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DIGITAL SIGNAL PROCESSING TECHNIQUES

APPLIED TO CHROMATOGRAPHY

by

Carl Daniel Villalanti

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A Dissertation Submitted to the Faculty of the

DEPARTMENT OF CHEMISTRY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read

the dissertation prepared by ___Carl Daniel Villalanti___

entitled ___Digital Signal Processing Techniques Applied to Chromatography.___

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and recommend that it be accepted as fulfilling the dissertation requirement

for the Degree of ___Doctor of Philosophy___.

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I hereby certify that I have read this dissertation prepared under my
direction and recommend that it be accepted as fulfilling the dissertation
requirement.

M F Burke _____ _5-5-80_____
Dissertation Director   **Date**

And now I see with eye serine

The very pulse of the machine.

        William Wordsworth
        (She Was a Phantom of Delight,
         Stanza 3)

ACKNOWLEDGMENTS

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ABSTRACT

The field of analytical chemistry has always taken advantage of advances from other fields. The application of digital signal processing techniques to chromatographic systems has been shown to enhance the chemical information concerning the solute-adsorbent interaction.

Applying the techniques of Fourier transforms, spectrum analysis, correlation, and convolution has expanded the scope of multiple injection type experiments. A review of these signal processing techniques relevant to analytical chemistry is given.

The effects of restricting the concentration range a solute undergoes was the impetus for a new type of multiple injection experiment, known as frequency modulated (FM) correlation chromatography. By limiting the deviation in concentration to a small portion of the isotherm, gaussian shaped peaks can be obtained even when working in a nonlinear portion of the isotherm.

The characterization of the experimental parameters in FM experiments (i.e. carrier frequency and modulation bandwidth) and the effects on peak shape, retention time, and signal-to-noise ratios was evaluated.

The FM multiple injection technique was then applied to the study of linear and nonlinear behavior on a homogeneous (i.e. Porapak) and heterogeneous (i.e. Durapak) chromatographic support. Specific types and strengths of solute-adsorbent interacts are proposed.

The implications of multiplex and modulated techniques to linear system chromatography and the isolation of extra-column band-broadening effect (via a Fourier deconvolution approach) in modern open tubular GC and HPLC conclude this work.

CHAPTER 1

INTRODUCTION

Separation Science is a broad discipline encompassing a
variety of techniques. A major branch of separation is chromatography,
an entire science based on a deceptively simple idea. The differential
migration of components of a mixture through a fixed medium according to
their mutual affinity. One of the first experiments using this method
was the separation of a mixture of plant pigments. A glass column
filled with limestone with xylene as the eluting liquid separated the
various chlorophylls into different colored eluting bands. Tswett (1903)
named the resulting phenomena chromatography or color writing. From
this simple beginning the important concepts were put forth:

1. A stationary phase (limestone)

2. A mobile phase (xylene)

3. Mixture introduction to the head of the column

4. A detector (the eye perceiving the colored pigments)

The groundwork for thin-layer chromatography (TLC), paper chromatography,
and liquid-liquid chromatography (LLC) evolved in the ensuing years. A
history of chromatography (Ettre and Zlatkis 1980) reviews these and
other developments.

A major advancement in chromatography came in 1941 (Martin and
Synge 1941) with the idea of using a gas for the mobile phase. From

these historic developments an entire field of endeavor, as varied as the compounds that need separating, has emerged.

The unique ability to identify and quantitate a complex mixture to trace level concentrations has placed chromatography in an essential role in environmental, energy, and medical research. While the 50s and the 60s witnessed the growth from infant to adolescence, the 70s and 80s will see the maturation of the art. The goals of better separations at lower levels appears to be permanent, especially when dealing with the many toxic agents in the biosphere (i.e. dioxins, PCBs, etc.).

Mechanisms of differential migration down a chromatographic column rely on a retarding force exerted on the solutes (mixture components) by the stationary phase.[1] The stronger the affinity of the solute for the stationary phase, the greater the amount of solvent or gas necessary to elute the species from the column. Mechanisms of the retardation force include adsorption, partitioning into a liquid phase, and molecular sieving. Each of these mechanisms has been exploited to achieve separations of closely related compounds.

An understanding of the multiple injection technique is helped by a review of the thermodynamics and kinetics involved in chromatography.

_____

1. Retardation may occur with the absence of a stationary phase by the application of a field-interaction with the solute. Gravitational, electrical, thermal, etc. fields have been used in this technique known as field-flow fractionation, pioneered by Giddings, Fisher, and Myers (1978).

Equilibrium Approach to the Elution Peak Position

If a solute is non-retained it will exist entirely in the mobile phase, if it is irreversibly bonded or adsorbed, it will never elute. Useful chromatography occurs between these limits. A quantitative measure of the affinity between the solute and the stationary phase is known as the capacity factor or k'.

$$k' = \frac{\text{amount of solute in stationary phase}}{\text{amount of solute in the mobile phase}}$$

All things being equal, this can be a measure of the amount of a liquid or bonded-phase present in the column. The capacity factor (k') is easily calculated from the retention times or volumes by:

$$k' = \frac{t_r - t_m}{t_m} \quad \text{or} \quad \frac{v_r - v_m}{v_m} \tag{1.1}$$

When the strengths of interaction are varied (i.e. $\Delta G°$s), the k's of the different solutes will differ enough to provide the separation. The quantitative measure of this difference is called the separation factor (α)

$$\alpha = k'_1/k'_2 \tag{1.2}$$

The Gibbs free energy (G) is related to the equilibrium constant (in this case the distribution constant K°) according to:

$$\Delta G° = -RT \ln K° \tag{1.3}$$

Being a true thermodynamic constant K° is the ratio of activities based on standard states of the solutes in the mobile and stationary

phase (Freiser and Fernando 1963, Conder and Young 1979). The retention volume V_r is related to the distribution constant according to their mechanisms, be it partitioning, adsorption, or size exclusion, according to:

$$V_r = V_m + KV_s + K'A_s + K''V_i \qquad (1.4)$$

where the distribution constant for partitioning, adsorption, and sieving are K, K', and K''. Other parameters include V_m, the column void or dead Volume, V_s the stationary phase volume, A_s the adsorbent's surface area, and V_i the interstitial or pore volume of an exclusion type packing material. Most chromatographic packings are designed for one particular type of mechanism, however, it is rare that only one mechanism is operating when using the variety of solutes on the same packing material. Examples of secondary adsorption occurring in a partitioning column are common. Deactivation of the surface can often minimize the problem.

If we assume only a simple case of adsorption on a homogeneous surface equation 1.4 simplifies to:

$$V_r = V_m + K'A_s \qquad (1.5)$$

Solving for the distribution constant yields:

$$K' = \frac{V_r - V_m}{A_s} = V_s^t \qquad (1.6)$$

where V_s^t is the corrected retention volume. Similar simplifications exist for partitioning and size exclusion cases.

The effect of temperature on the equilibrium constant is expressed in the Gibbs-Helmholtz equation:

$$\frac{\gamma(\Delta G^\circ/T)}{\gamma(1/T)} = H^\circ \tag{1.7}$$

substituting equation 1. and integrating yields:

$$\ln V_s^t = - \frac{\Delta H^\circ}{R}\left(\frac{1}{T}\right) + C \tag{1.8}$$

This equation fits the form $y = mx + b$, so a plot of $\ln V_s^t$ versus reciprocal temperature gives a line with a slope of $- H^\circ/R$ (Fig. 1.1). The Y intercept is equal to $\Delta G^\circ/RT$ or $\Delta S^\circ/R$. Thus the thermodynamic state variables can be precisely obtained by a series of chromatographic experiments.

Average values of H° or the heat of phase transition from the mobile to stationary phase are approximately 3-7 kilocalories per mole for partitioning and 9-15 kilocalories for adsorption.

The entropy values for the particular experiment can be solved from the equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad \text{yielding} \quad \Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \tag{1.9}$$

The entropy values obtained give an estimation of the difficulties that a molecule has in obtaining the proper orientation to make a fruitful interaction with the packing (e.g. a phase transition or adsorption). In partitioning or exclusion chromatography, the entropy values are very small because little or no orientation is necessary,

van't Hoff plot

$$\text{slope} = -\Delta H^{\circ}/R$$

In K
or
In V_s^t

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

$(\text{temperature})^{-1}$
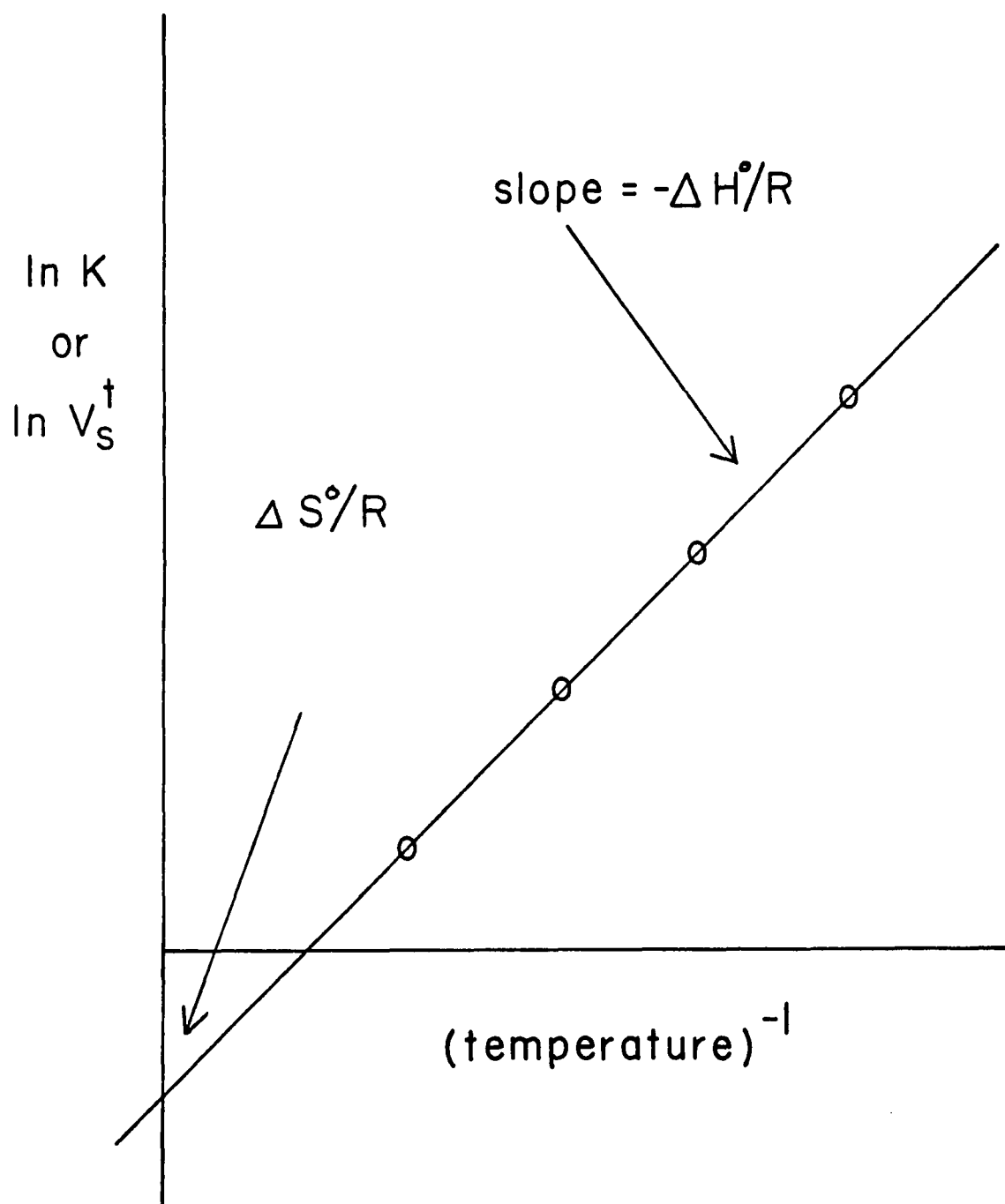
Figure 1.1. Thermodynamic measurements from chromatographic data, the van't Hoff plot.

however in adsorption, the molecule often needs a particular orientation to "fit" the adsorbent, and therefore entropy values are higher.

Intracolumn Band Broadening and the Quantitation of Column Efficiency

The thermodynamic parameters forementioned predict the retention time of any component on a specified chromatographic system. However, practical separations require that the physical nature of the differential migration be efficient. This means that the number of solute-stationary phase interactions be maximized and that intracolumn band-broadening be minimized.

The first approach to quantifying column efficiency was made by Martin and Synge (1941). Before suggesting a gas as the mobile phase and thus the birth of GLC, these two investigated liquid-liquid partition chromatography, and likened the migration of a solute down the column to the operation of a distillation column divided into N elements, each of which attains equilibrium of the gas phase with the stationary element. A similar approach to the separation of liquid-liquid systems described by Craig (1973) is essentially a series of solvent extraction experiments assembled in a manner providing automated operation. Again, equilibrium is established in each extraction element.

A complete description of the migration down a discrete distillation apparatus can be described by Newton's binomial distribution:

$$w(n,r) = \frac{h!}{r!\ (n-r)!}\ p^r\ q^{(n-r)} \qquad (1.10)$$

where:

n = # of incremental volume elements

$w(n,r)$ = proportion of original mass remaining after r volume

elements

p and q are related to the distribution of the gas between the

vapor and the liquid phase

As the number of volume elements or "plates" is increased, equation 1.10 approaches a normal or gaussian distribution. Thus, the broadening of a gaussian peak (i.e. the variance or σ^2) is a measure of the number of plates (n). Even when a gaussian shaped peak arises from a continuous process as in a chromatographic column, the plate model can still be used to measure n or number of theoretical plates corresponding to the standard deviation of a peak as a function of the retention time according to:

$$n = (t_r/\Sigma)^2 \qquad (1.11)$$

Transforming equation 1.11 by simple gaussian equalities yields:

$$n = 5.545 \ (t_r/w_{.5})^2 \qquad (1.12)$$

where $w_{.5}$ is the peak width at half height.

A correction for the dead time (t_m) or the dead volume (v_m) is often used to describe the effective number of theoretical plates N.

$$N = 5.545 \ [(t_r - t_m)]/w_{.5}] \qquad (1.13)$$

where N and n are related by the capacity factors by:

$$N = n(k'/k' + 1)^2 \qquad (1.14)$$

The importance of Martin's work (Martin and Synge 1941) is that the efficiency of any form of chromatography can be quantified from any peak on a chromatogram. A measure of column efficiency is not only good for column to column comparison, but can also serve as a diagnostic signal when column performance is deteriorating. Manufacturers now specify column performance in terms of plates per meter.

Another parameter often used is the height (or length) equivalent to one theoretical plate, abbreviated H.E.T.P. which is defined as

$$\text{HETP or H} = \text{column length/\# of theoretical plates} \qquad (1.14)$$

The major weakness of the plate model approach is that no mechanisms of the band broadening inside the chromatographic column are used to describe the system. A band broadening model in which the individual processes responsible for the overall shape of the peaks was proposed by van Deemter, Zuiderweg, and Klinkenberg in 1956. In this model the various band broadening mechanisms which contribute to the variance of the peak are assumed to be independent and thus additive:

$$\sigma^2 \text{ total} = \Sigma\sigma^2 \text{ individual processes} \qquad (1.15)$$

An overall, but simplified equation describing the most important band broadening processes according to the van Deemter approach is:

$$H = A + B/u + (C_g + C_1 + C_k)u \qquad (1.16)$$

or

$$Cu$$

(where u = flow rate in cm/sec.)

The A term arises from the eddy diffusion or the different path-lengths that a molecule must traverse on its tortuous journey through a packed column. By using small spherical particles of narrow size range the flow independent A term can be minimized. The B term describes the variance from the longitudinal diffusion of the solute in the mobile and stationary phase. Since the diffusion constants of a gas are approximately 10^5 greater than a liquid this term is of little significance in liquid chromatography. Further, since the B term is divided by u (flow rate) measured in cm/sec, increasing the flow minimizes the longitudinal diffusion by shortening the retention time of the solute. The C term describes the various mass transfer rates associated with the retention processes. These arise from the finite rate of equilibrium of the solute with the stationary phase. C_g, C_1, and C_k define the resistance to mass transfer: into and out of the mobile phase, the stationary phase for partitioning, and the surface for adsorption, respectively. In most cases, one of the C terms predominates and therefore simplifies equation 1.15. A detailed description of the components of the A, B, and C terms is included in the Littlewood text (1970).

The effect of flow rate on the efficiency has been a powerful tool in developing chromatographic theory. The simplified van Deemter equation describes a hyperbola (seen in the plot of H vs flow rate).

Setting the derivative of dH/du to zero, to obtain the minimum plate height H_m gives:

$$H_{min} = A + 2(BC)^{.5} \text{ and } u_{opt} = (B/C)^{.5} \qquad (1.17)$$

Typical values of the van Deemter equation variables in common GC packed columns are:

$A = 0 - 1$ mm $H_{min} = 0.5 - 2.0$ mm

$B = 10$ mm^2/sec $u_{opt} = 1 - 10$ cm/sec

$C = -.001 - 0.01$ sec

The great advances in chromatographic systems have each addressed the band broadening processes described in the van Deemter equation. Capillary or open tubular GC, having no packing material, has no A term. HPLC, now comparable in efficiency to packed column GC when 5-10 micron packing materials are used, essentially has no B term. Field-flow fractionation (FFF) has eliminated the packing and being a liquid technique has eliminated the A, B, C_1, and C_k terms. Work on a one piece porous silica matrix for HPLC is currently under way and this would eliminate the A term. Thus the goal of more efficient columns giving rise to better separations in less time has been greatly aided by the van Deemter approach.

Thermodynamic and Kinetic Considerations to Separations

The quantitative measure of the degree of separation or resolution is expressed as:

$$R = 2(t_{r2} - t_{r1})/(w_1 + w_2) \qquad (1.18)$$

where w_1 and w_2 are the baseline tangent widths.

The general problem of separating two similar compounds now comes down to:

1. obtaining the narrowest peaks (kinetics)

2. obtaining the maximum difference in the retention times or k's (thermodynamics)

3. optimizing the k'_2 or retention of the latter peak

These are summarized in the fundamental resolution (R_s) equation:

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{k'_2 + 1}\right) \qquad (1.19)$$

The current trends in capillary GC is to minimize the number of stationary phases and thus compromise the difference in retention times, but with the great permeability of the open tubular columns, longer lengths and thus greater efficiency have provided the baseline separation (i.e. R of 1.5 or greater for 99.9% separations) desirable for quantitative results.

The Two Edge Approach to Chromatographic Investigations

The purpose of conventional chromatography is the separation and quantitation of unknown mixtures. To affect this separation requires a knowledge of the underlying principles of the chromatographic process. Although static techniques can provide information about distribution ratios, thermodynamics, and capacity, the chromatographic system itself can be used as a dynamic method of studying the nature of the interaction

between solute and column packing. This diagnostic role is many times the method of choice in the characterization of chromatographic materials. The measurement of adsorption isotherms via a modulated solute approach will be presented later.

Theory of Adsorbents

Most forms of practical analytical chromatography assume linear isotherms. This simply means that the distribution constant K and the capacity factor k' are independent of solute concentration. Thus a plot of concentration of solute in the mobile phase versus concentration in the stationary phase will be a straight line. Basically, nonlinear isotherms arise from three different scenarios:

1. adsorption site overload caused by an excess amount of solute

2. non-homogeneous packing materials (i.e. two or more different types of adsorption sites or retention mechanisms)

3. non-equilibrium cases caused by limiting rates of adsorption and/or desorption

The most common type of nonlinearity produces a Langmuir isotherm. This can arise on an adsorbent when a significant amount of the available adsorption sites become covered with solute so that some of the molecules will not experience the same environment as the others. These molecules could interact with molecules of themselves already adsorbed to the surface, but these interactions are usually much weaker than the adsorption on the surface. (In a few cases these inter-molecular attractions are stronger than the original interactions with the surface, and a fronting peak with a sharpening front (Fig. 1.2).

types of isotherms
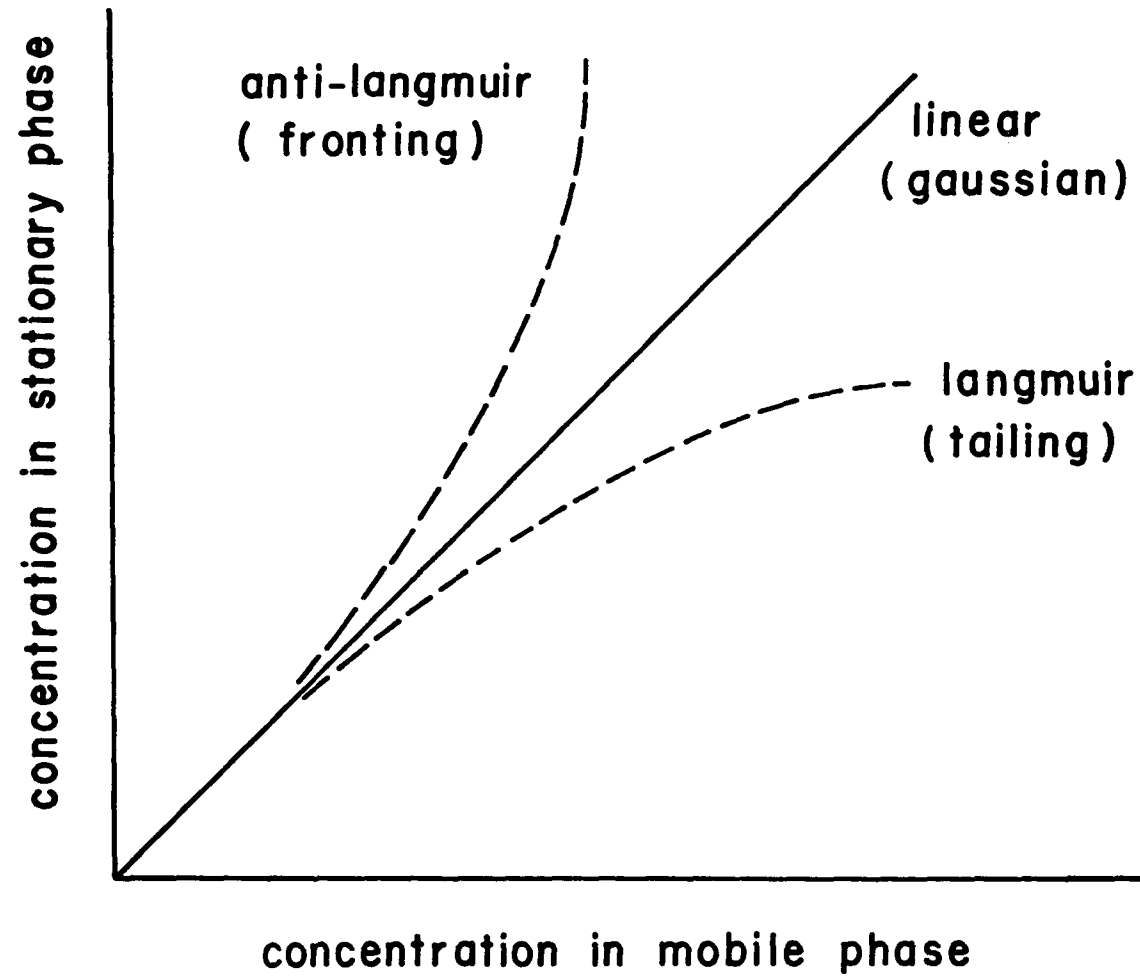
Figure 1.2. Types of isotherms.

With the advent of sensitive ionization detectors, the problems of nonlinear effects caused by site overload can be minimized by using lower concentrations. Nonlinearity caused by cases two and three is not cured so easily.

Peak asymmetry can arise even when using low concentrations if nonhomogeneous surfaces are encountered. This could be caused by residual silanols in GLC or reversed-phase LC, double bonds in graphitic type surfaces, etc. For the most part, designers of chromatographic packing materials try to eliminate the causes of the effects.

A quantitative measure of the asymmetry is shown in Figure 1.3. Peak Asymmetry (A_s) is defined as the ratio of the half peak widths at 10% of the peak height. Since peak asymmetry causes problems in quantitation and interpretation of unseparated peaks, some manufacturers imposed limits of 0.90-1.20 on the values of A_s.

In modern surface modified or bonded phase GC and LC packing materials, the use of monofunctional silyl reagents (one reactive Cl per molecule) prohibits undesirable polymerization and condensation reactions. Using this technique, mixed retention phenomena (i.e. the influence of unreacted surface silanols on solute retention) is minimized because of maximum coverage. In spite of these "homogeneous" hydrocarbon surfaces, polar solutes can still react with the residual silanols despite claims in the literature that no residual OHs are left on the surface.

One bonded phase packing material (^{R} Durapak N-octane) is actually classified as polar, even though a C_8 carbon chain is bonded to the surface. This seemingly contradictory classification was explained by a Waters scientist and explained in the Durapak literature (1974) to

measurement of asymmetry

$$A_s = a/b$$
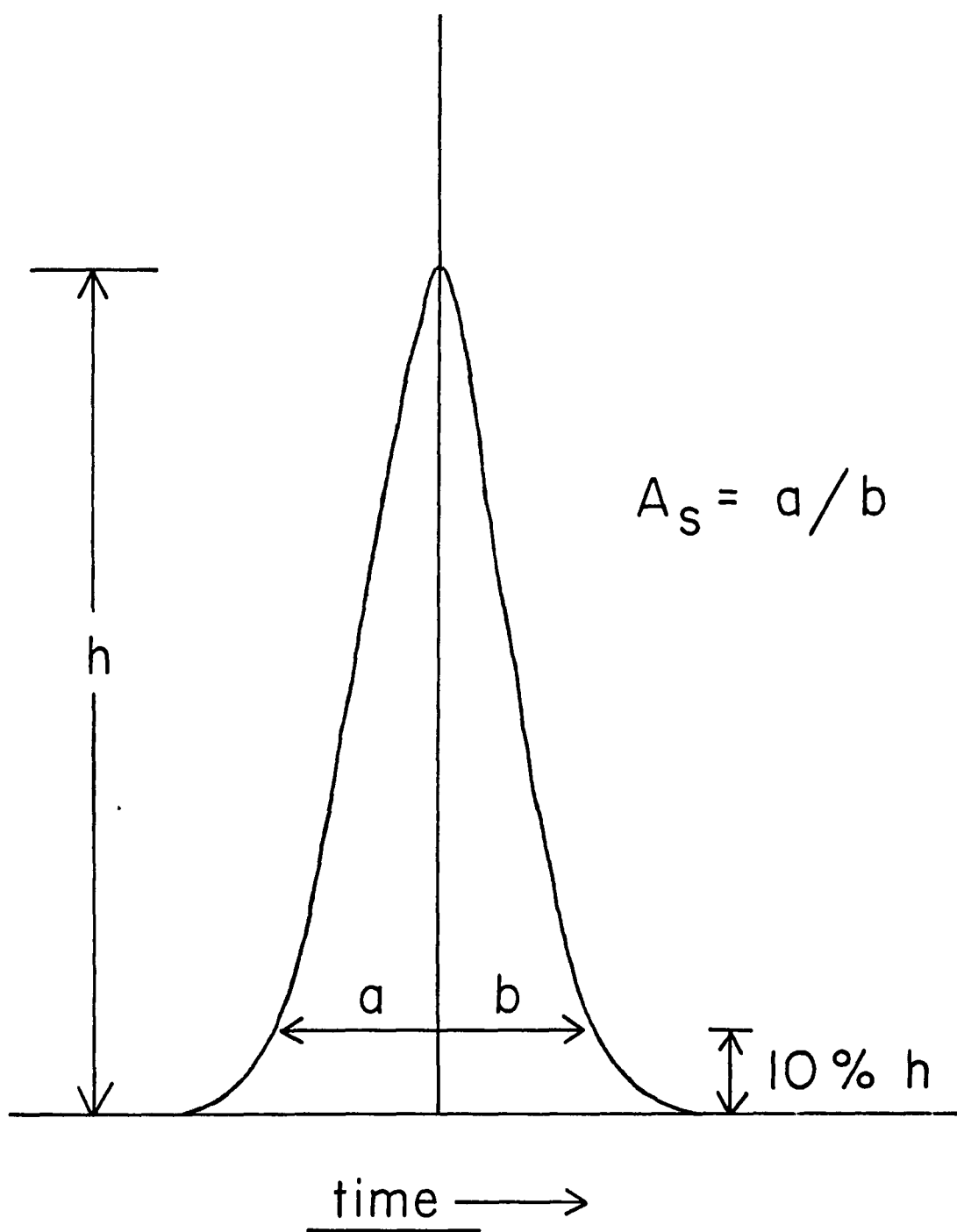
Figure 1.3. Measurement of peak asymmetry (A_s).

be polar <u>because of</u> the residual silanol groups. Thus a mixed reten-
tion mechanism is operating in this type of packing material. For this
reason, Durapak N-octane was selected as a material for study using the
modulated solute approach. Results showing the retention behavior to a
number of system parameters will be presented.

Trends in Modern Chromatography

The most obvious and apparently permanent need in chromatographic
systems is greater sensitivity and selectivity. This has prompted the
development of more efficient columns, such as the 5 micron packing for
high pressure liquid chromatography (HPLC) and capillary columns for gas
chromatography. Work on still smaller columns (e.g. fused silica) and
more sensitive and selective detectors continues. Improvements in
separation science have yielded better separations in faster analysis
times thus utilizing the available manpower and facilities to better gain.

