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I am submitting herewith a dissertation written by Charles Nicholas Kettler entitled "Photothermal Detection Methods for Capillary Liquid Chromatography." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

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PHOTOTHERMAL DETECTION METHODS FOR
CAPILLARY LIQUID CHROMATOGRAPHY

A Dissertation
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Doctor of Philosophy
Degree

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ABSTRACT

The advantages of capillary liquid chromatography make it a very attractive alternative to analytical scale high performance liquid chromatography. Unfortunately, instrumental requirements for capillary liquid chromatography are very stringent. This is especially true for the detectors employed which require a volume on the order of one nanoliter for optimum performance. Laser based methods offer the capability to make sensitive measurements while probing very small volumes, making them ideal for capillary liquid chromatography detectors. Two laser based absorption detectors were investigated in order to determine their capability as detectors for capillary liquid chromatography.

A continuous wave argon ion laser based single beam thermal lens detector was shown to be a sensitive detector for nitroaniline compounds separated with an open capillary liquid chromatography column. A photothermal refraction detector employing a tunable excimer pumped dye laser was coupled with slurry packed capillary columns to detect nitrated polycyclic aromatic hydrocarbons. The detectors illustrated the capability of making sensitive absorption measurements within the extremely small volumes associated with capillary liquid chromatography solute bands.

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CHAPTER I

INTRODUCTION

Historical Considerations

Since its conception by Tswett in 1903 (1), chromatography has passed many milestones in evolving to its current status as a highly versatile technique for chemical separation. The development of adsorption chromatography began in 1931 when Kuhn, Winterstein and Lederer employed the process to isolate α and β carotenes (2). In 1941, Nobel laureates Martin and Synge introduced the technique of partition chromatography (3). They employed columns of silica containing definite amounts of water as the stationary phase, with organic developers as the mobile phase, to separate hydrophilic compounds. This development of partition chromatography inspired Martin and James to develop the technique of gas chromatography (4). Although the development of gas chromatography (GC) did reduce the growth of liquid chromatography (LC) during the 1950's, the variant techniques of classical LC experienced rapid growth during this period. For example, paper chromatography, thin layer chromatography, ion exchange chromatography, gel chromatography and affinity chromatography all underwent thorough investigations (5). Beginning in the 1960's liquid-column chromatography began its rapid growth and eventually became known as high performance liquid chromatography (HPLC). HPLC may have been the fastest growing analytical technique in the last 15 years (6).

Modern Capillary LC

GC and LC are both very powerful tools for chemical analysis and their theoretical basis is very similar. LC has two distinct advantages over GC: only a small fraction (approximately 15%) of the known compounds can be analyzed by GC due to low volatility or thermal instability (7) and LC enjoys an approximately 100 to 1000 fold advantage in the maximum number of theoretical plates available (8). With increased interest in resolving complex mixtures of nonvolatile and high molecular weight samples, such as those encountered in biological and environmental samples, there has been an increased interest in enhancement of column performance. The typical HPLC column available today offers only 10,000 theoretical plates. With a myriad of different stationary phases available and many mobile phase compositions possible, changes in selectivity can provide sufficient resolution for most samples. Unfortunately, as samples become more complex, the argument expressed by J. C. Giddings (9) that "changes in selectivity may do little more than scramble the already crowded chromatogram, with new overlaps replacing the old" may be borne out. Consequently the chromatographer is faced with the need to find new approaches.

A definite means to improve resolution is by increasing the number of theoretical plates. This approach to solving the problem of analyzing complex mixtures has led to interest in miniaturization of LC systems. Microscale, or capillary LC, offers the following advantages: (a) higher efficiency (more theoretical plates); (b) ability to analyze extremely small sample amounts; (c) reduced consumption of mobile phase

with a concomitant reduction in cost of acquisition and disposal; (d) compatibility with ionization, flame, and laser based detection techniques.

The process of making these advantages of capillary LC a reality has led to a large amount of development work by many laboratories (10-15). The biggest disadvantage of capillary LC is the very stringent demands placed upon the instrumentation required. All of the development work cited above includes discussions of the need for pumps, injectors, and detectors which are compatible with these newly developed capillary columns. At the Eighth International Symposium on Column Liquid Chromatography in 1984 J. H. Knox of the University of Edinburgh stated::

The dispersion of the injector-detector system has got to be extremely low to operate a 1-mm-bore column packed with 5 μm particles and get full performance out of it. The precautions you have to take to get down to the required degree of dispersion with conventional hardware, like a UV detector, are positively extreme. You can't put in any length of connecting tubing; you've got to take the column straight into the detector; you probably can't use a standard injection valve. Every component must be custom-built for this purpose. I think it's very much the case that microbore will lead to an improvement in equipment. In due course, design of equipment ostensibly for microbore operation will bring about substantial improvement in the performance achieved with normal columns. We're not, in fact, at the present time getting full performance from the normal 3 μm , 4.6-mm-bore columns.

Knox's comment provides some insight into the present status of commercially available LC equipment. The evolution of hardware associated with very small scale, high efficiency separations has not kept up with the interest of chromatographers for this type of system. In fact, the equipment available today has not been optimized. As the

demand for greater separation capability increases, capillary LC may finally be developed to the same extent as modern capillary GC. This will not occur without considerable research into the technical demands of the instrumentation required for this development.

The miniaturization of LC components is not without a controversy as far as nomenclature regarding column dimensions is concerned. For the purpose of clarity of the work presented here, the following definitions of columns and their respective dimensions will be used. Analytical (16) columns (referred to as normal columns in the above quotation) are the most widely used columns in HPLC. The usual dimensions of these columns are 4-5 mm i.d. x 25 cm in length with 3 to 20 μm particles as the packing. Microbore (17) columns will refer to columns which are 1 to 2 mm i.d. and are typically packed with 3 or 5 μm particles and are 10 to 50 cm in length. Capillary (15,18) columns include any columns less than 1 mm i.d. and may be slurry-packed with 3 to 5 μm particles or may be operated as open capillary columns in which case there is no packing material, instead the stationary phase is coated or bonded to the inside surface of the capillaries. The slurry packed columns are typically up to two meters in length while open capillaries can be up to 100 meters in length. The references cited for each column type will provide further information on the properties of the respective columns.

Band Dispersion and the Detector Volume Problem

The overall performance of any LC system is determined by the band dispersion that a solute band undergoes during the separation process.

The width of the solute band relative to neighboring bands determines whether the two solutes are resolved. The final band width of a solute is the result of individual dispersion processes which take place within the chromatographic system. The concentration profile of the solute band is often approximately Gaussian. For an assumed Gaussian distribution, the band variance (σ^2) is the square of the standard deviation (σ) of the solute band and is usually expressed in volume units. In order to determine the final solute band dispersion, as sensed by the detector, it is necessary to consider all the potential sources of band dispersion and their contribution to the total band dispersion. If each of the dispersion processes is independent of the development and progress of any of the other dispersive mechanisms, then the variances of these sources can be summed to give the final solute band variance. Sources of band dispersion occur within the chromatography column and by factors outside of the column. Within the column this band dispersion is caused by exchange between phases, simple diffusion, and other factors. The extracolumn sources of band dispersion can be attributed to mixing or dilution within the injector, detector, or connecting tubing. Dispersion can also be of a temporal nature stemming from the response of the detection electronics. The total solute band variance, σ_{total}^2 , is calculated by using equation 1.

$$\sigma_{\text{Total}}^2 = \sigma_{\text{column}}^2 + \sigma_{\text{inj}}^2 + \sigma_{\text{det}}^2 + \sigma_{\text{conn}}^2 + \sigma_{\text{temporal}}^2 \quad \text{Eq. 1}$$

The small dimensions and low volumetric flow rates associated with capillary LC make the reduction of contributions to band dispersion

extremely important. This importance becomes apparent when one considers the extremely small band volumes associated with capillary LC.

The volume of a band eluting from a chromatographic column (approximately 4σ) can be calculated from equation 2 (19):

$$V_{\text{peak}} = [4 V_m (1 + k')] / N^{1/2} \quad \text{Eq. 2}$$

where V_m is the volume of the mobile phase within the column, k' is the capacity factor or number of column volumes required to elute the solute band and N is the total number of theoretical plates.

Table I lists the calculated peak volumes for an assortment of capillary LC columns based on the above equation. As greater plate numbers are sought, the volume of the band decreases as the inverse square root of the number of plates. Hence the reduction of band dispersion becomes critical as greater numbers of theoretical plates are

TABLE I
PEAK VOLUMES ASSOCIATED WITH CAPILLARY LC COLUMNS

Column i.d. and Type	L	k'	V_m	N	Peak Volume
200 μm Slurry Packed Capillary	1 m	2	40 μL	100,000	0.6 μL
50 μm Open Capillary	1 m	2	8 μL	100,000	0.3 μL
20 μm Open Capillary	8 m	2	25 μL	100,000	64 nL
10 μm Open Capillary	3 m	2	0.9 μL	150,000	40 nL
10 μm Open Capillary	10 m	2	3 μL	500,000	50 nL

employed and peak volumes are correspondingly reduced. Each of the dispersive sources critical to capillary LC will be briefly discussed in order to assess the problems associated with capillary LC.

The variance in volume units provided by the column itself is given by equation 3 (20):

$$\sigma_{\text{column}}^2 = (\pi r^2 \epsilon_t)^2 H L \quad \text{Eq. 3}$$

where r is the column radius, L is the length, H is the height equivalent to a theoretical plate and ϵ_t the total porosity. For a given column, reductions in this term are limited to proper column dimension selection (r and L) and total system optimization to reduce H . The height equivalent to a theoretical plate is determined by the chemical nature of the phase system being employed and the physical properties of solute and solvent. Rather than attempt to discuss the long and involved theory of band dispersion within a chromatographic column (21), the extracolumn variances, which include detector volume, will be considered.

Dispersion due to connecting tubings are reduced by absolutely minimizing any connecting tubing. Connecting the column directly to the injector and detector is the best way to accomplish this; however, depending on what mode of detection is used, connecting tubing may be necessary. The variance due to connecting tubing is (20):

$$\sigma_{\text{conn}}^2 = [r_t^2 L_t u_t] / 24 D_m \quad \text{Eq. 4}$$

where r_t is the radius of the connecting tubing, L_t the length of the connecting tubing, u_t is the linear velocity within the tube and D_m is the mobile phase diffusion coefficient. This term, on first examination is dominated by the radius of the column. While this is true, the effect of reducing the tube radius will increase the linear velocity as the square of the tube radius reduction. Also alignment and connection of capillaries can distort the flow profile of the system and result in distortion of the chromatographic band. For capillary LC applications there is virtually no choice but to eliminate the use of connecting tubing between the injector, column, and detector due to the potentially large increase in variance due to this term.

Band dispersion which occurs when introducing a sample onto the column is very critical and has been discussed at length by a number of authors (22-25).

Katz derived a very simple equation (26) to calculate the maximum solute volume that can be injected onto a column without causing appreciable injection related band dispersion:

$$V_{inj} = 0.60 V_o / (N)^{1/2} \quad \text{Eq. 5}$$

where V_o is the column dead volume and N is the total number of theoretical plates. Using realistic values (27) for a very efficient open capillary column ($d_c = 10\mu\text{m}$, $L = 7.7\text{m}$, $N = 300,000$) a very small injection volume of 7 nL is calculated.

There are a large number of factors affecting the process of injecting small volumes onto capillary columns. Injector geometry,

washout problems, poor alignment of injector outlet and column inlet, and tailing due to rough edges within the injection system can all provide unacceptable dispersion which can inflate the desired injection volume to be in excess of the maximum volume as calculated by equation 5. Many different injection methods have been devised for capillary LC including internal loop valves (27,29), split injections (30-32), heart cut injections (33), sampling tube (34), and direct on column procedures (35). All of these techniques have various advantages and disadvantages which must be considered when choosing which injection method is optimal. The truth of the matter here is that there does not yet exist one best method for sample injection for capillary LC.

The primary consideration for whether a capillary LC column can be successfully used with a given injector-detector system is dependent upon the volume of an eluted peak and volumetric dispersion provided by the detector. This consideration was analyzed by Knox and Gilbert (27) who concluded that the detector volume should be less than one half the standard deviation in volume units for an unretained peak. The condition necessary for this to occur is:

$$2V_{\text{detector}} = 1/4 (N)^{1/2} \pi d_c h \quad \text{Eq. 6}$$

where d_c is the column diameter and h is the reduced plate height. The reduced plate height, h , for open capillary columns is defined as $h = H/d_c$ with d_c the column diameter and for packed columns $h = H/d_p$ with d_p the particle diameter. In their analysis of the utility of open and packed capillary columns compared to HPLC columns when both are

operated under conditions of minimum h , they showed that, if the detector volume were 100 nl, then open capillaries of 30 μm i.d. are required and will provide better performance than packed columns only when $N > 300,000$. In a situation where the volume of the detector is reduced by two orders of magnitude to 1 nl, then open capillaries with 10 μm i.d. are needed and will be better than packed columns when $N > 30,000$. The time required to elute a peak with 1 million theoretical plate efficiency is 2 hours using the open capillary column and 55 hours using packed columns (27). Thus it is imperative that if the advantage of obtaining more theoretical plates in a shorter period of time is to be realized with open capillary columns the problem of detector related band dispersion is paramount. If detector volume could be conveniently reduced to 1 nl or less, the use of open capillary columns would increase greatly due to their inherent advantages.

The Role of Lasers In LC Detection

As discussed in the previous section, in order for capillary LC techniques to be useful, injectors capable of placing low to sub-nanoliter volumes onto the column with minimal dispersion and detectors with volumes on the order of a fraction of a nanoliter will have to be developed. The injection of such small volumes places another constraint on the detector. The detection method employed must be sufficiently sensitive to detect the very small amounts of analyte within these extremely small volumes. Hence, low volume capability with high sensitivity are the key characteristics of a successful capillary LC detector.

Lasers offer the capability to enhance optical measurement techniques and probe very small volumes. These capabilities are embodied in the unique properties of lasers compared to conventional light sources. It is important to consider these properties to acquire a better understanding of how lasers can be beneficial in LC detection. Exhaustive details on lasers and their properties are available in the literature (35-38).

Power. This property of lasers can be considered in two different ways depending on the type of laser at hand. Average power is the property of interest for a continuous wave (CW) laser. For example, a CW Ar^+ laser can provide up to 20 W adjustable average power for a number of discrete wavelengths. For a pulsed laser, the energy available during the time of the laser firing (typically in the micro to nanosecond range) is referred to as the peak energy. The corresponding peak powers for large Nd-YAG or excimer lasers can exceed 100 MW. These power levels are useful for spectroscopic studies and detection methods. Greater power levels have been exploited for strategic defense scenarios, metal cutting, and other industrial applications.

The replacement of conventional sources with lasers has enhanced fluorescence measurements due to the corresponding increase in signal when excitation power increases. This signal enhancement via increased power also assists such measurement techniques as Raman scattering, photoionization and photoacoustic spectroscopy.

Spatial Coherence. The quality of spatial coherence refers to the orderly propagation of laser light in a well defined, collimated beam. These collimated beams are readily focused to extremely small spots. Under the laws of geometrical optics a Gaussian beam converging at a given angle should collapse to a point; however, diffraction limits this proposition to a finite spot size determined by the divergence of the beam.

For a beam with a half angle divergence of θ , the approximate beam radius or waist (ω_0) of the beam when focused with a lens of focal length f can be estimated from equation 7 (40):

$$\omega_0 = f \theta \quad \text{Eq. 7}$$

For example, a HeNe laser that has a half angle divergence equal to 0.4 mrad can be focused with a 25 mm focal length lens to a beam waist of only 40 μm . This spot size is maintained over a specific distance known as the confocal parameter (also referred to as the Rayleigh range). The confocal parameter (40) can be calculated from the equation:

$$z = \pi \omega_0^2 / \lambda \quad \text{Eq. 8}$$

In the above case ($\lambda = 633\text{nm}$), the confocal parameter is calculated to be approximately 2 mm. The volume defined by the 2 mm Rayleigh range and the 40 μm spot is only 2.5 nL. With the proper lenses and optical setup, subnanoliter volumes can be probed.

Monochromaticity. Most lasers have emission lines which have a typical bandwidth that is narrower than that observed in liquid phase fluorescence emission (41). This narrow bandwidth is useful for many spectroscopic techniques; however, it is not a particularly useful property for LC detection applications. A narrow excitation band can be useful for observing fluorescence emission or Raman scatter which occurs near the exciting wavelength.

Temporal Coherence. Pulsed lasers can deliver high photon fluxes in a short well defined period of time. Fluorescence detection can benefit from this property if the pulsed laser excitation is combined with gated detection techniques. Since Raman and Rayleigh scatter are essentially coincident with the laser pulse and fluorescence has a lifetime depending on the analyte (42), by observing emission at a set interval of time after excitation, fluorescence can be distinguished from scattered radiation and signal-to-noise (S/N) can be enhanced.

By employing fixed frequency excitation, lock-in amplifiers and gated detectors can be employed to assist in rejection of background and elimination of noise which arises at frequencies other than the excitation frequency.

