COMPUTER ASSISTED INVESTIGATIONS
OF CHROMATOGRAPHIC PROCESSES

by
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ABSTRACT

Chromatography is an important analytical technique applied in a wide range of scientific fields. Continued advances in the fundamental understanding of chromatographic processes provide appropriate applications. Full utilization of computer capabilities will allow the development of new experimental approaches to provide information otherwise not readily available.

Deconvolution techniques based on Fourier transform have been applied to isolating column from extra-column contributions to band broadening. Injector and detector contributions are considered together and separated both experimentally and mathematically. The injector is shown to play a major role in system band broadening, diminishing with retention, with a minimal contribution from the detector.

A system based on a probabilistic approach to digital simulation which describes the retention mechanism in terms of discrete events has been developed. The utility of the system for quantitatively studying both linear and non-linear chromatographic processes is demonstrated in characterizing HPLC column packings.
Two new methods for obtaining fundamental chromatographic information have been discussed. The band broadening considerations and the digital simulations are two very different approaches to chromatographic investigations. In both cases, however, a better understanding of the dynamic nature of the solvated stationary phase has resulted.
CHAPTER 1

INTRODUCTION

The popularity of and demand for separation techniques is continually increasing. Separations, especially chromatographic methods, are finding application in a wide range of scientific fields. The variety of applications has put technological demands on chromatographic systems which has led to improvements in all chromatographic techniques, with particular emphasis on high-performance liquid chromatography (HPLC) where remarkable advances have been made. The amount of progress to date has shown the necessity of a great deal of future research.

The development of bonded phases may be the single most important advance in the area of column technology. Initially used in gas chromatography to replace coated stationary phases, bonded phases were applied to liquid chromatography with great success. Bonded phases exhibit greater stability, efficiency, a higher capacity, and a greater degree of selectivity.

Although advances in column technology and instrumentation have kept pace with application demand, relatively little is known about the bonded phase structure and the
composition of the corresponding stationary phase. It is this limitation that restricts a fundamental understanding of the retention mechanism. Without an accurate picture of the stationary phase, a separation scheme must still be empirically determined.

Establishing a comprehensive mechanism of retention is of fundamental importance. The information needed to define many of the parameters can be determined chromatographically. To arrive at a quantitative mechanism, accurate data must be available. As column efficiencies improve, extra column effects may add a significant error to the accuracy of the data, and therefore a correction is necessary.

Even with the major advances that have been made in the areas of column technology and basic instrumentation, the most significant advances to come may very well be in the area of computer applications to data analysis and information retrieval. The currently routine use of computer data systems and microprocessor-controlled instruments is a great aid in operator convenience; but more importantly, the information available increases while the data reduction time decreases. This is a definite advance, but still does not fully utilize computer capabilities.

Chromatographic experiments which require computer interaction can now be easily undertaken. Multiplex
techniques, using Fourier transform techniques for data deconvolution, have been used to increase the information available from a chromatographic signal. Another area which virtually requires the use of a computer is in the modelling and simulation of a chromatographic system. These techniques have a common denominator: they use computers to increase the information available from and about chromatographic processes.

New chromatographic experiments which utilize computer control and data analysis are limited only by imagination. The current level of column technology and rapid development of computers, makes it possible to exploit these areas for the purpose of obtaining information not previously available. This can provide a better understanding of the fundamental chemical processes.

A quick review of the fundamental concepts of chromatography is now appropriate. All chromatographic methods have the following similarities. The system is composed of two immiscible phases, a mobile phase which flows over a stationary phase, and a third component, the sample of interest which distributes itself between the two phases when it is introduced into the system. The forward mobility of the sample is dependent on this distribution. A sample with a preference for the stationary phase will be retained
relatively longer. This differential migration is the basis of chromatography as a separation technique.

Resolution, the ability to resolve two components, is of fundamental importance in separation science. Improving resolution is a primary goal in chromatographic development. Resolution is the interrelationship of three independent factors: efficiency, selectivity and capacity or the retention ability. The efficiency of a chromatographic system is determined by dynamics or the rate of processes occurring on the column. This is measured by the amount of band broadening of a chromatographic peak. Selectivity and capacity are thermodynamic or equilibrium properties of the system which are measured by chromatographic peak position. Chromatographic theory in each of these areas has been progressing since the development of gas chromatography by James and Martin (1) in 1952. When the physical parameters affecting separation are well defined, then appropriate measures can be taken to optimize resolution. This progress will be reviewed briefly from both the band-broadening and peak position points of view.

**Band Broadening**

Tswett (2) was the first to recognize that the chromatographic process consists of sequential adsorption-desorption interactions. This was easily seen in his
separation of plant pigments into colored bands on a column of calcium carbonate with petroleum ether as the eluent. About thirty years passed before liquid-solid chromatography was established as an analytical technique when Kuhn and Lederer (3) separated carotene and xanthophyll isomers.

Wilson (4) described the chromatographic process mathematically in 1940. Complete adsorption desorption equilibria was assumed. The elution process was treated as the passage of a concentration profile through the chromatographic system. The dependence of band spreading on packing efficiency, diffusion, and finite adsorption-desorption rates was recognized.

In 1943, de Vault (5) improved on Wilson's model of the chromatographic process by using a continuous distribution of solute between the stationary and mobile phase to show that liquid-solid chromatography band shapes could be predicted from adsorption isotherms. This improvement still could not solve the elution behavior of overlapping solutes. This was solved by Glueckauf (6) who, along with coworkers, made many advances in the study of physicochemical applications to chromatography culminating in 1955 with the first generalized equation that quantitatively described the chromatographic process.

There are two peak broadening models. The plate model was first reported by Martin and Synge (7). The
second, more useful model is the continuous or rate model. It was first introduced by van Deemter, Zuiderweg and Klinkenberg (8). Both models lead to the same result and assume linear chromatography which implies continuous equilibrium, linear isotherms and negligible longitudinal diffusion.

The plate model imagines a chromatographic column divided into a number of separate zones, each of which is of a length that allows complete equilibration of the solute between mobile and stationary phases. The zones are called theoretical plates, and their length is referred to as the height equivalent to a theoretical plate, HETP. This model and terminology have their origin describing distillation columns.

A descriptive view of the plate model follows. Upon injection, the sample is contained in the first plate. As the mobile phase passes through the column, some of the sample is swept into the second plate where it equilibrates with the stationary phase. In the first plate, what remains of the sample equilibrates with fresh mobile phase. This procedure is repeated as the whole zone moves along the column.

The "plate theory" has often been criticized as a discontinuous (plate-to-plate) theory of the chromatographic
distribution process. However, it offers a simple description which qualitatively fits experimental data. Martin and Synge made several observations from this: for example, N, the number of theoretical plates, is dependent upon diffusion, flow rate, particle size, column pressure drop, uniformity of packing, and isotherm linearity. They recognized that since the chromatographic process consists of repeated partitioning of a solute between two phases, the mobile phase could be gas and the stationary phase a liquid. They also measured partition coefficients in terms of retention, which was the first physico-chemical application of chromatography.

Rate-controlled kinetic processes were investigated by many, including Drake (9), who considered the role of molecular diffusion in band spreading. Lapidus and Amundson developed a mathematical model that introduced the concepts of eddy diffusion, longitudinal diffusion, and nonequilibrium mass transfer. This led to a mathematically simplified expression known as the van Deemter equation (8). This clarified what is now known as the rate theory of chromatography and set specific parameters for improving column efficiency, which allowed Golay (10) to theoretically predict the efficiency of capillary columns.