The goals of multiple injection chromatography closely match
these modern trends. By its nature, increased sensitivity and S/N ratios
should be realized. Characterization of linear and nonlinear packing
materials and isotherm determinations have also been studied. As
micro-techniques such as the silicon chip capillary system of Terry (1979)
become popular, the need for repetitive or semi-continuous sampling
regimes for signal enhancement and error averaging will be utilized.

Direction of Research

The basic stratagem of this thesis is the application of signal
processing techniques to the field of chromatography. To this end, the
chromatographic system is viewed as an operator on a chemical system.

This operator can behave in linear and nonlinear ways depending on the system under study. By viewing the chromatographic process in this way, the traditional biases of single injection sampling, infinite dilution concentration ranges, and extracolumn broadening effects can be approached in a new light.

Multiple injection experiments provide the possibilities of:

1. studying nonlinear systems in a quasi-linear state

2. enhanced sensitivity, S/N ratios, and improved analysis time

Digital signal processing allows the analysis of the time dependent signals (i.e. solute concentrations) to be studied in a logical and convenient way. By choosing appropriate input signals, the influence of every perturbing effect on a chemical concentration signal (be it sampling, detection, column phenomena, etc.) can be isolated and characterized.

CHAPTER 2

SIGNAL PROCESSING

Chromatographic Systems from the Data Domain Viewpoint

Modern chemical instrumentation utilizes new electronic devices to present data in sensitive and accurate ways. To the uninitiated, modern computer controlled instrumentation is quite foreboding. To understand a complex system, the building block approach of breaking down a large problem into smaller, simple subunits is of great value. This analysis of complex instrument systems into Data Domains was explained by Enke (1971).

The basic concept of Data Domain analysis is that measurement data in an instrument at any point in time can be expressed by either a physical or chemical property or some property of an electrical (or other information carrying) signal. As the data proceeds through the instrument, changes in the characteristics or properties of the data occur and are called Data Domain conversions. Finally, all measurement data can be expressed in three basic types of electrical signals: 1) analog, 2) time varying, and 3) digital.

Analyzing the gas chromatographic system used in this research from the data domain viewpoint illustrates the above principles. As the different components of a mixture elute from the column (chemical concentration signal) and enter the FID, a data domain conversion to an ion concentration occurs. The ion concentration is converted to a

current (an interdomain conversion) and the current is converted to a voltage (electrical domain conversion). The analog voltage can be displayed on a recorder or again converted via a A/D to a digital signal in time. From the retention time and peak area the desired qualitative and quantitative information is derived for the unknown by the analyst.

Communication Systems

A chromatographic system therefore is easily likened to a communication system. Its basic purpose is the transmission of information. This might be the qualitative and quantitative content of an unknown mixture, the thermodynamic nature of the sorption-desorption process, kinetic rates of column phenomena, or other information containing events.

Communication is defined as the transfer of information from one point in time and space (i.e. the source) to another point in different time and/or space (i.e. the destination). What is transferred is the message. Although most communication systems today are electrical in nature, many other encoding techniques are possible. Whether we talk of smoke signals through the sky, photons through a fiber optic, or trace organics down a packed column, the purpose is the same; information transfer.

Figure 2.1 shows the basic elements of a communication system. The input message contains the information. In most communication systems the message is known at the source, however in a chromatographic system this is not the case when working with an unknown. The input must then be transduced to a form compatible with the transmitter, in my case,

source

input message

input
transducer

input signal

transmitter

transmitted signal

transmission
channel

interference
noise, and
distortion

received signal

receiver

output signal

output
transducer

output message

destination
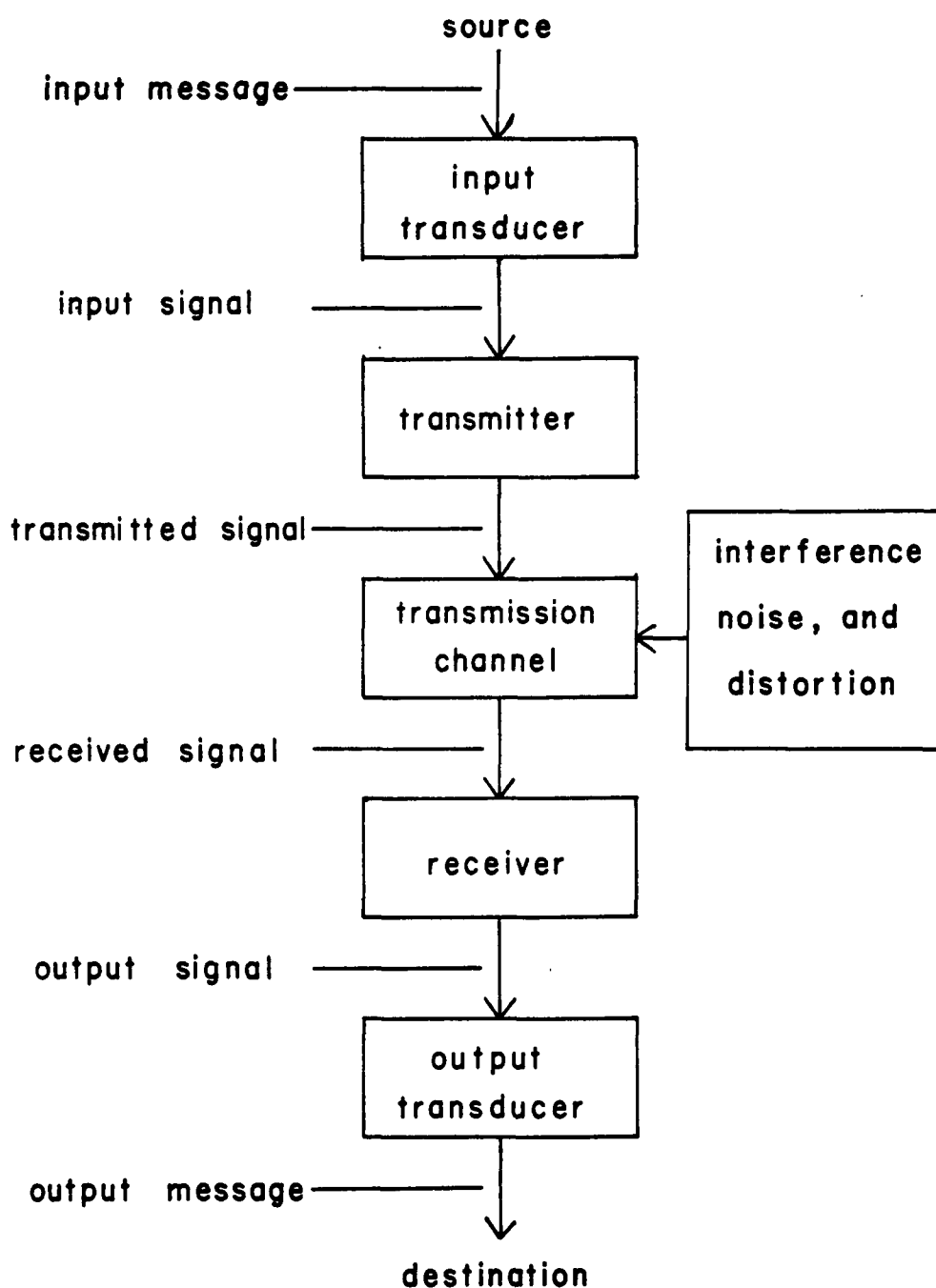
Figure 2.1. Basic elements of a communication system.

this means loading the sample loop of a valve with the vapors of interest. From here, the input signal is applied to the column by firing the sampling valve. Many types of input signals are possible. They range from a single concentration spike or delta function to a frequency modulated carrier wave and anything in between.

Between the transmitter and receiver lies the transmission channel or the chromatographic column. In most communication systems this medium's influence upon the signal is considered a contamination, and something to be minimized. In a chromatographic system, this medium is of central importance because of the selectivity for different solutes in the mixture of interest. Therefore the interaction of the signal with the transmission channel is optimized not minimized.

From the transmission channel the signal arrived at the receiver, where the information is converted back to an electrical signal; the flame ionization detector, hardware and software necessary to deliver a usable signal constitute the receiver in this set of experiments.

Figure 2.1 also shows possible sources of contamination such as interference, distortion, and noise entering the system via the transmission channel. This is certainly true, but these problems can also creep in at other places along the communication chain, and one must always be on guard against these culprits, especially the different types of noise.

The purpose of modulating a signal is to match the signal to the transmission media. In a chromatographic system large changes in the concentration from the sampling system to the column, many times create undesirable nonlinear effects. By modulating a signal, certain

complications such as tailing and site overload can be minimized. This concept is similar to impedance matching in electrical systems.

The method of signal impression upon the system is called modulation. This means a change in the amplitude, phase, frequency, etc., of the signal with time. The two basic types of signal modulation are continuous wave (CW) (i.e. a simple sine wave) and pulse modulation, in which the carrier is a periodic train of discrete pulses. Both methods of signal encoding have been used in chromatographic systems, but the discrete sample volumes of syringes and most sampling valves favor the pulse modulation form.

In any communication system two fundamental limitations always exist. These are the limitations of bandwidth and noise. To communicate a certain amount of information through a communication network, a signal must be changed or modulated a finite amount or number of times. If the rate of change in the signal is constant then the time necessary to relay the information is proportional to the length of the message. If the time to transmit the message is set, then the rate of change in the signal must be increased to accommodate the additional information. The property of a communication system to handle varying rates of data transfer is known as the system bandwidth. This parameter is the sum of all the rate limiting elements in a communication system.

An example of system bandwidth importance in chromatography is seen by comparing the bandwidth requirements of the sampling system and detectors for gel permeation chromatography to those used in capillary or open tubular gas chromatography. In size exclusion chromatography,

the sample is often layered on the top of a one inch diameter column in the mass range from milligrams to grams. The elution times can sometimes be measured in days, with peak widths of three hours or more. A detector dead volume of 500 microliters would not degrade many GPC systems. At the other extreme lies the work of Guiochon 1963) with fluidic sampling valves on gas capillary columns. With a fluidic sampling device, sampling times of 5-10 milliseconds have been recorded. These signals are delivered to a column only 65 microns in diameter and are recorded with a custom FID with a dead volume of approximately one microliter. Amplification with a two msec. time constant displayed on a storage oscilloscope shows peak base widths of 22 msec. with retention times under 600 msec.

The system bandwidth or frequency response requirements for most chromatographic systems lies somewhere between these extremes, however, one must always be suspicious that the influence of the associated injection and detection devices common to all forms of chromatography are not degrading the efficiency or accuracy of the column's separation capability.

The other major limitation imposed on any communication system is noise. Any parameter measured by a continuously varying signal (i.e. the FID current) is a combination of signal and noise. The ratio of the signal to noise or S/N often defines the limits of detection in many analytical measurements. At least six or seven different types of noise exist and techniques for the signal enhancement have been developed to minimize the contribution of each type of noise.

A more detailed account of the different types of noise relies on their frequency composition and will be presented in the context of Fourier signal processing techniques.

Digital Signal Processing

Signal processing techniques using the Fourier transform in chemistry have been limited to phenomena that collect data in the frequency or Fourier domain. These include interferograms (i.e. x-ray crystalography) and free induction decay signals (i.e. pulsed FT NMR). Recently, chemists have realized that data taken from conventional instruments can also be enhanced via transform techniques. The falling prices and increasing capabilities of the paraphernalia needed for these experiments; such as A/D converters, minicomputers, and integrated circuit technology, have prompted the applications of Fourier transform techniques in many areas of modern Analytical Chemistry.

Past and recent articles in Analytical Chemistry have demonstrated that transform techniques can enhance data acquisition, conversion, reduction, storage, smoothing, convolution, correlation, and deconvolution. In this work, the latter three techniques have been applied to linear and nonlinear chromatographic systems in order to better understand the solute-adsorbent interactions as well as a signal enhancement technique. The multiplex and throughput advantages enjoyed by all FT techniques promise that the current popularity of the technique is not a fad, but an important concept with which the modern analytical chemist should be familiar. A brief review of Fourier techniques, correlation,

convolution, and deconvolution are necessary for an appreciation of the approach to chromatography which has been developed in this work.

Fourier Transforms

When a chromatogram emerges from the strip chart recorder, a time domain signal results from the plot of detector current vs. time. However, every signal associated with a physical measurement also has a different but equally valid display in the frequency domain or S-plane. This plot of amplitude vs. frequency is known as the Fourier spectrum. The transformation from the time to frequency domain and back was established in an elegantly concise mathematical form by Baron J. B. Fourier, a Frenchman. Basically, Fourier discovered that any signal can be approximated by a DC level and the superposition (i.e. linear combination) of sinusoidal components.

The concept of expressing a signal in the frequency domain at first seems odd, since we are prejudiced by the way we have observed signals in the past. But, after working in the field of signal analysis for a short time, the conversion from the time to frequency domain and the inverse becomes second nature; at least for simple functions. The insight of viewing a signal in both domains is well worth the initial confusion especially when operations in the frequency domain, such as correlation, proceed by the mere multiplication of the two signals.

Spectrum Analysis

The concept of the spectrum is of central importance in signal analysis theory (Lynn 1973). One straightforward approach (Fig. 2.2) to
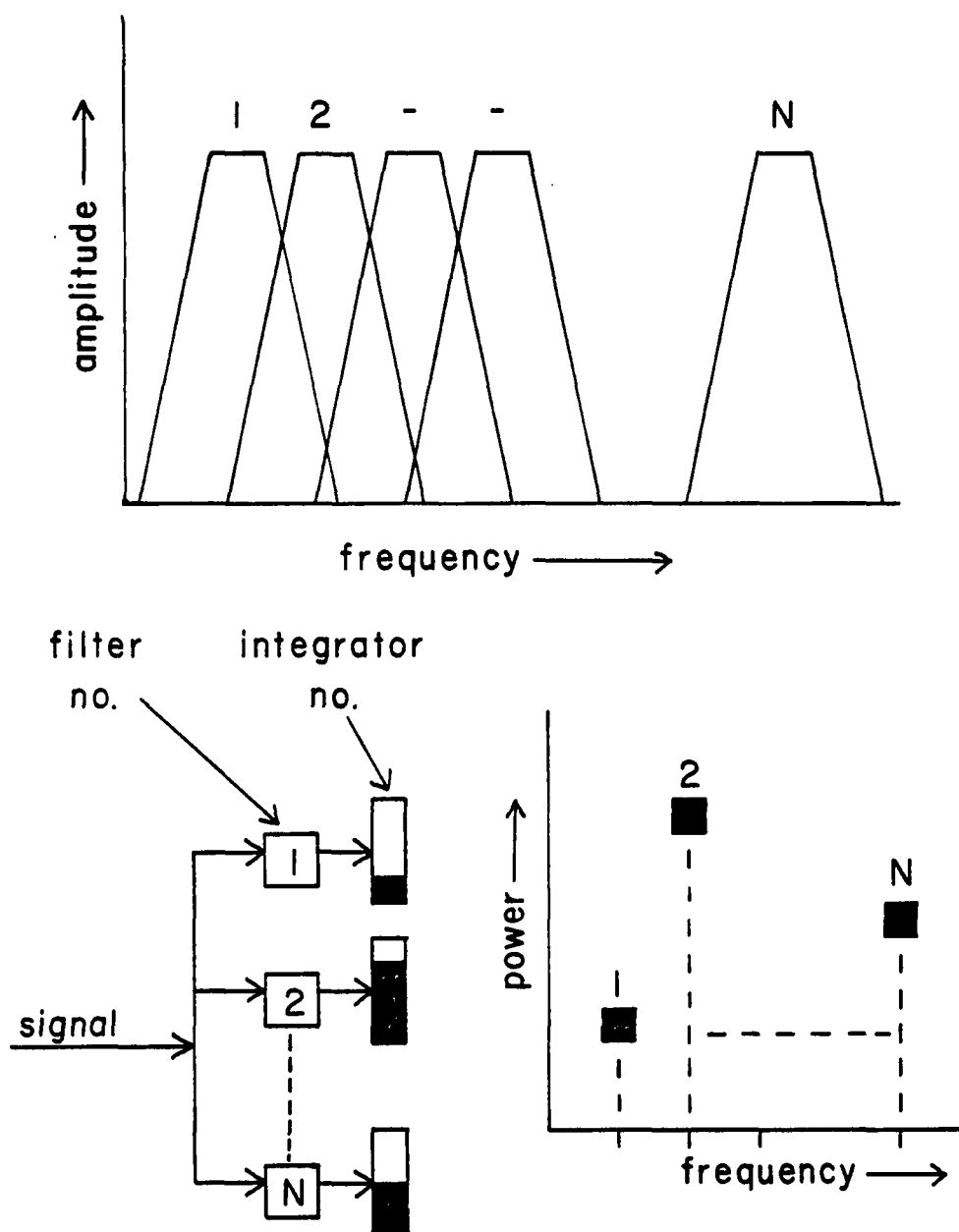
Figure 2.2. Power spectrum determination by the bank of filters approach.

measuring a spectrum is to run a signal through a series of narrow bandpass filters, each connected to an integrator (Rockland Systems 1977). As the frequency of the signal intersects the bandpass of a certain filter, that integrator starts counting. As the frequency of the signal changes, the original integrator stops and the integrator corresponding to a higher or lower frequency starts counting. The integration rate is equal to the power of the signal at any instant (i.e. for a voltage varying signal, the power $P = E^2/R$ or I^2R). At the end of a specified time the signal terminates and the integrators are read off. The value of each summing element is then plotted vs. the frequency that each filter was tuned in on. Figure 2.2 illustrates this bank of filters approach. When the number of integrators goes to infinity and the bandwidth of each filter goes to zero, the resultant plot is a frequency spectrum. Using state of the art electronics and Rube Goldberg techniques in complexity, this approach could be made to work. Fourier, however, had a much better approach.

Figure 2.3 shows a Fourier Approximation of a gaussian peak using the superposition of nine sine waves. Starting with zero frequency and progressing to one hertz in steps of 0.1250 Hertz, the resulting linear combinations of sine waves gives a close fit after only nine superpositions.

Any function of time with even symmetry around the origin can be synthesized by a series of cosine functions according to:

$$S(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} a(f) \cos 2\pi ft \, df \qquad (2.1)$$
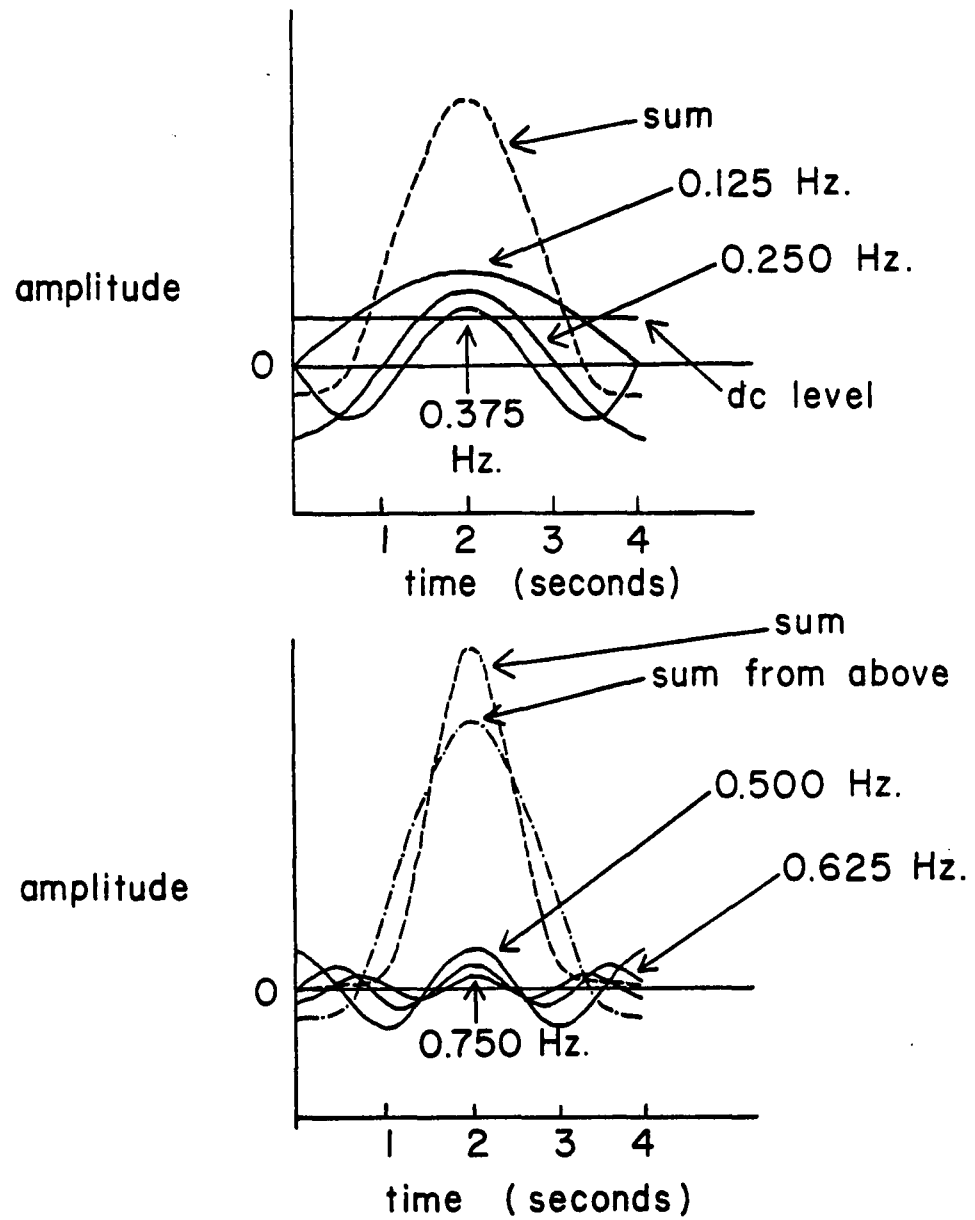
Figure 2.3. Fourier approximation of a Gaussian peak.

Similarly, any function with odd symmetry around the origin can be synthesized by a series of sine functions by:

$$S(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} a(f) \sin 2\pi ft \, df \qquad (2.2)$$

Most signals in nature are hardly perfectly symmetric much less symmetric around an origin. Luckily, by the combination of the above sine and cosine transforms any arbitrary signal can be synthesized by simple superposition.

The definition of an even function is one which has the same value when the operation is on the negated independent variable. The cosine is such a function:

$$h_e \, (t) = h_e \, (-t) \qquad \text{or} \qquad \cos \, (\pi) = \cos \, (-\pi) = -1 \qquad (2.3)$$

The combination of sine and cosine transforms to synthesize a waveform are valid but cumbersome. Fourier noticed a shorthand method of expressing this combination which is seen from the following Taylor expansions:

$$\sin \, (x) = x - \frac{x^3}{3!} + \frac{x^5}{5!} + \ldots \qquad (2.4)$$

$$\cos \, (x) = 1 - \frac{x^2}{2!} + \frac{x^4}{4!} + \ldots \qquad (2.5)$$

$$\exp \, (x) = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \ldots \qquad (2.6)$$

Therefore the equality between exp and the sum of sin and cos is established:

$$\exp \, (Z) = \exp \, (i\theta) = \cos \, (\theta) + i \sin \, (\theta) \qquad (2.7)$$

The combination of the sine and cosine transform can be simplified to:

$$\int_{-\infty}^{\infty} h(t)(\cos 2\pi ft - i \sin 2\pi ft)dt = \qquad (2.8)$$

$$\int_{-\infty}^{\infty} h(t) \exp (-i\ 2\pi ft)dt = H(f)$$

The inverse relationship to get back to the time domain is:

$$h(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} H(f) \exp (i\ 2\pi ft)\ df \qquad (2.9)$$

The way is therefore set to move data back and forth from the time to frequency domain. The shorthand expression is:

$$h\ (t)\ \frac{\text{Fourier transform}}{\text{Inverse Fourier transform}}\ H\ (f) \qquad (2.10)$$

A problem still existed with the use of the Fourier transform. Only simple type functions could be solved for the integral to simplify, thus its use on most real data was limited. The true value of the Fourier Transform came of age in 1965, when Cooley and Tukey, from Bell Labs, devised the algorithm for the Fast Fourier Transform (FFT). This is a discrete form of the FT (first discovered by the mathematician Runge in 1903, long before any computer implementation) and therefore takes a signal or function at discrete points in time (t) and converts them to amplitudes in discrete units of frequency (f). The relationship between time and frequency units is:

$$\text{delta } f = 2*\pi/N*\text{delta } t \quad \text{or} \quad \text{delta } t = 2*\pi/N*\text{delta } f \qquad (2.11)$$

Therefore if 1024 data points are taken at a rate of 10 Hz, the values of frequency on the abcissa would range from zero to 62.8 Hz in increments of 0.0614 Hz. The only limitations of the Fast Fourier Transform or FFT are that the data must be equally spaced in time during the acquisition and for best run time and memory efficiency, an even power of two worth of data must be taken (i.e. 2^n). These two requirements are usually easy to meet.

The FFT is not perfect because it is finite and discontinuous. It can, however, be applied to any signal which meets the above requirements and it is a very good approximation. The approximation can be made more accurate by using fast data acquisition rates and thus more data points for the same period of time, at the cost of more computer facilities. In summary, all of the techniques of signal analysis using transform principles can now be applied to any signal using the FFT.

The practical use of the FFT requires that the highest frequency that can be studied is one-half the sampling rate. This "Nyquist rate" or "Shannon limit" warns that the acquisition rate be at least twice that of the highest frequency of interest. Harry Nyquist and Claude Shannon of Bell Labs, pioneers in information theory, state that a low pass filter of steep rolloff be used to remove those unwanted high frequencies and prevent signal distortion known as aliasing.

Now that a signal is represented in the Fourier Domain, a number of useful signal enhancement methods can be easily implemented. The basic operations of convolution and correlation are the basis of

selective smoothing of data, differentiation, integration, deconvolution and other signal enhancement techniques.

Correlation and Convolution

In the normal or time domain, the convolution of two signals or functions is done by holding one of the functions stationary and sliding the other function past it. The area of intersection of the two functions define the resultant convolution. The mathematical shorthand for expressing convolution is:

$$con_{ab}(T) = \int a(t) \ b(-t \pm T) \ dt \qquad (2.12)$$

where functions a and b are convolved together to yield con_{ab}. If the values of the moving function is reversed or slide the opposite way, then the operation is known as correlation (fig. 2.4). When the signals a and b are the same, the process is called autocorrelation.

The autocorrelation of random waveforms (i.e. noise) is of central importance in digital signal processing. The noise will contribute to the autocorrelation function at a time shift of only zero, where it is one, and at all other points along the time shift its value will be zero.

When a finite amount of data is used, the value of the auto-correlation will decrease rapidly about delay times of zero. If a periodic signal (i.e. a multiple injection chromatogram) is present with the noise, then the periodic signal will be present at large values of delay time and will be a result of the average of all the signal collected during the sampling period. Autocorrelation is
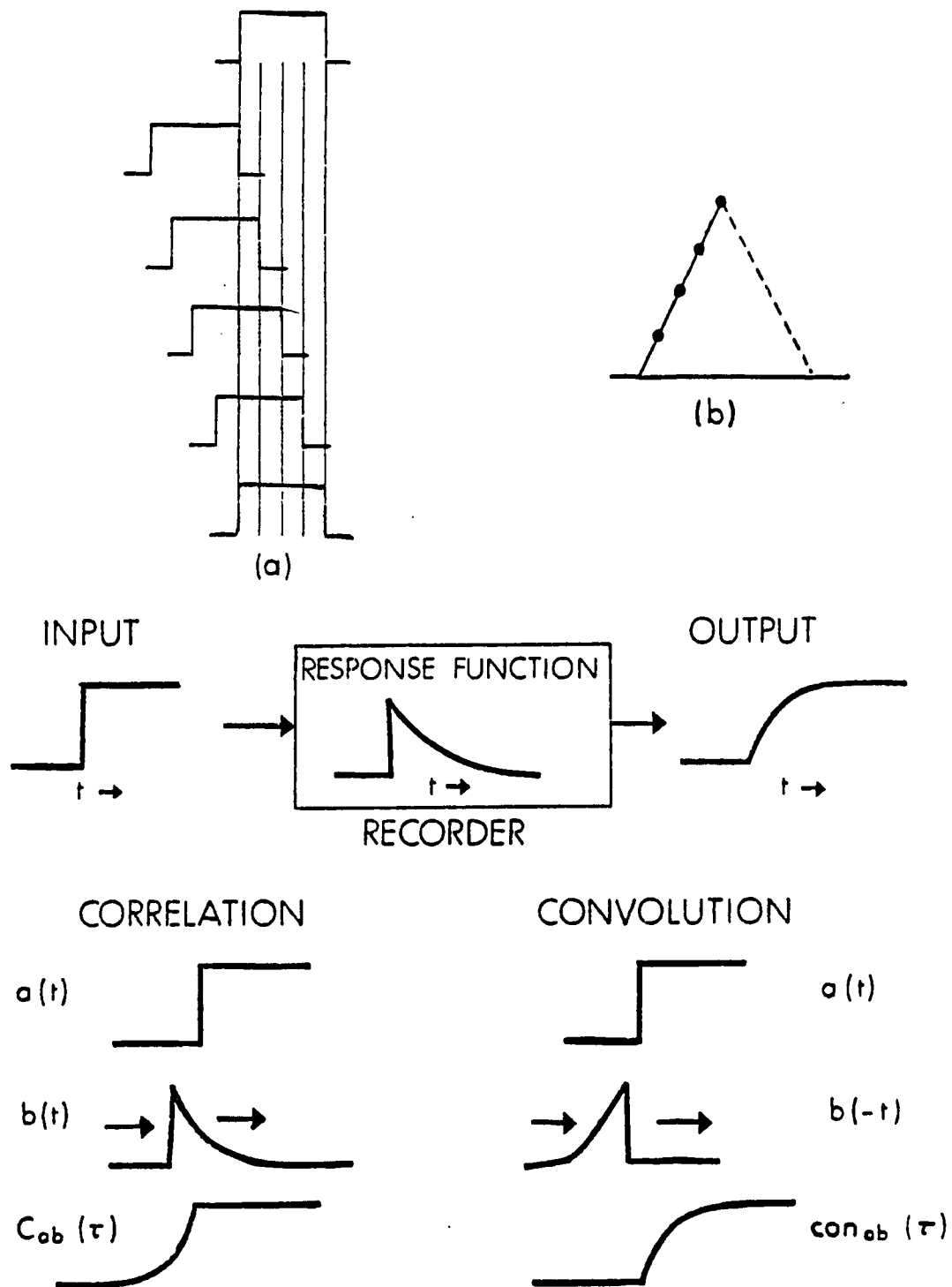
Figure 2.4. Graphical method of correlation and convolution.

therefore an extremely powerful tool for the extraction of periodic signals from high level of noise.

