGuffin, M. Novotny; *Chromatographia* 17, 303 (1983).
2. C.E. Evans, V.L. McGuffin; *Proc. Int. Symp. Capillary Chromatogr.* 11, 802 (1990).
3. C.E. Evans, V.L. McGuffin; *Anal. Chem.* 60, 573 (1988).
4. V.L. McGuffin, C.E. Evans; *J. Microcol. Sep.* 3, 513 (1991).
5. V.L. McGuffin, C.E. Evans, S.H. Chen; *J. Microcol. Sep.* 5, 3 (1993).
6. J.C. Sternberg; *Adv. Chromatogr.* 2, 205 (1966).
7. A. Tchaplá, S. Heron, E. Lesellier, H. Colin; *J. Chromatogr.* 656, 81 (1993).
8. A. Tchaplá, H. Colin, G. Guiochon; *Anal. Chem.* 56, 621 (1984).
9. J.F. Wheeler, T.L. Beck, S.J. Klatte, L.A. Cole, J.G. Dorsey; *J. Chromatogr.* 656, 317 (1993).
10. L.C. Sander, S.A. Wise; *J. Chromatogr.* 656, 335 (1993).
11. R.K. Gilpin; *J. Chromatogr.* 656, 217 (1993).
12. J. Chmielowiec, H. Sawatzky; *J. Chromatogr. Sci.* 17, 245 (1979).
13. K.B. Sentell, A.N. Henderson; *Anal. Chim. Acta* 246, 139 (1991).
14. B.A. Bidlingmeyer, R.P. Hooker, C.H. Lochmuller, L.B. Rogers; *Separ. Sci.* 4, 439 (1969).
15. E. Katz, K. Ogan, R.P.W. Scott; *J. Chromatogr.* 260, 277 (1983).
16. L.C. Sander, S.A. Wise; *Anal. Chem.* 59, 2309 (1987).
17. L.C. Sander, S.A. Wise; *LC-GC Magazine* 8, 378 (1990).
18. K.B. Sentell, J.G. Dorsey; *Anal. Chem.* 61, 930 (1989).
19. L.A. Cole, J.G. Dorsey; *Anal. Chem.* 64, 1317 (1992).
20. B. Buszewski, Z. Suprynowicz, P. Staszczuk, K. Albert, B. Pfeleiderer, E. Bayer; *J. Chromatogr.* 499, 305 (1990).
21. S.H. Chen, V.L. McGuffin; *J. Chromatogr.* 762, 35 (1997).
22. V.L. McGuffin, S.H. Chen; *Anal. Chem.* 69, 930 (1997).
23. L.A. Cole, J.G. Dorsey, K.A. Dill; *Anal. Chem.* 64, 1324 (1992).
24. W. Melander, D.E. Campbell, C. Horvath; *J. Chromatogr.* 158, 215 (1978).
25. A. Tchaplá, S. Heron, H. Colin, G. Guiochon; *Anal. Chem.* 60, 1443 (1988).
26. G. Guiochon, M.J. Sepaniak; *J. Chromatogr.* 606, 248 (1992).
27. S.H. Chen, V.L. McGuffin; *J. Phys. Chem.*, manuscript in preparation.
28. R.P.W. Scott, C.F. Simpson; *Faraday Symp. Chem. Soc.* 15, 69 (1980).
29. R.M. McCormick, B.L. Karger; *Anal. Chem.* 52, 2249 (1980).
30. L.A. Cole, J.G. Dorsey; *Anal. Chem.* 62, 16 (1990).
31. M.E. Montgomery, M.A. Green, M.J. Wirth; *Anal. Chem.* 64, 1170 (1992).
32. R.B. Bird, W.E. Stewart, E.N. Lightfoot; *Transport Phenomena*; Wiley, New York, NY (1960).
33. T.K. Sherwood, R.L. Pigford, C.R. Wilke; *Mass Transfer*; McGraw-Hill, New York, NY (1975).
34. J.C. Giddings; *Dynamics of Chromatography*; Marcel Dekker, New York, NY (1965).
35. S. Golshan-Shirazi, G. Guiochon; *J. Chromatogr.* 603, 1 (1992).
36. J.C. Giddings; *J. Chromatogr.* 2, 44 (1959).