The rate model has the advantage of relating band broadening to parameters which can be experimentally
determined and controlled. It assumes that the various contributions to band broadening are independent of each other and their variances are additive, which can be written in the following manner:

$$\sigma_{\text{Total}}^2 = \sigma_A^2 + \sigma_B^2 + \sigma_C^2 + \ldots \ldots (1.1)$$

It can be also expressed as a simplified form of the van Deemter equation as a function of linear flow velocity, $u$:

$$\text{HETP} = A + B/u + Cu \ldots \ldots (1.2)$$

The A-term arises from the multiple path through the packing material taking geometry, size and uniformity of packing into account. The B-term, which is less important at high flow rates, takes into account longitudinal diffusion of the solute in either phase. The C-term which predominates at higher flow rates contains the kinetic effects of the transfer of solute between phases. The C-term is also called the resistance to mass transfer or non-equilibrium term.

Probability concepts were applied to the rate theory beginning with Giddings and Eyring (11) in 1955. Giddings then developed a stochastic description of chromatographic adsorption-desorption and diffusion processes. The treatment of diffusion was presented in terms of random walk statistics. This random walk approach to the chromatographic process has been used as the basis for the discrete event simulation models.
Both the plate and rate models use the number of plates, N, as a measure of column performance. Plate height can be determined experimentally from the equations:

\[ H = \frac{L}{N} \quad (1.3) \]

\[ N = \frac{5.545}{(t_r/w_{1/2})^2} \quad (1.4) \]

where \( L \) is the column length, \( t_r \) the retention time from point of injection to peak maximum, and \( w_{1/2} \) is the width of the peak at one-half height.

The previous discussion included only column band broadening considerations. With increasing column efficiency through new technology, injection and detection methods have not kept pace. Extra column band broadening plays an increasingly important part on limiting the efficiency of the system. Because of this, quantitation of extra column effects and separating them into their individual components is highly desirable. A modified band broadening expression to include extra column considerations follows:

\[ \sigma_{\text{Total}}^2 = \sigma_{\text{inj}}^2 + \sigma_{\text{col}}^2 + \sigma_{\text{det}}^2 + \sigma_{\text{tubing}}^2 \quad (1.5) \]

The band broadening due to tubing, connectors, the injector and detector will be discussed in depth in Chapter 2.

Minor modifications in the van Deemter equation are necessary for different forms of chromatography. For open
tubular columns, with no multiple paths, there is no $A$-term as shown in the following equation:

$$H = B/u + C_m u + C_s u$$

(1.6)

Diffusion in liquids is approximately five orders of magnitude slower than gaseous diffusion and therefore in liquid chromatography the $B$-term can be neglected, as shown here:

$$H = (\frac{1}{A} + \frac{1}{C_m u})^{-1} + C_s u$$

(1.7)

where $A$, $B$, and $C$ have the same meaning as in the general form of the van Deemter equation previously mentioned (Equation 1.2). The subscripts, $s$ and $m$, on the $C$-term signify stationary phase and mobile phase interactions, respectively.

Various attempts have been made to relate the $C$-term to the nature of the adsorbent. This is especially important when dealing with porous material. Kiselev and coworkers (12, 13) have shown the HETP, and therefore the $C$-term, increased with decreasing pore radius and increased as pores with constant radius were made deeper. Van Berge and Pretorius (14) have confirmed this work. Peak broadening measurements are capable of providing information about molecular probe-stationary phase interactions as well as the stationary phase itself. Peak broadening data helps explain
rates of adsorption, desorption, diffusion, film thickness and stationary phase homogeneity.

**Peak Position**

Thermodynamic properties which help to describe chromatographic processes, can be derived from the partition coefficient which is a concentration equilibrium constant. James and Martin (1), in the first paper on gas-liquid chromatography (GLC), emphasized the valuable information available from an analysis of retention behavior. Hoare and Purnell (15) demonstrated that vapor pressures, boiling points, heats of vaporization, solution and mixing and activity coefficients could all be measured by GLC. In 1955, Littlewood, Phillips and Price (16) determined free energies and entropies by GLC. Many workers have carried out thermodynamic studies of GLC and GSC by using frontal, elution and displacement techniques. In several studies (17), band shapes were correlated with adsorption-desorption isotherms, and subsequently isotherms were determined for other solutes from their elution behavior.

The distribution of a solute by the mobile and stationary phases is at equilibrium when the solute free energy is at a minimum. The chemical potential, $\mu$, which is defined as the partial molar free energy in a given phase, is equal in both phases:
\[ \mu_s = \mu_m \]

where

\[ \mu_i = \mu^0_i + RT \ln a_i \tag{1.8} \]

\( a_i \) is the solute activity in the ith phase and \( \mu^0_i \) is the chemical potential at unit activity. Equation expressions for the free energies gives this expression:

\[ \mu^0_s + RT \ln a_s = \mu^0_m + RT \ln a_m \tag{1.9} \]

which rearranged gives:

\[ -RT \ln \left( \frac{a_s}{a_m} \right) = \Delta \mu^0 \tag{1.10} \]

The activity ratio, \( a_s/a_m \), is the thermodynamic distribution constant, \( K^0 \); therefore Equation 1-10 can be expressed:

\[ -RT \ln K = \Delta \mu \]

The distribution coefficient, \( K \), is easy to measure chromatographically while the thermodynamic \( K^0 \) is easier to derive theoretically. The two values can be related by substituting concentration for activity. This approximation is valid at infinite dilution where most elution studies are carried out.

Chromatographic distribution coefficients are of fundamental importance in determining equilibrium thermodynamic properties. In elution chromatography, the volume
required for the center of gravity of the solute band to pass completely through the column is the retention volume, \( V_r \), which is a function of the amount of time spent in each phase. This relationship is the distribution coefficient, \( K \). Another important quantity is the capacity or retention factor, \( k' \), which is defined as the total amount of solute in the stationary phase divided by the total amount of solute in the mobile phase. This can be expressed as:

\[
k' = \frac{C_s V_s}{C_m V_m} = K \frac{V_s}{V_m} \tag{1.11}
\]

A solute migrates down the column only when it is in the mobile phase. The fraction of solute in the mobile phase or the probability that a specific solute molecule will be in the mobile phase at any given time is expressed by \( R \), where:

\[
R = \frac{n_m}{n_m + n_s} \tag{1.12}
\]

Since the capacity factor has been defined as \( k' = n_s/n_m \):

\[
R = 1/1+k' \tag{1.13}
\]

When \( R \) is equal to zero, there is no solute migration and when \( R \) equals one, there is no retention. The retention volume therefore is the mobile phase volume divided by \( R \),

\[
V_r = V_m/R \tag{1.14}
\]
The basic retention equation is arrived at by substituting in Equation 1-13 and rearranging:

\[ V_r = V_m + KV_s \]  \hspace{1cm} (1.15)

It should be noted that similar expressions can be derived for adsorption and exclusion as well as the partitioning shown above. A general expression would be as follows:

\[ V_r = V_m + KV_s + K'A_s + K''V_i \] \hspace{1cm} (1.16)

where \( V_s \) is the volume of the liquid or bonded stationary phase for partition chromatography, \( A_s \) is the surface area for adsorption chromatography, and \( V_i \) is the interstitial volume for exclusion chromatography.

The previous discussion has dealt with retention assuming a linear distribution isotherm. In practice, however, nonlinear isotherms are often encountered. In non-linear elution chromatography, retention time can shift and peak shapes become asymmetric. This behavior has been studied by many workers as indicated by Conder and Purnell (18, p. 35-53). Frontal analysis is a finite rather than infinite concentration technique of nonlinear elution chromatography which was first treated quantitatively by Glueckauf (6). In the technique, pure mobile phase is initially passed through the column. At some time later, a stream of the solute is added to the mobile phase which
produces a finite concentration step change at the inlet. The solute breaks through and the recorded profile rises to a plateau corresponding to the initial solute concentration. The shape of the front and rear boundaries gives an indication of the retention mechanism and isotherm type. Frontal analysis and frontal analysis by a characteristic point (FACP) have been discussed as methods of obtaining the adsorption isotherm (18, chapter 9).