Tunability. Most CW and pulsed lasers emit at fixed wavelengths and are only tunable over a series of discrete emission lines. However, these lasers can be used to pump dye lasers with an efficiency of between 1 and 20% (43). With the use of appropriate laser dyes and harmonic generation optics any wavelength between 200 and 800 nm can be accessed.

The use of laser based techniques for detection in LC has received considerable interest (44). Among the laser based techniques used for LC are: fluorescence (45), absorption (46), Raman scattering (47,48), light-scattering (49,50), photoionization (51), refractive index (52), photoconductivity (53), optical activity (54,55), and photoacoustic spectroscopy (56).

Although all of this interest in laser based detection for LC exists, very few investigators have shown an interest in employing the unique properties of lasers for detection in capillary LC.

Statement of the Problem

As discussed in an earlier section, open capillary LC using columns with an inner diameter of less than 10 μm and coupled with a detector with a volume of 1 nl or less can provide better performance than packed columns. An inherent problem has been the ability to develop a low volume detector with sufficient sensitivity. Most of the reported research in open capillary LC has utilized absorbance or fluorescence detection with the measurement being performed either on-column (57-59) or in miniaturized flow cells (60-62). Unfortunately, when absorbance or fluorescence detection is used in conjunction with capillary columns, short optical pathlengths, large amounts of stray radiation, and poor light throughput can seriously limit detector performance. These problems should be less significant when a laser, with its high power and unique spatial properties, is employed as the radiation source.

The sensitivity of laser-excited fluorescence makes that mode of detection desirable whenever the compounds of interest have reasonably

large fluorescence quantum efficiencies or can be made to fluoresce through derivatization. The use of laser fluorometric detection in HPLC has been reviewed (45) and it has been applied to microbore LC detection (63) as well as capillary LC (64).

Absorbance detection is much more versatile than fluorescence detection due to the fact that all molecules will absorb a finite amount of light at some wavelength. However, the sensitivity of the technique is limited by the dependence of Beer's Law on the pathlength of the sample cell. Laser based photothermal techniques have been employed to make sensitive absorbance measurements with pathlengths on the order of tens of microns (65). Utilizing the laser property of spatial coherence, these techniques are ideally suited as low volume detection methods and would be applicable to capillary LC. The research presented in this dissertation will investigate the use of two photothermal techniques, thermal lens and photothermal refraction, as low volume sensitive detection methods for capillary LC.

The initial laser based photothermal detector developed employed the single beam thermal lens technique coupled with open capillary LC. The excellent spatial properties of the Ar^+ laser employed in that work allowed sensitive detection of the very low volume solute bands eluted from the open capillary columns. Although sensitive absorbance measurements were accomplished, the limited number of emission lines of the Ar^+ laser restricted the types of samples which could be analyzed. Subsequently, an excimer pumped dye laser system was obtained which provided a greater wavelength tuning range and allowed the use of sensitive

lock-in detection. This laser was used in combination with a HeNe laser to perform double beam photothermal refraction detection. The open capillary columns used in the first study were replaced by slurry packed capillary columns. The slurry packed columns were less susceptible to the clogging and breakage problems encountered with the open capillary columns. This system was used to investigate the presence of nitrated polycyclic aromatic hydrocarbons in environmentally important samples. A detailed discussion of the research developing these two sensitive, low volume, capillary LC compatible, detection systems is provided in the following chapters following a brief introduction to laser based photothermal measurement techniques.

Introduction to Photothermal Methods

Photothermal methods measure the absorbance of a liquid sample via the deposition of energy from a laser. When a liquid solution is irradiated with a laser beam that has a radial Gaussian intensity profile, a temperature rise associated with the absorption of radiation occurs. Unless the solutes in the sample possess a fluorescence quantum efficiency of 1, a portion of the energy absorbed will be lost via radiationless transition processes which will cause the irradiated sample to increase in temperature. This temperature increase will also have a Gaussian profile with the largest temperature rise occurring at the center of the irradiated volume and diminishing radially. Concomitant with this temperature gradient a refractive index gradient is formed, due to the direct relationship between temperature and

refractive index. The result of this refractive index gradient is the formation of a lens-like optical element within the sample. For most liquids a rise in temperature produces a reduction in refractive index. For the refractive index gradient formed this means that the optical pathlength is shorter at the center of the gradient than at the edges. This is the equivalent to a diverging lens. This phenomena is known as the thermal lens effect. The effect that the thermal lens has on the propagation of a laser beam through the absorbing solution is illustrated in Figure 1.

In Figure 1 the solid line is the divergence of the beam when no thermal lens is present. The dashed line illustrates the increased divergence, also known as "thermal blooming", which occurs when a thermal lens has formed. The formation of the thermal lens is monitored in the far field by observing changes in intensity at beam center with an appropriate detector.

The thermal lens effect as illustrated in Figure 1 was first discovered by Gordon and coworkers in 1964 (66). The thermal lens was the first in a series of techniques based on the photothermal formation of an optical element within a sample. Indirect absorption measurements have been accomplished by photothermal formation of an optical element in one arm of an interferometer (67-74) and also by the generation of a prism (75-77), diffraction grating (78), curved mirror (79) or cylindrical lens (80) within the sample. Each of these techniques provides the opportunity to make indirect absorbance measurements and still enjoy the sensitivity advantage which is normally affiliated with fluorimetry. An

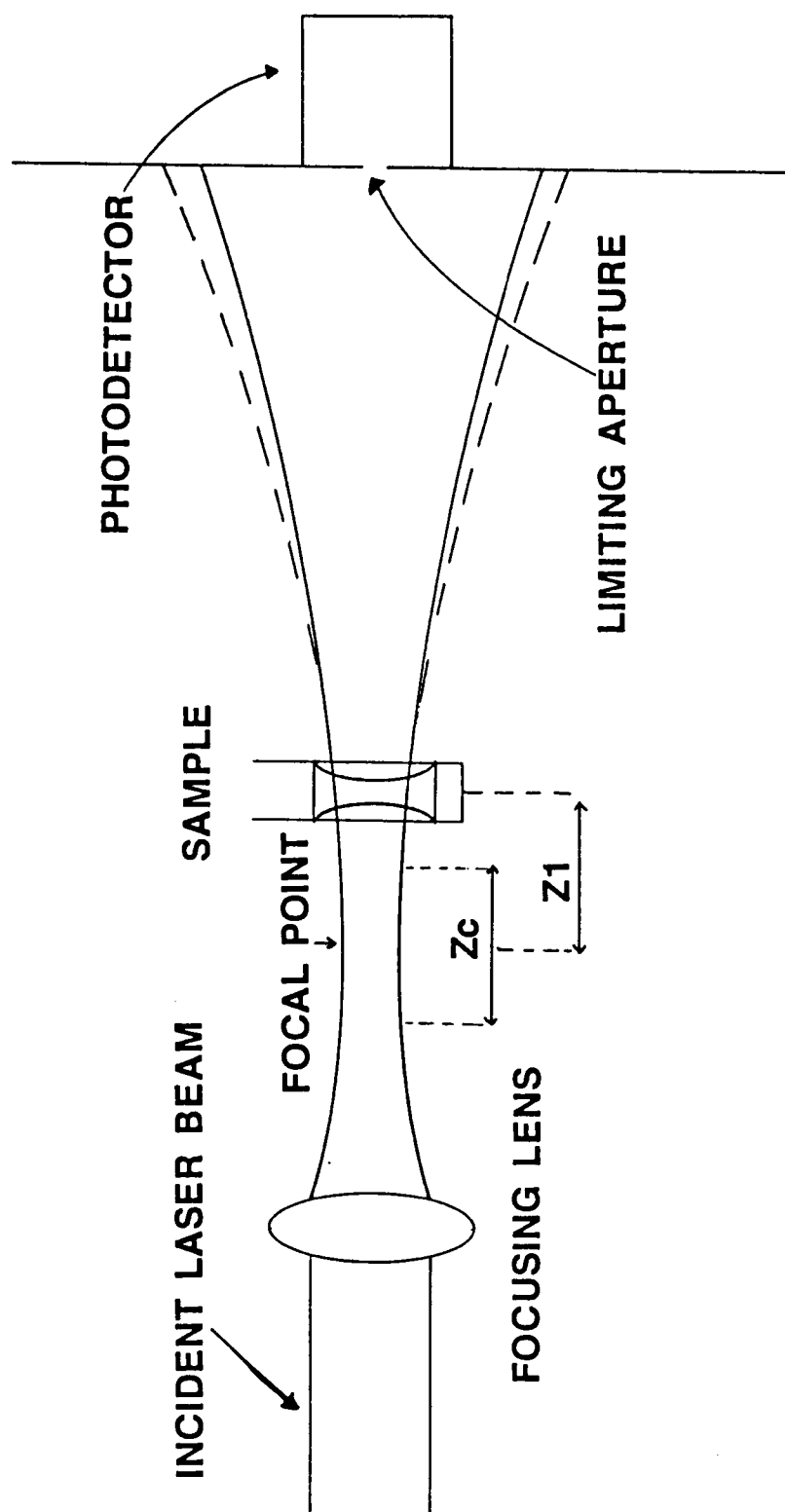


Figure 1. Effect of thermal lens on the propagation of a laser beam. Solid line depicts beam divergence without thermal lens present, dashed line is increased divergence due to thermal lens. Z_c is the confocal parameter of the focused laser, Z_1 is the distance from the focal point of the laser to the sample.

absorbance measurement based upon Beer's Law is accomplished by taking the difference between two large signals. As the need for sensitivity increases the ability to find a very small difference between two large numbers becomes limited by the relative concentration error and noise of the detector (81). In contrast, photothermal methods offer the opportunity to make sensitive absorbance measurements in short pathlengths, with the pathlength restriction imposed by Beer's Law being compensated for by the enhancement offered by the photothermal technique. The photothermal measurements also provide the same source power dependence that is typically associated with fluorescence measurements. These laser based absorption measurement techniques are ideally suited for detection in capillary LC.

CHAPTER II

THERMAL LENS DETECTION FOR OPEN CAPILLARY
LIQUID CHROMATOGRAPHYIntroduction to Thermal Lens Spectroscopy

As briefly described in the previous section, the thermal lens effect is an indirect method of making absorbance measurements. The experimental arrangement is similar to single-beam absorption spectrometry with the exception that the laser light passing through the sample is detected in the far field only at the center of the beam by restricting the field of view of the photodetector with an appropriate limiting aperture. The sample placed in the beam causes a decrease in radiation at beam center via thermal defocusing. The light absorbed by the analyte is converted to heat by nonradiative relaxation and thus increases the temperature of the bulk sample. The temperature rise is greatest at beam center which causes a lowering of refractive index and forms the divergent thermal lens illustrated in Figure 1.

The effect of the thermal lens formed on the laser beam is a time dependent process. The formation of the thermal lens within the sample requires a finite amount of time to come to a steady state. The steady state is reached when the rate of laser heating equals the rate of heat loss. The rate of heating depends on the laser power density and sample absorbance and rate of heat loss is dependent on solvent thermal conductivity and temperature change produced.

The focal length of a steady state thermal lens $f(\infty)$ is given by (82,83):

$$f(\infty) = \pi k \omega^2 / 2.303 P (dn/dT)A \quad \text{Eq. 9}$$

where k is the thermal conductivity in $\text{W cm}^{-1} \text{K}^{-1}$, ω is the beam radius at the sample in cm, P is the laser power in watts, (dn/dT) is the change in refractive index with temperature and A is the sample absorbance. This expression assumes all absorbed radiation is converted to heat. If the analyte possess a finite fluorescence quantum efficiency a correction can be made (84).

The time dependent approach to steady state by the thermal lens is governed by equation 10 (82):

$$f(t) = f(\infty)(1 + t_c/2t) \quad \text{Eq. 10}$$

where t is the time since the laser irradiation of the sample began and t_c is a time constant given by:

$$t_c = \omega^2 \rho C_p / 4k \quad \text{Eq. 11}$$

where ρ is the density of the sample in g cm^{-3} and C_p is the specific heat in $\text{J g}^{-1} \text{K}^{-1}$. Time constants can range from microseconds to seconds depending on the spot size of the laser and thermo-optical properties of the solvent.

The actual measurement of a thermal lens is accomplished by measurement of the intensity at the center of the laser beam in the far field when there is no thermal lens present and when the lens is

present. In the case of small absorbances the initial and final intensities are governed by equation 12 (85):

$$\frac{I(0) - I(\infty)}{I(\infty)} = - \frac{2.303 P (dn/dT)}{\lambda k} A \quad \text{Eq. 12}$$

$$= 2.303 EA$$

where $I(0)$ is the intensity at image center at time $t = 0$, $I(\infty)$ is the intensity when a steady-state thermal lens has formed, λ is the laser wavelength and A is the sample absorbance. Beer's Law for small absorbances also involves a change in relative intensity: $(I_0 - I)/I = 2.303A$ where I_0 and I are the incident and transmitted intensities respectively. E therefore represents an enhancement in sensitivity of the thermal lens measurement relative to a normal spectrophotometric measurement (86). The degree of enhancement is thus governed by the laser power and thermo-optical properties of the solvent. Table II lists the thermo-optical properties for a number of solvents (87). Nonpolar solvents provide the largest enhancements due to their low thermal conductivity and high dn/dT values. The magnitude of these values can have an important impact on chromatographic detection.

Thermal lens measurements are advantageous in that they provide an enhanced measurement capability relative to normal spectrophotometric determinations. When this advantage is coupled with the linear relationship of the signal to concentration, thermal lens can be considered as an excellent detector for capillary LC.

TABLE II

THEMAL CONDUCTIVITY AND DERIVATIVE OF INDEX OF REFRACTION
WITH RESPECT TO TEMPERATURE FOR LC MOBILE PHASE SOLVENTS

Solvent	Thermal Conductivity $10^4 k$ (cal/sec cm °C)	Change in RI with Temperature dn/dt
Hexane	2.95	-5.2
Methanol	4.80	-3.9
Benzene	3.45	-6.4
Toluene	3.18	-5.6
Methylene Chloride	3.3	-5.3
Acetone	3.80	-5.0
Water	14.6	-0.8

Instrumentation and Experimental Considerations

Chromatography. The open capillary liquid chromatographs used in this work are shown in Figure 2. Capillary columns, 6-8 m x 20 μ m i.d., were drawn from stock Pyrex glass tubes by using a glass drawing machine (Shimadzu, model GDM-1, Kyoto, Japan). The inside surfaces of the columns were etched and bonded with dimethylocta decylchlorosilane (ODS) using a procedure similar to that reported by Jorgenson and Guthrie (57) and discussed in Appendix A. Five nanoliter sample volumes were injected with an on-column injection procedure reported previously (88). Mobile phase was pumped through the columns at a linear velocity of approximately 0.5 cm/s with a Haskel (Burbank, CA) model 27502, pneumatic amplifier pump.

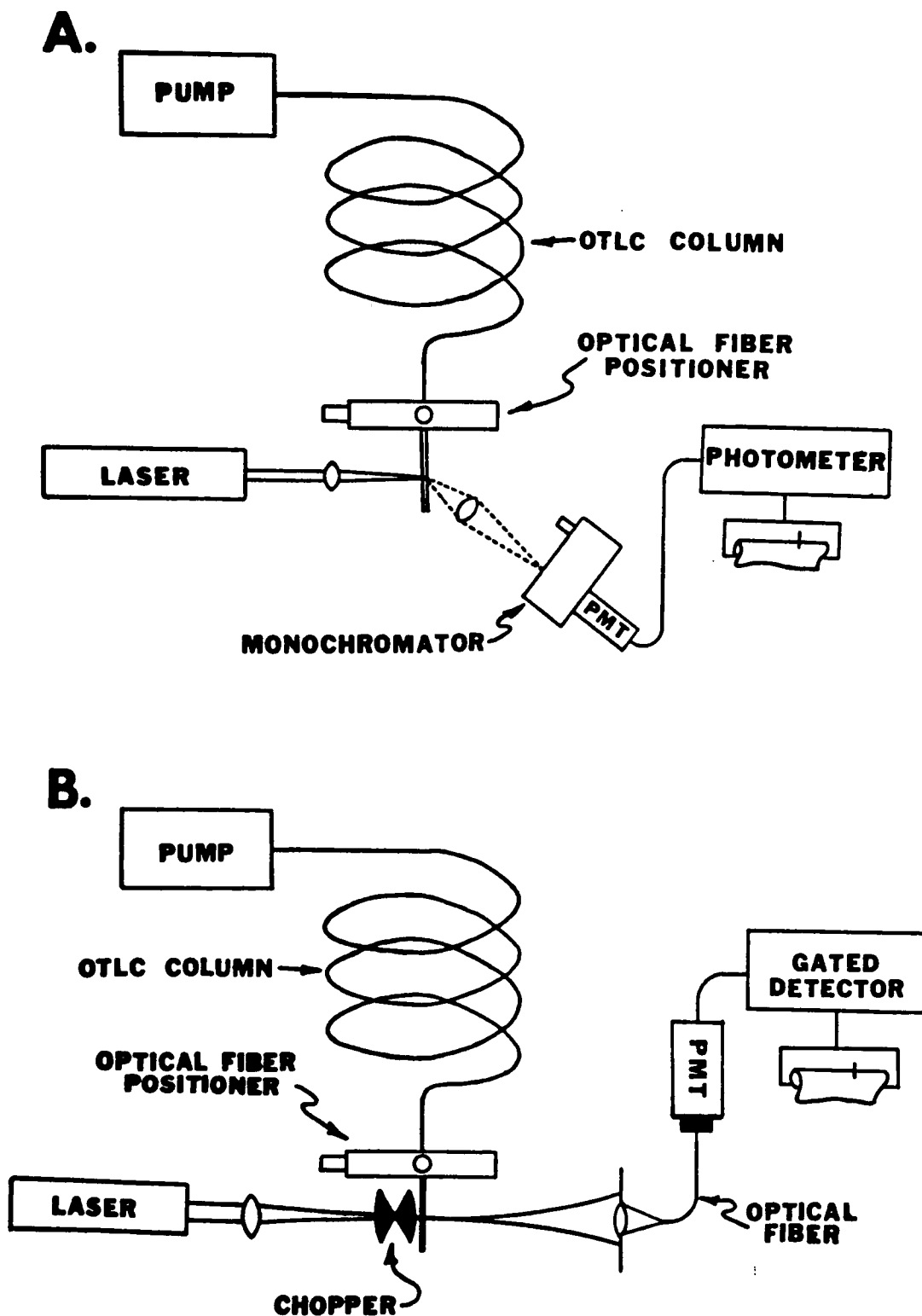


Figure 2. Schematic of instrumental arrangements used for open capillary LC detection. Arrangement A is used for on-column laser fluorescence detection. Arrangement B is for thermal lens detection.