37. J.C. Giddings; *J. Chromatogr.* 3, 443 (1960).
38. C. Lee, V.L. McGuffin; *Anal. Chem.*, manuscript in preparation.
39. D.B. Marshall, J.W. Burns, D.E. Connolly; *J. Chromatogr.* 360, 13 (1986).
40. D.B. Marshall, J.W. Burns, D.E. Connolly; *J. Am. Chem. Soc.* 108, 1087 (1986).
41. J.F. Baret; *Prog. Surf. Membr. Sci.* 14, 291 (1981).
42. A. Ulman; *An Introduction to Ultrathin Organic Films*; Academic Press, Boston, MA (1991).

43. C.M. Knobler, R.C. Desai; *Ann. Rev. Phys. Chem.* 43, 207 (1992).
44. C.E. Evans, V.L. McGuffin; *Anal. Chem.* 63, 1393 (1991).
45. C.E. Evans, V.L. McGuffin; *J. Liq. Chromatogr.* 11, 1907 (1988).
46. C.E. Evans, J.S. Yoo, V.L. McGuffin; *Anal. Chem.*, manuscript in preparation.
47. P.J. Schoenmakers, H.A.H. Billiet, L. de Galan; *Chromatographia* 15, 205 (1982).
48. J.G. Dorsey, K.A. Dill; *Chem. Rev.* 89, 331 (1989).
49. V.L. McGuffin, P. Wu, D.L. Hopkins; *Proceedings of the International Symposium on Chromatography*; H. Hatano and T. Hanai, Eds.; World Scientific Publishing, River Edge, NJ (1995), pp. 45-69.
50. V.L. McGuffin, P. Wu; *J. Chromatogr.* 722, 3 (1996).
51. D.L. Hopkins, V.L. McGuffin; *Anal. Chem.* 70, 1066 (1998).
52. V.L. McGuffin, P.E. Krouskop; in *Unified Chromatography*; T.L. Chester, J.F. Parcher; ACS Symposium Series, in press (1999).
53. W.H. Press, B.P. Flannery, S.A. Teukolsky, W.T. Vetterling; *Numerical Recipes: The Art of Scientific Computing*; Cambridge University Press, Cambridge, England (1989).
54. A. Einstein; *Ann. Phys.* 17, 549 (1905).
55. R.C. Reid, J.M. Prausnitz, T.K. Sherwood; *The Properties of Gases and Liquids*; McGraw-Hill, New York, NY (1977).
56. W. Feller; *Probability Theory and its Application*; Wiley, New York, NY (1950), Ch. 14.
57. P.E. Krouskop, Michigan State University, unpublished research (1998).
58. G. Taylor; *Proc. Roy. Soc. (London)* A219, 186 (1953).
59. A.L. Hines, R.N. Maddox; *Mass Transfer: Fundamentals and Applications*; Prentice-Hall, Englewood Cliffs, NJ (1985).
60. C.L. Rice, R. Whitehead; *J. Phys. Chem.* 69, 4017 (1965).
61. G. Guiochon, M. Martin; *Anal. Chem.* 60, 1837 (1988).
62. M. von Smoluchowski; *Bull. Intern. Acad. Sci. Cracovic* 1903, 184 (1903).
63. E. Huckel; *Physik. Z.* 25, 204 (1924).
64. J.P. McEldoon, R. Datta; *Anal. Chem.* 64, 230 (1992).
65. P.W. Atkins; *Physical Chemistry*; Oxford University Press, Oxford, England (1978).
66. R.A. Robinson, R.H. Stokes; *Electrolyte Solutions: The Measurement and Interpretation of Conductance, Chemical Potential, and Diffusion in Solution of Simple Electrolytes*; Butterworths, London, England (1959).