As mentioned, at delay time of zero, the value of the autocorrelation function is equal to the power of the noise and (if present) the periodic signal. However at large delay time the autocorrelation is equal only to the power of the periodic signal (S). By dividing the values of the autocorrelation at zero delay (S+N) by the values at appropriate delay times (S), a method of accurately and conveniently determining the signal to noise (S/N) ratios can be solved (Horlick and Hieftje 1978) (Fig. 2.5).

The definition of cross-correlation is:

$$cor_{ab} (T) = \int a(t) \, b \, (t \pm T) \, dt \qquad (2.13)$$

When working on a discrete digitized signal the cross-correlation is expressed by:

$$cor_{ab} (n\Delta T) = \sum_{t}^{n=1} a(t) \, b(t \pm n\Delta t) \qquad (2.14)$$

where n is the number of data points and delta t is the time between data points.

The cross-correlation of two functions can thus be solved on a computer by computing n^2 multiplications and summations. For large amounts of data (i.e. greater than 1 or 2 K) the exponential growth of the calculations required puts large demands on the computer facilities.

Fortunately, Fourier techniques provide a simple and efficient method for solving correlation and convolution functions. Cross-correlation in the Fourier domain proceeds by a mere multiplication of
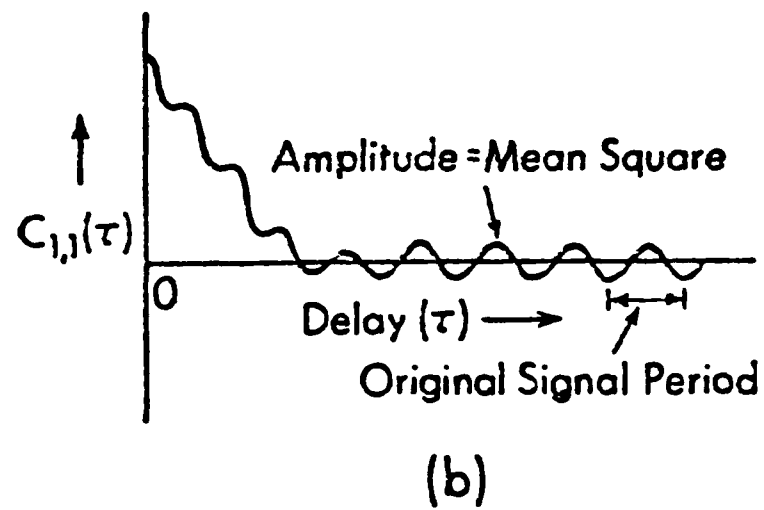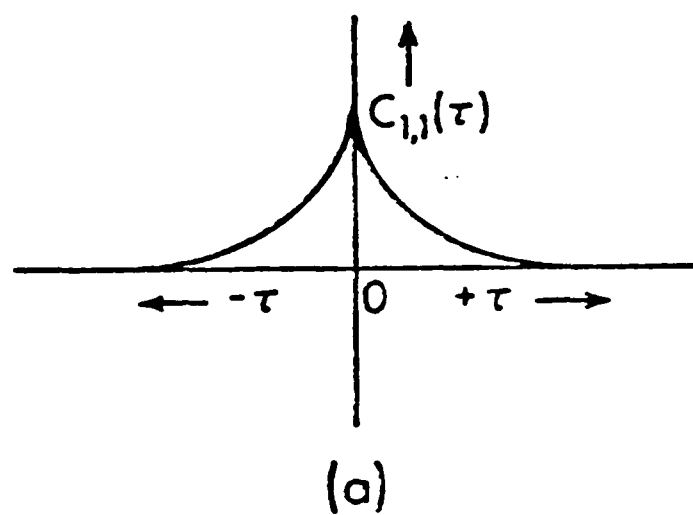
Figure 2.5. Determination of signal-to-noise ratios by autocorrelation.

each frequency element. A conversion back to the time domain via the inverse Fourier transform yields the cross-correlation. Schematically this is represented:

$$(2.15)$$

$$
\begin{array}{cccccc}
 & a(t) & \circledast & & b(t) & = & & c(t) \\
\text{fourier} & \big\downarrow & & \text{fourier} & \big\downarrow & & \text{inverse} & \big\uparrow \\
\text{transform} & & & \text{transform} & & & \text{fourier} \\
 & a(f) & X & & b(f) & = & \text{transform} & c(f)
\end{array}
$$

Thus a cross-correlation of two arrays of n elements requires only n multiplications and three Fourier transforms. The use of the Fourier transform is easily justified in terms of its simplicity of use and speed of execution. With the advent of minicomputers with 100K bytes plus of semiconductor memory, 4-16 K data arrays can be solved in less than 30 seconds. When larger arrays are needed several array processors are available, and can be easily interfaced to a small minicomputer to give the performance of a large mainframe computer at about a tenth the cost.

Linear Systems

In order to use most digital signal processing techniques, a system must be linear. A linear system is one which responds to different inputs in a uniform linear fashion. If a sine wave function is transmitted thru a linear system only two modifications can occur to the input:

1) the amplitude of the wave may be magnified or attenuated by a factor B

2) a phase lag of θ radians may be introduced

If the modifications to a signal of any amplitude can not be expressed in the above terms, then the system is nonlinear.

To study the frequency response of a linear system a special function is often used as the input function. It is known as the unit impulse or Dirac delta function, (i.e. $\delta[t]$), and ideally has a width of zero, a height of infinity, and an area of one. The Fourier transform of the Dirac function reveals a flat line of finite amplitude in the frequency domain. This means that the unit impulse contains an equal amount of all frequencies. Thus, the Dirac delta function is an ideal input function for studying linear systems. The familiar syringe or sampling valve input to a chromatographic system is a close approximation to the Dirac function. Convolving a function with the unit impulse function yields the same function back:

$$a(t) \ \circledast \ \delta(t) \ = a(t) \tag{2.16}$$

Thus, if a linear system is perturbed by a unit impulse, the resulting output defines the complete frequency response of the system. This response is known as the transfer function of a linear system [H(t)]. Once the transfer function of a system is known the output, Y(t) of a linear system can be calculated by convolving the input function X(t) with the transfer function H(t) according to:

$$Y(t) \ = X(t) \ \circledast H(t) \tag{2.17}$$

Perturbing a system with a Dirac function (i.e. injecting a concentrated plug of an organic solute onto a chromatographic column) can often drive it into a nonlinear range because of the large

amplitude modulation. This fundamental limitation of the Dirac func-
tion as a means of obtaining the transfer function has been circumvented
by the use of random inputs. A random waveform contains all frequencies
but distributes them over a large time period. Therefore, to sample all
frequencies would require an infinitely long random input. Any
finite random sequence will have a finite frequency content, however,
all chromatographic information can be sampled with relatively low
frequencies (i.e. less than 100 Hz).

Random Sequence Generation

To ensure that the random input contains the proper frequencies
and is a regular length, a special random pattern is often used. It is
known as a Psuedo Random Binary Sequence or PRBS (de Visme 1971).
Whereas any finite random sequence will have local inhomogeneities in
the frequency domain, the PRBS self cancels these effects to provide a
powerful probe to use as the input function to study linear systems.
The autocorrelation of an infinitely long random sequence or a
Psuedo random binary sequence (PRBS) both produce a sharply peaked
function at delay times of zero. The use of this sequence for an input
function eliminates the problem of correlation noise and is therefore a
preferred input function. Another advantage of the PRBS approach is the
simplicity of its generation. Figure 2.6 shows a complete PRBS FORTRAN
subroutine implemented in only 13 lines of code.

The point of using a PRBS is to spread the frequency components
necessary to derive the transfer function over a finite time period and
avoid the sudden, explosive nature of the Dirac pulse. This method

```
                Page 0001          (FTN4--Release 24177B--July, 1971)

0001                Subroutine PRBS(IPROB)
0002     C
0003                Dimension IPRBS(20)
0004                Common IPRBS
0005     C
0006     C
0007     C
0008                N=5
0009                NS=2
0010                IPPROB=IPRBS(N)
0011                ITEMP=1
0012                IF(IPRBS(N).EQ.IPRBS(NS)) ITEMP=0
0013                DO  10 I-N,2,-1
0014      10        IPRBS(I)-IPRBS(I-1)
0015                IPRBS(1)=ITEMP
0016                Return
0017                End
```

** No Errors*

The number of array elements is 5

The feedback connection is 2

Element # 1	PRBS = 1	Element # 17	PRBS = 0
Element # 2	PRBS = 1	Element # 18	PRBS = 0
Element # 3	PRBS = 1	Element # 19	PRBS = 1
Element # 4	PRBS = 1	Element # 20	PRBS = 0
Element # 5	PRBS = 1	Element # 21	PRBS = 1
Element # 6	PRBS = 0	Element # 22	PRBS = 0
Element # 7	PRBS = 0	Element # 23	PRBS = 1
Element # 8	PRBS = 1	Element # 24	PRBS = 1
Element # 9	PRBS = 1	Element # 25	PRBS = 1
Element # 10	PRBS = 0	Element # 26	PRBS = 0
Element # 11	PRBS = 1	Element # 27	PRBS = 1
Element # 12	PRBS = 0	Element # 28	PRBS = 1
Element # 13	PRBS = 0	Element # 29	PRBS = 0
Element # 14	PRBS = 1	Element # 30	PRBS = 0
Element # 15	PRBS = 0	Element # 31	PRBS = 0
Element # 16	PRBS = 0		

Figure 2.6. Software generation of a pseudo-random binary sequence.

often results in a clear, more resolved, more noise free record of the impulse response function. The input and output signals may then be digitized and cross-correlated in the normal manner to solve for the impulse response. A more versatile and simpler method of solving for the impulse response will be given when the frequency modulation experiments are described.

Signal Enhancement

Another important concept in Fourier techniques is their inherent signal enhancement advantages (Phillips 1980):

1) the Fellgett or multiplex advantage

2) the Jacquinot or throughput advantage

Fellgett (multiplex) Advantage

The Fellgett advantage stems from the fact that in Fourier techniques more than one parameter is responsible for the signal at any point in time. An example of the multiplex advantage in correlation chromatography is that during one time period the signal recorded at the FID is the summation of the overlap of many chromatographic peaks. The signal-to-noise enhancement from the multiplex advantage is therefore the same as averaging N chromatograms, where N is the number of overlapping peaks. Quantitatively, a S/N ratio increase of $N^{.5}$ is expected. The same advantage exists in Fourier spectroscopic techniques using an interferometer, because the signal measures more than one wavelength at the same time as in a conventional slit scanning mono-chromator type spectrometer.

Jacquinot (throughput) Advantage

The Jacquinot or throughput advantage is seen because during a certain time period the amount of singal can be drastically increased by Fourier techniques. In spectroscopic terms the amount of light reaching a detector in a conventional spectromoter is limited by the entrance and exit slits (areas) of the monochromator. When an interferometer is used the slits are opened up and more light passes thru to the detector. In a normal chromatogram (elution mode) only one injection is made, but in a correlation chromatographic experiment, ten to one hundred injections often occur during the time period of a single chromatogram. A corresponding throughput advantage is manifested in an increased S/N ratio.

The increased S/N ratios and signal averaging capabilities of multiple injection analysis have potential applications in the process stream and trace analysis fields. These advantages might also prove useful in a new computer controlled pocket sized chromatograph being developed by Terry (1979) at Stanford for NASA. In this instrument a spiral capillary column is etched into a 5 centimeter silicon wafer along with a microcomputer and associated peripherals, using integrated circuitry photolithography techniques. The open tubular coated column measures only 200 by 20 microns and is equipped with a thermal conductivity detector with a dead volume of 200 nanoliters. A servo driven sampling valve injecting gas samples down to one nanoliter has also been developed. The onboard computer would make

good use of the signal averaging and enhancement techniques multiple injection chromatography would provide to permit lower detection limits and more accurate measurements.

CHAPTER 3

EXPERIMENTAL METHODS

This chapter describes the experimental apparatus, instrumentation, chemicals, computer hardware and software necessary for successful multiple injection chromatography experiments. The details of the particular experiments which were conducted for frequency modulated correlation chromatography will be presented in Chapter 4.

Carrier Gas and Chromatographic Solutes

The use of fine sampling valves, 1/16" packed columns, and water labile packing materials requires pure, clean, and dry carrier gases. Carrier gases used were nitrogen and helium supplied by Airco (Montvale, New Jersey) and Liquid Air Inc. (Phoenix, Arizona).

All carrier gases were further purified by an Alltech Gas Purifier Model 8121 (Alltech Assoc., Arlington Heights, Illinois). This clear tube contained equal amounts of indicating Drierite and Molecular Sieves 5A to remove moisture, oil, and other foreign materials from the carrier system. A stainless steel frit prevents dust from the filter from entering the system.

Immediately before all gas inlets to the chromatograph and sampling valves, 2 micron sintered stainless steel inline filters (Nupro Corp., Willoughby, Ohio) were installed to protect the fine instrumentation.

All solutes samples used were either Analytical Reagent or
Spectrochemical Grade and were used without further purification.

Chromatographs

Two chromatographs were used during the course of this work.
The earlier phase utilized a HP model 5720A equipped with a HP model
18790A oven programmer and flame ionization detector (FID, Hewlett-
Packard, Avondale, Pennsylvania). During the latter phase of the
research a Varian model 1700 was refurbished to good working order
before use (Varian Aerograph Inc., Walnut Creek, California). Both
were equipped with flame ionization detectors, flow controllers, and
solid-state field effect electrometers. The Varian instrument also has
two meters for monitoring the column head pressures. Both oven
temperature controllers were checked with a quartz thermometer and were
reproducible to ±0.5° C. Injectors and detectors were maintained at
either 150 or 200 °C depending on the solute and column.

Electrometers

Three electrometers were used during the research. The
importance of the time constant in the chain of detector, electrometer,
and integrator or recorder was studied by Glenn and Cram (1979) and
Smit and Waig (1975). The HP electrometer provides three outputs; a
recorder output of 1 millivolt, an integrator output of 1 volt, and a
computer output of 10 volts full scale. The respective noise bandwidths
for the three outputs are 4, 2, and 10 Hz. The computer output was
chosen because its higher bandpass would not distort fast peaks. The

Varian electrometer has only one output of 10 millivolts, but will operate at 1 volt full scale with a time constant about a tenth of a second, corresponding to a 10 Hz bandwidth. The Varian electrometer's FET operational amplifier failed during the research and a separate Keithley model 417 high speed picoammeter with a model 4170 input head was substituted (Keithley Instruments Inc., Cleveland, Ohio). This electrometer is equipped with a variable output filter from about 0.5 to 250 Hz at 3 volts full scale, corresponding to a 4 msec. time constant (Keithley Catalog 1979). This damping was always used in the off position during data acquisition.

Sampling Systems

Sampling devices used in column chromatography can be separated into two basic types:

1) stream switching

2) pressure injection

The common syringe is an example of the latter, as the sample loop injector is typical of the former. The quality of the sampling system defines not only the accuracy of the recorded retention times but also influences the bandspreading. A quantitative study of this effect was studied by Guiochon (1963) and is summarized in Appendix A.

Experiments in correlation chromatography place special requirements on the sampling system. Among these requirements are 1) accurate and reproducible injection volumes, 2) accurate injection timing, 3) fast recycle times for multiple injections, 4) low hysteresis, 5) automation capability, and 6) reliability. Other

features such as heatability, cost, maintenance, and small volume injections are desirable but not as important for research in correlation chromatography. Table 3.1 summarizes the advantages and weaknesses of 12 different injection systems used in gas chromatography. Four of these valves are non-commercial prototypes designed for the special requirements of high speed (i.e. small plug width) by Cram's hybrid fluidic (Wade and Cram 1972, Bowen et al. 1973) and Guiochon's fluidic valves (Gaspar, Arpino, and Guiochon 1977; Gaspar, Olivo, and Guiochon 1978), or a design with no mechanical parts by Phillips-Villanti's linear motor (Phillips and Villalanti 1980).

The donation of a Seiscor model VIII high speed sampling valve from Mr. Ben Keller was appreciated and provided the sampling capability for my correlation experiments (Seismograph Service Corporation, Seiscor Division, Raytheon Company, Tulsa, Oklahoma, Analytical Instruments Catalog, 1979). A comparison of sampling valves by Oberholzer and Rogers (1969) demonstrates the Seiscor valve to be the most reproducible and accurate valve commercially available. Some of the more important specifications of the Seiscor valve include a 0.20% elution time accuracy, a 10 millisecond switching time, 300 PSI operating pressure, 450 °C operating temperature, half-million cycle life, easily changed sample loop volume, and easy interface to standard air-actuated soleniod valves.

The Seiscor valve came with 24" sample loop tubing which contained approximately 500 microliters. To _sweep and refill_ this loop at 30 ml/min would take two seconds. To shorten this time the

Table 3.1. Characteristics of sampling valves used in gas chromatography.

	Injection accuracy	speed	reproducibility	versatility	maintenance	life	cost	automated	hysteresis	small volumes	injection mode	heatable
gas syringe	-	--	-	++	+	+	++	--	-	++	pi	-
annular O-ring	+	-	+	+	-	+	+	-	--	-	ss	+
Carle micro valve	++	+	+	++	--	-	--	+	++	+	ss	++
Seiscor valve	++	++	++	++	+	++	---	+	++	+	ss	++
Rheodyne	+	+	+	+	-	+	--	+	+	-	ss	-
Altex	+	+	+	+	+	+	-	+	+	--	ss	-
Valco	+	--	+	-	-	-	-	--	+	+	ss	+
Cram's fluidic	+	+	+	-	--	--	n.a.	+	?	+	ss	-
Guiohchon's fluidic	-	++	+	--	---	-	n.a.	+	?	+	pi	--
Annino's speaker drive	-	+	+	+	-	-	n.a.	+	-	+	ss	--
Phillips, Villalanti linear	--	+	--	-	---	-	n.a.	+	-	--	pi	--
Clippard Solenoid	--	+	--	+	+	+	+	++	+	-	pi	-

pi = pressure injection
ss = stream switch

loop was shortened to about 2.4" or about 50 microliters. This shortens
the sweep time to about 0.10 second.

At speeds faster than 5 Hz, the carrier gas does not have time
to completely sweep the sample loop, therefore smaller sample sizes are
delivered. If faster sampling rates are needed, a Seiscor valve with a
half microliter internal sample loop could be used to provide a 10 to
30 Hz upper response limit. This is not necessary for anything except
the most exotic capillary systems.

Since the sampling valve is of such importance in correlation
experiments a schematic of the Seiscor valve is presented in Figure
3.1. The sampling valve was installed outside the Varian chromatograph
using the two flow controllers and pressure gauges already in the
instrument. The sample loop was maintained at the same pressure as
the column by use of a Nupro needle valve acting as a restrictor
(Nupro Corp., Willoughby, Ohio). This simplified the plumbing require-
ments and also enabled manual injection for diagnostic purposed via
the septum injection device on the front of the instrument. The
relatively large dead volume of the Varian injectors did not degrade
the new modified sampling system because all performance degrading
components were upstream from the sampling valve. All tubing to and
from the sampling valve was 1/16" thin wall stainless to minimize
extracolumn effects. With these sampling system modifications any
high quality chromatograph could be used for multiple injection work.

Figure 3.1. Operation of the Seiscor gas sampling valve.

Sampler Interface

The interface from the computer to sampling valve consists of the assembly language software for driving the D/A converter (a programmable Digital Voltage Source, HP model 6131B, Hewlett-Packard, Cupertino, California) which triggers the Exact function generator, which provides a variable pulse width to the nitrogen powered solenoid which drives the Seiscor valve. The decision to hardware control the pulse-width was made to simplify the already multiprogramming, multi-tasking software necessary for experimental control, data collection, and data transfer. Since the function generator is free running (i.e. induces an uncertainty of ± half a cycle of the pulse width) and has a limited current capability, and was not always available, a custom circuit for varying the sampling pulse width was designed. The circuit utilizes a monostable one shot and the schematic is shown in Figure 3.2. This circuit eliminates any timing and current-limiting problems experienced with the previously used function generator.

Solute Introduction

A constant reproducible amount of solute must be injected repeatedly and accurately for any successful correlation experiments. It is also desirable to be able to change the concentration with minimal trouble, but more important is reproducibility. If some injections in a sequence are larger than others, correlation noise will show up in the correlogram.

Using neat ethane and a short empty 1/16" column, the sampling valve was evaluated when driven by the HP 2115 computer via a D/A

PULSE WIDTH CONTROL
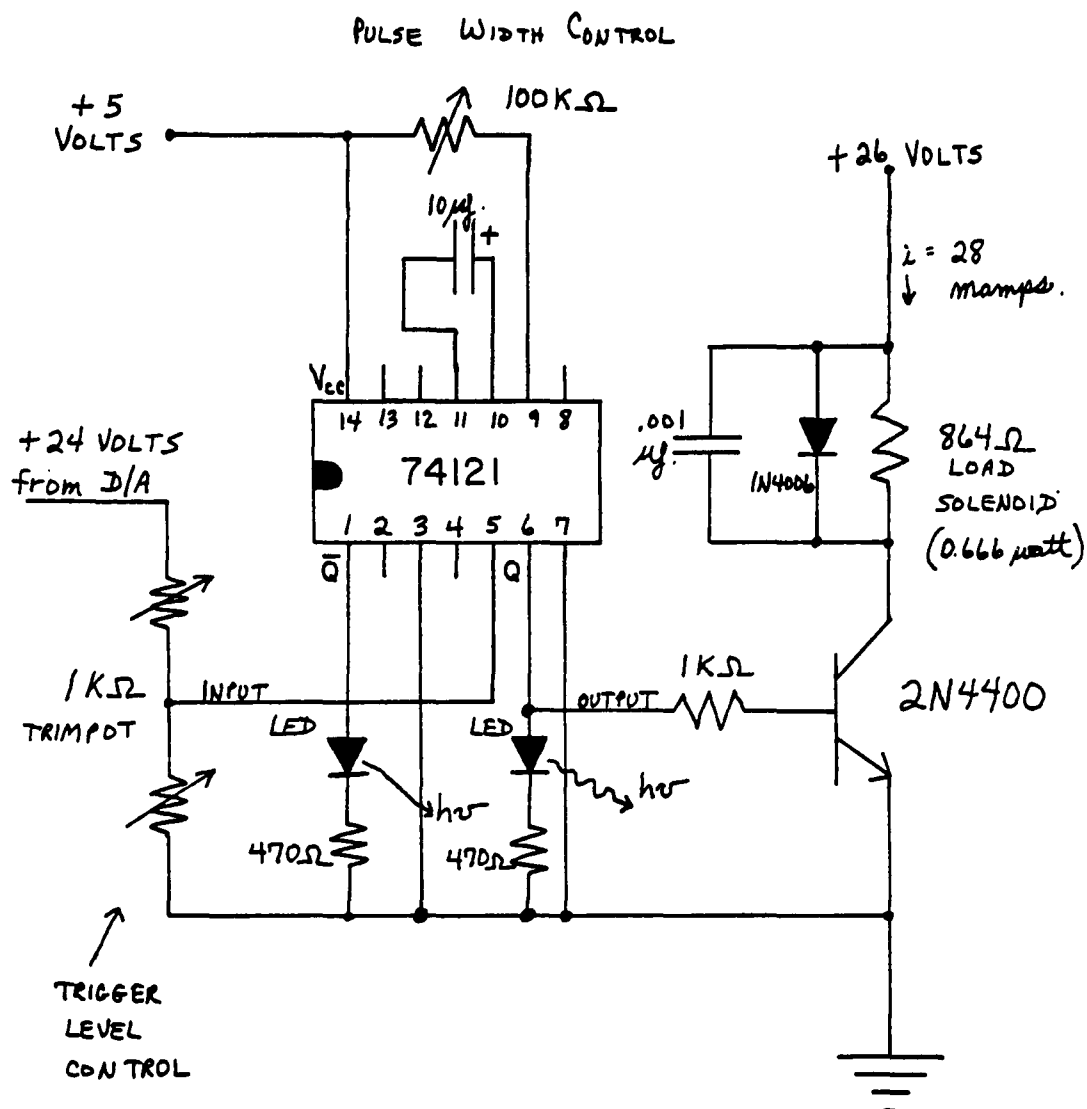
Figure 3.2. Schematic diagram of circuits to drive sampling valve from computer output.

converter powered by an Exact function generator (Exact Electronics Inc., Hillsboro, Oregon). Software for this evaluation is contained in PULSA (Fig. 3.3), an assembly language program to drive the HP digital voltage source. As seen in Figure 3.4, the reproducibility at any speed below 4 or 5 Hz is virtually perfect. A slight degradation in response was noticed when using the Exact generator, caused by the slight delay in opening and closing the Clippart Minimatic model EVO-3 solenoid (Clippard Instrument Laboratory Inc., Cincinnati, Ohio) by the limited current (about 10 milliamps) of the function generator.

The simplest method of delivering a trace gas to the sampling valve is merely flowing a gas stream past a volatile liquid. By varying the flow rate of the gas, the surface area of the liguid, the diffusion geometry of the cell, or the vapor pressure of the solute (i.e. either changing the solute or changing the temperature), the concentration of the solute of interest can be changed. This method of sample introduction was implemented by filling a melting point capillary tube (Kimax #51, 0.90 mm x 90 mm) or a 1/8" nylon tube with the solute of interest, then placing it in a 1/4" O.D. x 6" glass tube in series with the sampling stream. By varying the solute levels in the two tubes, a concentration range of 400 was achieved with diethylether.

By carefully controlling the temperature, reproducible data was recorded and successful correlation chromatograms were computed. A careful look at Figure 3.5, the raw data from a multiple injection experiment using this type of sampling device shows a slowly downward drifting concentration profile. This is an unavoidable problem caused by

SPULA SS   0006

      ASMB,A,B,L,T,X

      NAME OF SOURCE: SPULA, IE, PULSE ABSOLUTE.

      NAME OF SUBROUTINE = PULSE
      SUBROUTINE TO FIRE THE SIESCOR SAMPLING VALVE VIA THE HEWLETT-
      PACKARD DIGITAL VOLTAGE SOURCE.

      WRITTEN BY DAN C. VILLALANTI.