Once the adsorption isotherm is known, the equation put forth by Brunauer, Emmett and Teller (19) can be used to determine surface areas. One form of the BET equation is:

\[
\frac{P_1}{V(P_i^0-P_1)} = \frac{1}{V_m C} + \frac{(C-1)}{V_m C} \frac{P_i}{P_i^0}
\]

where \(V\) is the total amount of adsorbed solute at vapor pressure \(P_1\), \(V_m\) is the volume required for monolayer coverage and \(C\) is a constant. By graphing this equation, the slope and intercept both yield \(V_m\) from which the surface area is calculated. Surface area determinations by chromatographic methods have been described by Nelson and Eggertsen (20) and Tremaine and Gray (21).

Chromatographic peak position studies can provide the following information. Thermodynamic parameters such as activity coefficients, distribution coefficients, free energy,
heats of solution and entropy can be determined. Characterization of the solid support by surface area determinations and adsorption isotherms can be done using the finite concentration methods. Peak shape studies are used to relate kinetic processes to stationary phase characteristics.

An understanding of these fundamental concepts concerning both peak position and peak broadening are essential for further development in the field of chromatography. Recent advances have followed an orderly progression which include expanded applications, increased resolution and column efficiency stemming from a better knowledge of bonded phase reactions and column technology. These improvements have brought chromatography to the point where a computer can either completely control an experiment or entirely simulate a chromatographic system to provide information not previously available by other experimental methods.

Peak deconvolution using fourier transform techniques enables the study of extra column band broadening processes. The removal of extra column band broadening from a chromatogram leaves only the column-induced band broadening which is essential for theoretical studies. From the practical point of view, this allows the calculation of the true column efficiency and also resolution can be improved for two peaks which are not well resolved.
Digital simulation of chromatographic processes approaches computer utilization from a different perspective. This work involves investigating the theoretical basis of chromatography. One method of studying these basic processes involves the development of a descriptive mathematical model. The model can be tested by using a digital computer to simulate the processes. A flexible computer language was developed for the simulations (22). This includes a hierarchial threaded code approach which, when implemented in microcode, decreases computer run time two to ten times and allows complex adsorption-desorption models to be studied in a reasonable length of time. Simulation of these probabilistic discrete event models would not be feasible without the use of a high-speed computer.

It is these computer-assisted investigations of chromatographic processes which are the basis of this thesis, and are presented in the next two chapters.
CHAPTER 2

DECONVOLUTION OF INSTRUMENTAL
AND COLUMN BAND BROADENING

Column efficiency in high performance liquid chromatography has improved during the last decade with the advent of 5 and 10 μm packing materials, improved bonded phases and better packing techniques. As better columns are made and efficiencies approach their theoretical limit, the importance of instrumental considerations in evaluating column performance will increase. This may occur with the microbore columns discussed recently by Reese and Scott (23) and Knox (24). The columns still have technological difficulties but have the potential for extremely high efficiencies. The ability to accurately measure these high efficiencies is directly related to the minimization of extra column band broadening or its quantitation and elimination. Instrumentally minimizing the extra column effects is the ideal; however, when using a commercial instrument, the latter approach may be the most realistic.

The band broadening due solely to column effects in liquid chromatography has been well characterized by Horvath and Lin (25), while the extra column contributions have been discussed in detail by Sternberg (26).
potentially limiting factor in HPLC has been addressed by several workers. Smuts et al. (27), stated that "there is reason to believe that the inlet system can ultimately be the limiting factor." Kirkland et al. (28) noted that "sampling can be a major cause of band broadening in HPLC." Recently, Colin, Martin and Guiochon (29) have examined the theoretical aspects of the injection in HPLC. Even with the considerable interest in injection-caused band broadening, the detector contribution to system band broadening has not been ignored. Detectors have been examined by many workers including Low and Haddad (30) and Stewart (31), who have discussed the detector time constant and its effect on peak shape. Although extra column contributions to band broadening have long been recognized, only recently have they become a significant factor with continually increasing column efficiencies.

Band broadening is a measure of the column and overall system efficiency. The sum of the variances of all column- and extra column band broadening processes is shown in the equation below:

\[
\sigma^2_{\text{Total}} = \sigma^2_{\text{Inj}} + \sigma^2_{\text{Col}} + \sigma^2_{\text{Det}} + \sigma^2_{\text{Tubing}} \quad (2.1)
\]

In order to understand the mechanisms of extracolumn band broadening, the effects of each source of variance must be known. So that all extracolumn effects can be considered
only as a function of the sampling and detection systems, the band broadening contributed by the connecting tubing will be included in the injector and detector terms of Equation 2.1. Because injection techniques do not interfere with the detection system, their variances can be treated independently. (The approach of defining extra column effects as variances is limited, however, because it assumes that the band broadening processes are gaussian in nature.)

This paper deals with deconvolution techniques based on Fourier transform (FT) which were used to examine extra column contributions to band broadening. Fourier transform deconvolution techniques do not imply a fixed function and have been applied to the isolation of column phenomena in chromatography (32) and resolution enhancement in steric exclusion chromatography (33). Recently, the use of Fourier transforms has been suggested as a technique to increase the information available in a multiplex chromatographic system (34). These techniques have also been used to correlate information from a frequency modulated multiple injection gas chromatographic system (35). The advantage that Fourier transform brings to this work is not the signal to noise improvement or the throughput advantage generally associated with FT, but the ability to deconvolve the extra column effects by using the frequency domain to define the individual components of the chromatographic peak.
Background

A general approach to the separation of extra column band broadening utilizes the convolution principle. Normally, we assume that the output chromatogram \( Y(t) \), is a result of the column transfer function \( H(t) \), convolved with the input function, \( X(t) \). Further, assuming that the input function \( X(t) \) is a perfect Dirac or unit impulse function, then:

\[
Y(t) = X(t) \otimes H(t)
\]

\[
\begin{align*}
&\text{FT} & &\text{FT} & &\text{FT} \\
\Rightarrow &Y(f) = X(f) & H(f) \\
\end{align*}
\]

and since the Fourier transform of a Dirac function is unity, then:

\[
Y(f) = 1 \times H(f) = H(f)
\]

\[
\begin{align*}
&\text{IFT} & &\text{IFT} \\
\Rightarrow &Y(t) = H(t) \\
\end{align*}
\]

Therefore, the chromatographic peak is equal to the transfer function of the column when the injection and detection systems are ignored. Long retention and good instrumentation makes this a reasonable approximation. As column efficiencies approach their theoretical limits, the extra column effects will become more apparent and correction will be necessary to accurately evaluate the column. A more complete description of a generalized chromatographic system is now presented.
Y(t) = X(t) \ast H_s(t) \ast H_c(t) \ast H_d(t) \quad (2.2)

where:

Y(t) is the detector output (the recorded chromatogram)
X(t) is the unit impulse or Dirac delta function
H_s(t) is the transfer function of the sampling system
H_c(t) is the transfer function of the column for a particular solute and temperature
H_d(t) is the transfer function of the detection system
t is a time domain operation
f is a frequency domain operation
\ast is the convolution operation

The fourier transform of Equation 2.2 yields:

Y(f) = X(f) \ast H_s(f) \ast H_c(f) \ast H_d(f) \quad (2.3)

Solving for the transfer function of the column in the frequency domain from Equation 2.3:

H_c(f) = Y(f) / [X(f)H_s(f)H_d(f)] \quad (2.4)

This equation now describes the interaction of the column with the solute of interest, independent of the extra column effects. However, only two of the four functions on the right-hand side of the equation are currently known (Y(f) and X(f)). The transfer functions of the sampling and detection systems
H_s(f) and H_d(f) can be solved either by a theoretical or an empirical approach.