Detection. Fluorescence detection was accomplished either on-column or in optical flow cells constructed from thin-wall, 100 μm i.d., quartz capillary tubes (Vitro Dynamics, Rockaway, NJ). The capillary tubes were epoxied into short pieces of needle gauge stainless steel tubing. The stainless steel tubing served to protect the fragile flow cells during the connection procedure and also helped to match the outer diameter of the flow cell to that of the column (about 600 μm for the columns used in this work). The latter condition is important in assuring that there are no void spaces between the column and the flow cell and to ensure that the column and flow cell openings are properly aligned. The flow cells were attached to the column by butting the column and flow cell together, then using heat shrinkable PTFE tubing to hold them in place. Thermal lens detection was accomplished with 100 or 200 μm square capillary flow cells, obtained from Vitro Dynamics, and connected to the columns by using the procedure described above. The flow cells were positioned in the focused laser beam by use of a Newport (Fountain Valley, CA) model FP-1 optical fiber positioner. A short piece of narrow bore capillary column was connected to the outlet of the flow cell to provide back pressure, thereby minimizing degassing problems.

The laser employed in this work was a Spectra Physics (Mountain View, CA) model 171, argon ion laser, operated at 488 nm and 300 mW for the fluorescence work and at 458 nm and 500-800 mW for the thermal lens work. During fluorescence detection the laser radiation was focused

into the flow cell with a 25 mm focal length lens or into the capillary column for on-column detection with a 19 mm focal length lens. A 25 mm diameter f/1 lens was used to collect the fluorescence emission and image it onto the entrance slit of a monochromator (Instruments SA, Metuchen, NJ, model H-10). The monochromator was set to 540 nm and had a 16 nm bandpass. Signals were monitored with an RCA 1P28 photomultiplier tube (PMT) and a photometer (Gencom, Plainview, NY, model 1012, picoammeter).

During thermal lens detection the laser radiation was focused into the 100 or 200 μ m flow cells, perpendicular to a side, using 25 or 60 mm focal length lenses, respectively. The laser beam was modulated at 500 Hz with a mechanical chopper (Ortec Brookdeal, Princeton, NJ) model 9479. The chopper was positioned in the path of the laser beam approximately 0.3 cm in front of the flow cell. The thermal "blooming" of the laser beam was monitored by placing in the path of the diverging beam, approximately 1 meter from the flow cell, a 100 mm focal length, 20 mm diameter lens with an optical fiber at its focal point. The diameter of this lens was about 1/15 that of the expanded laser beam. The collected radiation was monitored with the PMT after suitable attenuation with neutral density filters.

The signal arising from this single beam thermal lens configuration was processed with a gated detector (Quanta-Ray, Mountain View, CA) model DGA-1. The 100 ms gate duration of this detector was triggered by the synchronous output of the chopper. In order to adjust the time of the gating of the detector, relative to the exposure of the flow cell to

the laser radiation, the chopper was spatially translated perpendicular to the laser beam. This permitted temporal inspection of the formation of the thermal lens. For the recording of chromatograms the chopper was positioned so that $I(\infty)$, the steady state thermal lens intensity, was measured. The gated detector did not provide a means to conveniently measure $I(0)$ and thus the signal measured did not correspond directly to equation 12.

Reagents. Fluorescence detection was performed for several alkyl amines (Alltech Associates, Inc., amine analytical standard kit) derivatized with 7-chloro-4-nitrobenz-2,1,3-oxadiazole (NBD-Cl). The amines were derivatized by adding a weighed amount of amine to an acetonitrile solution containing approximately a 10-fold excess of NBD-Cl. The amine-NBD derivatives were separated on a 8.3 m x 20 μ m i.d. column using a mobile phase that was 20% acetonitrile (HPLC grade) in water.

Preliminary thermal lens studies were performed in 200 μ m square flow cells using alkaline, aqueous solutions of phenolphthalein. Thermal lens detection for actual open capillary separations was performed in 100 μ m square flow cells using three nitroaniline compounds (o-nitroaniline, 4,5-dimethyl-2-nitroaniline, and N,N-dimethyl-3-nitroaniline). The nitroaniline compounds were obtained from Aldrich Chemicals. A 7.5 m x 20 μ m i.d. column and a mobile phase that was 40% methanol (HPLC grade) in water were used to separate the nitroaniline compounds.

Flow Cell Considerations

The calculated volume of a peak eluted from an open capillary column that is typical of the columns used in this work (20 μm i.d. x 8 m) is given in Table 1. This volume of 64 nl illustrates the need for a detector which will not disperse these narrow solute bands, thus allowing retention of efficiency and maximizing detection capability by reducing dilution of eluting bands.

The optimal method of detection in capillary LC is on column detection. On column fluorescence detection has been used in open capillary LC (57). There are two principal advantages in this type of detection. First, since there is no flow cell, there is no band dispersion due to the detector, providing the effective illuminated volume is much smaller than the smallest solute band volume, and the detector response time is sufficiently short. Second, the high sensitivity of fluorimetric detection permits detection of very small injection quantities.

On column laser fluorimetric detection was employed in evaluating the open capillary LC columns prepared. Laser fluorimetric detection in flow cells was compared to on column detection in order to assess the contribution of the flow cells to solute band variances. This evaluation is important since the utility of on column optical detection is limited by the composition and geometry of most open capillary LC columns. The materials which have been most successfully employed in the preparation of bonded phase columns (e.g. soft and borosilicate

glasses) contain impurities which absorb UV radiation and fluoresce. With these column materials, on column detection is restricted to the near-UV and visible region of the spectrum. Moreover, the large wall thickness and narrow inner diameter of most open capillary columns make it difficult to efficiently focus the incident laser radiation into or through the column. This is especially important in thermal lens detection. The Gaussian intensity profile of the laser beam must be maintained at the column (or flow cell) and in the far field.

A chromatogram illustrating the efficiency of the open capillary LC columns prepared in this work and the sensitivity of laser fluorimetric detection is shown in Figure 3. This chromatogram was obtained by using a 20 μm i.d. x 8.3 m, ODS bonded phase column and employing on column detection. The mobile phase was 20% acetonitrile in water. Minimum detectable quantities of the separated amine-NBD derivatives were in the low picogram range. Figure 4 illustrates the importance of employing sensitive detectors in open capillary LC. The figure contains a plot of plate height vs. amount injected for a five component mixture of amine-NBD derivatives. For some of the derivatives, increases in plate height due to column overload occur at injected amounts less than 1 ng. The sensitivity to detect these small amounts of injected solutes is possible with laser fluorimetric detection.

Initial attempts at on column thermal lens detection were unsuccessful due to a severe distortion of the Gaussian laser beam profile after passing through the column. Better far field beam profiles were observed when thin walled round flow cells were employed. However, the

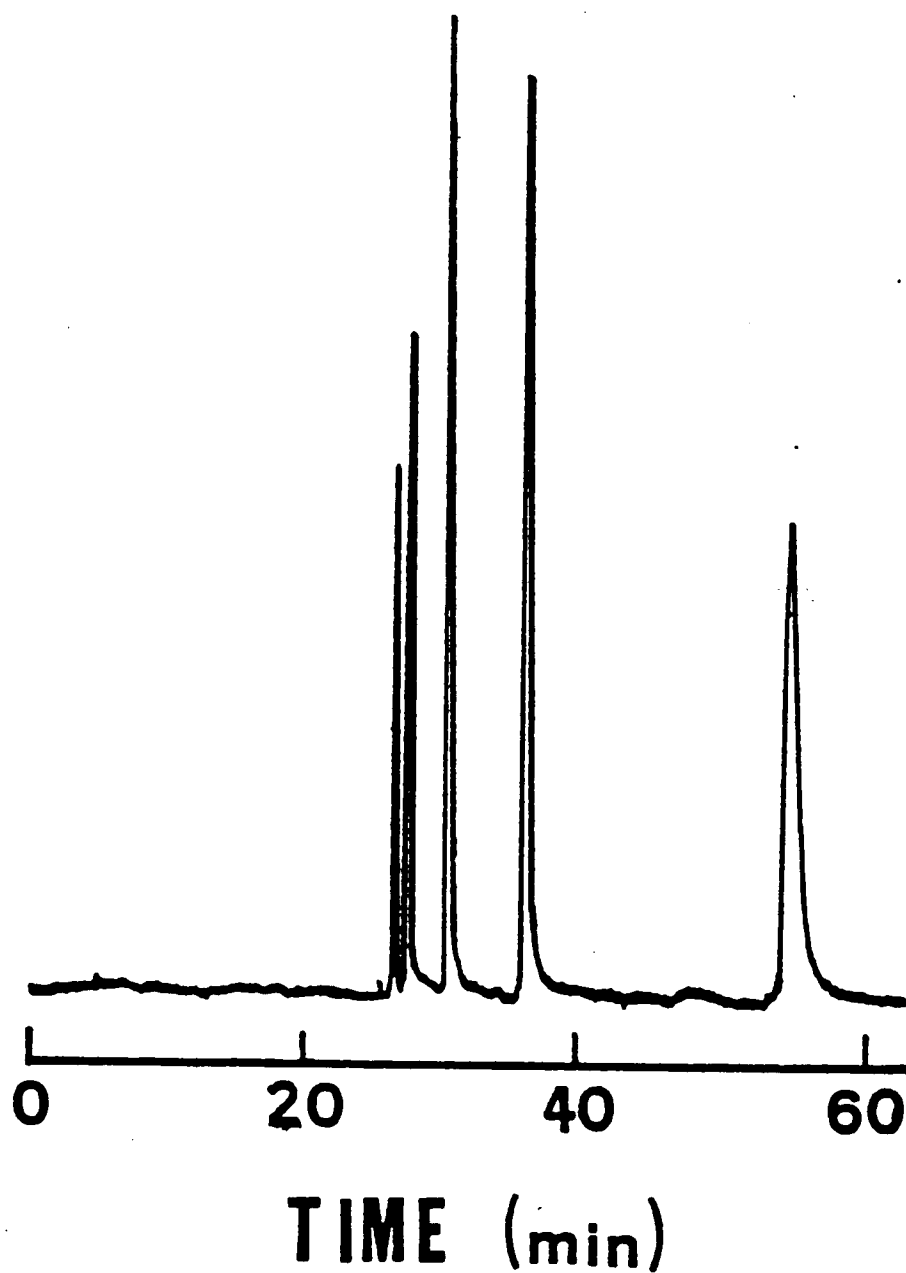


Figure 3. Open capillary LC separation of several amine-NBD derivatives, using on-column fluorescence detection. The derivatives in order of elution are ethyl amine, n-propylamine, n-butylamine, cyclohexylamine, and n-hexylamine.

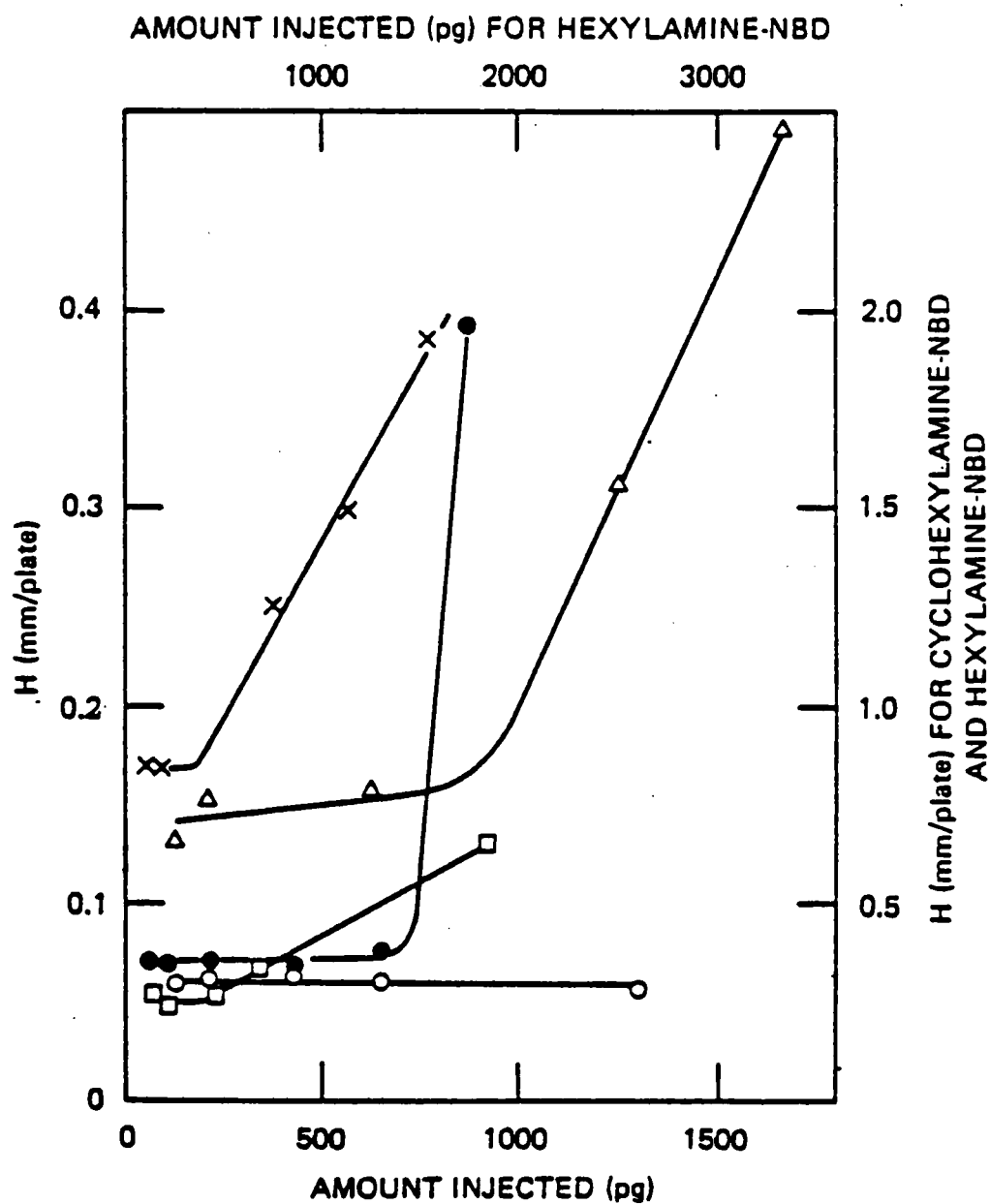


Figure 4. Relationship between plate height and amount of sample injected for a mixture of amine-NBD derivatives. A 20 μ m i.d. x 8.6 m, ODS bonded phase column and a 23% acetonitrile in water mobile phase was employed. The mixture contained ethylamine (o), propylamine (•), n-butylamine (x), cyclohexylamine (◻), and n-hexylamine (Δ).

best beam profiles were observed with square flow cells. A study was initiated to determine the magnitude of the contributions of these flow cells to solute band variance.