      NO PARAMETERS ARE PASSED IN THIS VERSION OF PULSE

```
            ORG 100B
DVS         EQU 11B     D/A CONVERTER IN CHANNEL 11 OCTAL.
PULSE       NOP
START       HLT 77B
            CLC DVS     PREPARE THE A/D CONVERTER.
            LDA VOLTS   GET THE PROPER VOLTAGE.
            OTA DVS     SEND THE VOLTAGE OUT.
            STC DVS,C
            LDA CONT
            OTA DVS
            LIA 1       GET A PARAMETER FROM THE SWITCH REGISTER TO
            CMA,INA     CONTROL THE DURATION OF THE VOLTAGE PULSE WIDTH
WAIT        LDB PULS1
            INA,SZB     INTERNAL TIMING LOOP FOR PULSE WIDTH CONTROL.
            JMP *-1
            INA,SZA
            JMP WAIT
            CLC DVS
            CLA         PREPARE TO SEND D/A CONVERTER ZERO VOLTS.
            OTA DVS,    SEND 0.0 VOLTS.
            STC DVS,C
            LDA CONT
            OTA DVS
            JMP START
VOLTS       OCT 012000  VOLTAGE WORD CORRESPONDING TO +24.00 VOLTS.
CONT        OCT 50      CONTROL WORD TO SET THE PROPER VOLTAGE RANGE
*                       AND CURRENT LIMITING PARAMETERS.
PULS1       DEC-100     SIMPLE TIMING DOWN COUNTER.
            END
      LIST END
```

Figure 3.3.   Assembly language software to drive the D/A or HP digital
              voltage source.
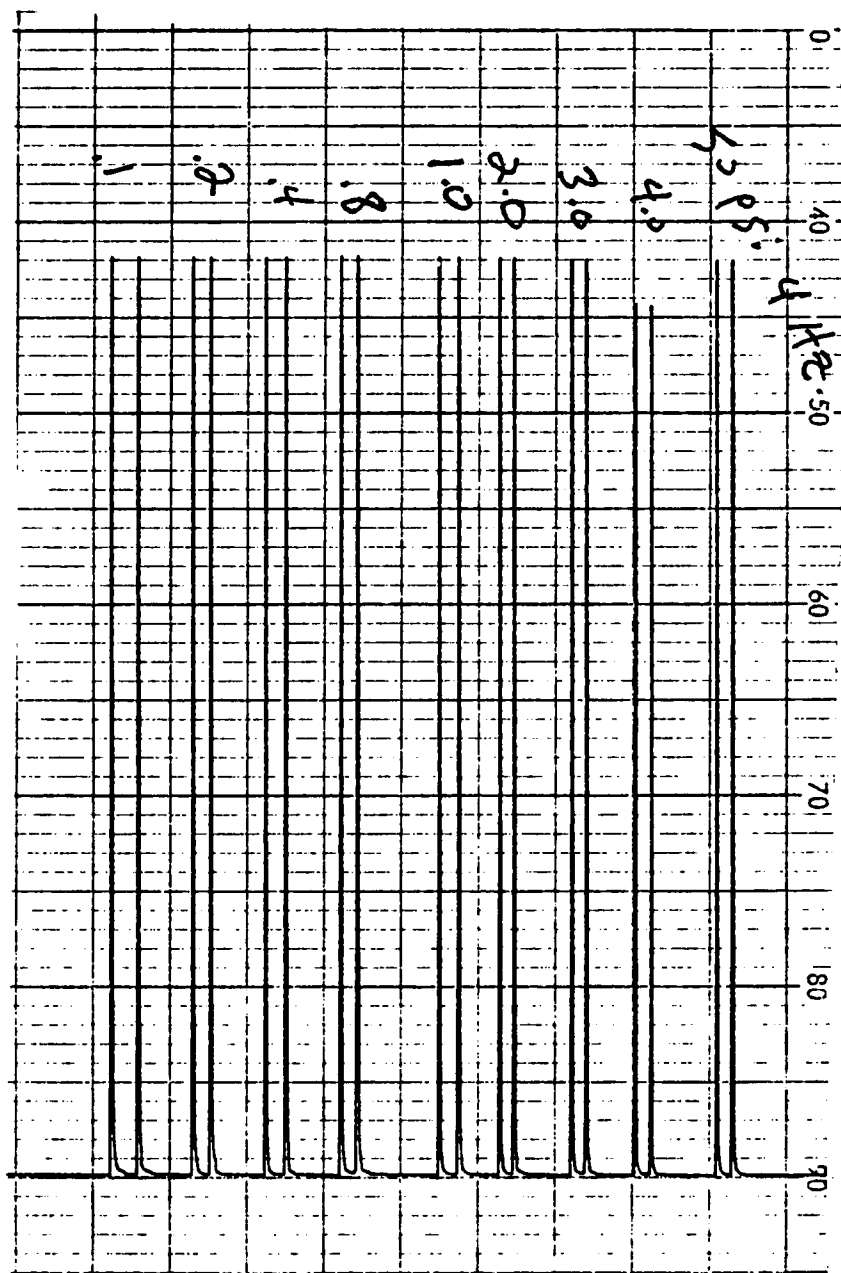
Figure 3.4. Replicate injections of neat ethane at various sampling
speeds (0.1 to 4.0 Hz).
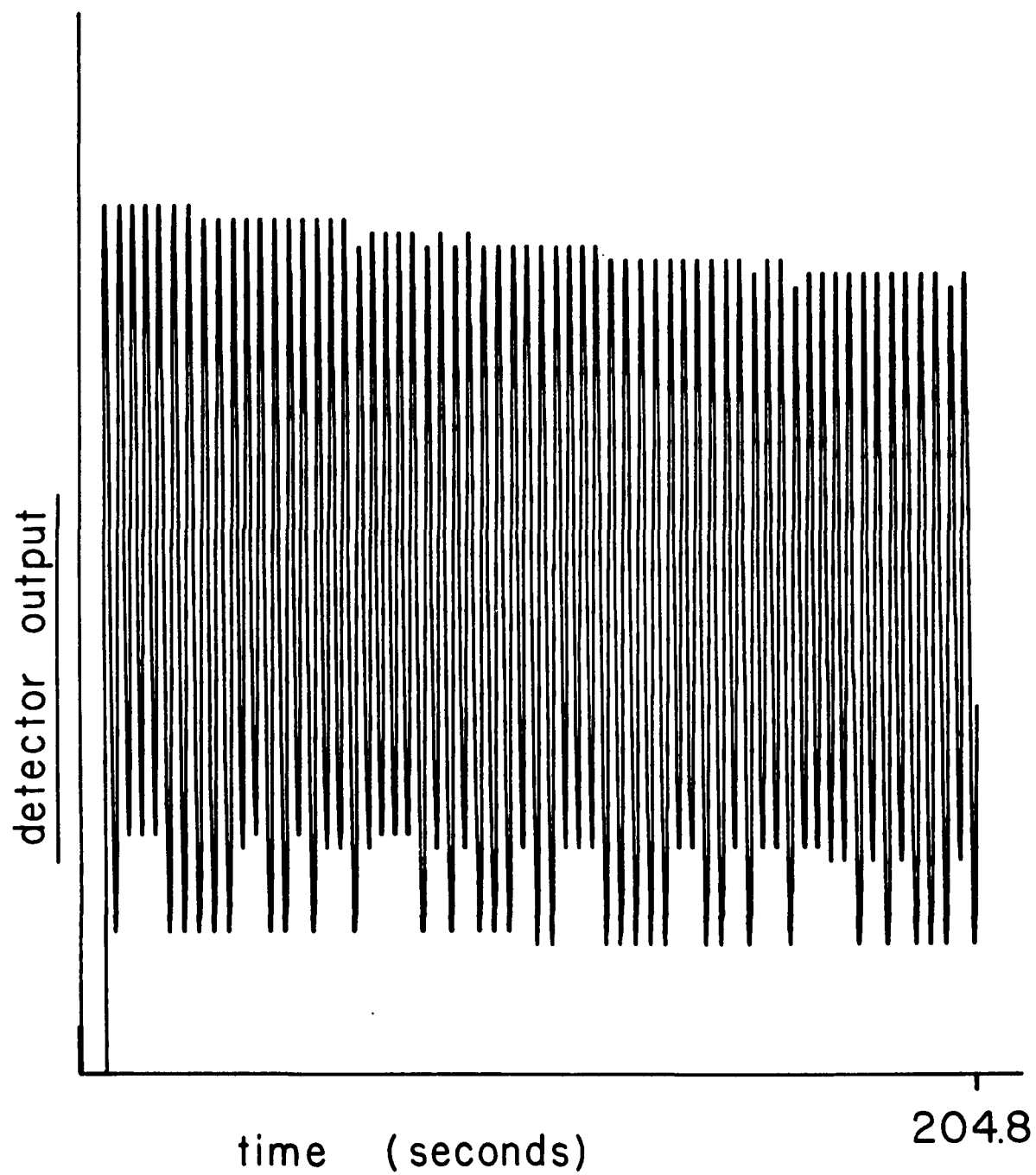
Figure 3.5. Raw data from multiple injection experiment showing drifting solute concentration.

a finite amount of solute evaporating and changing the diffusion pathway to the gas stream. This drift, which contributes to correlation noise, is accentuated when using highly volatile solutes or elevated temperatures.

The most reproducible sample injections were made with neat ethane from a pressure regulated lecture bottle. In an effort to obtain better chromatograms, a pressurized tank of trace hexane in an inert gas was ordered from Matheson (Cucamonga, California). Lyle Seely and Gary Harper of Matheson instructed me in the method of making your own calibration gas mixtures.

The first step is determining the vapor pressure of the desired solute at room temperature according to the formula (CRC Handbook of Chemistry and Physics 1974):

$$\log_{10} P = \frac{0.05223\ A}{K^{\circ}} + B$$

where A and B are constants for each solute. Using this formula for hexane at 20 °C gives a vapor pressure of 119.4 torr or 2.308 PSI. For a 30% safety factor ensuring that all the solute remains in the vapor phase only 70% of this vapor pressure will be used as a maximum solute concentration.

To determine the PSI of the solute, the total pressure of the tank is multiplied by the volume % accordingly:

$$\text{PSI (inj.)} = \text{PSI (total)} * \text{volume\%}$$

or

$$1\ \text{PSI} = 1000\ \text{PSI} * 0.001\ (1000\ \text{PPM})$$

Now determining the weight of the solute:

$$\frac{\text{volume of container (liters) * molecular weight of solute * PSI (inj)}}{354.85}$$

equals the grams of solute. For a one liter container with 1000 PPM of hexane, 0.2429 grams or 368 microliters are required.

Although special sampling cylinders are available for the above application, lecture bottles (Matheson lecture bottle model 9M76-# 457 DOT SE 1800 0.44 liter) were used because of the availability and cost. Preparation of the lecture bottles included a general cleaning of the valves, two vacuum degassings and two nitrogen purges at 1000 PSI.

After the amount of solute was calculated, the lecture bottle valve was removed to allow the liquid to be injected with a 50 micro-liter syringe (Hamilton Corp., Reno, Nevada) and quickly reassembled. The lecture bottle was then refilled from a large nitrogen tank to the desired total pressure. A Matheson lecture bottle regulator (model 3320) was used to set the pressure on the sample loop. A total pressure of 1000 PSI was never exceeded although the bottles are rated to 1800 PSI.

The gas mixtures were prepared for one and two component systems and gave extremely reproducible results even with the temperature changes in the laboratory. When a more versatile and certified solute introduction system is needed, a permeation device which uses a liquid-vapor equilibrium with a permeable membrane is commercially available (Metronics, Santa Clara, California).

Columns

The chromatographic columns were eighth inch copper and stainless steel and 1/16" thin wall (i.e. 0.05" I.D.) stainless. All were cleaned by solvent polarity staircases and packed straight using the tapping method. Column lengths varied from 10 centimeters to 1 meter. Although most of the work used porous polymers of the Porapak series; plain silica (Porasils), and bonded-phase packings (Durapaks) were also investigated. Discussion of results from the different solute-adsorbent interactions follows in a later chapter.

Experimental Computer Hardware

A Hewlett-Packard model 2115 computer provided experimental decision, data acquisition, and data transfer. The computer was equipped with 8K (16K bytes) of ferrite core or non-volatile memory, a paper tape photoreader (HP model 2737a, 300 baud), a storage type high resolution graphics terminal Tektronics model 4010 (Tektronixs Inc., Beaverton, Oregon), an analog-to-digital converter (A/D) (HP model 2401c Digital voltmeter DVM), and a digital-to-analog converter digital voltage source (D/A HP model 6131b).

Although the HP 2115 is an antiquated machine, it is still a very useful and relatively easy machine for experimental interface. A vectored priority interrupt system is scheduled by interface slot assignment. This allows peripherals to be serviced in a logical sequence according to their priority and data transfer rates. The second essential feature of the 2115 is a programmable real-time clock (HP model 12539a time base generator or TBG). This provides the timing for

data acquisition, transfer, and experimental decision making, program-
mable rates from 0.1 msec to 256 seconds per interrupt are available.

The A/D converter was a digital voltmeter which provides good
common-mode rejection (i.e. noise immunity) of 140 db. at the expense of
conversion speed. The A/D has three sampling periods (windows) of 1.0,
0.1, and 0.01 seconds. The resolutions of each window are respectively,
one part in 10^5, 10^4, and 10^3. These resolutions compare to 16, 13, and
9 bits A/D converters. Because of the internal delays inside the A/D
converter, any data acquisition rates of 10 hertz or higher require a
window of 0.01 seconds, the resulting 9 bit resolution is a limiting
contributor in the precision of recording fast peaks. When greater
resolution was needed, rates of 9 hertz were used to gain 13 bit resolu-
tion at the cost of speed.

The D/A converter used provided ± 100 volts at zero to .5 amps
with a settling time of 300 microsec. Although the D/A was used to only
trigger the sampling valve, many other interesting waveforms (i.e.
exponential, sine wave, etc.) can be approximated by using small
increments in time and voltage. Other programmable features of the D/A
included voltage range, current limiting, and polarity (the operating,
service, and programming information for this D/A are available in the
HP manual #06131-90002). The configuration of the peripheral devices
used on the two experimental computers is shown in Table 3.2.

Table 3.3 outlines the features of the programming to control the
experiments. Assembly language subroutines were used to control the
peripherals. The main program was written in FORTRAN IV, as well as the
PRBS random number generator.

Table 3.2.   Configuration of input-output channels for experiment
computers.

Name of file=C2115   Configuration and other operation parameters
As of July 1977.

Abbreviations: 16 Bit duplex register = GPR Card
                                          + True In/Out   = MC Card
                                          Universal Interface   = UI

   Select Codes (in OCTAL) for:

   HP 2100   HP 2115

   Channel #   Channel #

   10 GPR   10 TBG   D.41   I.41
   11 MC   11 DVS
   12 UI(comm. net.)   12 UI(comm. Net.)
   13 MTD   13 GPR   D.45   I.45
   14 MTC   14 DVM   D.43
   15 PHOTO   15 PHOTO
   16 PUNCH   16 PUNCT
   17 TTY   17 TTY
                                          20 MC (not available only 8 slots)

   Function Codes for .IDC.   Unit Ref. # (Drivers for .IDC. 2115)

   00 Clear   1 Keyboard input
   01 Read   2 TTY output
   02 Write   3 Photo (Lib. input)
   03 Control   4 Punch
   04 Status   5 Input (photo)
                                          6 List (TTY)
                                          7 TBG
                                          10 GPR (Fire)
                                          11 DVM
                                          12 Photo
                                          13 Punch
                                          14 TTY Driver

Table 3.3. Capabilities of the Experimental Software.

---

1. User control of experimental parameters via the graphics terminal
   a) data acquisition rate
   b) total number of data points in the experiments
   c) modulation bandwidth
   d) carrier frequency

- -

2. Pseudo-Random number generation
   a) Pseudo-random binary sequence generation (feedback OR configuration)
   b) Feedback shift register random generator

- -

3. Timing and control of injections via the sampling valve

- -

4. Data collection, injection encoding on the data, and data transfer to the disc operating system.

- -

5. Normal and manual termination of experiment

In all multiple injection experiments the input and output must be available for correlation and deconvolution calculations. Since the 2115 has only 8K of memory and 5K was used for the programming, it was necessary to transfer the data to the disc-operating system (HP 2100 DOS) computer in real-time (i.e. during the course of the experiment). To minimize the amount of data transferred, the input data (i.e. whether or not an injection took place) was encoded onto the output. A negative value of output voltage meant that an injection occurred and a positive value meant no injection. Every 64 floating point words (256 bytes) or one disc sector, the data were transferred to the larger system without missing a tick via the interrupt system.

The communication network included bidirectional 16 bit data lines on both computers which were connected by a communication network selector box. The network is capable of sending and receiving data at high baud rates (100,000 words per sec.) but to date has been used only for sending data to the disc-operating system. A useful reason to receive data would be to load programs directly from the larger system, instead of loading via paper tape.

All program development and compilation was carried out on the disc operating system because of its convience and speed. Relocatable binary tapes were then linked together (using the basic control system BCS) on the NSF 2100 tape operating system (16K). The configuration of peripherals on the two smaller computers were made compatible for programming usage on both computers.

Mainframe Computer Hardware

All data reduction and display utilized an HP 2100 a computer equipped with floating-point hardware, extended core storage (Fabri-tek model A100, Minneapolis, Minnesota) to 65K bytes, a HP model 7900A 10 megabyte replaceable disc, a HP model 2767A line printer (300 lines/min), a HP model 2795A paper tape photoreader, a HP model 2795A paper tape punch, a HP model 7200A graphic plotter, and a Tektronix model 4002A joystick equipped high resolution graphics terminal. Although a 5 megabyte discpack was provided for each graduate student, mass storage onto magnetic tape via the communication network to a HP model 7970A 9 track tape drive was necessary because of the large arrays of data.

Data Reduction Software

Since methods of signal analysis and enhancement are of central importance in this research effort, the reason for and implementation of various signal processing techniques will be presented in some detail.

Whenever using laboratory minicomputers for digital signal processing, memory limitations become critical. Since one data point in the Fourier domain requires a floating point complex number (4 words or 8 bytes) arrays of only one or two thousand points can be processed. This is enough to derive the required precision for most experiments. To perform Fourier transforms of this size requires extensive use of virtual memory to keep enough core memory available. Since the operating system used (DOS-M) is primitive, virtual memory routines written by John Phillips (1977) were used. These are cumbersome and difficult to

use compared to commercial systems like RT-11 or UNIX operating system

approaches, but worked well after debugging (Table 3.4).

Methods of correlation in multiple injection chromatography until

now have used the conventional computation method to approximate the

impulse response function.

$$\phi_{XY}(\tau) = \frac{1}{N} \sum_{k=1}^{n} Y(k\Delta T) \ X \ (k\Delta T - \tau) \tag{3.1}$$

where X = input data, Y = output data, τ = delay time, n = number of data

points, ϕ = cross correlation, T = time.

For a block of 2K data points, two thousand multiplications must

be solved for every single point in the correlogram. Thus for a 2K

array of data, N^2 or four million multiplications must be performed. As

the amount of data increased, the exponential growth in the number of

calculations imposed by equation 3.1 taxes the computer facilities

quickly. Equation 3.1 also shows that the Y data (output) is shifted by

various delay times. This requires that either two cycles of the PRBS

be run or a complex fold-around of the output data be computed. These

so-called "boundary problems" necessitate cumbersome computer code for

calculating the cross correlation.

For the above reasons all correlation and deconvolution

procedures, in the latter portion of the research, were solved by the

Fourier method. The Fourier approach not only saves computer time but

also simplifies the coding because boundary problems are eliminated.

After the two matrices, X and Y, are loaded into complex arrays with

Table 3.4. Subroutine structure for the main data reduction software.

ND2 the main FORTRAN manager calling program.

GETF gets the file name and file length.

GETP gets experimental data parameters including number of data points, carrier frequency, and modulation bandwidth.

GETY gets the raw output data (Y) or the chromatographic detector current and loads it into virtual memory.

GETX gets the input data X or the injection sequence from the incoded y output data (i.e. if an injection occurred then the output was negated), loads it into virtual memory, and regrooms the decoded output data.

GETC loads the complex arrays with the input and output data, X and Y, calculates the FOURIER transforms, solves the deconvolution, and then inverse transforms back to the time domain.

PLOTZ generalized plotting subroutine (i.e. Plot-10 package) for displaying any of the data arrays onto the graphics terminal. This routine autonormalized both the absissa and ordinate.

MAG magnifies current plot on the graphics terminal for easier interpretation via user interactive graphics.

Real function calls X.Y,C, and P. Virtual memory routine calls for automatic data retrieval.

Real subroutine calls DMX,DMY,DMC, and DMP. Virtual memory calls for temporary data storage.

---

the imaginary portions set to zero, the forward Fourier transform of
each is solved. This is accomplished in one line of FORTRAN code:

CALL FOURT (DATA,NN,NDIM,ISIGN,IFORM,WORK)

where the complex array is stored in DATA, NN is the number of data
points, NDIM is the number of dimensions of array DATA, ISIGN of -1 for
a forward Fourier transform and +1 for an inverse transform, IFORM of +1
for non-zero imaginary data, and WORK is a complex array if NN is not
an even power of two.

Once the forward (into the frequency domain) transform is
calculated (about 1 second for a 1K array), the correlation or deconvolu-
tion can be solved by a complex multiplication or division of the output
by the input elements. Not only are the boundary problems mentioned
earlier eliminated but the coding is only three FORTRAN lines. An
inverse transform back to the time domain finishes the procedure.

If the output array Y (i.e. raw FID current) is displayed after
the first Fourier transform, then a power spectrum is obtained. A
display of power vs frequency is ideal in studying the speed requirements
of detectors, amplifiers, and associated chromatographic equipment.

The entire data reduction software is contained in file SND2.
Since this program is over 1500 lines of FORTRAN and ASSEMBLY code, it
was essential that a clear and logical approach to its development and
maintenance be adopted. Although FORTRAN was not designed to be a
structured programming language (i.e. perhaps newer approaches like the
Univ. of California at San Diego's language named PASCAL, similar to the

DEC-10's RATFOR or rational FORTRAN will be the language of choice for laboratory programming in the future), the extensive use of over 15 subroutines, each not over one page (approximately 60 lines per page) were used in the data reduction programming. I can not stress enough the necessity of approaching major program development in this structured approach.

An example of one of the subroutines (GETC) is included to demonstrate the modular software approach and the elegant simplicity of the FOURIER deconvolution approach (Table 3.5).

Table 3.5. Fortran Fourier deconvolution subroutine (GETC).

```
1/3/80
SND2  SS   00033

0202          SUBROUTINE GETC
0203   C
0204   C
0205   C      LOAD THE CROSS-CORRELATION ARRAY.
0206   C
0207   C
0208   C
0209          INTEGER FNAME(3),PARA(128)
0210          DIMENSION PL1(128)
0211          DIMENSION A(1050),B(1050)
0212          COMPLEX A,B
0213          COMPLEX CTEMP
0214          COMMON PL1,FNAME,PARA
0215   C
0216          NPTS=PARA(71)
0217   C
0218          WRITE(1,15)
0219   15     FORMAT("NUMBER OF FOURIER TRANSFORM POINTS ?, PLEASE KEEP")
0220          WRITE(1,16)
0221   16     FORMAT("IT A POWER OF TWO, I.E. 2 ** N.")
0222          READ(1,*)NPTS
0223   C
0224   C      LOAD COMPLEX ARRAY A WITH THE DEMODULATED INPUT DATA, AND
0225   C      LOAD COMPLEX ARRAY B WITH THE DEMODULATED OUTPUT DATA.
0226   C
0227          DO 20 I=1,NPTS
0228           A(I)=CMPLX(X(I),0.0)
0229           B(I)=CMPLX(Y(I),0.0)
0230   20     CONTINUE
0231   C
0232   C
0233   C      GET THE FORWARD FOURIER TRANSFORMS OF THE DEMODULATED INPUT
0234   C      AND OUTPUT DATA.
0235   C
0236          CALL FOURT(A,NPTS,1,-1,0.0)
0237          CALL FOURT(B,NPTS,1,-1,0.0)
0238   C
0239   C
0240   C      CALCULATE THE CORRELATION MATRIX BY MULTIPLICATION OF THE
0241   C      FOURIER TRANSFORMS.
0242   C
0243          WRITE (1,25)
0244   25     FORMAT(" # OF CONVOLUTION POINTS ?")
```

Table 3.5. Fortran Fourier deconvolution subroutine (GETC)--Continued.

| 1/3/80 |
| SND2 SS 00033 |

```
0245          READ(1,*)ICP
0246          DO 30 I-1,ICP
0247           B(I)=B(I)/A(I)
0248   30     CONTINUE
0249   C
0250   C
0251   C
0252   C
0253   C GET THE INVERSE FOURIER TRANSFORM OF THE PRODUCT, TO YIELD
0254   C THE CORRELATION MATRIX
0255   C
0256          CALL FOURT(B,NPTS,1,1,1,0)
0257   C
0258   C      LOAD THE CORRELATION MATRIX INTO THE C DMEM ARRAY.
0259   C
0260          CO 50 I=1,NPTS
0261           TEMP=((REAL(B(I))**2)+AIMAG(B(I))**2)**.5
0262           CALL DMC(I,TEMP)
0263   50     CONTINUE
0264   C
0265   C
0266          RETURN
0267          END
* * * LIST END * * *
```

CHAPTER 4

FREQUENCY MODULATED CORRELATION CHROMATOGRAPHY

This chapter consists of a publication entitled "Frequency Modulated Correlation Chromatography" by Dan C. Villalanti and M. F. Burke of the Analytical Division of the Department of Chemistry at the University of Arizona in Tucson and J. B. Phillips of the Department of Chemistry and Biochemistry at Southern Illinois University, which appeared in Analytical Chemistry. It describes the first successful experiment using frequency modulated multiple injection approach applied to a chromatography system.

Introduction

In a benchmark article by Rielley, Hildebrandt, and Ashley (1962) the response of a chromatographic column to any sample input profile was discussed. The use of input profiles other than the usual step and spike functions, and their output responses were predicted for analytical work and for studying fundamental properties of chromato-graphic systems. To data, multiple injection profiles appear the most promising.

Correlation chromatography has been proposed as a method for continuous monitoring of process streams (Annino and Bullock 1973) and as a means of increasing signal-to-noise ratios for lower detection limits (Moss, Kipping, and Godfrey 1973; Smit 1970; Clough , Gibb, and

Littlewood 1972). Annino and Grushka (1976) proposed the use of a pseudo-random binary sequency (PRBS) as the input function for correlation chromatography. This function was chosen because it was simple to generate, its code length easily varied, and most importantly, it contributed no correlation noise when one entire code length sequence was used. A deconvolution approach to eliminating correlation noise by Phillips and Burke (1976) allowed other input regimes to be used since correlation noise was removed in data reduction.

However, in each of these cases an inherent problem, first pointed out by Annino and Bullock (1973) exists. Since correlation assumes a linear system, extreme base-line irregularities are created when the sample concentration is high enough to reach into the non-linear regions of the isotherm. For that reason, successful correlation experiments required dilute streams to keep the partition coefficient constant, or working in a comparison mode in which the two streams are of similar concentration. The latter solution seems impractical since concentration is the parameter that we propose to measure.

Frequency modulated correlation chromatography is a novel multiple injection technique which involves modulating an input signal a small amount around some fixed carrier frequency. This method is similar to standard FM radio broadcasting. A frequency modulated signal resulting from the convolution of the input signal with the impulse response function (transfer function) of the chromatographic system is then recorded at the detector output. The input signal is then deconvolved from the output signal to compute the impulse response

of the chromatographic system. This impulse response is equivalent to a normal chromatogram. Alternatively, the input and output signals may be demodulated and cross-correlated to give the same chromatogram.

The main advantage of the frequency modulated approach is that the amount of solute on the column at any time is almost constant, this being set by the carrier frequency. The input signal is impressed on the system by making small changes around the carrier frequency. By maintaining the solute concentration at an almost constant value, partition coefficients also remain constant even when working in a non-linear region of an isotherm (Fig. 4.1).

Theory

A frequency modulated signal can be input to a chromatographic column by slightly modifying a constant frequency multiple injection signal. Before each injection, a random number is generated to determine the magnitude and sign of the deviation from the carrier frequency for the next injection time. The next injection may come a little earlier or later than the time dictated by the carrier frequency.

The amount of deviation allowed from the carrier frequency (i.e., degree of modulation) is a theoretically important parameter. As it is increased, the bandwidth and information carrying capacity of the signal also increases. However, as the degree of modulation is decreased, the deviations from linearity inherent in a chromatographic system become less important because each cycle of the carrier experiences a more similar environment. A very large degree of modulation causes correlation noise in the computed chromatogram while a small modulation
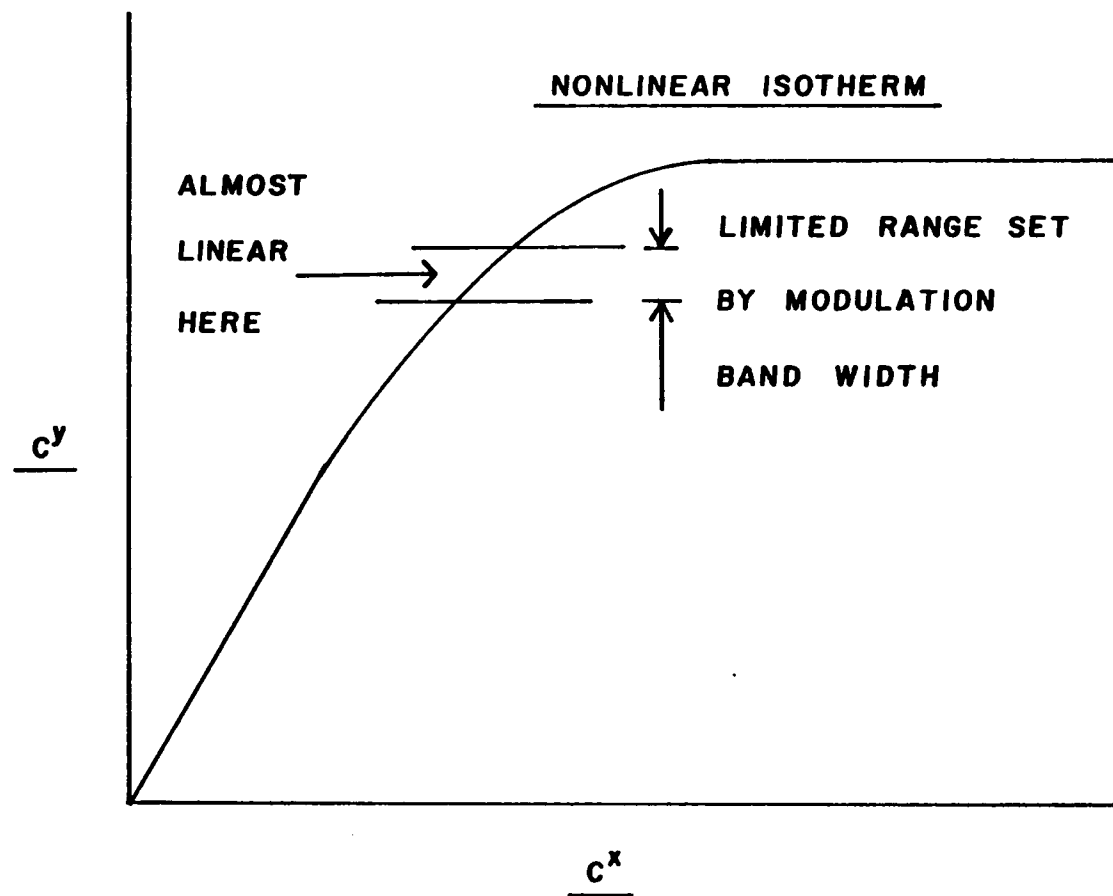
Figure 4.1. An example of a non-linear distribution isotherm approximating a linear system by restricting the change in concentration by frequency modulation

reduces the information content of the signal to the point where all but the carrier frequency is lost in the noise. Thus, there is some optimum degree of modulation which gives the best S/N ratio for a particular chromatographic system. The previously employed pseudo-random input signals have high degrees of modulation and therefore suffered from correlation noise unless sample sizes were small to minimize non-linearity.