In the theoretical approach, variables such as diffusion, geometry, time, tubing size, flow rate, and temperature are formulated in a complex equation to solve the response of a sampling valve or a detection system. This has been done in the case of both gas and liquid chromatography (36). These results could then be used directly for solving the above equations.

An empirical approximation of the transfer function of any one part of a chromatographic system can be made if the other sources of system response or variance can be minimized. For example, to learn the transfer function of the detection system, Equation 2.3 is rearranged:

$$H_d(f) = Y(f) / [X(f)H_c(f)H_d(f)]$$  \hspace{1cm} (2.5)

By eliminating the column and connecting the sampling valve directly to the detector, H_c(f) is eliminated (set equal to unity).

$$H_d(f) = Y(f) / [X(f)H_s(f)] \approx Y(f) / [1 \times 1]$$  \hspace{1cm} (2.6)

Since the fourier transform of a Dirac function is unity, then H_s(f) could closely approximate a unit impulse if the injection bandwidth is extremely short. If it is now assumed that H_d(f) is known, a similar approach to finding the
transfer function of the sampling valve is presented. Equation 2.3 can then be solved for \( H_s(f) \) yielding:

\[
H_s(f) = \frac{Y(f)}{[X(f) H_c(f) H_d(f)]} \quad (2.7)
\]

since \( X(f) \), the input generation signal is a Dirac function, and also eliminating the column results in:

\[
H_s(f) = \frac{Y(f)}{H_d(f)} \quad (2.8)
\]

The injection system contribution to the output signal can be solved since both terms on the right hand side of the equation are known. To check this contribution without assuming a knowledge of \( H_d(f) \), a detection system which would closely approximate a unit response to the signal would be needed. The transfer function of the sampling system could then be approximated by the recorded output signal according to:

\[
H_x(f) = \frac{Y(f)}{1} \quad (2.9)
\]

Once the transfer functions of the sampling and detection systems are solved, all of the variables in Equation 2.4 are known except for \( H_c(f) \), the column transfer function, or the elution chromatogram minus the extra column effects. Rewriting Equation 2.4 gives:

\[
H_c(f) = \frac{Y(f)}{[X(f) H_s(f) H_d(f)]} \quad (2.10)
\]

This equation is easily solved by two complex multiplications
and one complex division. An inverse fourier transform back to the time domain yields the corrected chromatogram.

\[
H_c(f) \xrightarrow{IFT} H_c(t) \tag{2.11}
\]

These mathematical manipulations do not mean that the actual solution is as easy. The real difficulty is in separating the various components of band broadening. Solving for one component generally assumes another is known. Approximations can be reasonably valid but are not a rigorous solution for each individual contribution.

An experimental gas chromatograph that minimized extra column band broadening was built by Gaspar, Arpino, and Guiochon (37). An extremely fast fluidic injection system with no moving parts was used, and the detector on the system was a flame ionization detector having a post column dead volume of only one microliter. High speed amplification kept the detector variance at a minimum. This system is not practical for commercial application, but the variance of either the injector or detector when compared with the complementary commercial system would be negligible.

Similar approaches to maximizing system efficiency must be done in HPLC to solve for the individual components of extra column band broadening. A high speed injection system such as the fluidic gate with a near unit input, when coupled with a commercial detector will approximate the
extra column band broadening of the detector. It then follows that a detector of minimal volume and virtually instantaneous response can be used to accurately describe a commercial injection system. A system with both of these features would have virtually no extra column contributions to band broadening and would accurately describe the band broadening of the column alone. One approach to separating the extra column contributions to band broadening will now be described.

Experimental

All experimental HPLC work was carried out using an Altex (Berkeley, CA) Model 332 Liquid Chromatograph equipped with a fixed wavelength UV detector set at 254 nm. Data collection was performed by a dedicated Hewlett Packard 2115 minicomputer which also transferred the data to a Hewlett Packard 2100A minicomputer for storage and analysis. This data system has been more completely described previously (35).

A μ-Bondapak C-18 column (Waters Associates, Milford, MA) was used in this work. The methanol and water used as the mobile phase were filtered through a 0.45 μm filter (Millipore) and vacuum degassed prior to use. Methanol was reagent grade (Fisher Scientific Co.). House-distilled water was redistilled from alkaline permanganate. The benzene
(Fisher Scientific Co.) and the series of alkyl benzenes (Aldrich Chemical Co.) were dissolved in methanol at approximately the 0.01% (v/v) level.

Results and Discussion

The chromatograph was configured with the injector and detector directly connected in order to obtain the total extra column band broadening. A corrected chromatogram was deconvolved from this overall measure of extra column band broadening and a system chromatogram. An example of the application of this technique is shown in Figure 2.1. Plot A is the apparent extra column contribution to the chromatogram, plot B is the uncorrected chromatogram and plot C is the deconvolved chromatogram. The retention time has shifted to a shorter time and the peaks are noticeably narrower. These are both expected results. The shorter retention time is due to the removal of extra column dead volume associated with the injector and detector which in turn narrows the peak.

This corrected chromatogram was analyzed for plate count and the results are compared in Figure 2.2, which shows the late eluting peaks have been overcorrected. Individual characterization of detector and injector is necessary in order to understand the contributions to band broadening. Using experimental and mathematical methods, the difficulty of separating the individual components physically for characterization can be circumvented.
Figure 2.1. Plot A is the apparent extra column contribution, plot B is the uncorrected chromatogram and plot C is the deconvolved chromatogram.
Figure 2.2. This plot shows the increase in plate count after applying a constant correction for extra column contributions to band broadening.
The following experiments were done to characterize the injector contribution to band broadening. First, the sample loop size was varied to examine the injection plug width contribution to band broadening. Four sample loops with volumes of 10 μl, 50 μl, 340 μl were used. The injector/detector profiles with no column in the system are shown in Figure 2.3. Second, the compression effect, which occurs at high k' values, was utilized to effectively eliminate any injector contribution to band broadening.

A mixture of benzene and a series of alkyl (C₂ to C₆) benzenes were separated, giving capacity factors which ranged from approximately 2 to 80. This injection was done with the four sample loops. The sample was diluted to maintain relative sample concentration as the sample loop volume increased. The results are shown in Figure 2.4. For short retentions, low k', the efficiency of the column is dependent on the sample size. By a k' of approximately ten, the number of plates has equalized, as plotted in Figure 2.5. This indicates an injection contribution to band broadening that decreases with increasing retention time, or k', as shown in Figure 2.6. This is the compression effect in which a long retained species tends to concentrate at the head of the column and negate any band broadening due to a large sample volume corresponding to a relatively long injection time.
Figure 2.3. Injection/detection profiles resulting from four different sample loops: plot A = 10 μl, plot B = 20 μl, plot C = 50 μl, plot D = 340 μl. — Flow rate = 2 ml/min.
Figure 2.4. Chromatograms resulting from injections using the four different sample loops. — Identification the same as in Figure 2.3.
Figure 2.5. Results of plate count analysis for each peak in Figure 2.4.
Figure 2.6. This plot shows the relationship of decreasing injection contribution to band broadening with increasing retention, or $k'$. 
Correction for the injector contribution must be done on an individual peak basis. This again is due to the compression effect which makes the correction for the injector band broadening a function of retention time. By assuming that the 10 µl injection is virtually ideal, the difference in the number of plates between the chromatograms using the 340 µl and 10 µl sample loops is injector effects. Chromatogram D in Figure 2.4 can be improved to mimic chromatogram A by deconvolving each peak with a gaussian function representing the injector contribution for that peak. The gaussian peak is representative of the sample plug width reduced appropriately to model the sample compression as shown in Figure 2.6.