67. J.N. Butler; *Ionic Equilibrium: A Mathematical Approach*; Addison-Wesley, Reading, MA (1964).
68. H.A. Laitinen, W.E. Harris; *Chemical Analysis*; McGraw-Hill, New York, NY (1975).
69. M.J.E. Golay; in *Gas Chromatography*; D.H. Desty (Editor); Academic Press, New York, NY (1958), p. 36.
70. J.I. Steinfeld, J.S. Francisco, W.L. Hase; *Chemical Kinetics and Dynamics*; Prentice Hall: Englewood Cliffs, NJ (1989).
71. S.W. Benson; *Foundations of Chemical Kinetics*; McGraw-Hill: New York, NY (1960).
72. P. Wu, V.L. McGuffin; *AIChE J.* 44, 2053 (1998).
73. V.L. McGuffin, P.E. Krouskop, P. Wu; *J. Chromatogr.* 828, 37 (1998).
74. P.H. Lukulay, V.L. McGuffin; *J. Microcol. Sep.* 8, 211 (1996).
75. H. Colin, G. Guiochon, P. Jandera; *Anal. Chem.* 55, 442 (1983).
76. A.C.J.H. Drouen, H.A.H. Billiet, L. de Galan; *J. Chromatogr.* 352, 127 (1986).
77. H.J. Issaq, D.W. Mellini, T.E. Beesley; *J. Liq. Chromatogr.* 11, 333 (1988).
78. A. Nahum, C. Horvath; *J. Chromatogr.* 203, 53 (1981).
79. W.E. Hammers, P.B.A. Verschoor; *J. Chromatogr.* 282, 41 (1983).
80. P.H. Lukulay, V.L. McGuffin; *J. Chromatogr.* 691, 171 (1995).