The carrier frequency should be high enough to cause severe peak overlap. A low frequency carrier offers no advantages and can slow the experiment by limiting the frequency of the modulating pseudo-random signal. A high frequency carrier is desirable because it gives a more uniform sample distribution minimizing non-linearity. Beyond some frequencies, however, the remaining non-linearity is caused by the degree of modulation and changing the carrier will have no effect.

The impulse response or transfer function (H) can be derived directly from a deconvolution procedure or can be approximated by computing the cross-correlation. The correlation method is described first with the deconvolution procedure following.

The output signal is demodulated by subtracting it from itself with a delay of one cycle of the carrier signal.

$$Y'(T) = Y(T + 1/2F) - Y(T - 1/2 F) \tag{4.1}$$

where $Y(T + 1/2F)$ is the output signal at a time T plus one half a carrier cycle and $Y(T - 1/2F)$ is the output signal one cycle earlier.

Similarly, the input signal, X, is demodulated by:

$$X'(T) = X(T + 1/2F) - X(T - 1/2F) \tag{4.2}$$

where $X'(T)$ is the demodulated input signal and $X(T + 1/2F)$ and $X(T - 1/2F)$ are the input signals separated by one carrier cycle.

The top portion of Figure 4.2 shows a frequency modulated FID signal followed by pure carrier frequency. This carrier wave is the result of overlapped peaks. The demodulated signal, calculated from equation 4.1, appears on the lower portion from about 75-850 and the demodulated carrier is essentially reduced to zero from 850-1500 on the abscissa.

The demodulated input and output signals are then cross-correlated in the usual way to approximate the transfer function or impulse response of the system.

$$\phi_{xy}(\tau) = 1/T \sum_{T=1}^{n} X'(T)*Y'(T - \tau) \tag{4.3}$$

where τ is the delay time and T is a data point. Figure 4.3 summarizes the important steps in a frequency modulated correlation type experiment.

The transfer function H can also be solved by the Fourier transform deconvolution method (Bracewell 1975; Carlson 1975; Horlick and Hieftje 1978). This not only saves computation time but also simplifies boundary problems encountered when using equation 4.3.

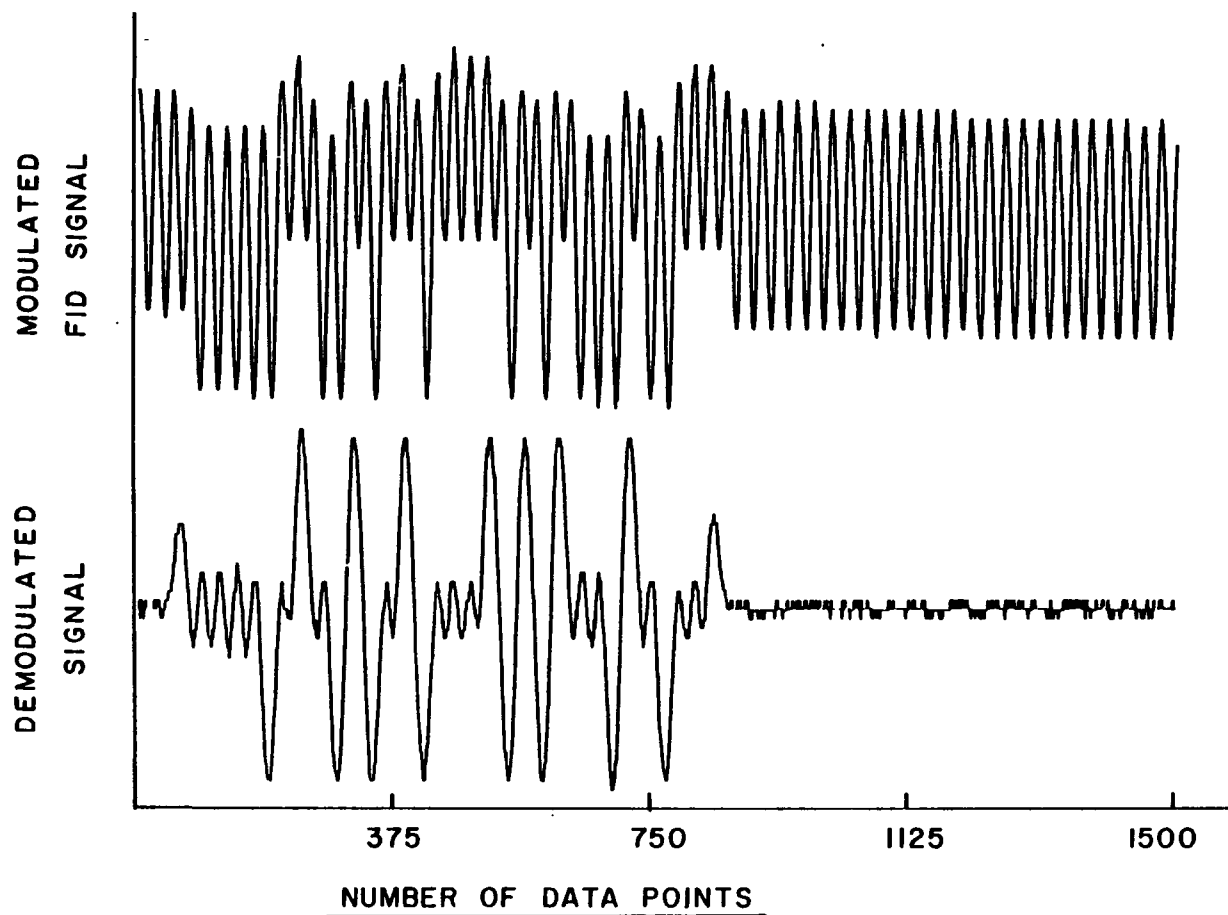**Figure 4.2.** Detector output and demodulated signal of hexane on Durapak-N-octane at 60° C. -- PRBS code length = 31. Data acquisition rate = 10 Hz. Carrier frequency = 0.400 Hz. Flow rate = 40 cc/min., Column head pressure 18 P.S.I. Modulation bandwidth = 0.416-0.384 Hz. or +/- 4.0 %.
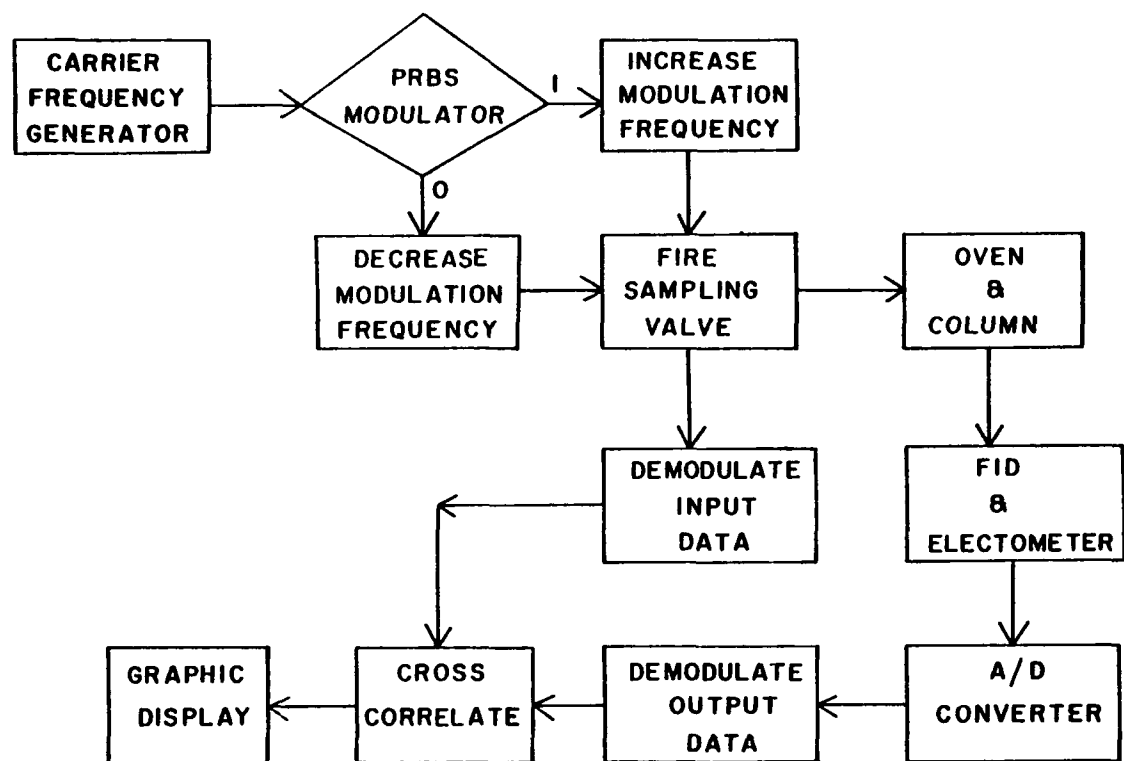
Figure 4.3. Flowchart diagram summarizing the basic steps of a frequency modulated (FM) correlation type experiment.

$$Y(T) = X(T) \; \boxtimes \; H(T) \tag{4.4}$$

$$\downarrow \qquad \downarrow \qquad \downarrow$$
$$FT \qquad FT \qquad FT$$
$$\downarrow \qquad \downarrow \qquad \downarrow$$

$$Y(F) = X(F) \; * \; H(F) \tag{4.5}$$

Solving for H(F):

$$H(F) = Y(F) \; / \; X(F) \tag{4.6}$$

$$| \qquad | \qquad |$$
$$IFT \qquad IFT \qquad IFT$$
$$\downarrow \qquad \downarrow \qquad \downarrow$$

$$H(T) = Y(T) \; \boxtimes^{-1} X(T) \tag{4.7}$$

or overall:

$$H(T) = IFT \; (FT[Y(T)]/FT[X(T)]) \tag{4.8}$$

where:

X = Input	T = Time Domain
Y = Output	F = Frequency Domain
H = Transfer Function	FT = Fourier Transform
$\boxtimes$ = Correlation	IFT = Inverse Fourier Transform
$\boxtimes^{-1}$ = Inverse Correlation	

Thus the entire demodulation and cross-correlation procedure is reduced to a mere division operation in the frequency domain. With a suffieiently high carrier frequency and a limited modulation bandwidth, the input signal can be limited to a small region of the isotherm of a non-linear system which will then behave as if it were linear.

Experimental

A Varian model 1700 gas chromatograph with a FID detector and a Linear model 261 recorder were used throughout these experiments. The column was a 30 cm by 1/8 inch copper tube, packed with 80/100 mesh Porapak P. The carrier gas was filtered dry nitrogen. The solutes were hexane and acetone. Sampling was done via a sample loop type, Seiscor model VIII sampling valve with variable resistance (Nupro model SG fine metering valve) to match sample loop and column head pressures. Total switching time was specified to be 10 miliseconds by the manufacturer. The sample loop was shortened from 24" to 2.4" to reduce the sample volume approximately 50 μl. This maximized the frequency response of the valve by shortening the sweep and refill times of the sample loop. These times, at a carrier flow of 30 cc/min, are approximately 100 milliseconds. Although other sampling methods have been proposed (Annino, Gonnord, and Guiochon 1979), the Seiscor valve provided the speed, accuracy, and reliability needed for these experiments.

Data acquisition and experimental control were under the reign of a dedicated Hewlett-Packard 2115A computer, with an integrating digital voltmeter (HP 2401C), and a digital voltage source (HP 6131B). Programming for the experimental side of the computer network was written in Fortran and HP assembly language. User selected experimental parameters included: data acquisition rate, pseudo-random binary sequence (PRBS), code length, carrier frequency, and modulation bandwidth.

Briefly, the experiment begins by the user inputting the desired operating parameters to the computer. When finished, the computer then

starts generating injections at the carrier frequency. This brings the
concentration up to a stable level and maintains it there. After a
specified time period, the program jumps to the PRBS subroutine which
returns with a 0 or a 1. With this number the computer will begin
frequency modulating the input signal to the chromatograph. This was
accomplished by sending out a pulse via the digital voltage source to an
EXACT model 128 function generator with a variable pulse width, which
then fired a Clippard Minimatic model EOV-3 nitrogen powered solenoid
connected to the Seiscor sampling valve. The following table (Table 4.1)
shows this procedure for a PRBS code length of 7, carrier frequency of
0.400 Hz, and 25% modulation. Figure 4.4 is a graphical representation
of this procedure.

Every 64 data points, input and output data were sent to a
larger disk-operating system over a high-speed communications network.
When the entire PRBS code was cycled, the carrier frequency reappears
for a short time and the experiment is terminated (see Figure 4.5).

Results and Discussion

The first correlation chromatograms using the frequency
modulated scheme were essentially pure noise. The reasons for this were
numerous and a systematic evaluation of the signal processing at every
step of the experiment was undertaken.

Initial experiments used a PRBS code length of 31 and a 0.400
Hertz carrier frequency. With a data acquisition rate of 10 Hertz, the
total number of data points in one cycle of the PRBS was 31/0.400 Hertz
times 10 Hertz or 775 data points. Because of memory limitations on the

Table 4.1. Generation of an FM signal for multiple injection chromatography using a pseudo-random binary sequence.

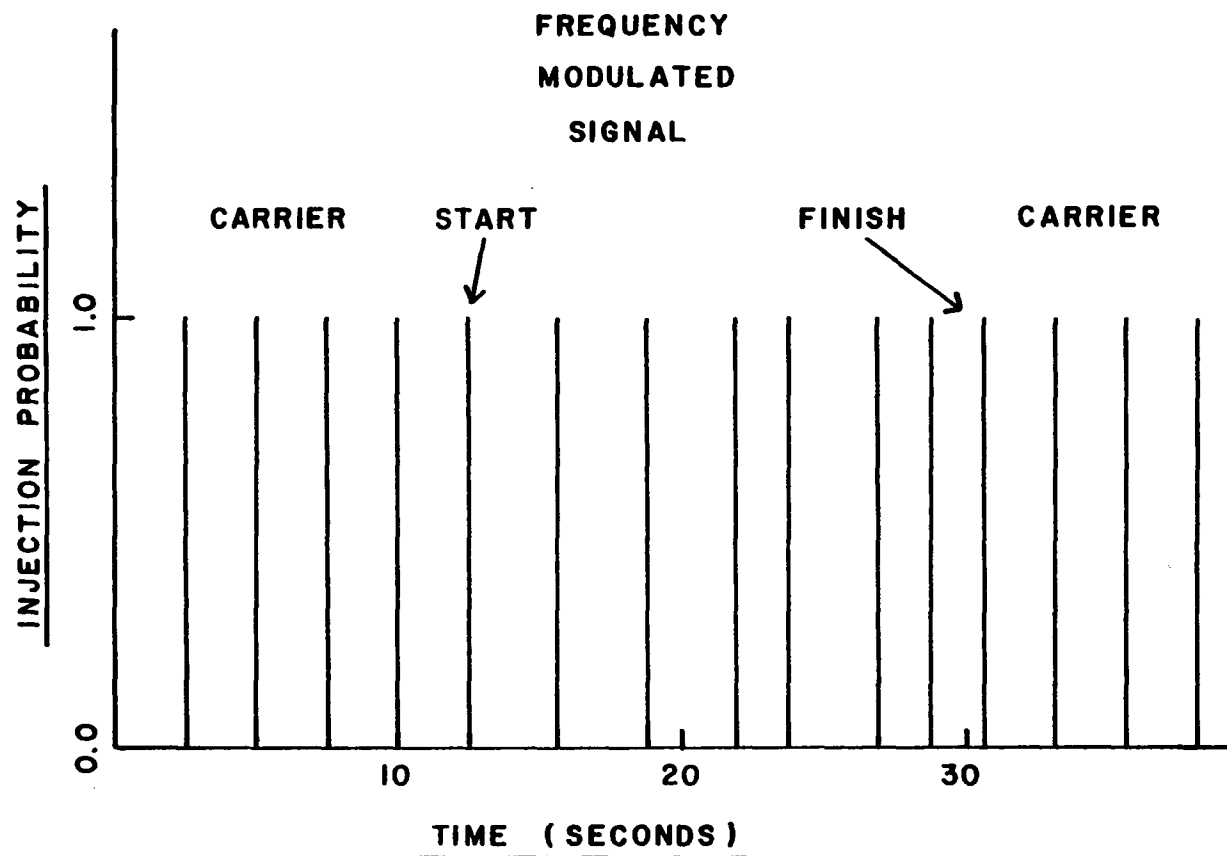| N	Output from PRBS	Deviation	ΔT between Injections (secs.)	Current Freq. in Hertz
N-1	-	0	2.500	0.400
N	-	0	2.500	0.400
N+1	1	+	3.125	0.320
N+2	1	+	3.125	0.320
N+3	1	+	3.125	0.320
N+4	0	-	1.875	0.533
N+5	1	+	3.125	0.320
N+6	0	-	1.875	0.533
N+7	0	-	1.875	0.533
End of Cycle	-	0	2.500	0.400
	-	0	2.500	0.400

Figure 4.4. Input profile of a simple frequency modulated experiment. — PRBS code length = 7.
Carrier Frequency = 0.400 Hz. Modulation bandwidth = 0.320–0.533 Hz. or +/– 25.0 %.
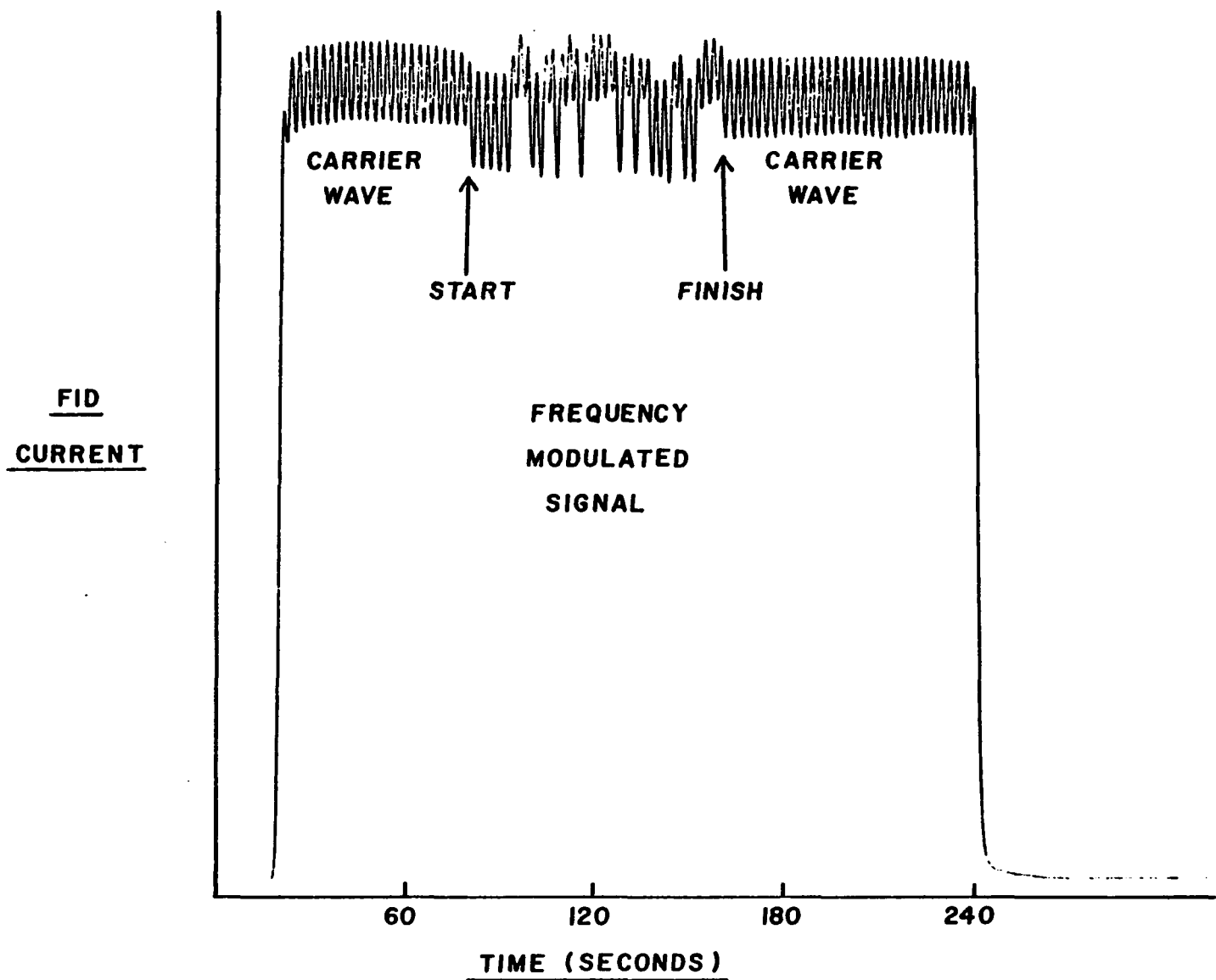
Figure 4.5. Entire detector output for an FM experiment (carrier FM carrier). -- Same conditions as in Figure 4.2.

computer, we were forced to use an even power of two in the Fourier routines. However, by using 2^9 or 1024 points, about one and one-third PRBS code lengths were covered. These results are seen in Figure 4.6. Although the major correlation peak at 10.5 seconds delay corresponds to the correct retention time, spurious peaks and a jagged baseline result because of the code length not matching the Fourier matrix dimensions. Although the PRBS code was not used to inject samples directly, it was used to modulate the carrier frequency and therefore complete cycles must be used to avoid correlation noise. By slightly reducing the carrier frequency to 0.303 Hertz or 33 points per PRBS call, 31/0.303 Hertz times 10 Hertz or 1023 data points were collected. This was very close to the 1024 points which the fast Fourier transform routine must use. Figure 4.7 shows a well defined baseline with a much better signal-to-noise ratio than Figure 4.6. These improvements are the result of using one entire PRBS code length sequence. Another solution to this problem would be to zero fill the Fourier matrix (Griffins 1978) in the above case from data point 776 to 1024, although this solution is wasteful of computer time and memory.

The second source of noise in these first experiments with FM chromatography involved the input scheme mentioned earlier. That was applying a carrier frequcney, then modulating a cycle, and then returning to the carrier, (i.e., carrier>FM>carrier). This scheme wasted some of the data points in the beginning, until the breakthrough time of the first injection, and at the end; that is, all data after the last injection were lost. These boundary problems are not peculiar to FM type experiments, but also exist for other correlation experiments.
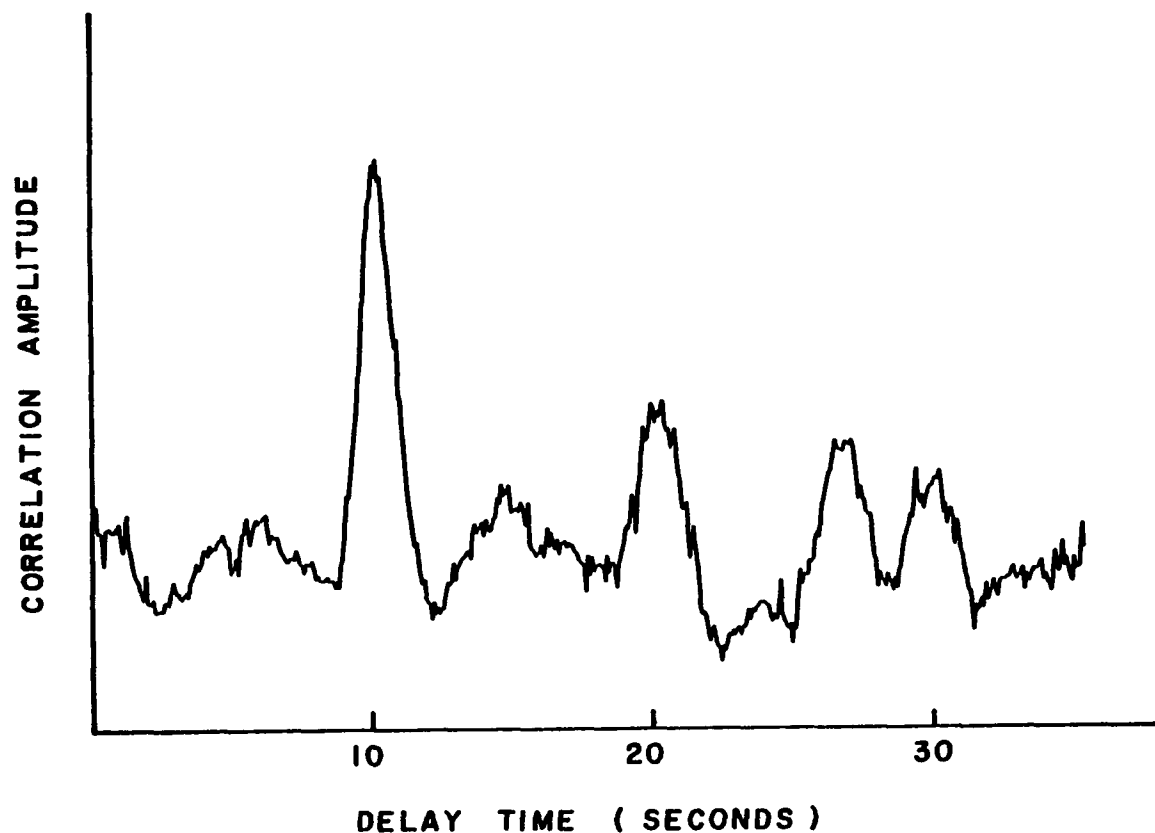
Figure 4.6.   Example of correlation noise due to PRBS cycle length. -- Column was a 50 cm long, 2 mm i.d. copper tubing packed with 100/120 mesh Porapak P.   Solute = acetone at 120° C. k' = 2.5.   Other parameters were the same as in Figure 4.2, except for +/- 20% modulation.
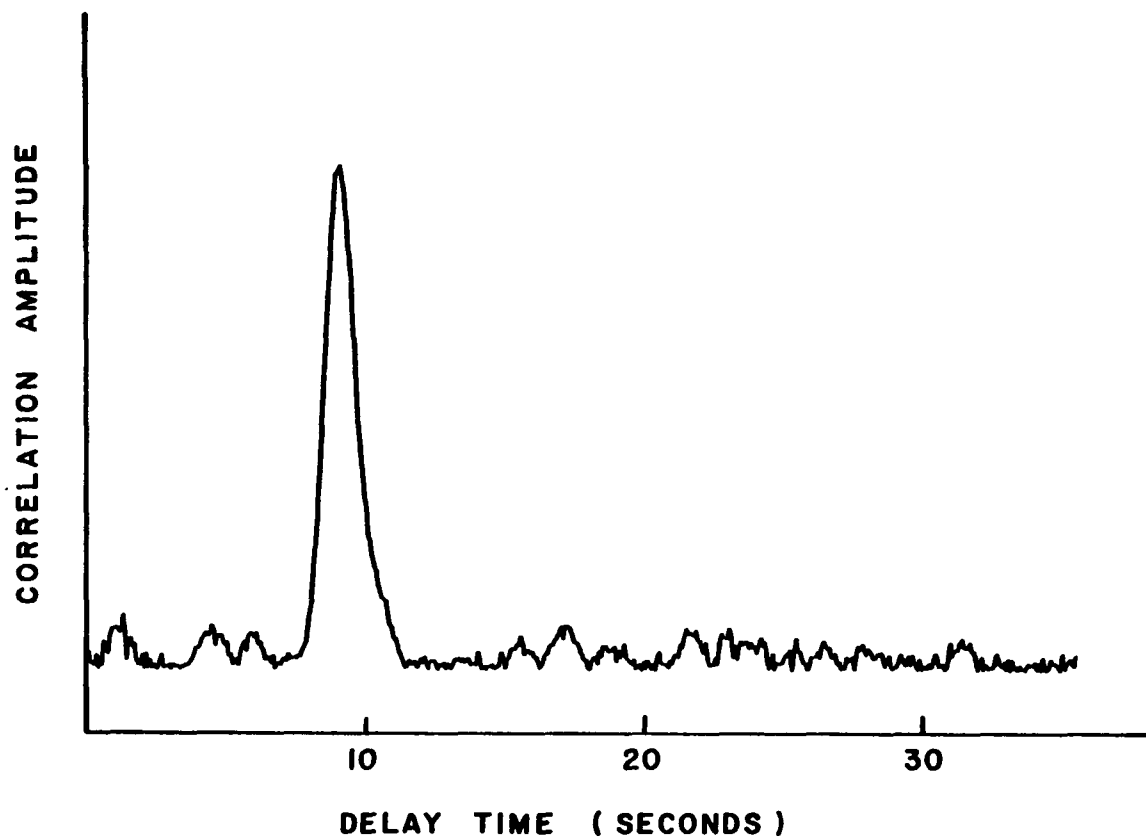
Figure 4.7. Same correlation chromatogram as in Figure 4.6, except that carrier frequency was reduced to 0.303 Hz. -- Correlation noise is greatly reduced because one complete PRBS code is used.