The detector contribution to band broadening is constant for every peak since the detector is at the end of the line. This contribution consists of band spreading due to the dead volume of the heat exchanger, the cell volume, and the detector response time. A small constant correction due to these factors is shown in Figure 2.7. The effect of the time constant on peak shape is negligible according to Stewart (9) who has shown that a peak with a width at half height ten times greater than the time constant does not significantly alter the peak shape. Benzene, the earliest eluting peak of the series meets this criterium. So, therefore, this is a small contribution to overcorrecting the chromatogram.
Figure 2.7. The detector contribution to band broadening is a small but constant correction.
Conclusions

This study was designed to show the utility of convolution techniques using Fourier transforms to help quantify and eliminate extra column contributions to band broadening. As long as column efficiencies are significantly below their theoretical efficiencies, extra column contributions to band broadening are relatively insignificant. However, as better columns are made and efficiencies do approach their theoretical limits, then extra column corrections will become important.

An important sidelight to this work came in demonstrating that a constant plate number could not be obtained for a column, when all extra column corrections were applied. The constant plate assumption was discarded after all of the above considerations. Scott and Kucera (38) have suggested a changing retention mechanism, on silica, at a $k'$ of approximately ten. This can be logically extended to a bonded phase retention mechanism. Each species in a sample sees a different stationary phase. The longer a molecule is retained, the more time it spends in the stationary phase. When the stationary phase is viewed as a solvated bonded phase, the composition varies depending on the distance from the solid support. This is consistent with the recent work of Stetzenbach (39). With this view in mind, there is no
reason to believe that a column should have a constant plate count because each molecule is seeing a different environment.

Minimizing extra column effects is an important consideration in instrument development as well as accurate column evaluation. Instrumentally minimizing extra column contributions will lead the way for the application of high-efficiency HPLC columns. The accurate evaluation of columns made with this type of system can provide additional information about the column and the intrinsic chromatographic processes.
CHAPTER 3

DIGITAL SIMULATION OF CHROMATOGRAPHIC PROCESSES

A quantitative description of the retention mechanism in reverse phase high performance liquid chromatography has eluded researchers to date. This lack of success appears to be due to limited knowledge of the composition and function of the stationary phase. The factors which contribute to the stationary phase as a whole are: the support material and its structural characteristics, the uniformity and degree of coverage of the bonded phase, and the interaction of these with the mobile phase.

There have been many physical and chemical methods used in attempting to define the chromatographic stationary phase. The support material can be defined by particle size, average pore size, and nitrogen surface area determination. The amount of bonded material can be determined by carbon analysis. Attempts have been made to measure the number of residual silanol groups by titration methods which have been generally unsuccessful. Chromatographic measurements which help define the stationary phase include \( k' \), the capacity or retention factor, and \( N \), the number of theoretical plates which is a measure of column efficiency. Accurate
characterization of these stationary phase parameters is necessary but an appropriate theoretical model is necessary to understand the retention mechanism. Regardless of the amount of information available to define the system, it can undoubtedly be viewed as complex.

Currently the solvophobic theory is the most widely accepted retention mechanism theory. The basis was suggested by Locke (40) as well as Horvath, Melander and Molnar (41) who studied the mobile phase and its influence on solute retention. According to the solvophobic theory, retention is primarily a function of the mobile phase composition, with the solute-solvent interaction the most important. The bonded phase is considered a passive receptor of the solute with a much smaller influence on the retention.

The solvophobic theory was shown to be incomplete by Scott and Kucera (42) who tested the formation of the solute-stationary phase complex. The bonded stationary phase was shown to be more of an active receptor in the retention mechanism. Blevins, Burke & Hruby (43) have shown that the elution order of certain peptide hormones varies from column to column using the same mobile phase. Recently Horvath, Nahum and coworkers (44, 45) used crown ethers to investigate the role of available surface silanol groups in retention. The term silanophilic interaction was coined for this extension of the solvophobic retention theory. Each of these
investigations demonstrate that the stationary phase must be included in an accurate retention mechanism for HPLC. This however, increases the number of variables involved in and complexity of the retention mechanism.

The understanding of complex systems can be greatly aided by the use of digital computer simulation. A model can be described and tested with the high speed computations that a computer provides. Chromatographic adsorption-desorption, or partitioning mechanisms, are the fundamental processes occurring in the column. Connecting these processes to experimental results such as retention time and peak shape must be made by a model of the chromatographic system. These processes are generally expressed in mathematical terms which allow for direct adaptation to computer simulation.

Individually, most of the basic processes involved in chromatography such as adsorption, diffusion and mobile phase flow are fairly simple to model. When these processes are combined, however, the resulting system may be much more complicated and at times exhibit unexpected behavior. Digital simulation is a way of performing experiments upon these models in an attempt to understand their simultaneous interactions and the corresponding chromatographic processes.

The primary goal of this work is to better understand the chromatographic stationary phase by the use of digital
simulations. Understanding current theory regarding HPLC retention mechanisms is helpful in formulating an appropriate discrete event model of the system. Previous work has shown the qualitative adequacy of the models (46, 47). In this work, the ability of experimental data to quantitatively fit the simulation parameters has been evaluated.

**Background**

Modelling peak shapes with combinations of analytic functions is one form of simulation which has been applied to chromatography. Chesler and Cram (48) generated simulated chromatograms by combining gaussian, triangular, and exponential functions in a study of moment analysis. Simulation using analytic functions has also been widely proposed as a method for recognizing and assigning areas to overlapping chromatographic peaks (49). The major limitation of analytic function simulations is that there is no relation between the mathematics that describe the peak shape and the chromatographic processes that are responsible for the experimental peak shape.

Chromatographic processes have been modelled using continuous system simulation. In this type of simulation the model is described in terms of differential equations. Given a set of initial conditions for the variables, the computer then moves the system in simulated time numerically.
solving the differential equations at each point in time. This kind of simulation has been widely used in testing theories of chromatography (50, 51). As the models become more complex and include nonlinear or non-linear equilibrium behavior, the simulation can become more difficult and much slower.

In a discrete event simulation, the model is expressed in terms of mechanisms and event occurrence probabilities. Computer generated random numbers determine what events occur and collect statistics on the results. Chromatography itself has recently been simulated using the discrete event technique (47). Previously, some of the physical processes which are involved in chromatographic systems have been. Nakagawa has written computer programs to simulate both the Langmuir and BET type adsorption processes (52, 53). Nagakawa also simulated the adsorption-desorption process for surfaces with two different kinds of sites and with access to some sites hindered by the presence of pores. This is significant because it shows how a complex structure which is difficult to accurately describe in most theories of chromatography can be modelled using a computer program.

The three varieties of digital computer simulation (analytic function, continuous, and discrete event) are useful in different situations and require different techniques
for their implementation. The analytic function approach is generally the simplest to implement since it just involves the calculation of formulas with sets of numbers plugged in. Continuous system simulation is next because it has problems maintaining accuracy while simulating the passage of time in addition to the problems of calculating formulas. Discrete event simulation is the most difficult to fit into a computer since, in addition, it has the problem of maintaining statistical significance.