The fluorescence peak profiles of ethylamine-NBD were obtained for 100 μm i.d. round and 100 μm inner dimension square flow cells, which were used with a 20 μm i.d. open capillary column. The observed solute band variances appear in Table III. The experimental flow cell

TABLE III
EFFECT OF FLOW CELLS ON SOLUTE BAND VARIANCES

Flow Cell	Solute Band Variance, nL^2		
	Obsd	Flow Cell Contribution	
		Exptl	Calcd
on column	41		
100 μm round	66	25	0.001
100 μm square	97	56	0.060

contributions to the observed variances were determined as the difference between the observed variances obtained by using flow cells and the observed variance for on column detection. Therefore the contribution to solute band variance by the 100 μm round flow cell is calculated by subtracting the on column solute band variance of 41 nL^2 from the observed variance of 66 nL^2 with the flow cell. The theoretical values for the flow cell contributions to the variance, σ_{det}^2 , were calculated by using

$$\sigma_{\text{det}}^2 = H_{\text{det}} L_{\text{det}} \quad \text{Eq. 13}$$

where L_{det} is the length of the flow cell up to the illuminated spot and H_{det} is the detector (i.e., flow cell) plate height. H_{det} for the round flow cell was calculated by using the following equation (89):

$$H_{\text{det}} = \frac{2D_m}{v} + \frac{1 + 6k' + 11k'^2}{(1+k')^2} \frac{d_{\text{fc}}^2 v}{96D_m} \quad \text{Eq. 14}$$

where D_m is the solute diffusion coefficient (typically $10^{-5} \text{ cm}^2/\text{s}$), v is the flow velocity in the flow cell, d_{fc} is the flow cell diameter, and k' is the capacity factor. For the calculation of the detector plate height for the round tube the diameter was $100\mu\text{m}$, the capacity factor was zero and the flow velocity was approximately 0.02 cm/s . For the square flow cell, H_{det} was calculated by using a similar expression derived by Golay for rectangular columns with end effects (89). The value of D_m for these flow cell plate height calculations was determined by using equation 14 and the on column detection results.

Changing the position of the illuminated spot along the length of the flow cell did not have noticeable effect on the observed solute band variances. Thus it was concluded that the very large difference between the experimental and theoretical contributions of the flow cells to the solute band variances is probably due to dispersion occurring at the column-flow cell connection. The theoretical expressions used to calculate the flow cell plate height assume a parabolic flow profile. The sudden jump in size and shape of the flow channel upon entering the flow cell results in a region of nonuniform flow, as well as regions in

the corners of the flow cells (especially the square flow cells) which were poorly swept or stagnant. The possibility of gaps between the column and the flow cell also exists. All of these factors contribute to the dispersion of solute bands.

Proper optical alignment is more difficult to achieve for optical detection in open capillary LC than it is for larger scale LC. In the case of thermal lens detection the focused beam waist, ω_o , of the Gaussian laser beam must be several times smaller than the inner dimension of the flow cell while the confocal parameter, Z_c , should be considerably larger than the optical path dimension of the flow cell. The beam waist for a focused Gaussian laser beam can be more accurately (more so than equation 7) calculated from the beam waist, $\omega_{o,1}$, and confocal parameter, $Z_{c,1}$, of the laser by using (84):

$$\omega_o^2 = \frac{\omega_{o,1}^2}{(Z_{c,1}/f)^2 + (1-Z_1/f)^2} \quad \text{Eq. 15}$$

where Z_1 is the distance from the laser beam waist to the focusing lens and f is the focal length of the focusing lens. Shorter focal length lenses yield smaller beam waist values. By use of the information supplied by the laser manufacturer, the calculated values for the 60 mm and 25 mm focal length lenses used in this thermal lens work are 8 μm and 3 μm respectively.

The confocal parameter is calculated using equation 8. Equations 8 and 15 point out the importance of choosing the proper lens for the flow

cell being employed. Unfortunately equation 15 does not consider the effect of spherical aberration of the lens and the actual beam waist is expected to be larger than that calculated by the equation. These equations do provide a good starting point in choosing a suitable lens and lens position. Subsequent adjustments of the lens focal length and position are usually necessary in order to achieve the clean focusing of laser radiation through the flow cell.

Thermal Lens Detection

A more rigorous consideration of the parabolic thermal lens model (84) provides the following equation:

$$I(t) = I(0) \left[1 + \frac{2.3EA}{1 + t_c/2t} \frac{2Z_1Z_c}{Z_1^2 + Z_c^2} + \left(\frac{2.3EA}{1 + t_c/2t} \right)^2 \frac{Z_1Z_c}{Z_1 + Z_c} \right]^{-1} \quad \text{Eq. 16}$$

in which all the terms are the same as in equation 12 and Z_1 is the distance from the sample cell to the focused laser beam waist. This equation takes into consideration the temporal and spatial properties of the thermal lens signal. The placement of the sample (actually the thermal lens) and its effect on the radius of curvature of the wavefronts of the laser beam as it diverges are important in determining the magnitude of the thermal lens signal. The quadratic term of equation 16 is particularly significant. When the difference between $I(\infty)$ and $I(0)$

is greater than 0.1, the quadratic term becomes significant (84). The response of the thermal lens is analytically useful if the ratio $I(\infty) - I(0)/I(\infty)$ is less than 1.5. The importance of the terms in equation 16 to thermal lens detection will be discussed.

Of considerable interest to thermal lens detection in chromatography is the effect of flow on the thermal lens signal. Under the flow conditions typically encountered in HPLC it has been demonstrated that small reductions in thermal lens enhancement factors are observed (89). For the flow cell flow velocities employed in this open capillary LC work, no reductions in signal, relative to stagnant solutions measurements, were observed. However, with the column removed it was possible to operate at flow velocities (greater than 10 cm/s) for which no thermal lens signal could be detected.

The enhancement, E , of the thermal lens signal relative to Beer's Law is dependent on a number of experimental parameters which can be manipulated to a certain degree when performing thermal lens detection in open capillary LC. Increasing laser power and decreasing wavelength lead to larger enhancements. The former is ultimately limited by the maximum power of the laser, while the latter is restricted to the absorption bands of the analyte. The thermo-optical properties of the solvent also influence the magnitude of the enhancement as illustrated in Table II. Solvents with low polarity generally have properties which yield the largest enhancements. Unfortunately the bonded, reversed phase open capillary columns employed for this research require very polar mobile phases.

Figure 5 illustrates the enhancement offered by the thermal lens effect over conventional absorbance detection for a flowing sample. The apparatus in Figure 2B (with a 200 μm square flow cell) was employed in this experiment except that the column was replaced by a section of relatively wide-bore tubing. The chopper was positioned so that the gate delay was relatively long and appropriate to observe the steady state thermal lens signal (I_{∞}). A 60 mm focal length lens was used in the experiment. The flow cell and the connective tubing were filled with an alkaline, aqueous solution, and the pump was filled with a 1 μM phenolphthalein in alkaline, aqueous solution. The laser was operated at 515 nm and a power at the flow cell of 0.4 W. I_0 in Figure 5 is the signal intensity for the alkaline, aqueous solution flowing at a very fast rate, I is the signal resulting from the arrival of the phenolphthalein solution at the flow cell, and I_{∞} is the signal resulting from the same phenolphthalein solution when the flow rate has been reduced to a value compatible with open capillary LC. Taking the case for fast flow to be a measurement without a thermal lens effect, there exists a situation which is equivalent to Beer's Law absorption. For the slow flow case, the system response is obviously enhanced. The increase in intensity, rather than a decrease, is a result of the placement of the flow cell before the beam waist of the focused laser beam (see equation 16). This corresponds to a negative value of Z_1 which produces a value of $I_{(t)}$ which is greater than I_0 , or a decrease in the far field spot size due to the thermal lens. Placing the flow

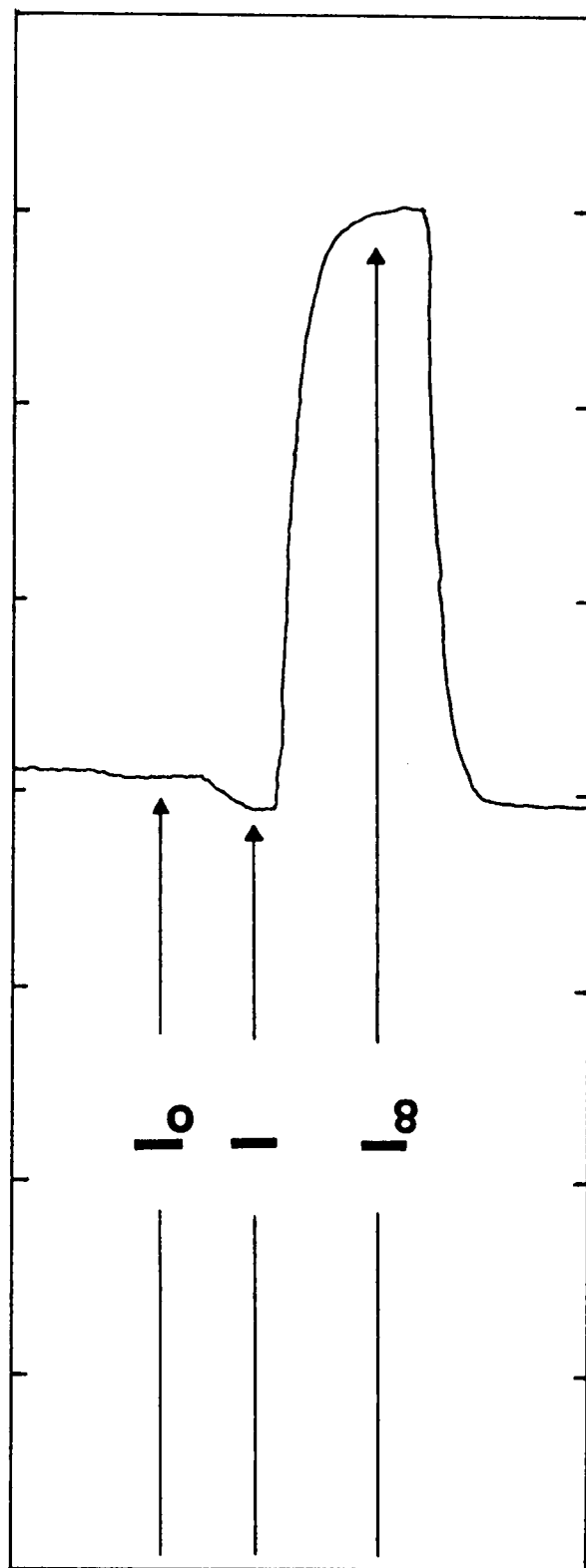


Figure 5. Thermal lens and conventional Beer's Law absorbance signals for a flowing sample illustrating the enhancement afforded by thermal lens detection.

cell after the beam waist has the opposite effect and a greater decrease in the signal would be experienced.

On the basis of the molar absorptivity and concentration of the phenolphthalein solution, the response at fast flow roughly corresponds with Beer's Law. The slow flow case, a thermal lens situation, provides an enhancement of approximately 20 over the Beer's Law measurement. While this is slightly less than half of the calculated enhancement for water of 0.11/mW (85), very little effort was made to fully optimize the critical position of the flow cell in the laser beam. This experiment clearly illustrated the ability to observe thermal lens enhanced absorption measurements under flow conditions which are compatible with open capillary LC.

The time constant for the formation of a thermal lens was given in equation 11. The experimental arrangement and phenolphthalein solution used to obtain the data for Figure 5 were also used to measure t_c . By translating the chopper perpendicular to the laser beam it was possible to observe the transient thermal lens signal. Fitting the observed transient signal to equation 16 yielded a value of 0.3 ms for t_c . However, the relatively large gate duration of the gated detector made it impossible to observe the very early stages of lens formation and this limited the accuracy of the time constant determination. The value of the time constant can also be calculated using equation 11. Using the previously calculated beam waist for the 60 mm focal length lens, and assuming the flow cell was positioned one confocal parameter from the beam waist, the value of w is calculated to be 11 μm (91).

Inserting this value into equation 11 with the appropriate solvent parameters results in a calculated time constant of 0.21 ms. Considering the limitations of measuring the time constant and possible errors in determining the beam waists, the agreement between theory and observation seems reasonable.

The positional dependence of the thermal lens signal can be discerned from equation 16. The equation predicts an optimum signal when the sample cell is positioned one confocal parameter before or after the focused laser beam waist (i.e. $Z_1 = Z_c$). Alternately, the aberrant theoretical model of the thermal lens effect (92) predicts an optimum when Z_1 is equal to $3^{1/2}Z_c$. The tight focusing that is necessary for thermal lens applications with open capillary LC results in small confocal parameters. This makes flow cell positioning critical. A plot of peak height vs. Z_1 for injections of an o-nitroaniline solution is shown in Figure 6. Optimum signals were obtained for Z_1 values of 0.15 mm. This study was performed with a 20 μm i.d. column, a 100 μm flow cell, and a 25 mm focal length lens. The symmetric nature of the curve is readily explained by considering the effect of a diverging lens on a focused laser. A diverging thermal lens placed to the right of the waist increases the divergence of the beam and therefore causes a decrease in intensity in the far field at beam center. Placing the thermal lens before the beam waist causes a decrease in convergence of the beam which increases the intensity at beam center. Assuming accurate models and using equation 11, the optimum flow cell position indicates that the beam waist for the experiment was 5 μm for the

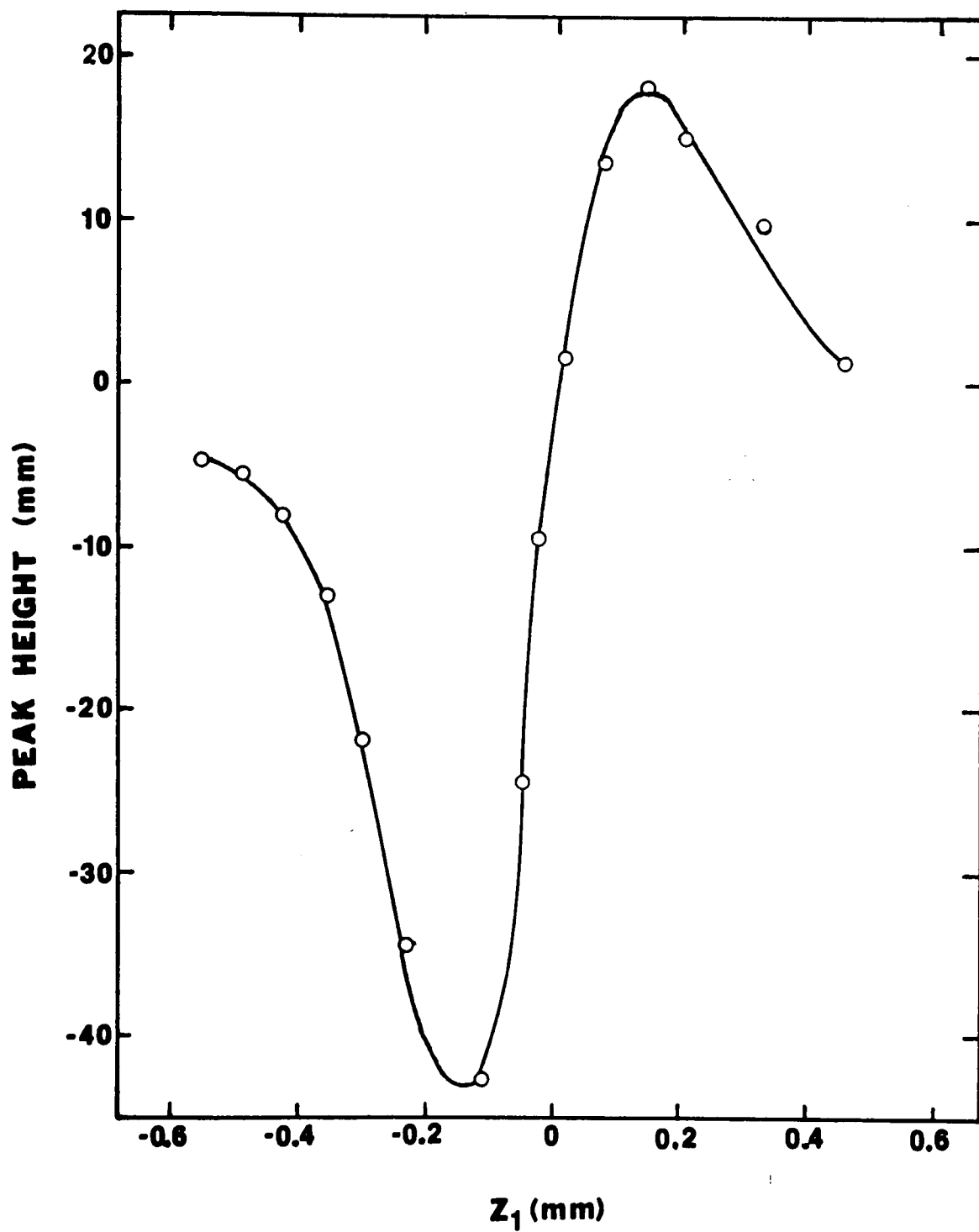


Figure 6. Positional dependence of the thermal lens signal for detection in open capillary LC.

parabolic lens model and 4 μm for the aberrant lens model. Employing the laser resonator parameters provided by the manufacturer, equation 15 yielded a beam waist of 3 μm . Considering the questionable ability to predict beam waists mentioned earlier, this agreement is reasonable.