The solution to this problem was to run through two cycles of the PRBS, i.e. (FM>FM), and then grab only the output during the last cycle. The time that was wasted until the breakthrough was now filled with the data that was missed after the last injection. Thus the entire output for one complete cycle of the PRBS was collected.

One other pitfall that was encountered occasionally involved the use of equation 4.6, the division of the output by the input in the frequency domain. This was done sequentially starting at DC and moving to the higher frequencies. The problem occurred when the input contained only very small amounts of those higher frequencies. The division then caused an overflow in the program. This was corrected by dividing only the lower frequencies, with no sacrifice in accuracy because all of the chromatographic information was concentrated in this area of the frequency domain. Once the above problems had been solved, frequency modulated experiments could be run in a routine manner.

The concept of information encoding and subsequent decoding, which allows frequency modulated experiments is summarized in equation 4.8. It is impressive in its simplicity and conciseness. From this equation any input to a chromatograph can be used to solve for the transfer function of the column, i.e. the spike elution response. Frequency modulation has been shown to expand the scope of potential applications of multiple injection chromatographic experiments by allowing finite concentration systems. Other encoding techniques such as pulse and phase modulation (Obst 1968) appear to have similar advantages. Work in these areas is currently being investigated.

CHAPTER 5

RESULTS AND DISCUSSION

Expectations of FM Multiplex
Techniques to Chromatography

As mentioned in Chapter one, multiple injection chromatography provides a method of:

1) studying solute-adsorbent interactions in non-linear chromatographic systems,

2) enhancing sensitivity, S/N ratios, and improving analysis time.

This chapter addresses the problem of studying linear and non-linear chromatographic systems using the multiplex method of sample introduction. The ability to restrict the sample concentration range (Fig. 4.5) and thus work in different regions of the adsorption isotherm (Fig. 4.1) provides unique information about the solute-adsorbent interaction.

Both homogeneous and heterogeneous adsorbent surfaces were used to provide information about non-linear chromatographic processes arising from:

1) single site overload (PORAPAK)

2) residual silanol effects on a bonded phase adsorbent (DURAPAK)

The case of site overload in normal single injection chromatography is characterized by shifting retention times and tailing peak shapes. This is explained by examining the effects on a solute concentration plug traveling down the column, in terms of the adsorption isotherm.

Assuming the sample is concentrated enough to drive the system into a non-linear region of the isotherm, the sites will be covered enough that the average molecule will not interact as many times with the surface (compared to a linear case) and thus have a shorter retention time. As the peak begins to broaden the amount of site overload will become less and less until the latter molecules are experiencing a linear chromatographic system. Thus, in the case of simple single site overloading, decreasing the concentration of the solutes will prevent non-linear behavior.

Non-linear behavior that does not diminish with the lowering of solute concentration is commonly a result of two (or more) site chromatographic surface. Thus, in the case of modern bonded phase GC and HPLC packing materials, tailing is often seen to some extent, especially with more polar solutes. The usual solution to this problem is to maximize the surface coverage of the bonded phase by covering the highly energetic silanol (Si-OH) groups. The use of mono-functional silanes such as dimethylchlorooctadecylsilane (i.e. for making reverse-phase C-18 packings) avoids troublesome polymerization problems and results in a more uniform, more derivatized surface. However, even the best reverse phase packings only provide approximately 60-70% coverage of the surface silanols, probably because of steric hindrance during the silylation reaction.

An example of single site overload is shown in Figure 5.1. Samples of 1.0, 0.5, 0.25, 1.125, 0.06 microliters of neat hexane and heptane and 20 microliters of the gas over the liquid were injected onto
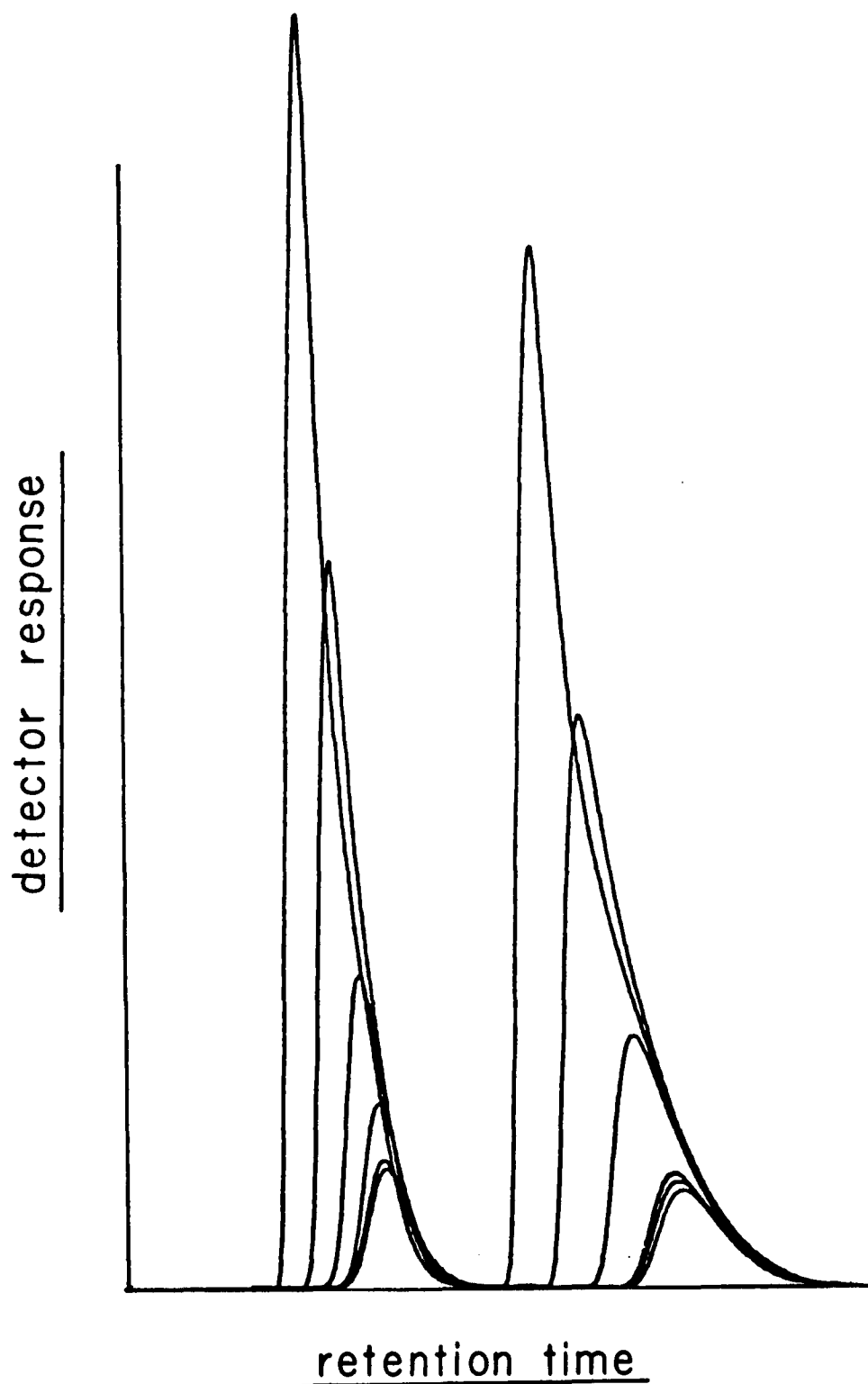
Figure 5.1. Changing retention times and peak shapes of hexane and heptane on Porasil B as a function of concentration. -- Taller peaks being higher concentration.

a 3 foot x 1/8" Porasil B, 80-100 mesh column at 120 °C. As expected, the peak shapes were highly skewed at the higher concentrations and exhibited sharp fronts and broad tails. As the concentration was decreased the peaks became more Gaussian in shape and the retention times approached a limiting value (i.e. infinite dilution).

Site overload with the resulting change in retention time is a phenomenon which can occur over a small range of sample concentration. Since the chromatographer is so used to thinking in terms of logarithmic changes of the parameters influencing chromatographic behavior (i.e. linear detector ranges of 10^6, plots of ln V_r, etc.), the idea that doubling the sample concentration can cause drastic non-linear effects seems strange.

Further discussion of the application of multiple injection chromatographic techniques to the characterization of non-linear systems requires an understanding of the experimental parameters which determines its useful range. These follow.

Characterization of Experimental Parameters in Multiple Injection Correlation Chromatography

Besides the obvious requirements of a high quality chromatograph with good temperature control, low dead volume injectors and detectors, other parameters also influence the quality of multiplex chromatograms. In order to explain the results and improve the technique, a study of the parameters which influence the results of multiple injection experiments was undertaken. These parameters include:

1) the carrier frequency or solute injection rate

2) modulation bandwidth

In order to utilize the frequency modulated approach to study non-linear regions of the isotherm conditions in the column must be such that the concentration profile be confined to a narrow range. This necessitates severe peak overlap so that excursions from the top of each peak to the minimum between peaks are small (Fig. 4.4). Thus, the narrower a peak, the greater the rate of injection or <u>carrier frequency</u> must be to maintain the proper peak overlap. Other parameters which influence peak spreading such as the temperature, column length, and flow rate, must be matched with the carrier frequency to determine the optimum injection rate.

The other fundamental parameter is the <u>modulation bandwidth</u>, or the instantaneous departures in time from the carrier frequency. In order to minimize the non-linear effects of changing concentration, these deviations from the carrier frequency should be kept as small as possible. However, the information carrying capacity of the signal is proportional to the bandwidth or modulation amplitude. Therefore, bandwidth is a trade-off between signal-to-noise and non-linear effects.

A series of experiments determining the optimum amount of modulation were run at 100 °C using hexane and heptane as the solutes, on a two foot x 1/16" O.D. stainless steel column packed with Porasil C. This packing material is a spherical silicous solid with average pore diameter of 300 $\overset{\circ}{A}$, and a surface area of 100 meters^2/gram. A data acquisition rate of one point every 0.12 seconds, with injections occurring, on the average, every 33rd data point, or every 3.95 seconds, gave a carrier frequency of 0.253 Hz.

A series of five experiments stored in data files FM041-FM045 with constant carrier frequency and variable modulation bandwidth is presented:

File name	ΔF	% Modulation = $\Delta F/33$
FM041	1	3.03
FM042	3	9.09
FM043	5	15.2
FM044	7	21.2
FM045	15	45.5

All of these experiments were run exactly two cycles of the pseudo-random binary sequence of 31. With the carrier injection frequency occurring on the average every 33 data points, a total of 31 x 33 x 2 or 2046 data points are collected during each experiment. Of these, only the first 1K or 1024 were used because of computer memory limitations using the FFT routine.

Figure 5.2 shows the raw FID output for the five FM experiments described. The lower plot corresponds to FM041 or the 3.03% modulation case; with the modulation increasing in the upward direction, FM045 being on the top. In FM041, the resultant detector current is essentially just the carrier wave of 0.253 Hz, with each peak being the super-position of the hexane and heptane components. As the percent modulation is increased, the time difference between injections also increases. If two injections are relatively close together, then the peaks will begin to merge. In the most extreme case (i.e. FM045, the
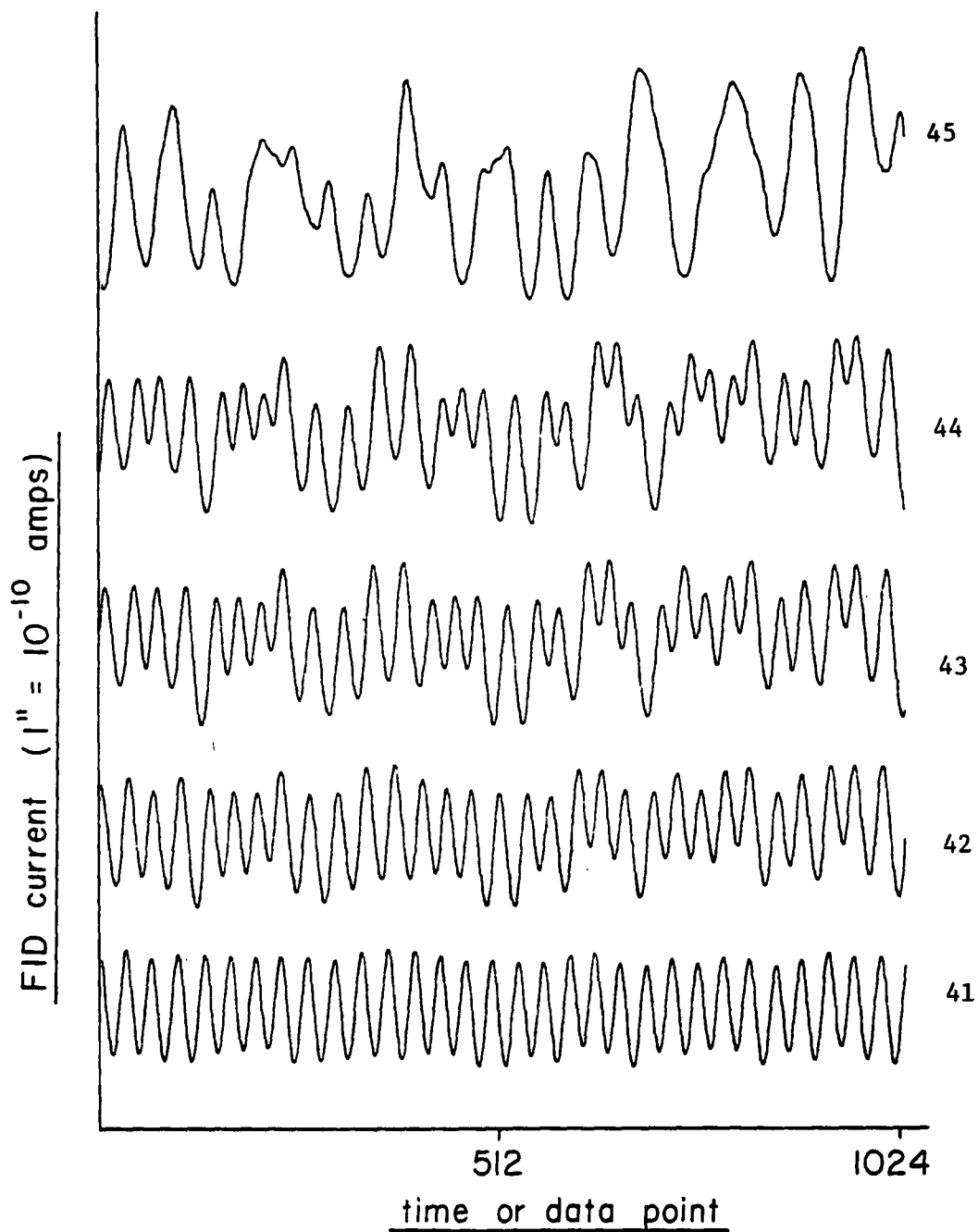
Figure 5.2. Effect of input modulation frequency on column response
as measured by detector output.

top plot of 45.2% modulation) the number of discernible peaks is decreasing and the amplitude modulation of the FID signal is increasing. Figure 5.3 shows the computed chromatograms of files FM042-FM045. The modulation bandwidth of file FM041 was too low for useful information encoding. Although the signal carrying capacity is greatest in FM045, the signal-to-noise ratio, as seen in the case of FM042 or FM043 (Fig. 5.3), is optimized in the lower modulation cases. This is the case because the concentration changes which occur in the higher modulation experiments are sufficiently large to cause non-linear behavior of the chromatographic system. The optimum modulation bandwidth appears to be in the 10% to 20% range. This represents the trade-offs between signal bandwidth and non-linear behavior.

The other parameter, kept constant until now, was the carrier frequency.

A series of experiments were run in which the modulation parameter was kept constant and carrier frequency varied. This was accomplished by changing the computer's interupt rate. This not the only method of changing the carrier frequency, but was selected because each experiment must contain 1024 data points. Since the data acquisition rate is changing in these experiments, artifacts could arise if the sampling rate drops below the Nyquist rate.

The input parameters for a series of four experiments (FM046-FM049) varying the carrier frequency are given in Table 5.1

Other experimental parameters for these experiments included: the solutes, hexane and heptane, the column, a 1/8" O.D. x 2 foot
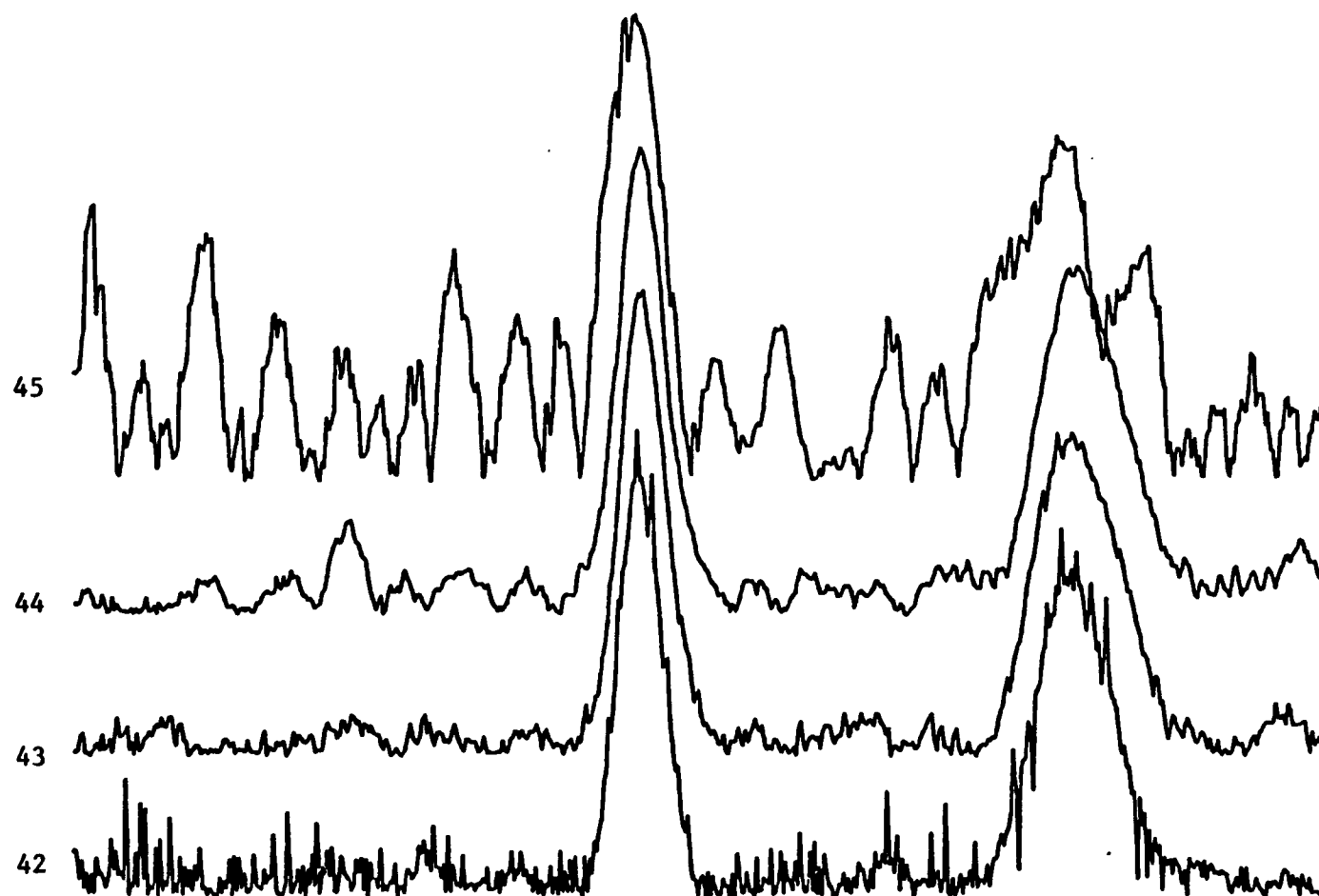
Figure 5.3. Effect of frequency modulation on signal-to-noise ratios for computed chromatograms of hexane and heptane on Porasil B.

Table 5.1.   Input parameters of experiments varying the carrier
frequency.

FM file	inter. skips	delta T	cycle time (sec.)	carrier freq. Hz	% mod.	data acq. rate Hz	total exp. time (sec.)
46	5	0.05	1.65	0.6061	12.12	20.00	51.20
47	8	0.08	2.64	0.3788	12.12	12.50	81.92
48	11	0.11	3.63	0.2755	12.12	9.09	112.64
49	14	0.14	4.62	0.2165	12.12	7.14	143.36

stainless steel tube filled with Porapak P 80-100 mesh, oven temperature 120 °C, electrometer, 1 x 10^{-9} amps sensitivity, detectors and injectors held at 150 °C, PRBS generator length = 31, and modulation of 4/33 or 12.12%. The output of these experiments is shown in Figure 5.4.

In order to superimpose the outputs (i.e. deconvolved chromatograms for S/N determinations) a different number of data points must be plotted for each file. This is because of the differing data acquisition rates. A forty second time window is displayed for each file, as shown below.

File #	ΔT or sec/data pt.	# of data points/40 sec.
46	0.050	800
47	0.080	500
48	0.110	364
49	0.140	286

$$= 40/\Delta T$$

It can be seen from Figure 5.4 that the signal-to-noise ratio is dependent on the carrier frequency ans is maximized in the region between 0.28 and 0.38 Hertz. As was the case with the modulation bandwidth, there exists an optimum region of carrier frequency which is a function of the band broadening and the retention time of the solute(s) being chromatographed.

Having established the useful range of the parameters which control FM experiments, the application to the two most common types of non-linear chromatographic systems was investigated.
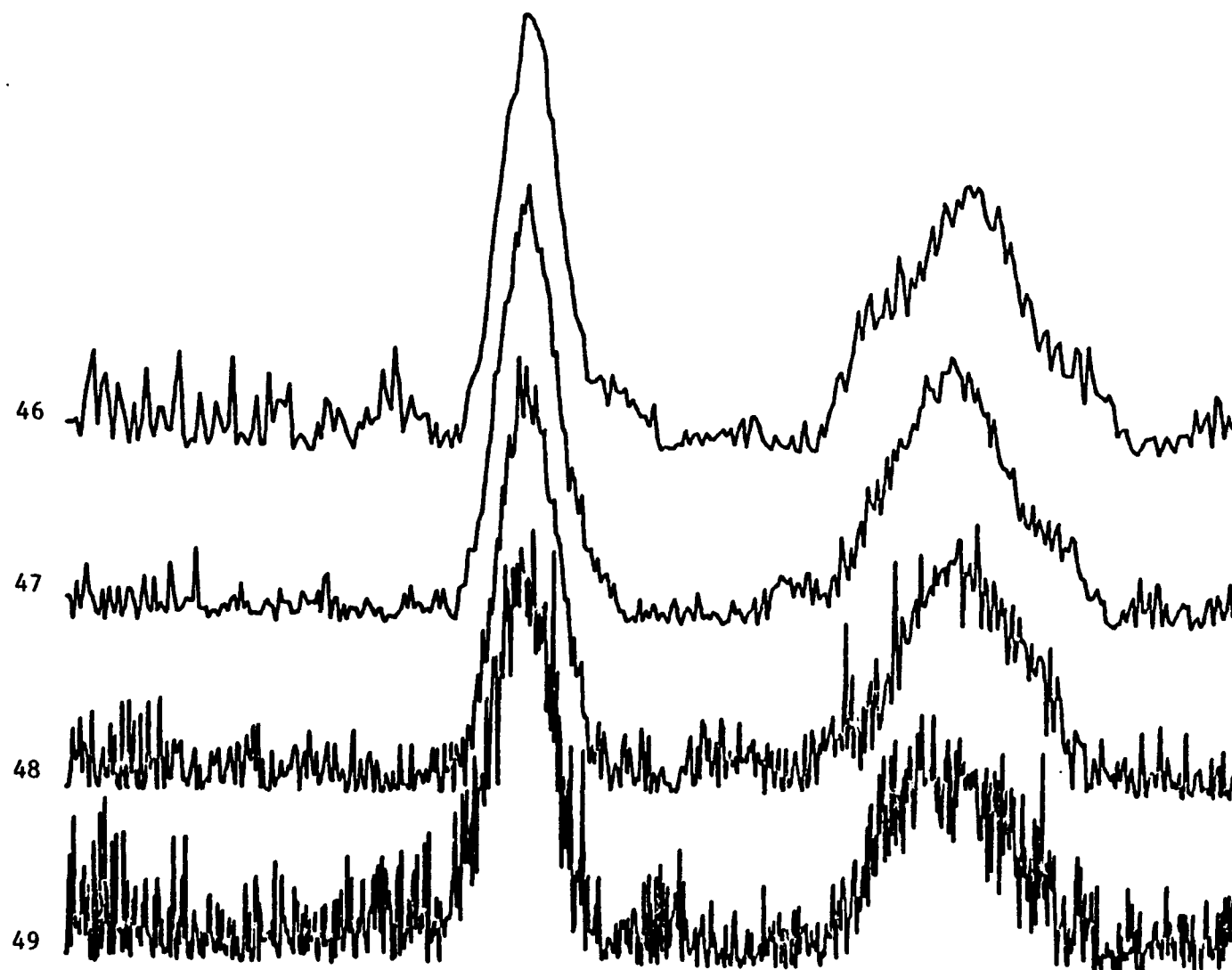
Figure 5.4.   Effect of carrier frequency on signal-to-noise ratios for computed
chromatograms of hexand and heptane on Porapak P.

Study of Non-linear Behavior on a Homogeneous Surface by Multiple Injection Chromatography

The first demonstration of the utility of the frequency modulated approach to studying non-linear chromatographic behavior was difficult because of the problems of getting enough solute (in the gas phase) onto the column for site overload. To produce non-linear effects (i.e. just the opposite of most chromatographers' problems) these changes were made:

1) A thin wall (0.05" I.D. 1/16" O.D. stainless steel column with Porapak P 100-120 mesh) was packed. This was approximately an order of magnitude less packing material than the regular 1/8" O.D. column and therefore should be about ten times easier to get into a non-linear range.

2) Changing solutes from acetone or hexane to diethyl ether, to get more solute onto the column per unit time. This however caused problems because the solute evaporated so fast that the level in the nylon sampling tube changed, thus changing the solute concentration during an experiment and decreasing the S/N ratio of the system.

Two expreiments FM025 (linear case) and FM026 (non-linear case) were run. Sample introduction in the former was with a melting point capillary tube and the latter case with a 1/8" nylon tube. Electrometer setting of 8×10^{-2} amps and 2×10^{-10} amps represent a 400 times concentration chang in the two experiments.

Single injection chromatograms are shown in Figure 5.5. A 8.9% change in retention time between the linear (R_T = 23.70) and non-linear case (R_T = 21.60) is seen, indicative of site overload. Also seen in Figure 5.5 is a change in peak shape; a badly tailing peak for the overloaded case and a Gaussian form for the linear run.

The deconvolved chromatograms resulting from 31 injections of a frequency modulated experiment run under the same conditions as the single injection experiments are presented in Figure 5.6. This, to my knowledge, is the first multiple injection experiment demonstrating non-linear behavior. It should be noted that the two peaks in Figure 5.6, although different in retention times, both have approximately Gaussian peak shapes. The reasoning for this will be explored.

A more systematic study of FM techniques applied to non-linear chromatographic systems required a more stable and reproducible experimental design. The calibration gas standard method of sample introduction prepared per instructions from Matheson provided a reproducible and drift free solute sample introduction method for the remainder of all FM experiments in this work. Also, modifications (Figure 3.3) to the sampling electronics, to eliminate any induced delays, were installed for all future runs. These two changes improved the reproducibility and precision of the system and made multiple injection experiments much easier for the experimenter.