81. P.H. Lukulay, V.L. McGuffin; *J. Liq. Chromatogr.* 18, 4039 (1995).
82. P.H. Lukulay, V.L. McGuffin; *J. Liq. Chromatogr.* 19, 2039 (1996).
83. P.H. Lukulay, V.L. McGuffin; *Anal. Chem.* 69, 2963 (1997).
84. T.D. Schlabach, J.L. Excoffier; *J. Chromatogr.* 439, 173 (1988).
85. J.H. Wahl, V.L. McGuffin; *J. Chromatogr.* 485, 541 (1989).
86. J.H. Wahl, C.G. Enke, V.L. McGuffin; *Anal. Chem.* 62, 1416 (1990).
87. J.H. Wahl, C.G. Enke, V.L. McGuffin; *Anal. Chem.* 63, 1118 (1991).
88. J.S. Yoo, V.L. McGuffin; *J. Chromatogr.* 627, 87 (1992).
89. M.F.M. Tavares, V.L. McGuffin; *Anal. Chem.* 67, 3687 (1995).
90. M.F.M. Tavares, V.L. McGuffin; *Anal. Chem.* 69, 152 (1997).
91. M.F.M. Tavares, V.L. McGuffin; *J. Chromatogr.* 686, 129 (1994).
92. V.L. McGuffin; *J. Liq. Chromatogr.* 18, 3787 (1995).
93. E.M. Kosower; *J. Am. Chem. Soc.* 80, 3253 (1958).
94. D.C. Dong, M.A. Winnik; *Can. J. Chem.* 62, 2560 (1984).
95. J.W. Carr, J.M. Harris; *Anal. Chem.* 59, 2546 (1987).
96. J. Stahlberg, M. Almgren, J. Alsins; *Anal. Chem.* 60, 2487 (1988).
97. S.H. Chen, V.L. McGuffin; *Appl. Spectrosc.* 48, 596 (1994).
98. M.E. Sigman, S.M. Lindley, J.E. Leffler; *J. Am. Chem. Soc.* 107, 1471 (1985).
99. C.R. Yonker, S.L. Frye, D.R. Kalkwarf, R.D. Smith; *J. Phys. Chem.* 90, 3022 (1986).
100. C.R. Yonker, R.D. Smith; *J. Phys. Chem.* 92, 235 (1988).
101. J.F. Brennecke, C.A. Eckert; *ACS Symp. Ser.* 406, 14 (1989).
102. J.F. Brennecke, D.L. Tomasko, J. Peshkin, C.A. Eckert; *Ind. Eng. Chem. Res.* 29, 1682 (1990).
103. L.M. Stephen, B.D. Olafson, W.A. Goddard; *J. Phys. Chem.* 94, 8897 (1990).
104. C. Reichardt; *Solvents and Solvent Effects in Organic Chemistry*, VCH Publishers: New York, NY (1988); p. 10-11.
105. V.L. McGuffin, J.V. Goodpaster; in *Encyclopedia of Environmental Analysis and Remediation*; R.A. Meyers, Ed.; Wiley, New York, NY (1998), pp. 3814-3831.
106. S.H. Chen, C.E. Evans, V.L. McGuffin; *Anal. Chim. Acta* 246, 65 (1991).
107. F.K. Ogasawara, Y. Wang, V.L. McGuffin; *Appl. Spectrosc.* 49, 1 (1995).
108. J.V. Goodpaster, V.L. McGuffin; *Appl. Spectrosc.*, in press.
109. J.V. Goodpaster, V.L. McGuffin; *Anal. Chem.*, manuscript in preparation.
110. J.V. Goodpaster, J.F. Harrison, V.L. McGuffin; *J. Phys. Chem.* 102, 3372 (1998).
111. J.W. Judge, V.L. McGuffin; *Anal. Chem.* 63, 2564 (1991).
112. J.W. Judge, V.L. McGuffin; *Appl. Spectrosc.* 48, 1102 (1994).
113. J.W. Judge, V.L. McGuffin; *Anal. Chem.*, submitted for publication (1999).

PUBLICATIONS OF THE RESEARCH PROJECT

1. C.E. Evans, V.L. McGuffin; "Retention and Dispersion Processes Along a Liquid Chromatographic Microcolumn", *Proc. Int. Symp. Capillary Chromatogr.* 11, 802-814, 1990. (DOE/ER/14056-5)
2. C.E. Evans, V.L. McGuffin; "Direct Examination of the Injection Process in Liquid Chromatographic Separations", *Anal. Chem.* 63, 1393-1402, 1991. (DOE/ER/14056-8)
3. V.L. McGuffin, C.E. Evans; "The Influence of Pressure on Solute Retention in Liquid Chromatography", *Proc. Int. Symp. Capillary Chromatogr.* 12, 90-99, 1991. (DOE/ER/14056-10)
4. V.L. McGuffin, C.E. Evans; "Influence of Pressure on Solute Retention in Liquid Chromatography", *J. Microcol. Sep.* 3, 513-520, 1991. (DOE/ER/14056-13)
5. V.L. McGuffin, C.E. Evans, S.H. Chen; "Direct Examination of Separation Processes in Liquid Chromatography by Laser-Induced Fluorescence", *Proc. Int. Symp. Capillary Chromatogr.* 14, 417-426, 1992. (DOE/ER/14056-17)