Figure 6 also shows larger signal when the flow cell is positioned before the beam waist. This is contrary to predictions based on equation 16 and previously reported thermal lens studies in large sample cuvettes, which predict a symmetric curve about the focal point. However, the theory of thermal lens formation assumes a thin thermal lens in which the sample pathlength is much less than Z_c . This is not valid for experiments performed in the very small flow cells required by open capillary LC.

Figure 7 illustrates a chromatogram of nitroaniline compounds separated by open capillary LC with thermal lens detection. A 7.5 m x 20 μm i.d., ODS bonded phase column was used with a mobile phase that was 40% methanol in water. The peaks correspond to 410 pg of o-nitroaniline, 310 pg of 4,5-dimethyl-2-nitroaniline, and 3.6 ng of N,N-dimethyl-3-nitroaniline. The base line noise level for this chromatogram is approximately 3×10^{-5} absorbance units. Figure 8 shows a calibration plot for o-nitroaniline. The plot yielded a limit of detection (LOD) of 30 pg (S/N=2) and a linear regression constant of 0.994 from the LOD to 1.2 ng injected. The thermal lens detector developed for this work proved that it is possible to make sensitive absorbance measurements in the small volumes eluted from open capillary LC columns. In order to extend photothermal detection to the UV

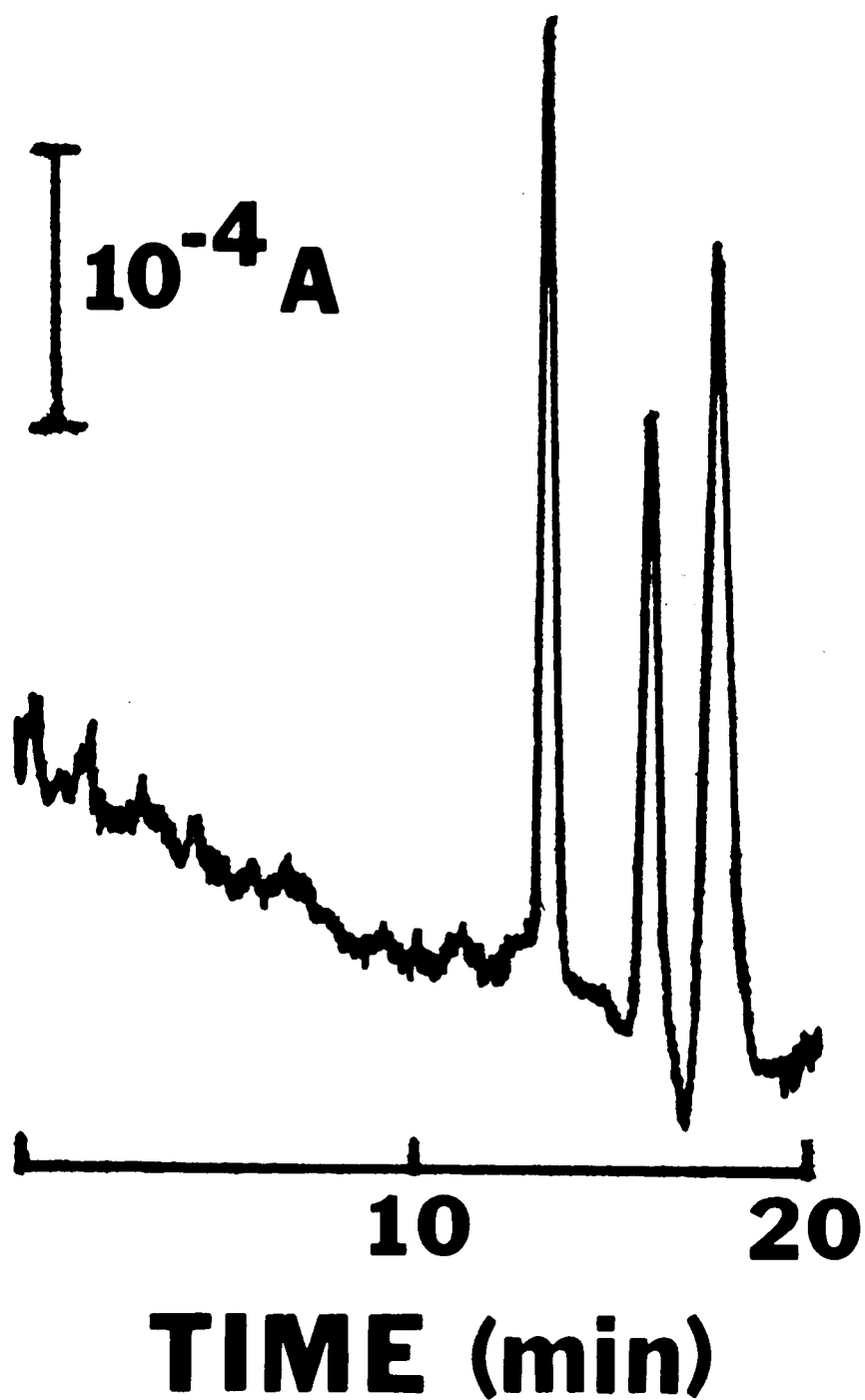


Figure 7. Open capillary LC separation of nitroaniline compounds using thermal lens detection. The compounds in order of elution are o-nitroaniline, 4,5-dimethyl-2-nitro-aniline, and N,N-dimethyl-3-nitroaniline.

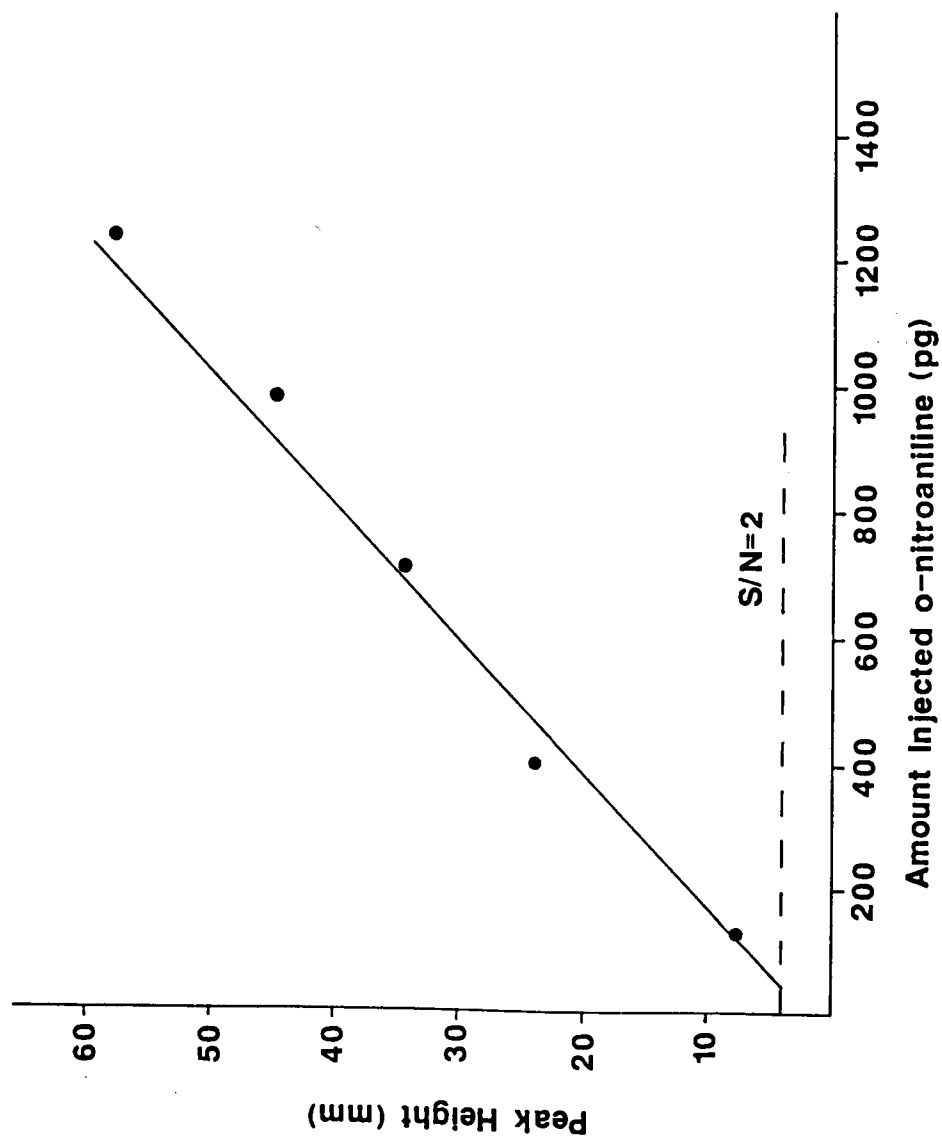


Figure 8. Calibration plot for o-nitroaniline. Limit of detection at a signal-to-noise ratio of 2 is 30 pg.

spectral region, an excimer pumped dye laser was obtained and double beam photothermal measurement schemes were investigated. The results of these investigations are discussed in the next chapter.

CHAPTER III

PHOTOTHERMAL REFRACTION DETECTION IN

SLURRY PACKED CAPILLARY LC

Introduction to Photothermal Refraction

Photothermal refraction is a laser based measurement technique which is similar to the thermal lens technique discussed in the previous chapter. The optical element in photothermal refraction is a cylindrical lens which is investigated by a second (probe) laser. Figure 9 illustrates the experimental apparatus for both double beam thermal lens and photothermal refraction measurements. The principal difference between the two techniques is that the probe beam for thermal lens is colinear with the pump beam while in the photothermal refraction technique the lasers intersect perpendicularly. The thermal lens optical element is a circular lens, while the photothermal refraction optical element is a cylindrical lens. Both optical elements provide a defocusing of the probe beam and are detected by monitoring the probe beam intensity in the far field.

The double beam thermal lens and photothermal refraction techniques were both investigated for potential use as capillary LC detectors. The pump laser used for these studies was an excimer laser pumped dye laser. Using a pulsed laser rather than a CW laser for photothermal measurements offers the following advantages (93): insensitivity of signal levels to flow, removal of the deleterious effects of thermal convection by the use of low average powers, and a greater theoretical enhancement

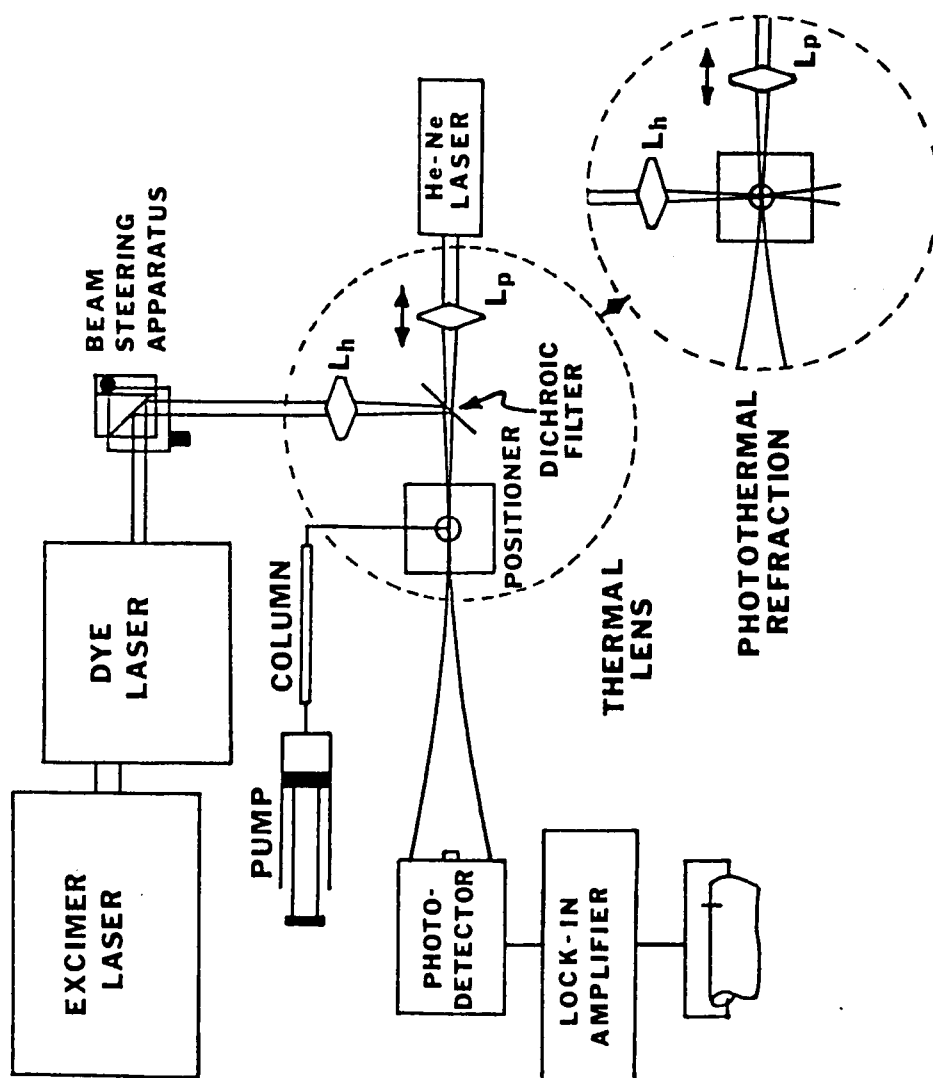


Figure 9. Schematic of instrumental arrangement for pulsed laser double beam thermal lens and photothermal refraction detection for capillary LC.

factor. In addition, the use of a tunable dye laser allows access to the analytically useful UV portion of the spectrum by using the appropriate laser dyes and frequency doubling optics.

Photothermal refraction proved to be a technique which was easier to align than the double beam thermal lens experiment. As illustrated in Figure 9, the thermal lens experiment requires the pump and probe beams to be combined at the dichroic filter before entering the sample. The task of combining the focused pump and probe beams was nontrivial. This problem was exacerbated by the need to employ short focal length lenses and a thick, high power dichroic filter. The short focal length lenses were needed in order to obtain the small beam waists required to probe small volumes and the high power dichroic was necessary to combine the high peak power dye laser with the HeNe probe laser. Conventional dichroic filters did not tolerate the high peak powers provided by the pulsed laser system.

The thermal lens setup proved to be very susceptible to any change in lens position, however small, and was very tedious to realign due to the difficulty in determining what optical component had become misaligned. An additional problem was the closeness of the lenses, filter, and sample cell. Any ineptness by the researcher when moving the sample cell or any other components was often rewarded with a loss in signal.

The beam steering and focusing apparatus shown in Figure 10 was developed and allows a focused laser beam to be translated through 3 axes in space. The high quality translational stages used in the construction of this device allowed the focused probe beam to be moved

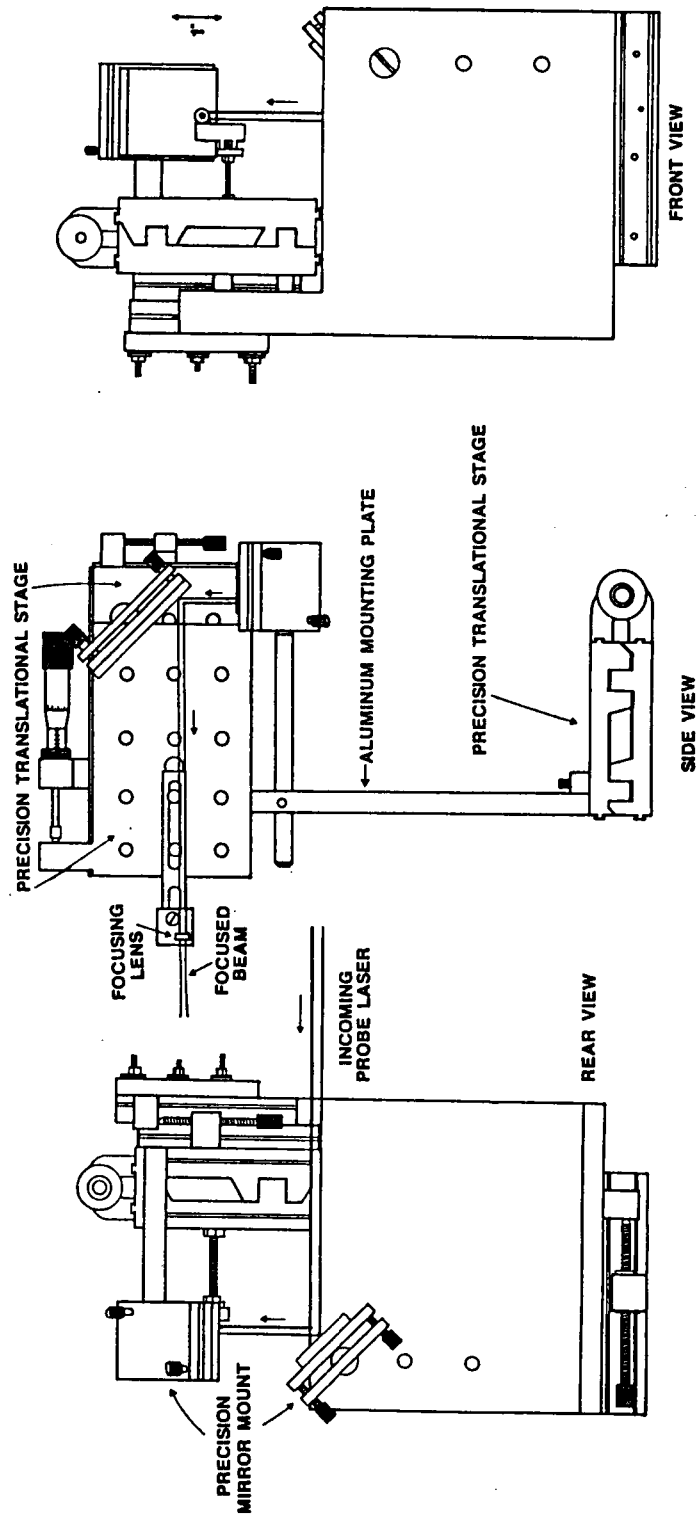


Figure 10. Schematic of beam steering and focusing device capable of translating a focused beam through 3 axes.

along any of the three principal axes in micrometer size increments. Fixing the location of the focused pump laser beam on one optical axis (the direction of beam propagation) allowed the use of the beam steerer to probe any point along the pump laser beam path with any size beam waist. Using the crossed beam photothermal refraction technique was the method of choice over the thermal lens arrangement. Eliminating the need to align the pump and probe beam colinearly allowed for a rapid alignment and optimization of the photothermal refraction signal. Once aligned and optimized, the photothermal refraction detector remained aligned from day to day, requiring only occasional "tweaking" of the signal.