The most important reason a discrete event model was chosen to simulate the behavior of the chromatographic system is because, of the three varieties, it is the most natural way of thinking about the basic chemical processes involved. Complex nonlinear retention mechanisms inside a column are much easier to describe and understand in the form of discrete event computer algorithms than they would be as differential equations. The mechanisms of adsorption and desorption are commonly visualized by chemists as individual molecules approaching and interacting with individual surface structures as illustrated in Figure 3.1. Here a molecule in the mobile phase has some probability of encountering the surface and becoming adsorbed. Some time later, it comes loose and returns to the mobile phase in a desorption event. As in the work of de Boer (54), this approach can lead to simple and understandable models of retention processes.
Figure 3.1. This is an informal model of a one-step retention mechanism. -- The dot matrix represents the solvated stationary phase.
It is an easy step from this visualization to defining a formal model which can be simulated by computer. A discrete event simulation, however, requires the most work out of a computer and therefore the most care in the design of the simulation algorithms. The amount of computer memory available for tracking the chromatographic behavior during a simulation is very limited, which in turn limits the number of computations a computer can perform in a reasonable amount of time. Therefore, the model must be carefully described to efficiently simulate the intended system. This model system has been drastically reduced in both scale and the amount of detail included to meet these criteria.

For the above reasons, a liquid chromatographic system is a logical choice to model. Diffusion in the mobile phase is negligible and can be neglected along with all flow effects. The model concentrates on describing the surface composition and the sequence of interactions of the molecular probe. The model of the bonded stationary phase presented here assumes two main types of surface interaction. The most predominant and preferred interaction is with the bonded material. This is a simple linear system which implies that there is no competition between molecules for interaction sites. The second site is a residual surface group such as a silanol. The number of these sites is assumed to be
limited and therefore this interaction is characterized as nonlinear or Langmuir. A discrete event picture of this model is shown in Figure 3.2. The shaded area represents the stationary phase where the initial interaction is made. Then the solute molecule may possibly find a silanol group available on the surface where it can spend additional time. After either case, it returns to the mobile phase.

The necessity for two separate models is demonstrated by the chromatographic behavior of benzene and aniline on an Ultrasphere ODS column. A mobile phase composition of 60% methanol/40% water was used and the resulting chromatograms are compared in Figure 3.3. The retention and asymmetry are virtually identical with the results summarized in Table 3.1. The column efficiency was radically different for benzene and aniline however, indicating the likelihood of two retention mechanisms.

Of course more is involved in a chromatographic experiment than just the retention processes. As a minimum, the model must also provide for molecules being injected into a column, moving through the column while undergoing adsorption and desorption, and then being detected as they reach the end of the column. Figure 3.4 is a flow chart modelling the behavior of a molecule in a chromatographic system.
Figure 3.2. This is an informal model of a two-step retention mechanism. The dot matrix is the solvated stationary phase. The secondary interaction is shown to be specific with a surface silanol group.
Figure 3.3. Chromatograms of benzene and aniline on Ultrasphere ODS are shown. — The mobile phase consisted of 60% methanol/40% water.
Table 3.1. Summary of the experimental HPLC results.

<table>
<thead>
<tr>
<th>Column and Probes</th>
<th>Mobile Phase Composition Methanol/Water</th>
<th>Capacity Factor $k'$</th>
<th>Theoretical Plates $N$</th>
<th>Asymmetry Factor $A_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultrasphere</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>60/40</td>
<td>5.21</td>
<td>1160</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.61</td>
<td>21</td>
<td>1.63</td>
</tr>
<tr>
<td>Aniline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Spherisorb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>60/40</td>
<td>1.90</td>
<td>266</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>30/70</td>
<td>7.26</td>
<td>338</td>
<td>1.27</td>
</tr>
<tr>
<td>Aniline</td>
<td>60/40</td>
<td>1.00</td>
<td>158</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>30/70</td>
<td>3.09</td>
<td>93</td>
<td>3.04</td>
</tr>
<tr>
<td><strong>μ-Bondapak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>60/40</td>
<td>2.41</td>
<td>1190</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>30/70</td>
<td>15.63</td>
<td>1831</td>
<td>2.83</td>
</tr>
<tr>
<td>Aniline</td>
<td>60/40</td>
<td>0.62</td>
<td>911</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>30/70</td>
<td>2.76</td>
<td>969</td>
<td>2.69</td>
</tr>
<tr>
<td><strong>Lichrosorb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>60/40</td>
<td>3.91</td>
<td>1170</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>30/70</td>
<td>20.81</td>
<td>720</td>
<td>1.95</td>
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<td>Aniline</td>
<td>60/40</td>
<td>3.43</td>
<td>65</td>
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</tr>
<tr>
<td></td>
<td>30/70</td>
<td>4.20</td>
<td>124</td>
<td>5.37</td>
</tr>
</tbody>
</table>
Figure 3.4. This flow chart shows the sequence of events for a two-site chromatographic retention mechanism.
Experimental

All experimental HPLC work was carried out using an Altex (Berkeley, CA) Model 332 Liquid Chromatograph equipped with a fixed wavelength UV detector set at 254 nm. Data collection was performed by a dedicated Hewlett Packard 2155 minicomputer which transferred the data to a disk-based HP 2100A computer for storage and analysis. The HPLC columns used in this work are described in Table 3.2. The methanol and water used as the mobile phase were filtered through a 0.45 μm filter (Millipore) and vacuum-degassed prior to use. Methanol was reagent grade (Fisher Scientific Co.). House-distilled water was redistilled from alkaline permanganate. The benzene (Fisher Scientific Co.) and the freshly distilled aniline (Mallinkrodt Chemical Co.) were dissolved in methanol at approximately the 0.01% (v/v) level.

All simulations were also performed on the above-mentioned HP 2100A which has the following peripherals: a HP 7900A 2.5-million-word disk drive, a Tektronics 4002A graphics terminal, line printer and plotter. The computer includes 32K of core memory, hardware floating point instruction set, and writable control store. Special microprograms stored in writable control store greatly increase the speed of this computer. Typically, a simulation
<table>
<thead>
<tr>
<th>Column</th>
<th>Particle Size ($\mu$m)</th>
<th>Column Diameter (mm)</th>
<th>% Carbon (wt)</th>
<th>Endcapped</th>
<th>Surface Area $^f$ ($m^2/gm$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$-Bondapak C-18$^a$</td>
<td>10</td>
<td>250 x 4.6</td>
<td>10</td>
<td>Yes</td>
<td>325</td>
</tr>
<tr>
<td>Lichrosorb RP-18$^{be}$</td>
<td>10</td>
<td>250 x 4.6</td>
<td>20</td>
<td>No</td>
<td>150</td>
</tr>
<tr>
<td>Spherisorb ODS$^c$</td>
<td>10</td>
<td>250 x 3.1</td>
<td>5</td>
<td>No</td>
<td>220</td>
</tr>
<tr>
<td>Ultrasphere ODS$^d$</td>
<td>5</td>
<td>250 x 4.6</td>
<td>12</td>
<td>Yes</td>
<td>200</td>
</tr>
</tbody>
</table>

a. Waters Associates, Milford, MA.
b. E. M. Laboratories, Darmstadt, Germany.
c. Phase Separation Ltd., Queensferry, Clwyd., U. K.
d. Altex Associates, Berkeley, CA.
e. Slurry-packed in this laboratory.
f. Manufacturers values (55).
requires 3-6 hours of computer time. A detailed description of threaded programming as applied to these simulations has been dealt with previously (22,46). The parameters used in this model include a probability of the probe molecule encountering a stationary phase site (solvated bonded moiety), transferring to a high energy site (solvated surface silanol group), and subsequent probability of the probe returning to the mobile phase. The sample size and number of surface interaction sites of each type, bonded and residual, is also included in the parameters. The simulation parameters are related to the experimental data in the following manner:

1. The surface encounter probability, SEP, is calculated from the column plate count and the dimensions of computer memory being used (32K).