6. S.H. Chen, C.E. Evans, V.L. McGuffin; "Selective Fluorescence Quenching of Polynuclear Aromatic Hydrocarbons in Microcolumn Liquid Chromatography"; *Anal. Chim. Acta* 246, 65-74, 1991. (DOE/ER/14056-20)
7. J.S. Yoo, V.L. McGuffin; "Determination of Fatty Acids in Fish Oil Dietary Supplements by Capillary Liquid Chromatography with Laser-Induced Fluorescence Detection"; *J. Chromatogr.* 627, 87-96, 1992. (DOE/ER/14056-21)
8. V.L. McGuffin, C.E. Evans, S.H. Chen; "Direct Examination of Separation Processes in Liquid Chromatography: Effect of Temperature and Pressure on Solute Retention"; *J. Microcol. Sep.* 5, 3-10, 1993. (DOE/ER/14056-22)
9. S.H. Chen, V.L. McGuffin; "Temperature Effect on Pyrene as a Polarity Probe for Supercritical Fluid and Liquid Solutions"; *Appl. Spectrosc.* 48, 596-603, 1994. (DOE/ER/14056-29)
10. J.W. Judge, V.L. McGuffin; "Comparative Study of Photoconductive and Photovoltaic Detection Methods for Liquid-Phase Photoionization"; *Appl. Spectrosc.* 48, 1102-1110, 1994. (DOE/ER/14056-30)
11. V.L. McGuffin, P. Wu, D.L. Hopkins; "Three-Dimensional Computer Simulation of Chromatographic and Electrophoretic Separations", in *Proceedings of the International Symposium of Chromatography*, H Hatano, T. Hanai, Editors, World Scientific Publishing: River Edge, NJ, 1995, pp. 45-69. (DOE/ER/14056-32)
12. M.F.M. Tavares, V.L. McGuffin; "Separation and Characterization of Tetracycline Antibiotics by Capillary Electrophoresis", *J. Chromatogr.* 686, 129-142, 1994. (DOE/ER/14056-34)
13. F.K. Ogasawara, Y. Wang, V.L. McGuffin; "Quantitative Evaluation of Selective Fluorescence Quenchers for Polynuclear Aromatic Hydrocarbons", *Appl. Spectrosc.* 49, 1-7, 1995. (DOE/ER/14056-35)

14. P.H. Lukulay, V.L. McGuffin; "Solvent Modulation in Liquid Chromatography: Extension to Serially Coupled Columns", *J. Chromatogr.* 691, 171-185, 1995. (DOE/ER/14056-36)
15. M.F.M. Tavares, V.L. McGuffin; "Theoretical Model of Electroosmotic Flow for Capillary Zone Electrophoresis", *Anal. Chem.* 67, 3687-3696, 1995. (DOE/ER/14056-40)
16. V.L. McGuffin; "Photoactivation: A Novel Means to Mediate Electrophoretic Separations", *J. Liq. Chromatogr.* 18, 3787-3812, 1995. (DOE/ER/14056-41)
17. P.H. Lukulay, V.L. McGuffin; "Comparison of Solvent Modulation with Premixed Mobile Phases for the Separation of Corticosteroids by Liquid Chromatography", *J. Liq. Chromatogr.* 18, 4039-4062, 1995. (DOE/ER/14056-42)
18. V.L. McGuffin, P. Wu; "Three-Dimensional Molecular Simulation of Chromatographic Separations", *J. Chromatogr.* 722, 3-17, 1996. (DOE/ER/14056-43)
19. P.H. Lukulay, V.L. McGuffin; "Evolution from Univariate to Multivariate Optimization Methods in Liquid Chromatography", *J. Microcolumn Sep.* 8, 211-224, 1996. (DOE/ER/14056-44)
20. P.H. Lukulay, V.L. McGuffin; "Experimental and Computer Simulation Studies of Solute-Solute Interactions in Liquid Chromatography", *J. Liq. Chromatogr.* 19, 2039-2058, 1996. (DOE/ER/14056-45)
21. V.L. McGuffin, M.F.M. Tavares; "Computer-Assisted Optimization of Separations in Capillary Zone Electrophoresis", *Anal. Chem.* 69, 152-164, 1997. (DOE/ER/14056-46)
22. V.L. McGuffin, S.H. Chen; "Theoretical and Experimental Studies of the Effect of Pressure on Solute Retention in Liquid Chromatography", *Anal. Chem.* 69, 930-943, 1997. (DOE/ER/14056-47)