The theory and confirming experiments for photothermal refraction were presented by Dovichi and coworkers in 1984 (65,80). The equation governing the photothermal refraction signal response for pulsed laser excitation is given by:

$$\frac{I(t) - I(0)}{I(0)} = \frac{\theta E / t_c}{(1 + 2t/t_c)^{3/2}} \quad \text{Eq. 17}$$

where $I(0)$ is the probe beam intensity with no lens present, $I(t)$ is the probe beam intensity after time t , and t_c is the time constant of the lens decay (defined by equation 11) and E is the energy of the laser pulse.

The sensitivity of the effect is proportional to θ :

$$\theta = \frac{4.606 \epsilon C (dn/dT) Z_1}{(2\pi)^{1/2} \omega k} \quad \text{Eq. 18}$$

where ϵ is the molar absorptivity, C is the concentration, dn/dT is the change in refractive index with respect to temperature, Z_1 is the distance from the probe laser beam waist to the intersection of the pump and probe beams and ω is the pump laser spot size which, because of the cross beam configuration, is effectively the optical pathlength.

Inspection of this equation provides some important analytical considerations of photothermal refraction as a detection technique for capillary LC. Signal linearity with absorptivity and concentration are very desirable features for LC detection due to their facility in ascertaining the concentration of individual components in a mixture and the need for a detector with good linear dynamic range.

The fact that the sensitivity is inversely related to pump laser spot size implies that greater sensitivity is gained when smaller volumes are probed. While this is true in theory, in practice, concern must be taken for the destructive effects of tightly focused high peak power lasers. This concern also encompasses the fact that signal is directly proportional to laser pulse energy. These concerns will be discussed later in this dissertation.

The sensitivity of the measurement is predicted to increase linearly with the distance from the probe beam waist to the sample, Z_1 . The spot size also increases as given by equation 15. As Z_1 does increase, concern must be taken that the pump beam spot size remains larger than the probe beam spot as assumed in the theoretical derivation of the

photothermal refraction model (80). Thus only a limited improvement is possible by varying Z_1 .

The temporal dependence of the photothermal refraction signal allows for the use of lock-in detection which is useful in enhancing the S/N of the measurement. The frequency dependence of the thermal lens signal has been shown to be an important factor with lock-in detection (94).

Experimental Instrumentation and Considerations

A block diagram of the equipment used for photothermal refraction experiments and LC detection in this work is shown in Figure 9. The photothermal refraction detector consisted of a Lambda Physik (Acton, MA) FL 2000 dye laser pumped by a Lambda Physik EMG 50 excimer laser. The excimer laser uses a Xe/HCl gas fill to pump the PBB0 dye (Lambda Physik LC 4000) at 308 nm. The probe laser was a Spectra-Physics (Mountain View, CA) model 145-01 helium neon laser. The absorbed laser beam was focused with a 25 mm focal length quartz biconvex lens and the probe laser was focused with a 22 mm focal length lens. The intensity of the probe beam at beam center was monitored with a Clairex (Mt. Vernon, NY) model CL707HM photoconductive cell placed 67 cm from the flow cell. An Ithaco (Ithaca, NY) model 391A lock-in amplifier, referenced to the synchronous output of the excimer laser, was employed to monitor and amplify the photocell signal. The lock-in output was monitored with a Cole-Parmer (Chicago, IL) model 8376-20 recorder. The entire system was rigidly mounted on a Modern Optics (El Monte, CA)

pneumatically stabilized optical table which served to minimize vibration induced contributions to the background.

When the photothermal refraction signal was being investigated or optimized, the setup in Figure 9, p. 47, was modified by removing the chromatography column and replacing it with a flow cell of the desired dimensions. A sample solution was typically fed to the flow cell with a Harvard Apparatus Co (Dover, MA) model 600-000 variable speed syringe pump.

For the chromatographic separations either a conventional HPLC setup was employed or slurry packed fused silica columns were employed. The HPLC apparatus consisted of a Rheodyne (Berkley, CA) model 7120 injector, a BIO-SIL (Biorad Laboratories, Richmond, CA) ODS-10 column and a Altex (Oakland, CA) model 110A pump. The slurry packed capillary columns were fabricated as discussed in Appendix B.

Evaluation of Photothermal Refraction Detection

In order to determine the utility of photothermal refraction detection for capillary LC, experiments were conducted to investigate the parameters which could be manipulated to optimize the signal. Of particular interest were the factors which would affect the lock-in amplifier optimization. A study of the signal dependence on lock-in phase angle and pump laser frequency was accomplished by using the experimental setup in Figure 9 with a 500 μm flow cell in place of the column. A 9.8×10^{-6} M 1-nitropyrene (1-NP) solution in methanol was pumped through the flow cell. The dye laser was tuned to 397.5 nm and

the lock-in amplifier sensitivity was 10 mV full scale and the filter time constant was 4 seconds.

The relationship of signal intensity to lock-in phase angle was studied by using a fixed frequency of 20 Hz for the pump laser and varying the phase angle. The results of this study are shown in Figure 11. For a phase angle of between 10° to 30° and at 200° the signal was maximized. With the phase angle set at 200° , the frequency was varied between 10 and 100 Hz. As illustrated in Figure 12, the optimal frequency lies between 23 and 28 Hz. The degradation of signal at higher frequencies is due to the fact that the thermal gradient formed by the pump laser does not diminish completely before the next pulse arrives. This "piling up" of thermal gradients subsequently reduces the magnitude of the intensity change of the probe laser at beam center. Thus a reduction in signal occurs. After these studies were accomplished the lock-in amplifier was typically set up with a phase angle of 200° and operated at 23 Hz.

The parameter which can be manipulated with the greatest ease is the energy of the laser pulse. For the excimer pumped dye laser used in this work this can be accomplished by varying the thyatron voltage of the excimer between 25 and 32 kV or adjusting the photon flux between the oscillator and amplifier dye cells within the dye laser. A study of the effect of average pump laser power on photothermal refraction signal level was accomplished using the same experimental arrangement as was employed for the phase angle and frequency study. The dye laser average power was measured with a Scientech (Boulder, CO) model 362 energy power

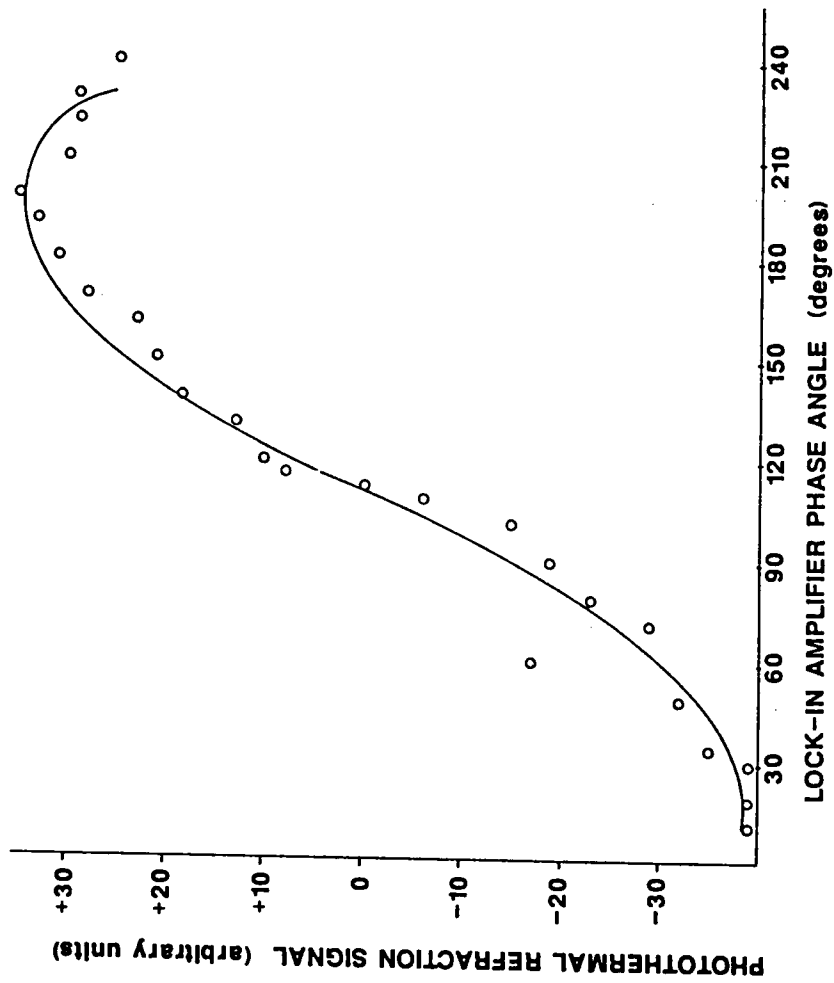


Figure 11. Relationship of photothermal refraction signal level to lock-in phase angle.

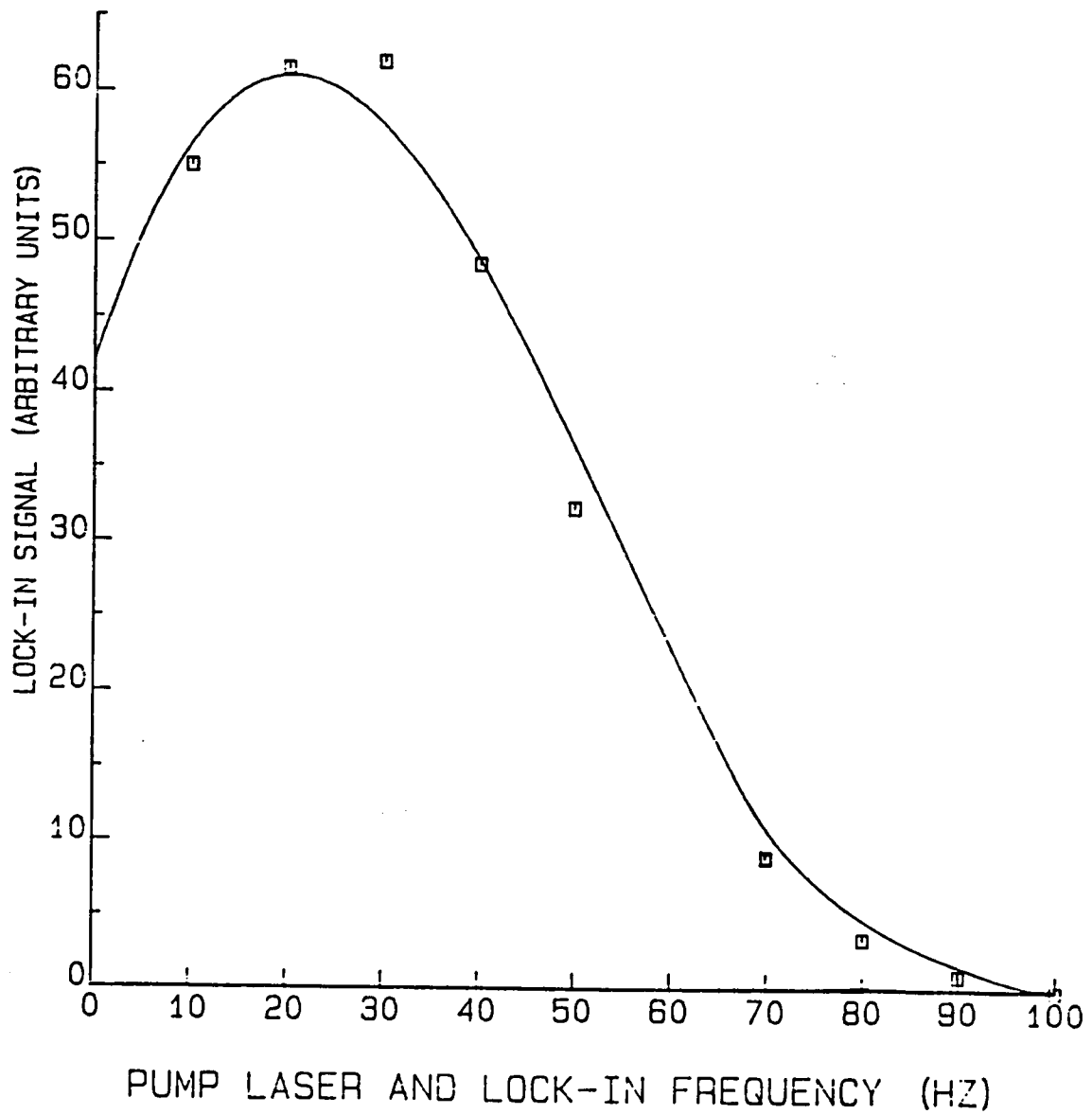


Figure 12. Relationship of pump laser frequency to photothermal refraction signal level.

meter. The results of this study are shown in Figure 13. For average laser powers above 3 mW, the flow cell often sustained damage in a very short period of time. The high photon fluxes were damaging to the flow cell and required the use of less than 2 mW average power for the extended measurement times that chromatograms required. Attempts to circumvent this flow cell damage problem by using a sheath flow cell are discussed later in this chapter.

The positional dependence of the probe laser was investigated in order to determine the approximate position where an optimal signal could be found. As shown in equation 18 the distance between the probe beam waist and the sample, Z_1 , is directly proportional to the signal intensity. This parameter was investigated by simply translating the probe laser focusing lens from a position where no signal was present back away from the flow cell and observing the behavior of the signal. The results of this crude experiment are illustrated in Figure 14. The signal shows the expected rise as Z_1 increases, but then falls to negative values where it flattens out to a constant value. The symmetric behavior of the signal with probe waist position is typical of photothermal methods as discussed in chapter 2. The fact that the signal does not rise back to zero at large Z_1 values is possibly explained by the fact that although the probe beam spot size is now probably greater than the pump beam spot, the violation of this assumption germane to the photothermal refraction theory does not destroy the signal altogether.