Since the lower hydrocarbons were used extensively, the vapor pressures at room temperature were calculated to four places to
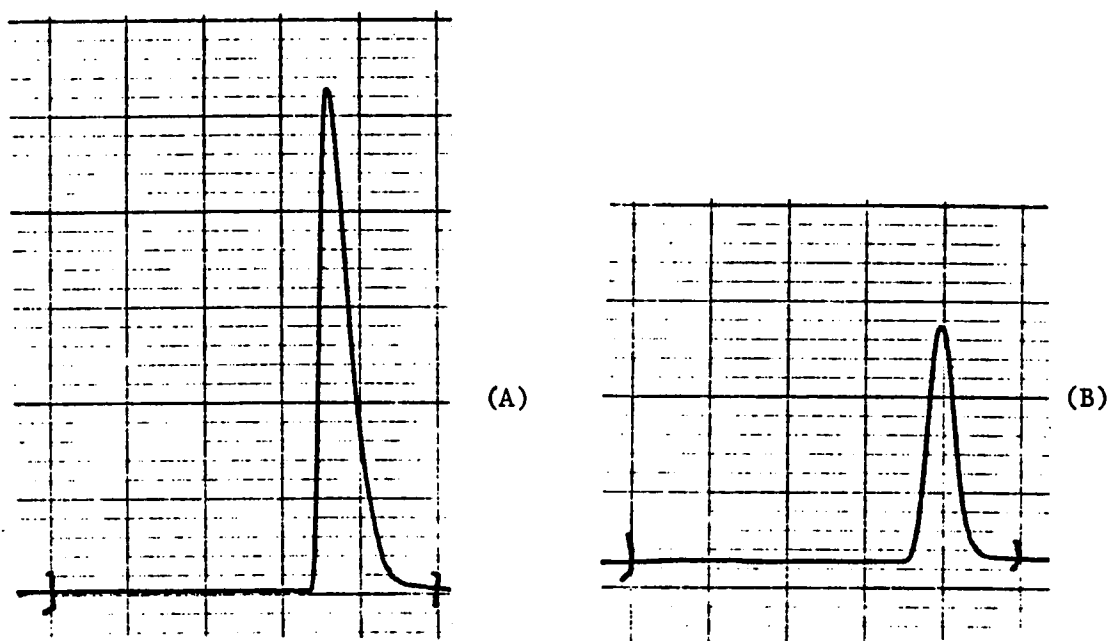
Figure 5.5. Single injection chromatograms of diethyl ether on
Porapak P demonstrating (A) nonlinear and (B) linear
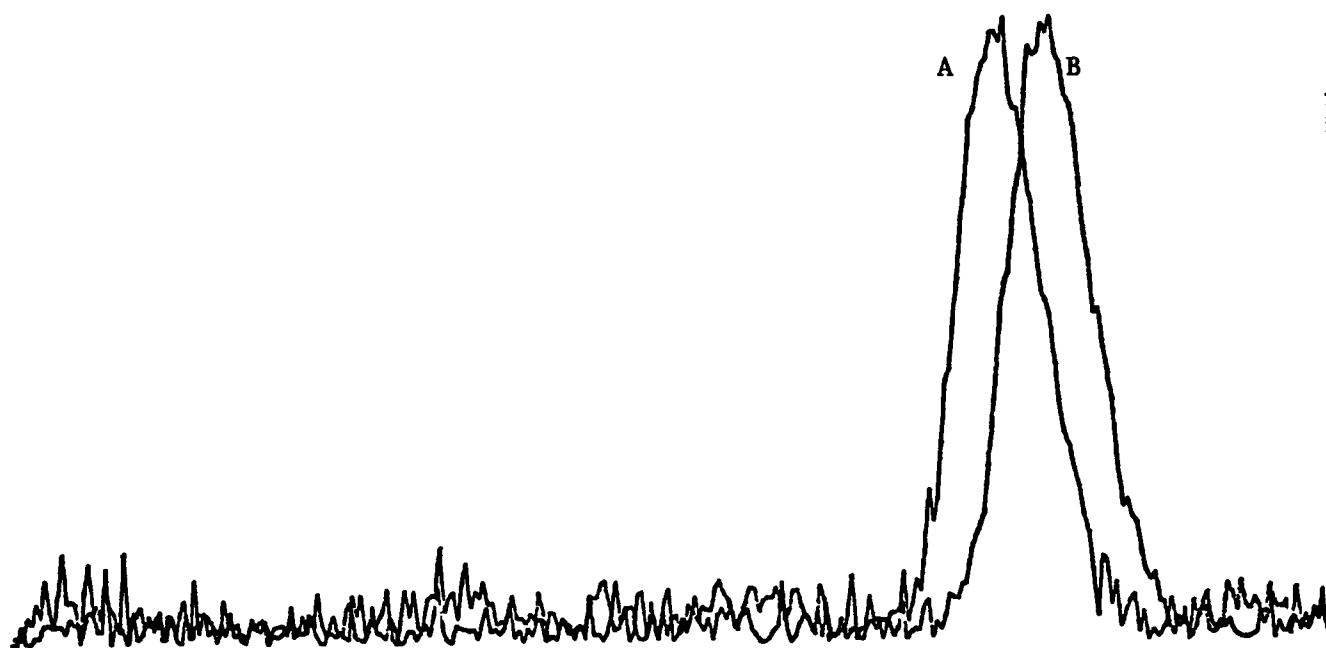behavior.

Figure 5.6. Deconvolved chromatograms of diethyl ether demonstrating Gaussian peak
shape for linear and nonlinear cases.

determine the maximum concentration allowed in the sampling cylinders

(CRC Handbook 1976). These were:

n-pentane	C_5	7.197 PSI	
n-hexane	C_6	2.070 PSI	at 293 K°
n-heptane	C_7	0.633 PSI	

Two pressurized lecture bottles of 100 and 10,000 PPM volume/
volume of pentane in clean dry N_2 were prepared by injecting 7.14 and
714.5 microliters of neat pentane into the sampling cylinder and
filling with nitrogen to a total of 500 PSI.

A series of six experiments were run as described below
(Table 5.2). The purpose of these experiments was to investigate the
modulation bandwidth effects on peak shape and retention time for a
finite concentration (i.e. non-linear system) and an infinite dilution
(i.e. linear) chromatographic system.

The raw output data for these six experiments is presented in
the following three figures (Figs. 5.7-5.9). The order is paired
concentrations of 100 and 10,000 PPM of pentane at 120°, 110°, and 100° C.

Figure 5.7 shows files 58 and 59, these waveforms show the
largest amount of modulation because the peak to peak overlap is least.
The relatively short retention times caused by the higher temperature of
these experiments restricts the longitudinal diffusion and thus peak
overlap is not great.

The extremes of output modulation would be an injection rate
which is so slow that the baseline occurs between eluting peaks. This
would be 100% signal modulation, and the entire range of the isotherm

Table 5.2. Experimental parameters for the study of modulation bandwidth effects on the peak shape and retention time for a frequency modulated multiple injection chromatographic system.

Figure #	File #	Conc. PPM	delta F	freq. % modul.	Data acq. rate	Temp. °C	Carrier f.	% Signal Mod.
7	FM058	10,000	5/33	15.2	8.33 Hz.	120	1.67 Hz.	68
7	FM059	100	5/33	15.2	8.33 Hz.	120	1.67 Hz.	63
8	FM060	100	4/33	12.1	10.00 Hz.	110	2.50 Hz.	25
8	FM061	10,000	4/33	12.1	10.00 Hz.	110	2.50 Hz.	33
9	FM062	100	4/33	12.2	10.00 Hz.	100	2.50 Hz.	19
9	FM063	10,000	4/33	12.1	10.00 Hz.	100	2.50 Hz.	21

from zero to the maximum concentration of the eluting peak would be covered. The other extreme case would be a column output signal with zero modulation, or a simple DC level. This would occur when the injection rate at a given temperature is so fast that the individual peaks are so merged together as to be totally obscured. Although this would restrict the concentration range to a single point of the isotherm, and thus completely eliminate non-linear peak asymmetry effects, the information carrying capacity of a DC signal is zero.

By varying the range of concentration of a particular solute (Fig. 4.1), retention behavior and peak shape can be explained and predicted.

These six experiments can be divided into two sets, (1) three linear isotherm cases, and (2) three non-linear isotherm cases. Each set was run at three temperatures, which, by influencing the retention times provides a means of controlling the output modulation.

As seen in Figure 5.7 (files 58 and 59), the highest temperature case, the signal modulation for both concentrations is over 60%. In the case of experiments 60 and 61, the intermediate temperature runs, the peak overlap is increased and the modulation is around 30%. The final case of Figure 5.9 (files 62 and 63) the lowest temperature, restricted the modulation to below 20%.

One item of note is that the non-linear cases of files 58, 61 and 63 all show slightly more output modulation than their linear counterparts because their retention times are less and the band broadening effects are minimized.
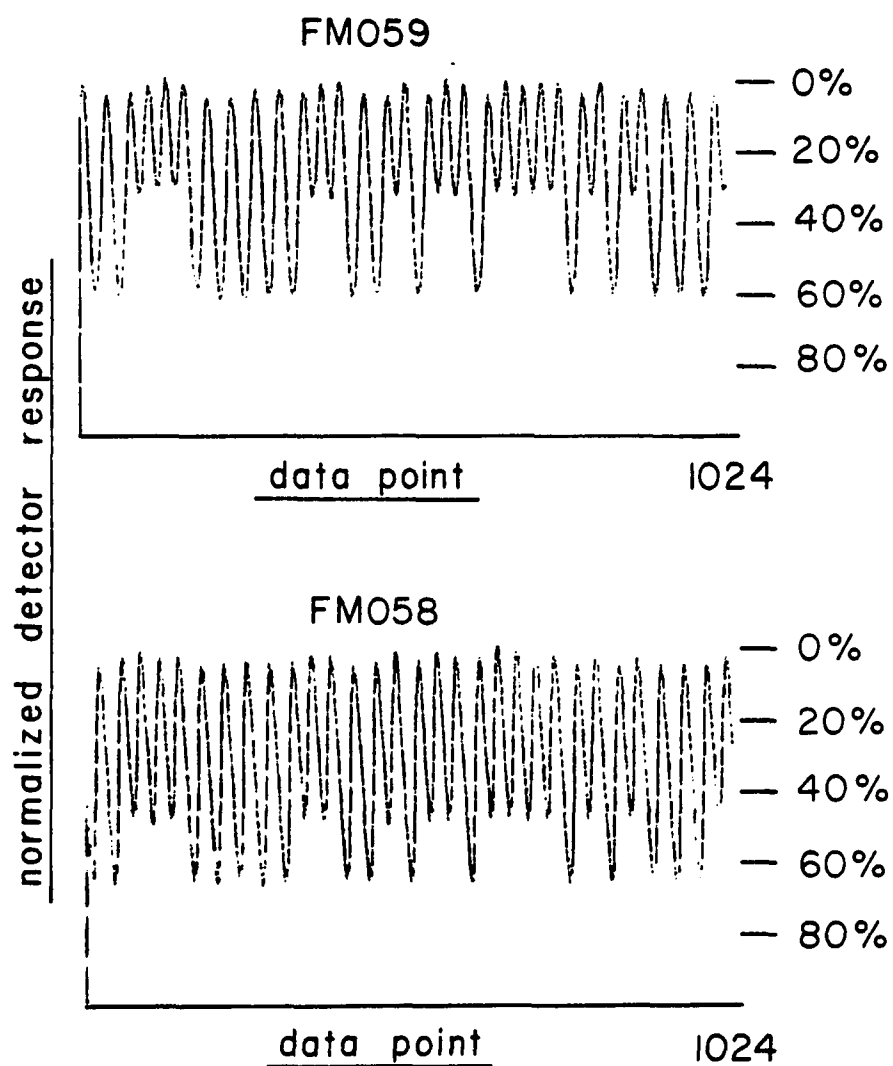
Figure 5.7. Effect of input modulation bandwidth at 120° C on solute concentration measured by detector output.

FMO6I

— 0%

— 20%

— 40%

— 60%

— 80%

data point   1024

FMO6O

— 0%

— 20%

— 40%

— 60%

— 80%

data point   1024

normalized detector response

Figure 5.8. Effect of input modulation bandwidth at 110° C on solute concentration measured by detector output.

FMO63

— 0%

— 20%

— 40%

— 60%

— 80%

normalized detector response

data point   1024

FMO62

— 0%

— 20%

— 40%

— 60%

— 80%

data point   1024
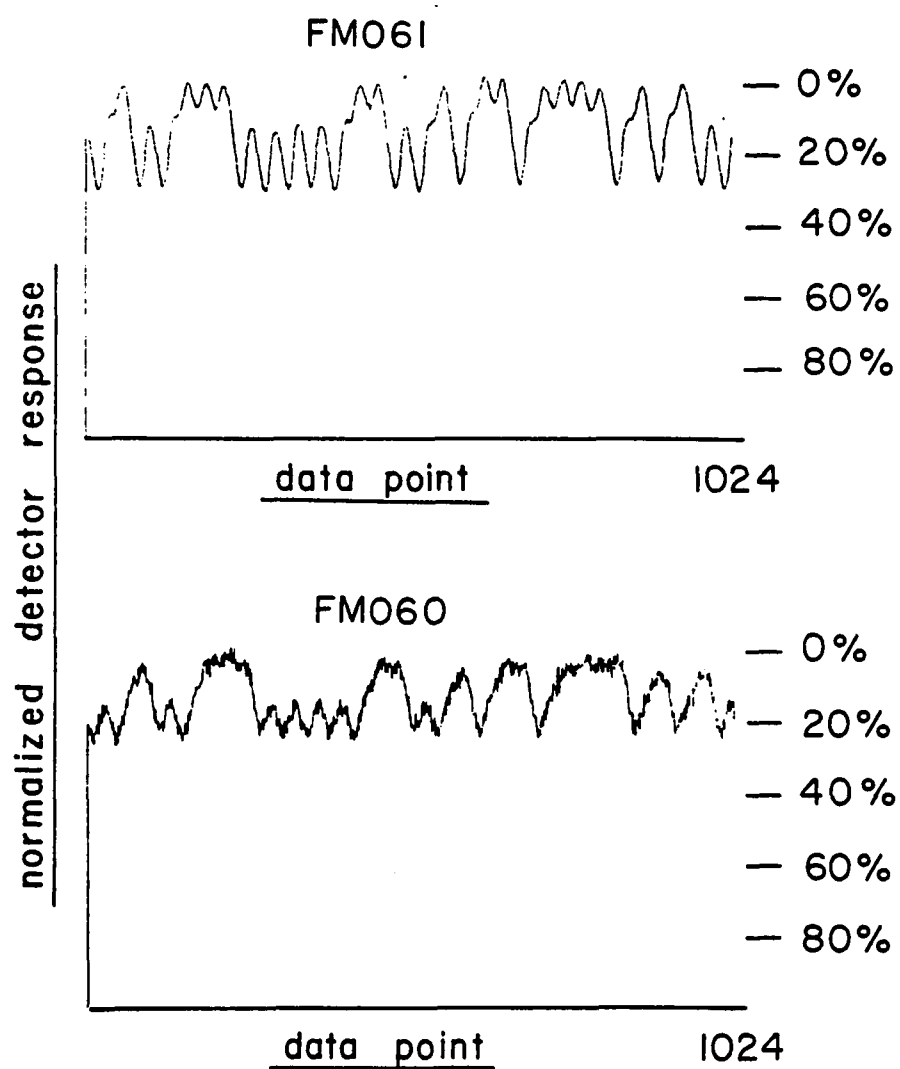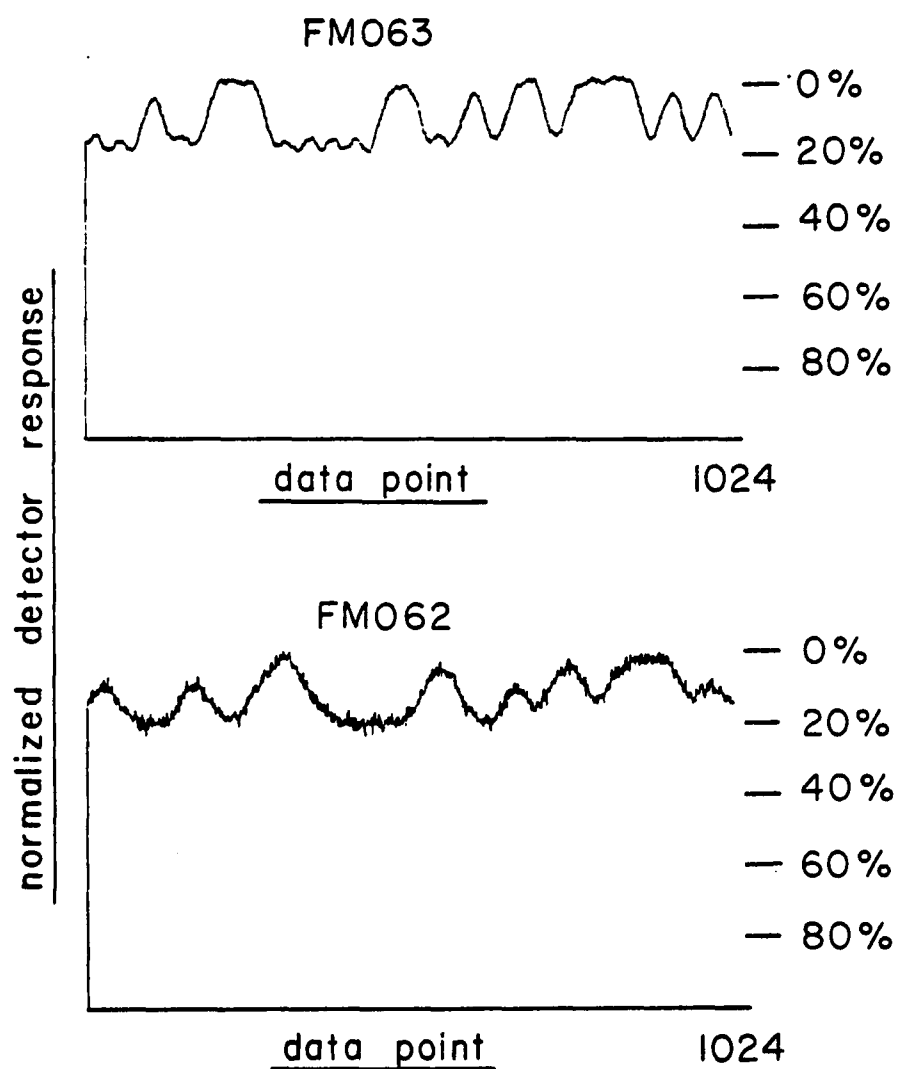
Figure 5.9.   Effect of input modulation bandwidth at 100° C on solute concentration measured by detector output.

The deconvolved chromatograms resulting from these experiments are presented in Figure 5.10 (linear case) and Figure 5.11 (non-linear case). Upon examination of these results the following observations are made:

1) The retention times at all three temperatures for the 10,000 PPM pentane case were all less than the 100 PPM case. This proves that the higher concentration was sufficient to ensure operation in a non-linear portion of the isotherm.

2) The peak shapes for all temperatures in the linear case (100 PPM pentane) were very nearly Gaussian and exhibited little tailing or asymmetry.

3) The S/N ratios for the most favorable case (i.e. linear isotherm at moderate modulation) the 100 PPM at 110 °C are about 50:1. A very good figure considering the system hardware and software limitations.

To quantitate the amount of non-linear behavior, the asymmetry factor A_s was calculated for the six experiments. Recalling $A_s = b/a$, where b and a are the peak widths at 10% peak height (Table 5.3).

As predicted from the isotherm considerations, file 58 shows the largest amount of asymmetry because of the wide modulation range and high solute concentration. In all of the other cases (linear and non-linear) the restricted modulation bandwidth was enough to minimize the non-linear effects on the peak shape, like sharp fronts and tailing. An asymmetry factor between 0.90 and 1.20 is indicative of linear column performance.
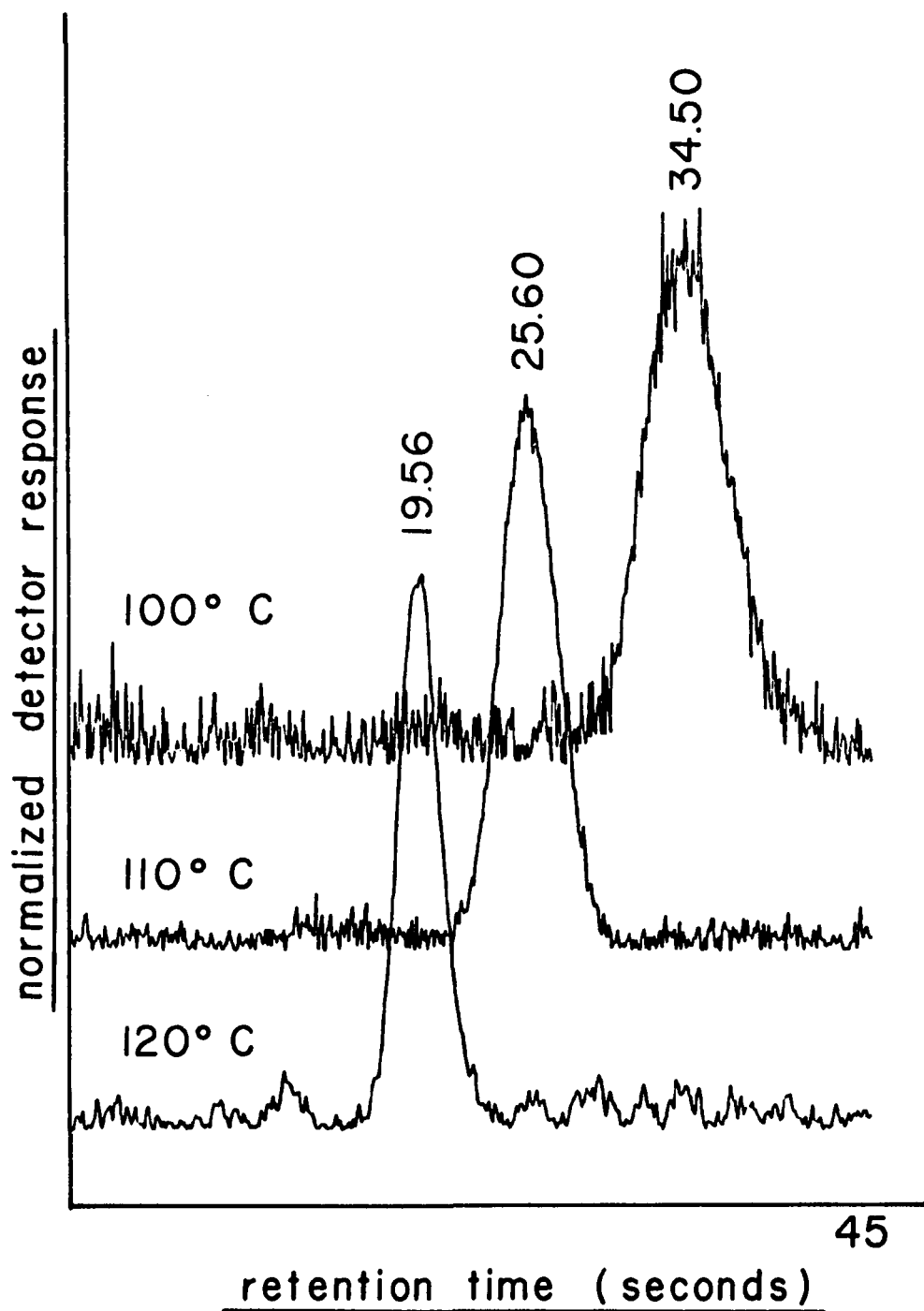
Figure 5.10. Deconvolved chromatograms of 100 PPM pentane on Porapak P (linear case).
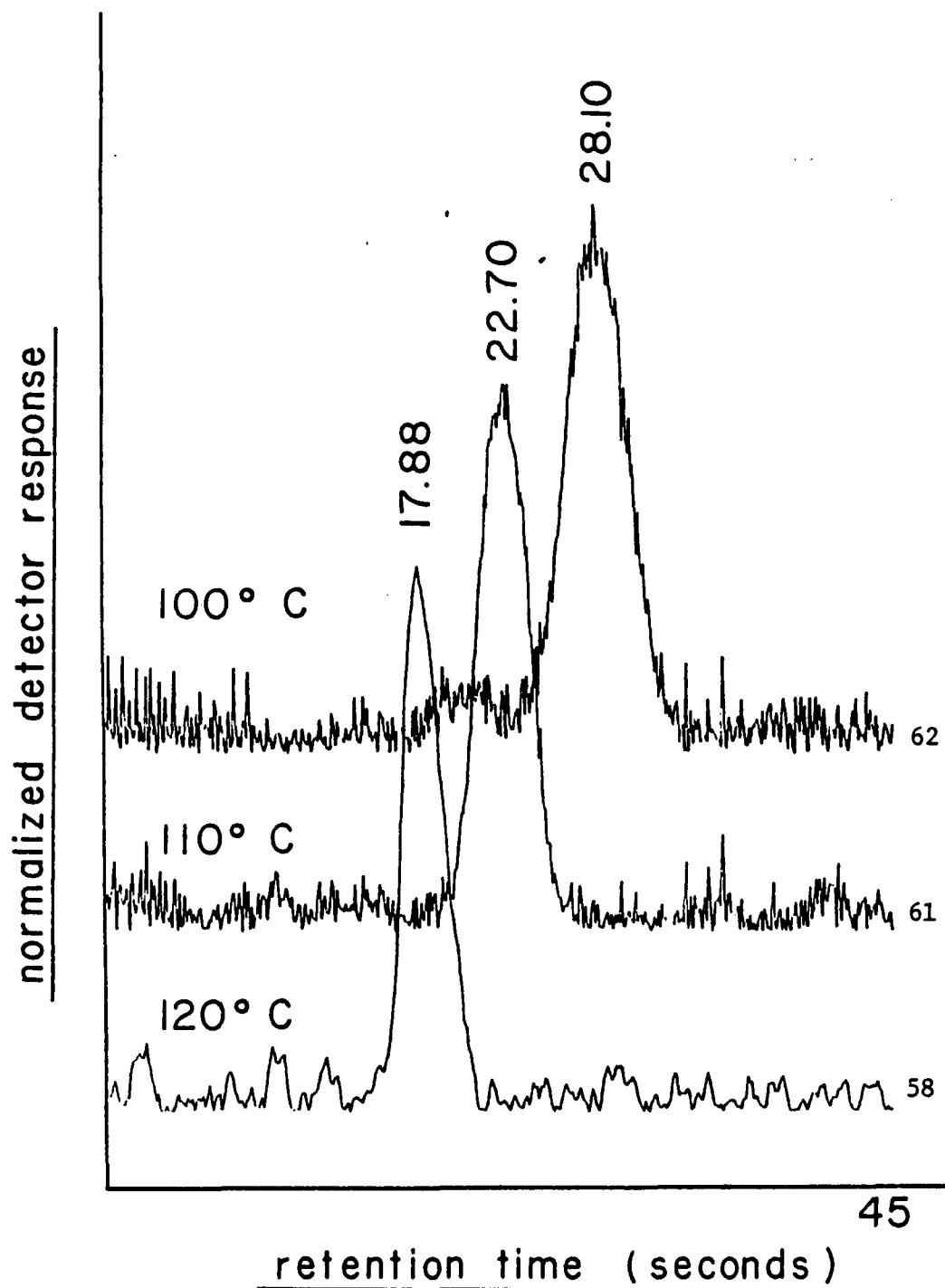
Figure 5.11. Deconvolved chromatograms of 10,000 PPM pentane on Porapak P (nonlinear case).

Table 5.3. Asymmetry factor calculated to quantitate the amount of non-linear behavior.

File	Temp. °C	A	B	A_s
FM058	120°	8.1	16.9	2.09
FM059	120°	12.3	15.1	1.23
FM060	110°	8.3	8.4	1.01
FM061	110°	9.9	9.9	1.00
FM062	100°	7.7	8.5	1.10
FM063	100°	10.5	10.4	0.99

It has been demonstrated that the FM technique enables operation on a controlled, restricted portion of the isotherm. The resulting symmetrical peaks, reproducible retentions and good signal-to-noise ratios yield good qualitative identification, and better quantitation for studying non-linear chromatographic processes.

Study of Linear and Non-Linear Behavior on a Heterogeneous Surface (^{R}Durapak-n-octane) by Multiple Injection Chromatography

The use of multiple injection frequency modulated chromatography provides a new method of characterizing a two site surface such as Durapak-n-octane. This packing material is classified as having medium polarity although the bonded functional group is an eight carbon alkane. This is because a signficant amount of residual silanols are still present. The advantages of bonded phases over traditional liquid-coated phases include: less column bleed and thus higher operating temperatures, no liquid puddling, longer column life, and less conditioning times.

In order to probe both sites, a solute which could interact specifically with the silanol and more generally with the bonded hydro-carbon chain was needed. Acetone fulfilled these conditions and also has the vapor pressure necessary to make calibration standards of the required concentration levels in the vapor phase.

Two cylinders of approximately 100 and 2000 PPM by volume were made by injecting 9.1 and 182 microliters of acetone into 0.44 liter lecture bottle cylinders and repressurizing to 700 PSI with clean dry nitrogen.

Single injections of both the dilute and concentrated acetone samples showed extensive tailing present. The uncovered active silanol sites are responsible for this tailing in dilute samples.

Two experiments using acetone on Durapak-n-octane in a two foot by 1/16" thin wall stainless steel column were run on a frequency modulated experiment with 31 x 3 injections, data acquisition rate of 10 Hz and at a column temperature of 150 °C (Table 5.4).

As seen in Figure 5.12, the retention times for the two concentration levels are significantly different. The computed chromatograms also illustrate other interesting effects using the FM technique. As mentioned, single injection chromatograms of the 100 PPM acetone run under the same conditions as file FM067 produced skewed peaks with long tails. The multiple injection experiment with the 100 PPM acetone produced the same retention time and also a nearly perfect Gaussian shaped peak. By maintaining a nearly constant amount of solute on the column at all times, the active silanol groups are covered and the retention mechanism is reduced to a single site interaction with the bonded phase. A well defined peak with an asymmetry factor of 1.18 results.

The more interesting case of non-linear two-site behavior is seen in experiment FM066. The evidence of site overload is immediately seen with the peak maximum shifting from 54.9 in the linear case to 48.0 seconds in the non-linear case. A more subtle effect is also seen in Figure 5.13, an enlarged view of the linear and non-linear peak shapes seen in Figure 5.12. Upon inspection and confirmed by the calculation of

Table 5.4.   Frequency modulated experiments.