2. A measure of the relative movement of a molecule down the column is the capacity factor, k', from which the desorption probability can be calculated. The simulation parameter version of a capacity factor is the SEP divided by the desorption probability.

3. The probability of the molecule desorbing from a surface silanol group must be experimentally ascertained from peak shape comparisons.

The simulation surface site parameters are in units of interaction sites per probe molecule. The number of sites
in a column can be estimated from the amount of the packing material in the column, the surface area, and assuming 4.5 silanol groups/nm$^2$. The number of molecules in a sample can be calculated from the sample volume. The site per molecule ratio can then be calculated. In a linear system, this ratio is on the magnitude of $10^5$ (56) while for a nonlinear system, it is two or three orders of magnitude less. The total number of bonded sites can be calculated from carbon percent and surface area determinations. The ratio of bonded moieties to residual silanols is based on the percent surface coverage.

The simulated data is evaluated in the same manner as any chromatographic peak. Retention and peak shape are the results of interest. In this case however, it is the relative and not the absolute value of the simulated results which are meaningful. The simulated results are in memory-size units which correspond to an idealized chromatographic system. Relating the benzene and aniline results by means of alpha values allows for a meaningful comparison of the simulations and experimental data. Once this relative peak position is set, the band broadening factors can be considered. The peak shape and degree of tailing can be compared using peak asymmetry as a measure.
Results and Discussion

Examples of the experimental chromatograms are shown for Spherisorb ODS, \( \mu \)-Bondapak and Lichrosorb in Figures 3.5, 3.6 and 3.7, respectively. The columns were evaluated in terms of both peak shape and peak position of selected probe molecules with the results summarized in Table 3.1. These experimental systems will now be discussed in terms of how the physical characteristics and experimental results fit into a retention scheme consistent with the models presented here.

Aniline was chosen as a probe molecule because it has a functional group with the ability to specifically interact with solvated surface silanol groups as well as the possibility of nonspecific interaction of its aromatic ring with the solvated hydrocarbon bonded phase. This is compatible with the two-site model presented in Figure 3.2. A one-site model such as shown in Figure 3.1, that only allows for nonspecific interaction between the aromatic ring and the solvated bonded stationary phase is appropriate for benzene, which has no functional group for specific interaction. A meaningful comparison of stationary phases of different composition can be made from a consideration of the relative retention of these two probe molecules.

The composition of the stationary phase for three different columns at two mobile phase compositions will be discussed in terms of the resulting retention and peak shape.
Figure 3.5. Examples of experimental chromatograms for benzene and aniline on Spherisorb ODS are shown.
Figure 3.6. Examples of experimental chromatograms for benzene and aniline on μ-Bondapak C-18 are shown.
Figure 3.7. Examples of experimental chromatograms for benzene and aniline on Lichrosorb RE-18 are shown.
The retention of benzene which follows the order; Lichrosorb > \(\mu\)-Bondapak > Spherisorb, corresponds directly to the percent hydrocarbon of the bonded phase of these columns as shown in Table 1. This pattern holds true as the mobile phase composition is changed from 60% methanol / 40% water to 30% methanol / 70% water. The order of elution for aniline is: \(\mu\)-Bondapak > Spherisorb > Lichrosorb for both mobile phase compositions. The peak asymmetry for both probes increases as the mobile phase is changed from 60% methanol / 40% water to 30% methanol / 70% water on all columns. The plate count for both benzene and aniline varies as a function of both mobile phase composition and stationary phase identity. This is indicative of the large differences in the types of interactions responsible for the retention of each of the probe molecules under these particular chromatographic conditions.

The retention behavior of aniline can be described in terms of a two-site model (Figure 2). Relative to benzene, aniline shows significantly less retention largely because of its strong hydrogen bond character which makes it extremely soluble in the methanol / water mobile phase. An increase of four times in percent hydrocarbon (Spherisorb to Lichrosorb) does not cause an increase in retention which could be expected if the nonspecific contribution of the aromatic ring dominated. A strong secondary interaction with solvated silanol groups is necessary to explain this retention
behavior. Although µ-Bondapak has an intermediate percent hydrocarbon coverage, it exhibits the shortest retention of the three columns. This is apparently due to the endcapping process (57) which covers residual surface silanol groups.

A mobile phase change to higher polarity shows an increase in the asymmetry as well as retention for aniline on each column. The plate count increased on the columns with the highest percent bonded phase (µ-Bondapak and Lichrosorb) but decreased on the Spherisorb column. The initial interaction of the aromatic ring with the solvated bonded phase is apparently more important at this mobile phase composition. The lightly bonded Spherisorb column shows an overall predominance of the solvated silanol interactions after limited interaction with the solvated bonded phase. The Lichrosorb column with 20% hydrocarbon shows increased plate count indicating a greater efficiency due to the increased possibility of interaction with the solvated bonded phase. The increase in asymmetry also indicates significant secondary interaction with the solvated silanol surface. The µ-Bondapak has the greatest efficiency with the least retention which is apparently due to the lack of solvated silanol groups, a function of endcapping, which presents a uniform surface to the aniline molecule. As a result of this uniformity, the µ-Bondapak exhibits the greatest degree of initial interaction with the solvated bonded phase. Even with the limited number of
available solvated silanol groups, asymmetry is an indication of significant retention relative to benzene when the secondary interaction occurs.

This discussion has detailed the experimental results in terms of the interaction of benzene and aniline with the various stationary phase compositions presented by the three columns. Qualitatively, the Spherisorb presents a surface which has the most polar nature because of low hydrocarbon coverage leaving the most available residual silanol groups. Lichrosorb has the highest percent of hydrocarbon coverage with residual silanol groups still available because of no endcapping. \( \mu \)-Bondapak presents the stationary phase most hydrocarbon in nature, because endcapping virtually eliminates residual silanol groups and shows the most uniform surface. From the physical data presented in Table 3.2. and assumptions made previously, the Spherisorb ODS has approximately 12% of the available surface covered with bonded material, the \( \mu \)-Bondapak C-18 has 17% of the sites bonded (without consideration of the endcapping), and the Lichrosorb RP-18 has 83% of the available surface covered with bonded material. These values can be used to initially set the simulation site type ratio of bonded sites to residual sites in order to mimic the experimental peak shapes.

The simulations presented here concentrate on the \( \mu \)-Bondapak and Spherisorb columns. From the information above,
the $\mu$-Bondapak column has high efficiency with relatively little second site interaction, while the Spherisorb column demonstrates the most straightforward application of the two-site model. Figures 3.8 and 3.9 compare examples of the experimental chromatograms and the best simulated results for benzene and aniline on $\mu$-Bondapak at the two mobile phase compositions. Figures 3.10 and 3.11 show the same comparisons of benzene and aniline on Spherisorb.