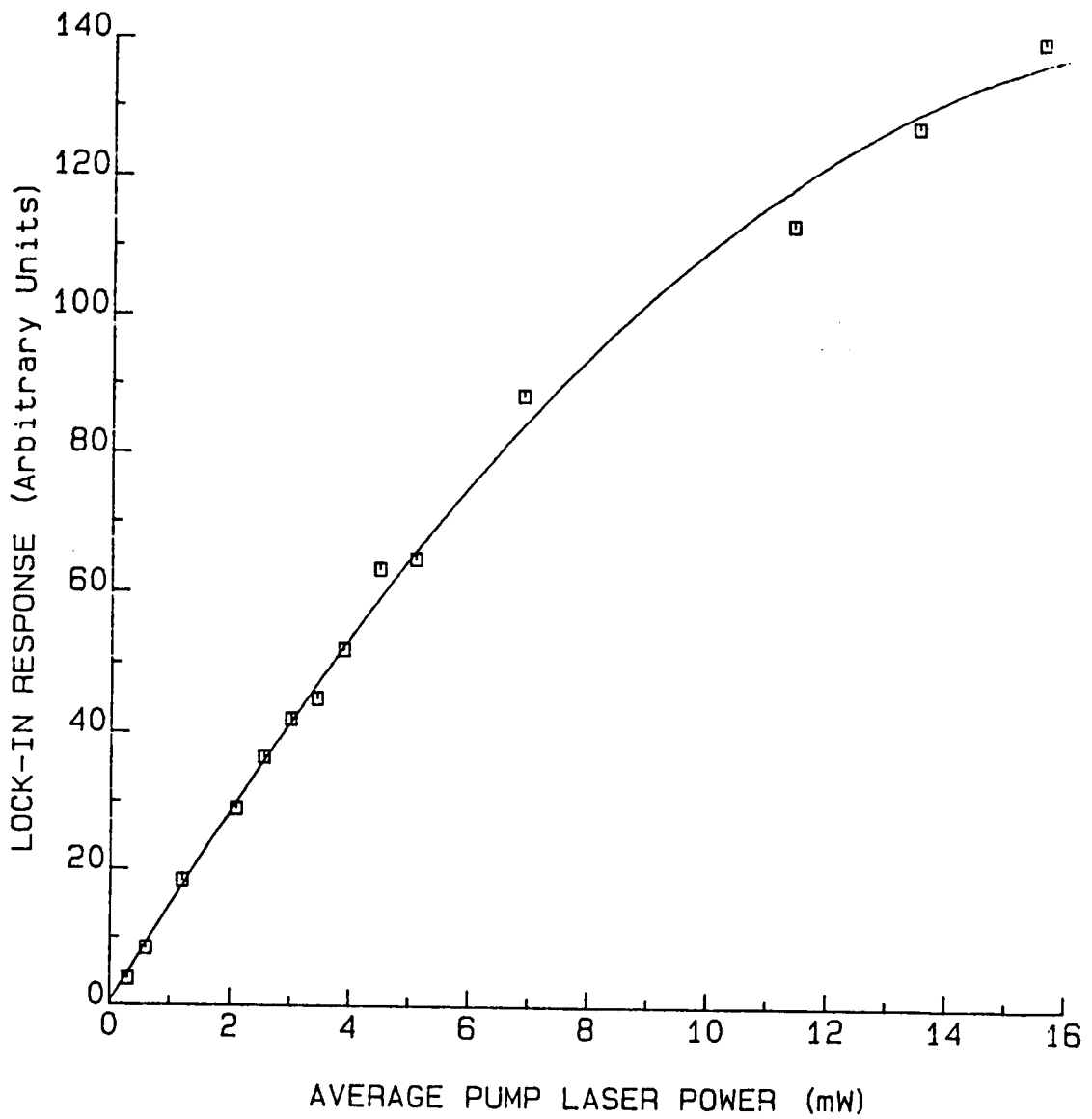


Figure 13. Photothermal refraction signal level dependence on average pump laser power.

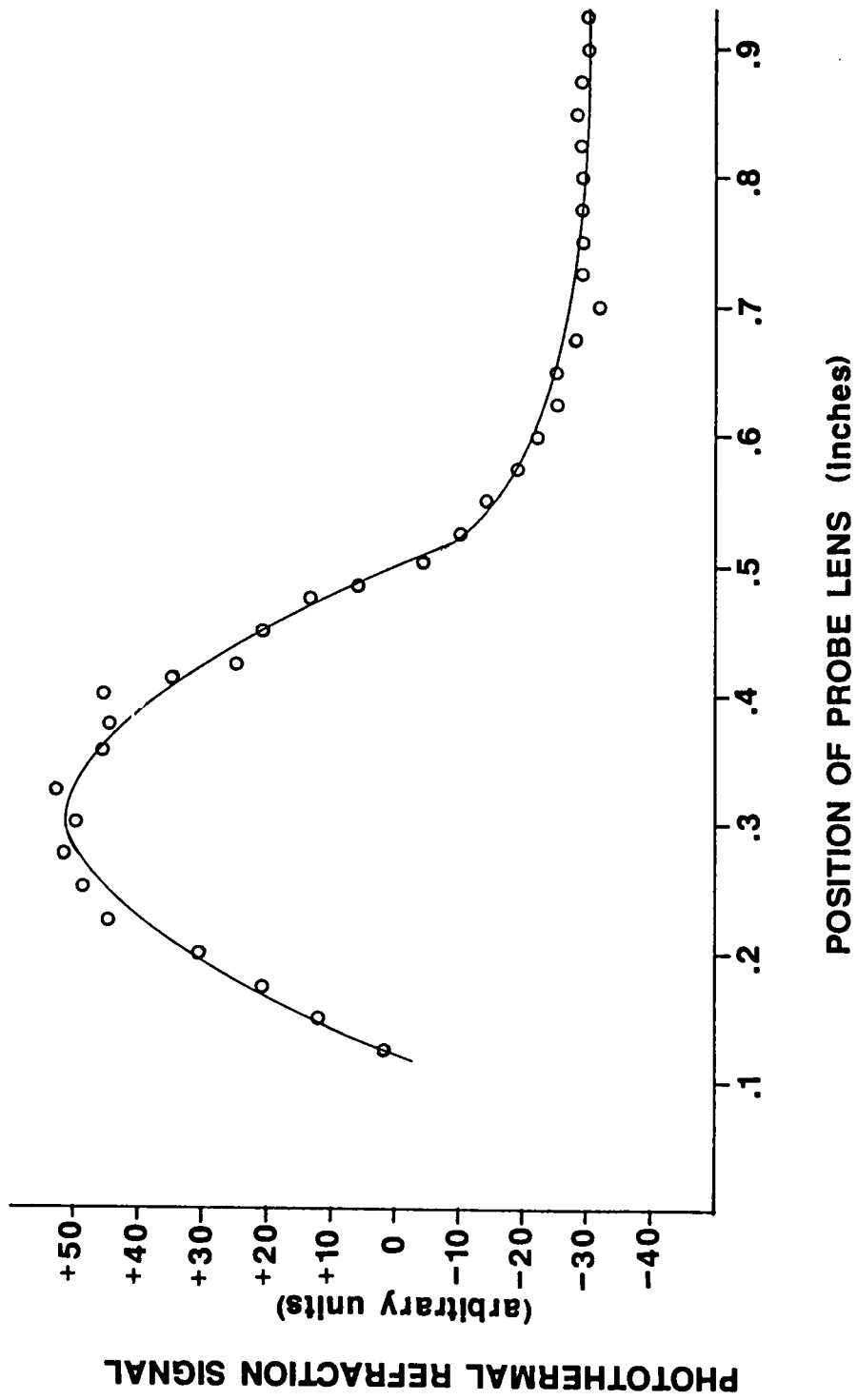


Figure 14. Photothermal refraction signal dependence on distance from probe beam waist to sample cell (Z_1).

With the optimization of detection variables, measurements were made to determine whether the sensitivity of this technique was sufficient to employ it as a capillary LC detector. Figure 15 shows photothermal refraction signals for a 7.8×10^{-7} M 1-NP solution flowing in a 500 μm square quartz flow cell. The signal-to-noise ratio for this measurement is 10 for the 4 second time constant and 40 for the 12 second time constant. Measurement of this dilute a concentration at this S/N level led to the conclusion that this technique was sensitive enough to use with capillary LC.

The linearity of the photothermal refraction signal was studied by employing a series of 1-NP solutions in hexane and measuring their signal level as they were pumped through a 200 μm flow cell. The results of this experiment are shown in Figure 16. The linearity extended from 5.7×10^{-4} M to 4×10^{-6} M with a correlation coefficient of 0.997. A linear dynamic range of nearly two and a half orders of magnitude was considered sufficient to employ this technique for LC detection.

Chromatography Detection with Photothermal Refraction

The use of photothermal refraction as a detection method for LC was initially carried out with analytical scale columns. These experiments were carried out in order to verify the optimum experimental conditions for LC detection. Once these experiments were accomplished the detection method was employed using slurry packed fused silica capillaries for the separations. The slurry packed fused silica columns

Photothermal Refraction Detection Signals

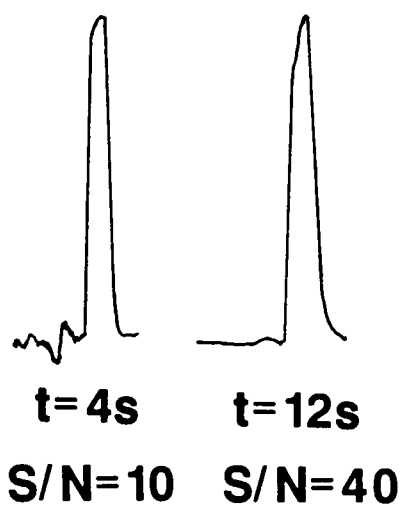


Figure 15. Photothermal refraction signals obtained detecting 7.8×10^{-7} M 1-NP solution flowing at approximately 1 mm/sec in a 500 μm square flow cell. Detection was at 400 nm.

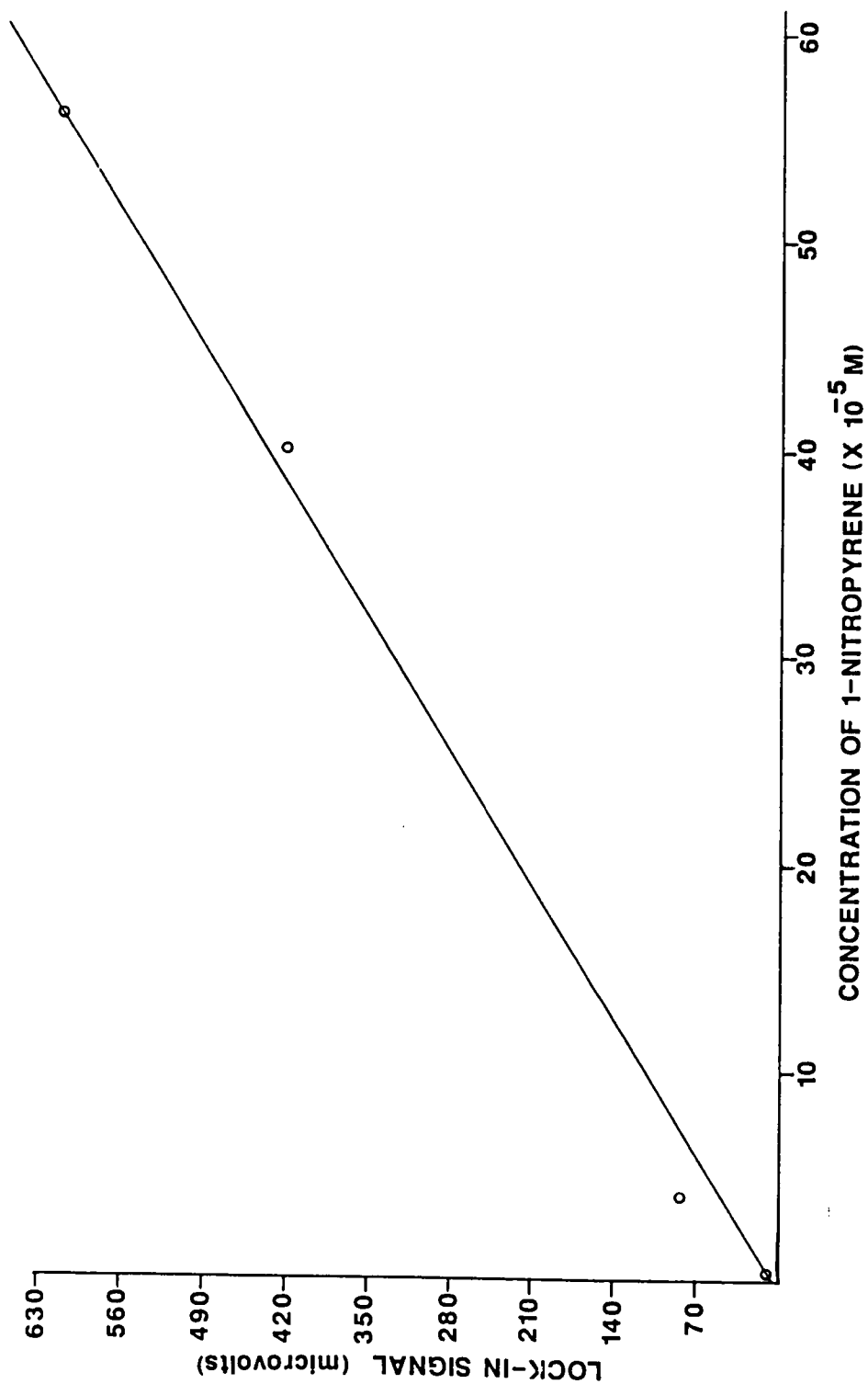


Figure 16. Linear response of photothermal refraction signal with respect to concentration.

were employed instead of open capillary columns because of problems encountered when trying to focus the pulsed dye laser to the very small beam waists required for open capillary LC detection. High gain lasers typically do not possess the excellent beam coherence properties and beam energy stability that CW lasers possess and thus capillary columns which did not have as small a peak volume as open capillary columns were used so that slightly larger beam waists were allowable.

Nitrated polycyclic aromatic hydrocarbons (N-PAHs) were used as test compounds. N-PAHs have generated considerable interest in analytical chemistry due to their mutagenic and carcinogenic properties (95-100) and the fact that they are found in many areas of the environment including air particulates (101-103), diesel and gas exhaust (104,105), cigarette smoke and coal burning power plants (106). In addition to the scientific interest in these compounds, they have high molar absorption coefficients and low fluorescence quantum efficiencies. These properties make them ideal for photothermal refraction detection.

Figure 17 and 18 illustrate the separation and detection of some nitrated compounds of interest. These chromatograms illustrate the potential of photothermal refraction as a detection method for LC. No attempt was made to optimize the overall sensitivity of the detection as the purpose of these experiments was limited to simply verifying photothermal refraction as a viable detection scheme.

The primary interest in the development of this technique is to address the problem of detection in capillary LC. Slurry packed capillary columns have received considerable interest as an alternative to

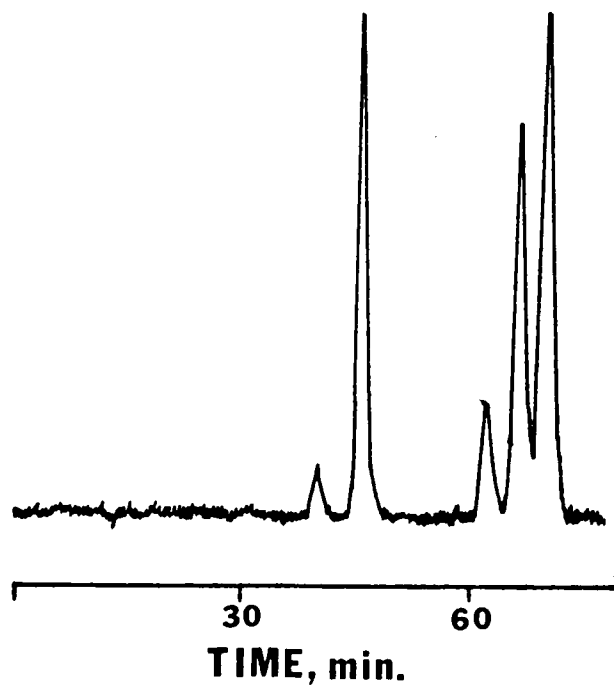


Figure 17. HPLC separation of nitroaniline compounds with photothermal refraction detection at 418 nm. Compounds in order of elution are: m-nitroaniline, 4,5-dimethyl-2-nitroaniline, 4-chloro-2-nitroaniline, 2,4-dimethyl-6-nitroaniline.

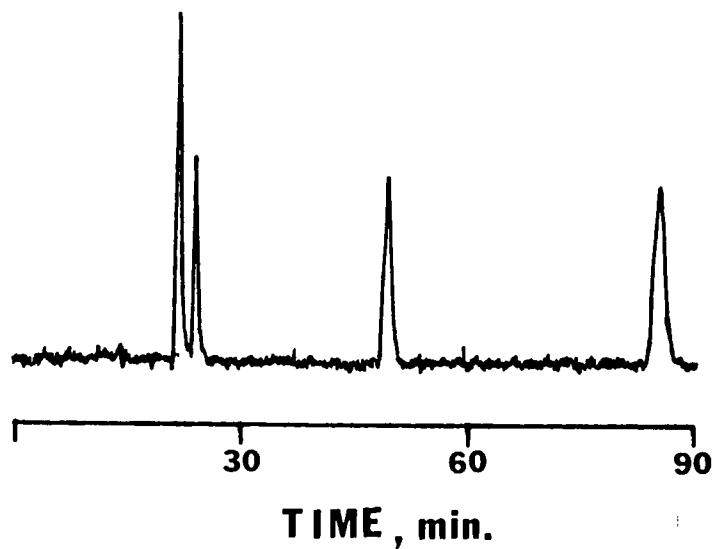


Figure 18. HPLC separation of nitroPAH compounds with photothermal refraction detection at 400 nm. Compounds in order of elution are: 1-nitronaphthalene, 1,4-dinitronaphthalene, 9-nitroanthracene, 1-nitropyrene.

open capillary columns. These columns are characterized by high efficiencies, better sample capacity than open capillary columns, and not quite as stringent requirements for injection and detection (107). Furthermore, these columns do offer an advantage over open capillary columns in that they are not as fragile as glass capillaries. The columns are usually constructed from fused silica which is polyimide coated. This makes the columns much less susceptible to fracture when compared with the glass capillaries employed for open capillary LC. Although these columns do not experience the clogging problems that open capillary columns often do during their preparation and use, their fabrication requires the absolute minimization of sources of extracolumn variance and large amounts of patience. The preparation of the columns used for this work is described in detail in Appendix B. An enlargement of the terminal end of the column where detection takes place is shown in Figure 19. Detection takes place in a short section of fused silica which has been epoxied to the end of the column. The polyimide coating has been removed leaving the thin wall fused silica as the flow cell walls.