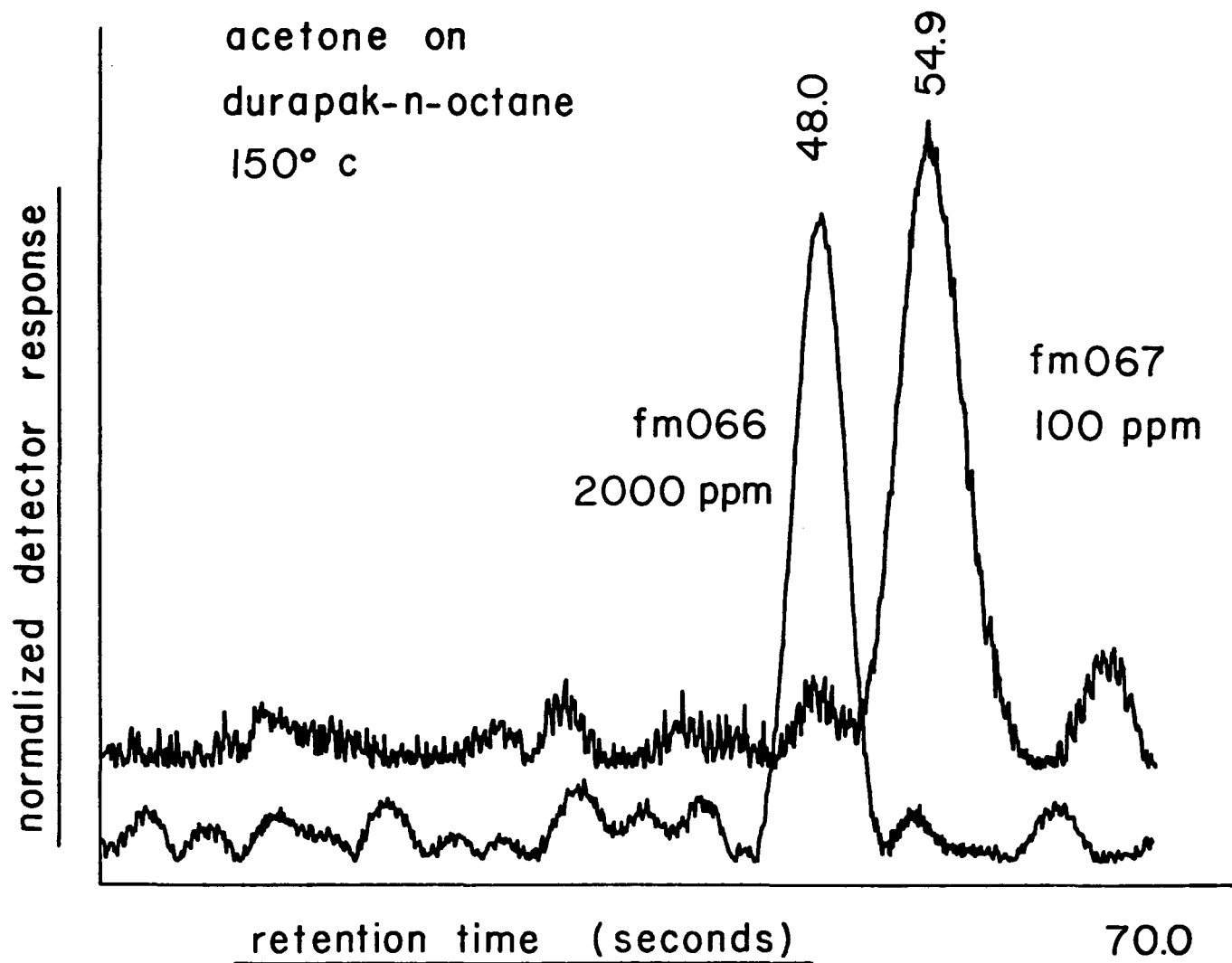| File	Concentration	Ret. Time (sec.)	A	B	A_s
FM066	2000 PPM	48.00	4.68	3.10	0.662
FM067	100 PPM	54.90	3.23	3.81	1.180

Figure 5.12. Deconvolved chromatograms of acetone on Durapak-N-octane for a linear and nonlinear case.
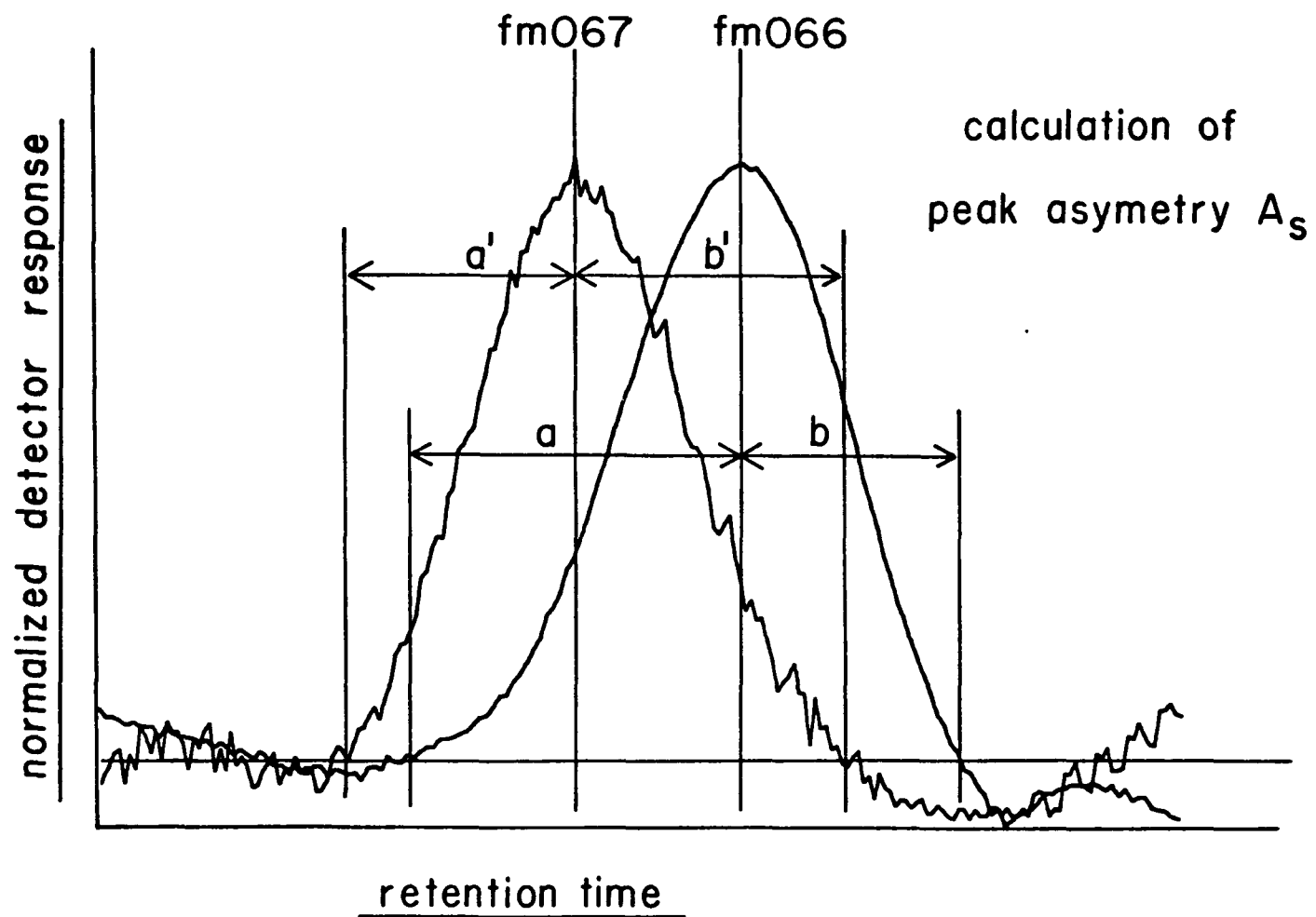
Figure 5.13.   Enlarged view of Figure 12 for the calculation of peak asymmetry A_s.

the peak Asymmetry value of 0.662, the uncommon fronting peak shape is seen in experiment FM066. This behavior was at first thought to be some kind of experimental artifact but replicate experiments under carefully controlled conditions proved the effect to be real.

The classical explanation of fronting in chromatography arises from a concave or anti-Langmuir isotherm. This isotherm shape arises from the interaction of solute molecules in the mobile phase with already adsorbed solute molecules on the stationary phase. It is also assumed that the strength of the solute(mobile) -- solute(stationary) interaction is as great or greater than the solute-clean stationary phase interaction. An example of this isotherm behavior is seen with water on carbon black. The hydrophobic surface of the carbon is not attractive to the water, but eventually a monolayer of water is adsorbed, and water vapor can then interact with the bonded water molecules; this is a more favorable interaction and therefore fronting is seen. If a single site homogeneous surface exhibits an anti-Langmuir isotherm, an increase in solute concentration should give rise to fronting peaks and increased retention times.

The case of acetone on Durapak is not as simple a case because of the two site complications. A model explaining the peak position and peak shape of acetone (2000 PPM) on Durapak must include at least three types of interactions (Fig. 5.14):

1) specific solute-silanol interaction via the O-H of the silanol and the O-C of the acetone to form a hydrogen bond.
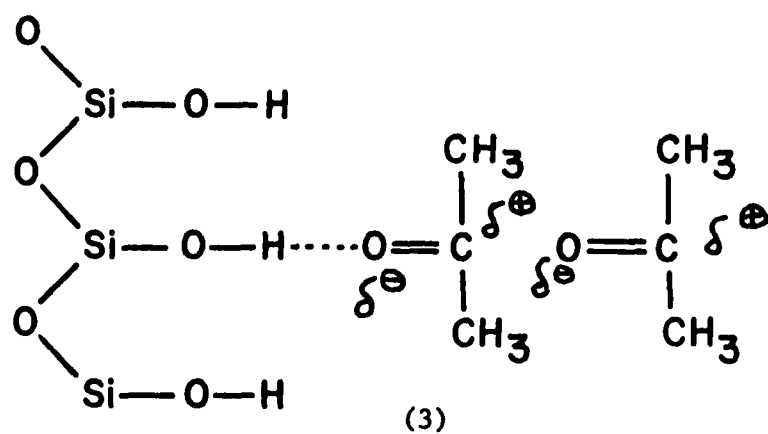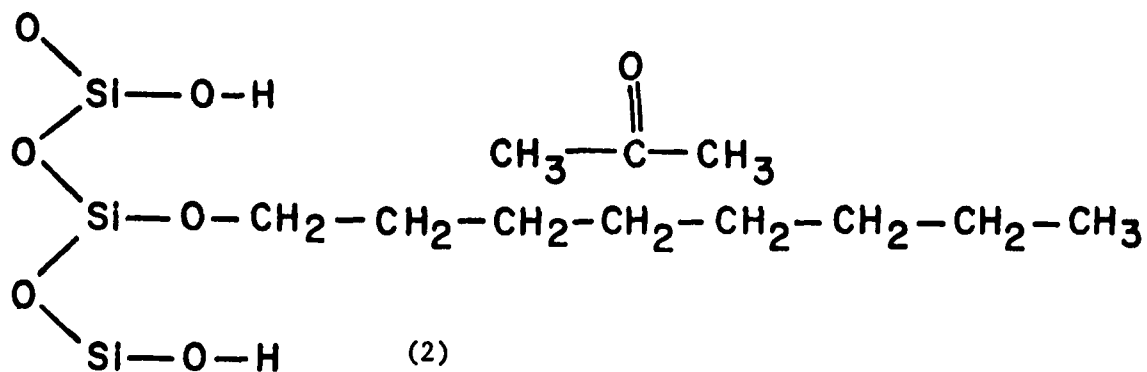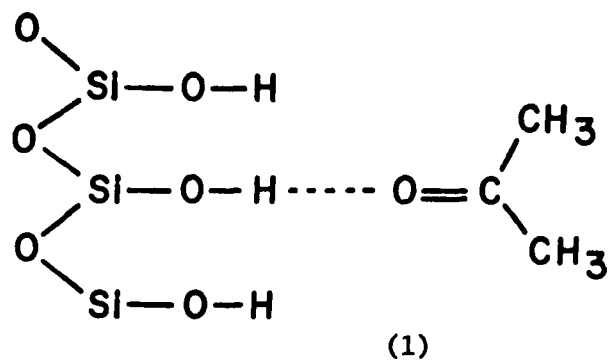
Figure 5.14. Retention mechanisms of acetone on Durapak-N-octane.

2) solute-bonded phase interaction, via the acetone carbon chain by London or Dispersion forces.

3) solute-bonded solute interaction via the net positive charge on the bonded phase acetone and the net negative charge on the O=C of the free acetone.

With three competing retention mechanisms a simple linear, Langmuir, or anti-Langmuir isotherm is not likely. It is assumed that the initial interaction involves the relatively strong adsorption of acetone directly by the silanol (Type 1). As the concentration is increased the competition for the acetone between interactions Types 2 and 3 become significant. The anti-Langmuir peak shape is indicative that Type 3 is preferred to Type 2. A more complete understanding could be pursued by careful variation of the surface (bonded phase coverage) with different solute probes at various temperatures.

The ability of the FM technique to allow control of not only the sample concentration, but also to vary this sample concentration over a preset range in the time frame of the chromatographic experiment provides a unique tool for the study of heterogeneous surfaces.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Now that the basics of FM and multiplex chromatography have been explored, applications to chromatographic systems where the through-put and multiplex advantages of multiple injection chromatography are needed should be investigated.

In addition to the demonstrated ability of multiplex chroma-tography to provide 1) greater signal-to-noise, and 2) insight into non-linear systems, Fourier techniques can also be used in a large variety of data handling situations. This chapter discusses briefly several of the implications which have resulted from the work developed during this research.

Implications of Multiplex and Frequency Modulated Techniques to Linear System Chromatography

The quantitation and identification of many environmentally important compounds pushes the detection limits of modern GC and LC technology to their limits. In order to analyze compounds at the nano-gram level and below, a pre-concentration step, in which a large volume of gas or liquid containing the dissolved compound is passed through a short column, quantitatively trapping the component(s) of interest. This technique is essentially the same as doing a frontal analysis experiment, but one must stop before the compounds of interest elute. After the required amount of gas has been concentrated on the pre-column

(sometimes held at sub-ambient temperature to increase the retention)
one would like to elute everything off the column in a small slug to
maintain good peak shape (i.e. high efficiency) in the final
chromatogram. In the GC case, heating the pre-column to thermally
desorb the material or in the LC case, using a strong solvent to wash
the column of all adsorbed components, are the most popular stripping
methods. The technique of pre-concentration is effective, but time
consuming.

Another way of increasing the amount of solute through the
column per unit time is the multiple injection approach. In the same
time period of one normal chromatogram, ten to a hundred injections
could take place in a multiplex experiment. Assuming a linear system,
the signal-to-noise levels should be improved by a factor of $N^{.5}$ (where
N is the number of injections) or from about 3.16 to 10 times the
single injection case. Overall system noise is a function of sampling,
system drift in temperature, flow control, detection, etc. The S/N
gain is a result of an averaging of these parameters.

Most of the correlation experiments were run on chromatographs
equipped with the FID. Being an extremely sensitive detector with
steady state currents of about 10^{-11} amps and a noise level of about
10^{-14} amps, the sensitivity limits (i.e. about 10^{-14} grams/second equals
the noise) were never approached. Therefore the detector noise was not
a significant contributor to the overall system noise.

However, the possible application of the multiple injection
approach to miniaturized gas chromatographs necessitates the use of

thermal conductivity type (TC) detectors, because of the low cost, small size, and no need for external gas sources. Although the noise of TC detectors is low, drift induced by temperature variations is a real problem. The uncertainties in the sampling systems of micro GC's (current models have solenoid type diaphragm valves that inject approximately 1 nanoliter) is also much greater than the larger (20-100 microliter) sampling valves used in the course of this work. Multiplex chromatography is a logical way of dealing with these limitations.

Isolation of Extra-Column Effects in Chromatography

With the advent of highly efficient columns, (i.e. 5 micron packings for HPLC and 200 micron fused silica capillary columns for GC) traditional methods of sampling and detection have not kept pace with chromatographic column technology. For this reason, the influence of extra-column effects should be quantified, and if not possible to minimize, at least taken into account.

In the past, many methods of sample introduction have been developed for gas and liquid chromatography. A review of twelve different types used in GC is included in the experimental section of this work (Table 3.1). From my experience and that of others, I conclude that no one sampling valve is ideal. A tradeoff must be made between convenience, reliability, speed, and cost. At the other end of the column lies detector technology. Again, no one detector can do it all. Similar tradeoffs must be made in terms of selectivity, sensitivity, convenience, and cost.

In order to understand the mechanisms of extracolumn band broadening, the effects of each source of variance must be known. The other condition of mutual independence of the various sources of extracolumn band broadening is easily met, because injection techniques do not interfere with the detection system.

Therefore the total variance of a chromatographic peak can be summarized by:

$$\sigma^2_{total} = \sigma^2_{sampling} + \sigma^2_{column} + \sigma^2_{detector} + \sigma^2_{tubing} \qquad (6.1)$$

Band broadening in the extracolumn tubing will be included in the first and third terms, therefore all extracolumn effects will be considered in terms of the sampling and detection systems. This approach of summing the variances assumes that the band broadening effects result in Gaussian type molecular populations. This is not necessarily the case (see Appendix A for a summary of the influence of injection time on efficiency in column chromatography).

A more general approach to the separation of extracolumn band broadening utilizes the convolution principle developed in Chapter 2. 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:

$$Y(t) \quad = \quad X(t) \quad \circledast \quad H(t) \qquad (6.2)$$

$$\downarrow FT \qquad \downarrow FT \qquad \downarrow FT$$

$$Y(f) \quad = \quad X(f) \quad x \quad H(f) \qquad (6.3)$$

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

$$Y(f) \quad = \quad 1 \quad x \quad H(f) \quad = \quad H(f) \qquad (6.4)$$

$$\downarrow \text{IFT} \qquad\qquad\qquad\qquad\qquad \text{IFT}$$

$$Y(t) \quad = \quad H(t) \quad \longleftarrow \qquad\qquad\qquad (6.5)$$

Therefore the chromatographic peak is equal to the transfer function of the column when the injection and detection systems are ignored. With a quarter inch GC column, long retentions, fast flow rates, and good instrumentation, this is not a bad approximation.

However, recent empirical observations have shown that the foregoing approximation is not always valid. For example, a researcher obtains a new 5 micron reverse-phase HPLC column and injects a test mixture to calculate the plate count to verify the column's efficiency. The manufacturer claims 50,000 plates per meter but the faster eluting peaks show only half this efficiency. The next incident involves a new fused silica column, designed for capillary GC work. Because of the new column's flexibility, ruggedness, and inertness, a smaller inside diameter of only 0.20 millimeter is used instead of the usual .5-.7 mm open tubular columns. This reduction in the column's ID should increase the efficiency because of a reduction in the diffusion path lengths. However, when the preliminary testing of these columns was evaluated, the improvements were not seen. Both of these cases represent not a failure of the columns, but of the associated sampling and detection systems.

A more complete description of a generalized chromatographic system is now presented:

$$Y(t) = X(t) \otimes H_s(t) \otimes H_c(t) \otimes H_d(t) \qquad (6.6)$$

where:

Y(t) is the detector output (i.e. 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(s) and temperature

$H_d(t)$ is the transfer function of the detection system

t is a time domain operation

f is a frequcney domain operation

$\otimes$ is the convolution operation

Taking the Fourier transform of equation 6.6 yields:

$$Y(f) = X(f) \times H_s(f) \times H_c(f) \times H_d(f) \qquad (6.7)$$

Solving for the transfer function of the column in the frequency domain from equation 6.7:

$$H_c(f) = Y(f)/[X(f)H_s(f)H_d(f)] \qquad (6.8)$$

This equation now describes the essence of the interaction of the column with the solute of interest, independent of the extracolumn effects. However, only two of the four functions on the right hand side of the equation are currently known (i.e. Y[f] and X[f]). The

transfer functions of the sampling and detection systems $H_s(f)$ and $H_d(f)$ can be solved by two methods:

1) The theoretical approach similar to Guiochon's where variables like diffusion, geometry, time, tubing size, flow rate, temperature, etc., are formulated into a complex equation to solve the response of a particular sampling valve or detection system.

2) An empirical approach to closely approximate the transfer function of any one part of a chromatographic system can be done, if the other sources of system response or variance can be minimized.

For example, to learn the transfer function of the detection system (solving from equation 6.7):

$$H_d(f) = Y(f)/[X(f)H_c(f)H_d(f)] \tag{6.9}$$

By eliminating the column, $H_c(f)$ is eliminated (i.e. set to unity):

$$H_d(f) = Y(f)/[X(f)H_s(f)] \tag{6.10a}$$

Since the Fourier transform of a Dirac function is unity, then $H_s(f)$ could closely approximate a unit impulse if the injection band width is extremely short. Equation 10a thus simplifies to:

$$H_d(f) = Y(f) \tag{6.10b}$$

By using a fluidic injection system (not practical for real world systems, but extremely fast because of the no moving part design), the total contribution of variance from the injection system can be only 1.5

milliseconds^2. Compared to the band broadening of any commercial

detector, this is virtually negligible and therefore equation 6.10b can

be used to solve for the detector transfer function with good accuracy.

Now that $H_d(f)$ is known, a similar approach to finding the

transfer function of the sampling valve is presented. Going back to

equation 6.7 and solving for $H_s(f)$ yields:

$$H_s(f) = Y(f)/[X(f)H_c(f)H_d(f)] \qquad (6.11)$$

eliminating the column results in:

$$H_s(f) = Y(f)/[X(f)H_d(f)] \qquad (6.12)$$

and since $X(f)$, the input generation signal is a Dirac function,

$$H_s(f) = Y(f)/H_d(f) \qquad (6.13)$$

Since both terms on the right hand side of the equation are now

known, $H_s(f)$, the injection system contribution to the output signal can

be solved. To check this contribution without assuming a knowledge of

$H_d(f)$, a detection system which closely approximates a unit impulse

response to a signal can be constructed. The flame ionization detector

designed by Gaspar et al. (1978), having a flame volume of only 0.5

microliter and a dead volume of another 0.5 microliter, gives a

detector contribution to the system variance of only 0.50 milliseconds^2

at a carrier flow of 5 cm^3 per minute. Using a high speed picoammeter

like a Keithley model 18000-20, the amplifier contribution to the

detector system's variance can be kept below 1 msec.^{2} if the current

range is above 10^{-8} amps. Since detector variances are independent and thus additive, the total variance of a real FID system can be about 1.5 msec.^{2}. This is negligible compared to any commercial sampling valve injection times. The transfer function of the sampling system could then be approximated by the recorded output signal according to:

$$H_s(f) = Y(f)/1 \qquad (6.14)$$

Similar approaches to the quantification of the transfer functions in the sampling and detection systems of modern HPLC equipment could be solved by using an annular flow detector cell (i.e. very low dead volume, thus approximately a unit response) to solve for the transfer function of the sampling valve, etc.

Once the transfer functions of the sampling and detection systems are solved, all of the variables in equation 6.8 are known except for $H_c(f)$, the column's transfer function, or the elution chromatogram minus the extracolumn effects. Rewriting equation 6.8:

$$H_c(f) = Y(f)/[X(f)H_s(f)H_d(f)] \qquad (6.15)$$

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

$$H_c(f) \xrightarrow{\hspace{1cm} IFT \hspace{1cm}} H_c(t) \qquad (6.16)$$

Although ideally one would like to work with equipment that would eliminate or minimize extracolumn effects, this is not always

possible. Deconvolving the known but unwanted effects of extracolumn band broadening can yield corrected results when using non-ideal equipment.

When only the extracolumn effects need be accounted for but not separated, the response of a chromatographic system without the column $[H_e(t)]$ can be recorded in computer memory. This combined response of all extracolumn effects $H_e(t)$ can then be used to solve for the column transfer function $H_c(t)$ by:

$$Y(t) = X(t) \otimes H_e(t) \otimes H_c(t) \qquad (6.17)$$

$$\downarrow FT \qquad \downarrow FT \qquad \downarrow FT \qquad \downarrow FT$$

$$Y(f) = X(f) \times H_e(f) \times H_c(f) \qquad (6.18)$$

for single injection work $x(f) = 1$ so equation 6.18 simplifies to:

$$Y(f) = H_e(f) \times H_c(f) \qquad (6.19)$$

Solving for $H_c(f)$

$$H_c(f) = Y(f)/H_e(f) \qquad (6.20)$$

and an IFT back to the time domain yields the corrected chromatogram:

$$H_c(f) \xrightarrow{\quad IFT \quad} H_c(t) \qquad (6.21)$$

Figure 6.1 shows a Fourier simulation of the removal of extracolumn effects. In this case the $H_e(t)$ is assumed to be a perfect square wave, the $H_c(t)$ a perfect Gaussian function, and the $Y(t)$ the convolution of
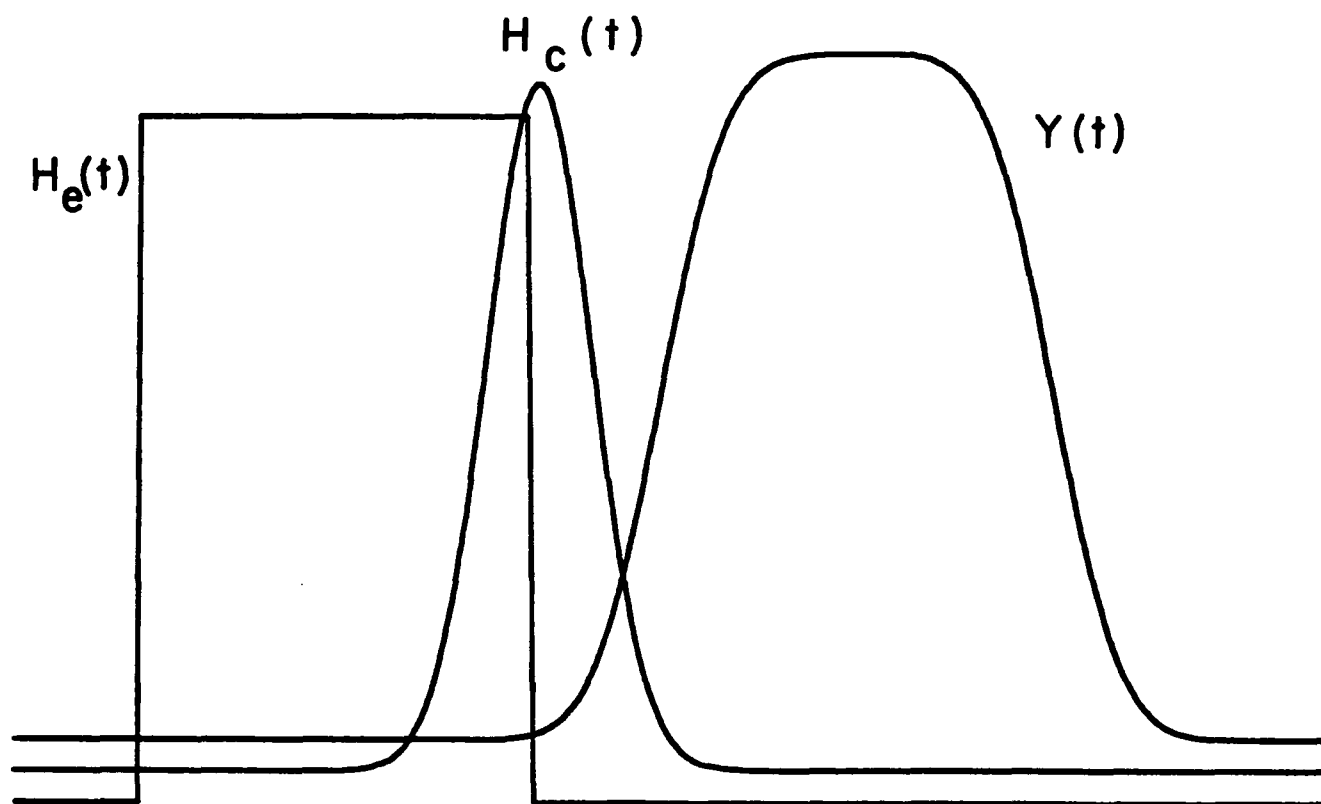
Figure 6.1.   Isolation of extra-column effects.

$H_c(t)$ and $H_e(t)$. As seen from the figure, the $H_c(t)$ is recovered easily from the distorted output $Y(t)$.

A microprocessor controlled chromatograph might easily record the $H_e(t)$ without the column and then automatically correct the chromatograms run on that particular sampling, tubing, and detector set-up, to yield corrected chromatograms free of extracolumn effects.

APPENDIX A

SAMPLING SYSTEMS

I. Influence at injection time on efficiency in column chromatography.
(Guiochon Approach)

<u>Symbols</u>

δ = delta function (defined as width = A amplitude = 1/a;

as A   0.)

σ = standard deviation of a peak due to chromatographic process

σ^2 = HL

H = HETP

L = column length

= f (diffusion, mass-transfer, eddy-diff, etc.)

Now assume that the input distribution is Gaussian. (Better than

or square-wave function.)

σ = TRu

T = standard deviation of injection time

R = ratio of zone velocity to u. or 1/k', i.e. R<1 and k'>1.

u = flow rate or gas velocity

To express H, we add the $\frac{2}{I}$ term to the convention Van Deemter

equation.

$$\sigma_I^2 = T^2R^2u^2$$
$$\sigma_t^2 = LH + T^2R^2u^2$$

$$H_t = H_o + \frac{T^2 R^2 u^2}{k'L} = H_o + H_i$$

or

$$H_t = H_o + \frac{T^2 u^2}{k'L}$$

Now the injection time (t) will be estimated as approximately 4-8 T. (assume 6) 50,

$$t = 6T$$
$$t^2 = 36T^2$$
$$T^2 = \frac{t^2}{36} \quad \text{or} \quad 0.0278t^2$$

so,

$$H = H_o + \frac{\lambda t^2 R^2 u^2}{L}$$

Summary:

$$H = f(t^2, R^2, u^2, 1/L)$$

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