A simulated column capacity can be defined using the experimentally determined relative retention between benzene and aniline. Once this has been established, a combination of the site type ratio, which is the description of the stationary phase composition, and the secondary interaction (residual silanol) desorption are taken into account in order to approximately shape each individual peak. Initially, the simulation parameters were estimated from the bulk physical data and experimental chromatographic results. It was necessary to modify these parameters in order to arrive at consistent results from the simulations. The results are summarized in Table 3.3. The initial estimates for the secondary desorption probability were determined from solution hydrogen bond interactions on the order of 7 kcal/mole which is two to three times greater than the energy of interaction of the solute with the solvated bonded material. The secondary desorption probability was varied between a factor of one
Figure 3.8. Comparison of experimental chromatograms and simulated results for benzene and aniline on μ-Bondapak. The mobile phase is 60% methanol/40% water.
Figure 3.9. Comparison of experimental chromatograms and simulated results for benzene and aniline on $\mu$-Bondapak. The mobile phase is 30% methanol/70% water.
Figure 3.10. Comparison of experimental chromatograms and simulated results for benzene and aniline on Spherisorb ODS. — The mobile phase is 60% methanol/40% water.
Figure 3.11. Comparison of experimental chromatograms and simulated results for benzene and aniline on Spherisorb ODS. -- The mobile phase is 30% methanol/70% water.
Table 3.3. A comparison of simulated and experimental stationary phase compositions.

| Column and Probe | Percent Surface Coverage |  
|------------------|-------------------------|-------------------------|-------------------------|
|                  | Bulk\(^a\) | 60/40\(^b\) | 30/70\(^b\) |
| Spherisorb ODS   | 12%        |             |             |
| Benzene          | 98%        | 97%         |             |
| Aniline          | 95%        | 88%         |             |
| \(\mu\)-Bondapak C-18 | 17%\(^c\) |             |             |
| Benzene          | 96%        | 98%         |             |
| Aniline          | 98%        | 92%         |             |

\(^a\) Calculated from physically available values.

\(^b\) Percentages from site type ratio for the listed methanol/water mobile phase composition.

\(^c\) Endcapping procedure not considered.
to ten less than the initial interaction. More consistent peak shapes were simulated when the difference was more pronounced (the factor of ten energy difference). A surface composition considerably different than that calculated from bulk data was also necessary for obtaining consistent simulation results. A considerably smaller number of available high energy sites was needed to give the appropriate peak shapes. These modifications are significant because they point out the limitations of using non-chromatographically obtained data to characterize chromatographic stationary phases.

Conclusions

This discrete event approach to simulation has shown the quantitative ability to consistently model a chromatographic system which can provide a better understanding of HPLC retention mechanisms. It has been demonstrated with the simple one-site and two-site models presented here, that each different molecule has a different retention mechanism. This is due to how the probe molecule interacts with the dynamic solvated stationary phase and changes in stationary phase composition.

The simulated results demonstrate the inaccuracy of applying bulk physical data to the characterization of chromatographic stationary phases. From a descriptive point of
view, this discrepancy can be attributed to the nitrogen surface area determination method. The surface available to the nitrogen molecule can be much greater than the corresponding surface area available to a chromatographic probe molecule because of the size difference, nitrogen being smaller. On a bonded column, the attached moiety may sterically hinder access to many residual silanol sites, thus creating an apparent stationary phase that is chromatographically more completely covered than the physical data suggests. It is apparent that accurate characterization of the stationary phase can only be accomplished by using carefully defined chromatographic methods.

The discrepancy brought out between the initial and secondary energies of desorption indicate that the molecular interactions are more complex than allowed by the simple one- and two-site solution type interaction models presented here. The initial interaction indicates a probably ternary solvent interaction. The solute molecule can interact with molecules of each component of the mobile phase as well as the bonded moiety. This initial interaction must be considered as a range of interactions throughout the depth of the solvated stationary phase, which itself varies.

Simulation results consistent with the experimental results demonstrate the applicability of these models. While they are admittedly simplistic, for these initial
investigations, they have shown their utility by showing the fundamental inaccuracy of stationary phase characterization methods. Additionally, the complexity of the retention mechanism in HPLC has been confirmed.
CHAPTER 4

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This work has demonstrated the value of computer-assisted chromatographic investigations. The use of the computer, in these cases, is not merely a convenience to the investigator; but experiments which otherwise could not be carried out profitably can now be done. Additional information leading to a better understanding of chromatographic fundamentals is the result of these experiments.

The work presented in the two preceding chapters is significantly different in conception and direction. Both require the use of a high-speed computer in order to perform the necessary calculations. The deconvolution of column and extra column band broadening could only be realistically undertaken using a fast fourier transform algorithm specifically designed for computer application. The digital simulations can be performed in a reasonable length of time only by the use of computers. Each of these investigations provides information virtually inaccessible without a computer.
Conclusions

The utility of convolution techniques using fourier transforms to help quantitate and eliminate extra column contributions to band broadening was shown in Chapter 2. As long as column efficiencies are significantly below their theoretical efficiencies, extra column contributions to band broadening are relatively insignificant. However, as better columns are made and efficiencies do approach their theoretical limits, then extra column corrections will become important.

Additional information resulting from this work showed that a constant plate number could not be obtained for a column, when all extra column corrections were applied. The constant plate assumption was discarded after all of the above considerations. A changing retention mechanism, on silica, at a $k'$ of approximately ten, has been suggested which can be logically extended to a bonded phase retention mechanism. Each species in a sample interacts differently with the stationary phase. When the stationary phase is viewed as a solvated bonded phase, the composition varies depending on the distance from the solid support. This is consistent with the recent work which supports the view that a column does not necessarily have a constant plate count because each molecule is seeing a different environment.
Minimizing extra column effects is an important consideration in instrument development as well as accurate column evaluation. Instrumentally minimizing extra column contributions will lead the way for the application of high-efficiency HPLC columns. The accurate evaluation of columns made with this type of system can provide additional information about the column and the intrinsic chromatographic processes.

The discrete event approach to simulation presented in Chapter 3 has shown the quantitative ability to consistently model a chromatographic system which can provide a better understanding of HPLC retention mechanisms. It has been demonstrated with the simple one-site and two-site models presented here, that each different molecule has a different retention mechanism. This is due to how the probe molecule interacts with the dynamic solvated stationary phase and changes in stationary phase composition.

The simulated results demonstrate the innacuracy of applying bulk physical data to the characterization of chromatographic stationary phases. From a descriptive point of view, this discrepancy can be attributed to the nitrogen surface area determination method not accurately reflecting a chromatographic surface area. On a bonded column, the attached moiety may sterically hinder access to many residual
silanol sites, thus creating an apparent stationary phase that is chromatographically more completely covered than the physical data suggests. It is apparent that accurate characterization of the stationary phase can only be accomplished by using carefully defined chromatographic methods.

The discrepancy brought out between the initial and secondary energies of desorption indicate that the molecular interactions are more complex than allowed by the simple one- and two-site solution-type interaction models presented here. The initial interaction indicates a probable ternary solvent system in which the solute molecule can interact with molecules of each component of the mobile phase as well as the bonded moiety. The initial interaction must be considered as a range of interactions throughout the depth of the solvated stationary phase, which itself varies.

**Future Research**

These experiments were designed to provide additional information about chromatographic processes available only by computer methods of analysis. As well as successfully returning the intended results, these studies provided additional information regarding fundamental characterization of the chromatographic process complementary to current parallel research. While the two chapters present significantly different approaches to investigating the chromato-
graphic problem, shortcomings demonstrated by the digital simulation can be investigated using FT correlation techniques. This shows the interrelationship of fundamental investigations.

The digital simulations have shown the inaccuracy of present bulk stationary phase characterization methods. FT multiple-injection techniques can dynamically modify the stationary phase with various probe molecules to provide accurate quantitative information. This procedure is now experimentally feasible for characterizing gas chromatographic packed and open tubular columns. Work is being done on a multiple-injection technique for HPLC. These experimental methods will allow for extended application of the simulations which can return even more reliable quantitative results. The controlled dynamic modification of the stationary phase will return information helpful in further defining the HPLC solvated stationary phase. The relationship of these independent experiments fully utilizing computers, will provide information essential to establishing a quantitative retention mechanism in HPLC.
REFERENCES