23. V.L. McGuffin, S.H. Chen; "Molar Enthalpy and Molar Volume of Methylene and Benzene Homologues in Reversed-Phase Liquid Chromatography", *J. Chromatogr.* 762, 35-46, 1997. (DOE/ER/14056-48)
24. V.L. McGuffin, P.H. Lukulay; "Parametric Modulation in Liquid Chromatography: Multivariate Optimization of Mobile Phase Composition and Temperature", *Anal. Chem.* 69, 2963-2971, 1997. (DOE/ER/14056-50)
25. D.L. Hopkins, V.L. McGuffin; "Three-Dimensional Molecular Simulation of Electrophoretic Separations", *Anal. Chem.* 70, 1066-1075, 1998. (DOE/ER/14056-51)
26. J.V. Goodpaster, J.F. Harrison, V.L. McGuffin; "Ab Initio Study of Polycyclic Aromatic Hydrocarbons in Their Ground and Excited States", *J. Phys. Chem. A* 102, 3372-3381, 1998. (DOE/ER/14056-52)

27. V.L. McGuffin, J.V. Goodpaster; "Fluorescence Quenching of Polycyclic Aromatic Hydrocarbons", in *Encyclopedia of Environmental Analysis and Remediation*, R.A. Meyers, Editor, Wiley: New York, NY, 1998, pp. 3814-3831. (DOE/ER/14056-57)
28. P. Wu, V.L. McGuffin; "Numerical Analysis of Kinetic Rate Constants Derived from Stochastic Computer Simulation", *AIChE Journal* 44, 2053-2067, 1998. (DOE/ER/14056-58)
29. S.A. Mehlman, P.D. Wentzell, V.L. McGuffin; "Prediction of Simple Physical Properties of Mixed Solvent Systems by Artificial Neural Networks", *Anal. Chim. Acta* 371, 117-130, 1998. (DOE/ER/14056-62)
30. V.L. McGuffin, P.E. Krouskop, P. Wu; "Stochastic Simulation of the Partition Mechanism under Diffusion-Limited Conditions in Chromatography and Electrochromatography", *J. Chromatogr.* 828, 37-50, 1998. (DOE/ER/14056-63)
31. V.L. McGuffin, P.E. Krouskop; "Three-Dimensional Stochastic Simulation for the Unified Treatment of Chromatographic and Electrophoretic Separations", *ACS Symposium Series*, in press.
32. J.V. Goodpaster, V.L. McGuffin; "Rapid and Accurate Determination of Stern-Volmer Quenching Constants", *Appl. Spectrosc.*, in press.
33. J.W. Judge, V.L. McGuffin; "Photovoltaic Detection System for Flow Injection and Liquid Chromatography", *Anal. Chem.*, submitted for publication.

PERSONNEL OF THE RESEARCH PROJECT

1. Christine E. Evans, graduate student 1985-1991, Ph.D. thesis "Fundamental Studies of Capillary Columns in Liquid Chromatography".
2. Jon H. Wahl, graduate student 1985-1991, Ph.D. thesis "Solvent Modulation in Liquid Chromatography: An Alternative to Premixed Mobile Phases".
3. John W. Judge, graduate student 1985-1992, 1997-1998.
4. Jong Shin Yoo, graduate student 1986-1992, Ph.D. thesis, "Temperature-Programmed Microcolumn Liquid Chromatography/Mass Spectrometry".
5. Shu-Hui Chen, graduate student 1987-1993, Ph.D. thesis "Fundamental Studies of Chromatographic Systems using Fluorescence Spectroscopy".
6. Marina F.M. Tavares, graduate student 1988-1993, Ph.D. thesis "Computer-Assisted Optimization of Separations in Capillary Zone Electrophoresis".
7. Yiwen Wang, graduate student 1989-1991, M.S. thesis "Application of Computer Simulation for Investigation of Fluorescence Quenching Mechanisms".
8. Patrick H. Lukulay, graduate student 1989-1995, Ph.D. thesis "Parametric Modulation: A Novel Computer-Assisted Optimization Method for Liquid Chromatography".
9. Daniel L. Hopkins, graduate student 1989-present.
10. Dr. Faye K. Ogasawara, postdoctoral research associate 1991-1993.
11. Dr. Peiru Wu, postdoctoral research associate 1994-1996.
12. John V. Goodpaster, graduate student 1995-present.
13. Peter E. Krouskop, graduate student 1995-present.