Another advantage of capillary LC is the ability to perform separations using extremely small sample volumes. The complex mixtures resulting from extraction of air particulate matter, soil samples, and other naturally occurring samples often contain many components which are at a concentration below the limit of detection. Concentration of extracts to microliter volumes prior to injection in capillary LC can often enhance the opportunity to detect these trace components and still

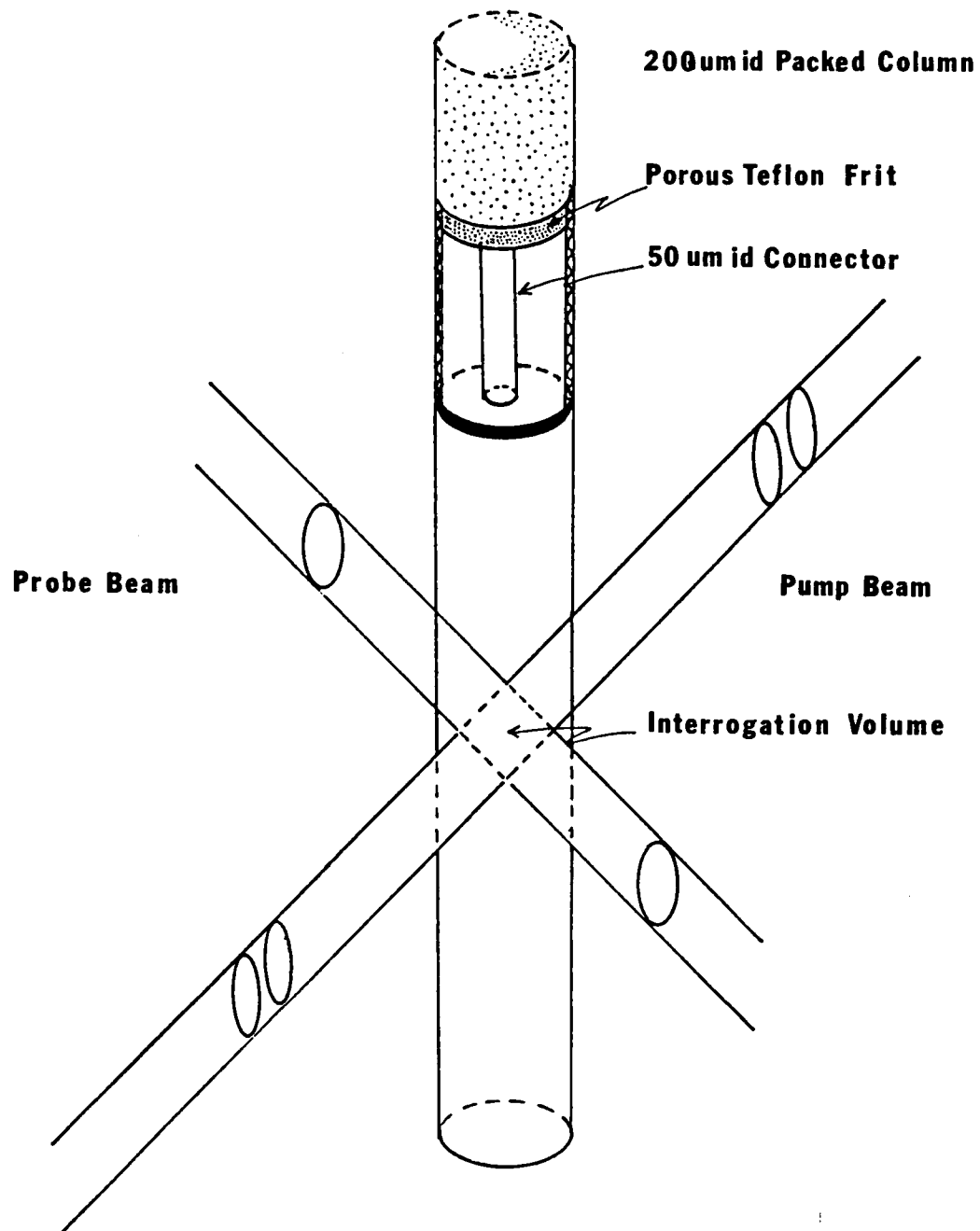


Figure 19. Expanded scale view of terminus of slurry packed fused silica capillary column.

have sufficient sample to carry out further analyses. The separation of the diesel exhaust extract illustrated in Figure 20 illustrates this advantage. The diesel exhaust extract was a sample of methylene chloride extract of diesel engine exhaust filters. The sample was obtained from Oak Ridge National Laboratory courtesy of Tuan Vo Dinh. The chromatogram shown was accomplished with a single 20 nL injection of an extract that had to be concentrated by a factor of 100 before the detection shown could be achieved.

The research performed to prepare Figure 20 also illustrates one of the problems with pulsed laser photothermal techniques. An attempt was made to analyze the same sample after spiking with 1-nitropyrene (1-NP). Unfortunately, the high laser energy which provided the excellent sensitivity in the chromatogram shown also caused a hole to be burned through the thin fused silica flow cell wall. Typically this caused problems with detection due to bubbles at the detection site. This required abandoning the column for a new one with an intact flow cell. This was a major problem with the technique. Often the zeal of the researcher for outstanding detection was the cause for the destruction of a column which had good efficiency. Regardless of the problems involved with this particular separation, the ability of this type of capillary column to analyze an extremely complex sample is illustrated in this chromatogram. The two major peaks of this chromatogram were suspected of being 9-nitroanthracene and 1-NP, respectively, due to their close match in retention time with standards; however, this could not be confirmed due to the destruction of the column with the application of too high a power level.

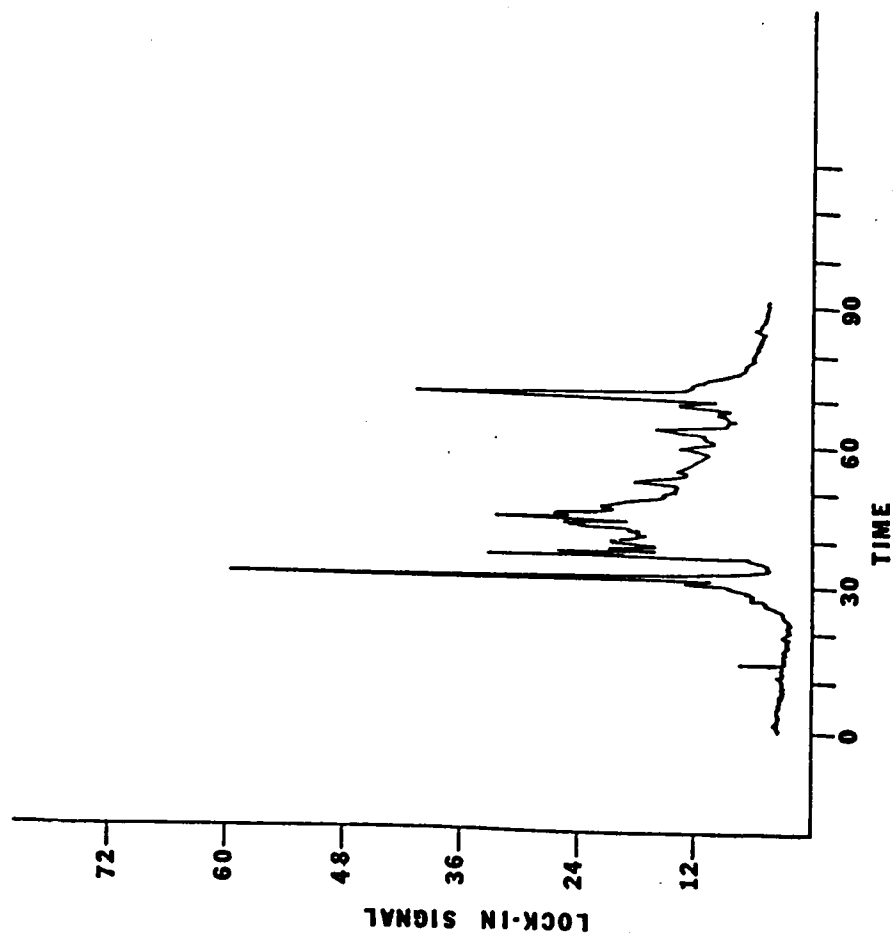


Figure 20. Slurry packed capillary column separation of diesel fuel exhaust particulate extract with pulsed laser photothermal refraction detection at 400 nm. Experimental conditions: column 200 μm i.d. x 90 cm, 5 μm CPS-Hypersil, isocratic elution with 95:5 n-hexane:isopropanol at 1350 psi constant inlet pressure.

Figure 21 contains a series of chromatograms illustrating the use of a slurry packed silica column with photothermal refraction detection to indicate the presence of nitropyrene isomers in a fly ash extract sample. The sample was an extract of coal fly ash which had been coated with pyrene and subsequently exposed to an atmosphere of NO_2 (108). The fly ash was then soxhlet extracted with toluene, concentrated, and injected onto the column. Again the capability to analyze small samples was advantageous. The sample studied was concentrated from 80 mL down to 10 μL before detection at the level shown in the chromatograms was attained. The use of split injection techniques provides minimal band broadening (31) and the greatest flexibility concerning the quantity injected. Unfortunately, when sample sizes are extremely small, as in this case, split injection is not possible. Using the nonmechanical injection procedure described in Appendix B, multiple injections were made without the concomitant wetting losses incurred when filling an injector. Chromatograms A and B are separations of the coal fly ash extract. Chromatogram C of the figure is the separation of a standard mixture of 1-NP, 1,3-dinitropyrene (1,3-DNP), 1,6-dinitropyrene (1,6-DNP) and 1,8-dinitropyrene (1,8-DNP). This chromatogram illustrates the separation capability of slurry packed capillary columns under isocratic elution conditions (analytical scale separations of this mixture typically require gradient conditions). The ability to accomplish this separation under isocratic conditions preserves the thermo-optical properties of the mobile phase, and, hence, serves to maintain a constant photothermal refraction background signal (65).

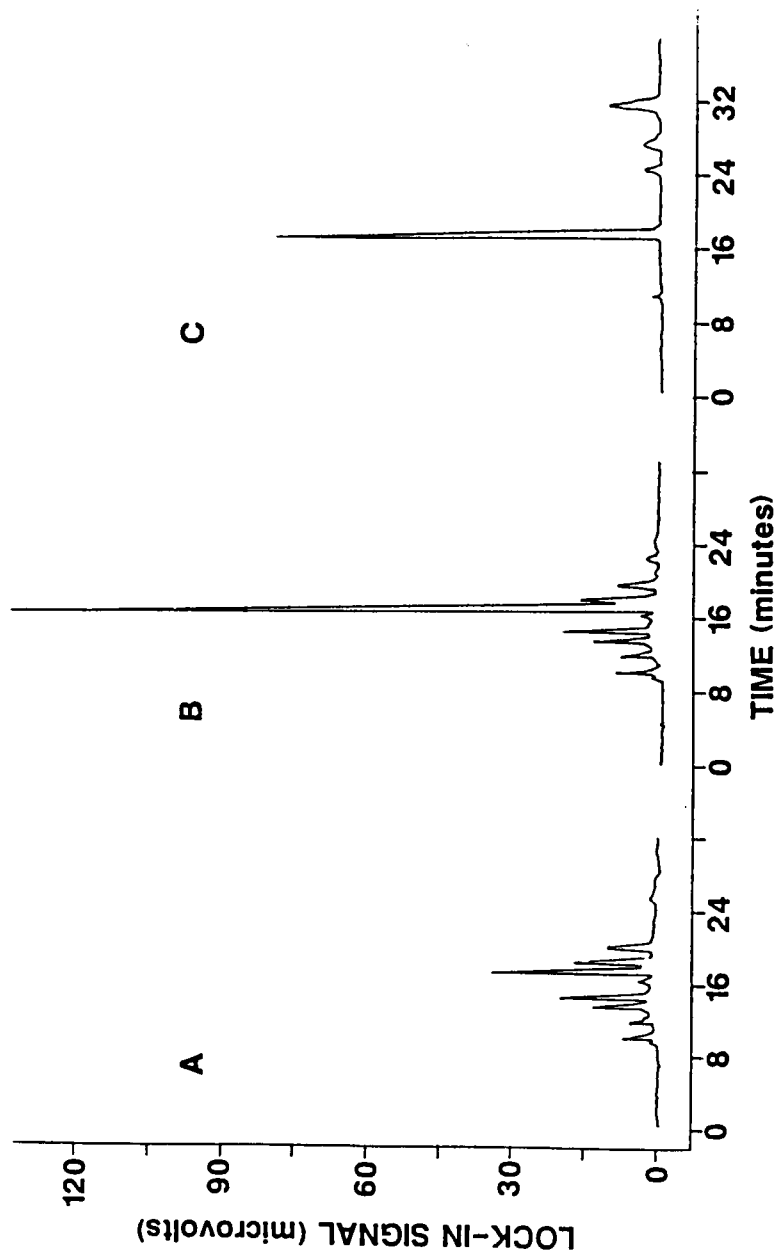


Figure 21. Chromatograms of a coal fly ash extract (A and B) and 1-nitropyrene and dinitropyrene isomers standard mixture (C). Experimental conditions: column, 200 μm x 62 cm, 5 μm CPS-Hypersil, isocratic elution with 95:5 n-hexane:isopropanol at 1000 psi constant inlet pressure, detection at 400.0 nm with 0.6 mW average pump laser power. Chromatograms A and B described in text, chromatogram C contained in order of elution: 3.0 ng 1-NP, 0.31 ng 1,3-DNP, 0.80 ng 1,6-DNP, and 1.3 ng 1,8-DNP.

All three chromatograms in Figure 21 were obtained using the same column and conditions. The tallest peak of chromatogram A was suspected of being 1-NP due to its match in retention time with 1-NP in the standard mixture. Spiking the extract with 1-NP provided an increase in peak height for the suspect peak while the remaining peaks remained the same height (Figure 21B). The presence of 1-NP in this extract has been confirmed by direct inlet probe mass spectrometry (108). The additional peaks in the chromatogram could include different positional isomers of nitrated pyrene.

The linear response of photothermal refraction detection was illustrated in Figure 16, p. 62. The reproducibility of response for chromatographic detection is illustrated by the detector response to the amount of 1-NP added to the complex mixture. The chromatogram of the standard mixture containing 3.0 ng of 1-NP (Figure 21C) yielded a lock-in amplifier response of 80.0 μV , or a response factor of 26.7 $\mu\text{V}/\text{ng}$ injected. The separation of the unspiked fly ash extract (Figure 21A) yielded a 1-NP peak of 34.5 μV . A spike of 3.9 ng/injection yielded a 1-NP peak of 137 μV after separation (Figure 21B). Taking the difference of the detector response for 1-NP for the spiked and unspiked chromatograms yields a response factor of 26.2 $\mu\text{V}/\text{ng}$. This good correlation indicates the possibility of employing standard addition in order to quantify analytes present in a sample when using this type of detection.

The photothermal refraction measurement is independent of pathlength (65). Rather than attempt to relate these measurements to Beer's Law absorbances, the measurements at the limit of detection are

best expressed as the product of the concentration and molar absorptivity (ϵC). The 1-NP peak in Figure 21C has a Gaussian profile and therefore the concentration at peak center for the 3 nanograms injected is calculated to be 2.7×10^{-5} M. Assuming a direct proportionality between signal and amount injected, the limit of detection at a signal-to-noise of 3 is $\epsilon C = 7.4 \times 10^{-3} \text{ cm}^{-1}$. This value is relatively poor with respect to previously reported values in the literature (64,107) and with good reason. One problem in using a tightly focused pulsed laser is the destructive effects of the large power densities employed as mentioned earlier. The average power used to obtain the chromatograms in Figure 21 was 0.6 mW. Figure 22 shows a chromatogram of the synthetic mixture of 1-NP and dinitropyrene isomers with an improved S/N. Detection was obtained using an average power of 1.0 mW and a peak power density of approximately 0.2 GW/cm^2 . This incident power did eventually burn a hole through the flow cell. Extrapolation of the signal for the 3.0 ng injection of 1-NP for this chromatogram to a signal-to-noise of 3 yields $\epsilon C = 4.6 \times 10^{-3} \text{ cm}^{-1}$. Heavy wall 500 μm i.d. square quartz flow cells have been employed to obtain limits of detection of $2.7 \times 10^{-4} \text{ cm}^{-1}$. While this value exceeds the value obtained in the only previous report of pulsed laser thermal lens detection of flowing liquids (110), it is a factor of 50 less than the extrapolated value of $5 \times 10^{-6} \text{ cm}^{-1}$ reported for the detection of dabsyl-glycine (109) using CW excitation. In order to attempt to improve detection the use of a sheath flow cell was investigated. It was expected that the sheath flow cell could solve the problem of flow cell

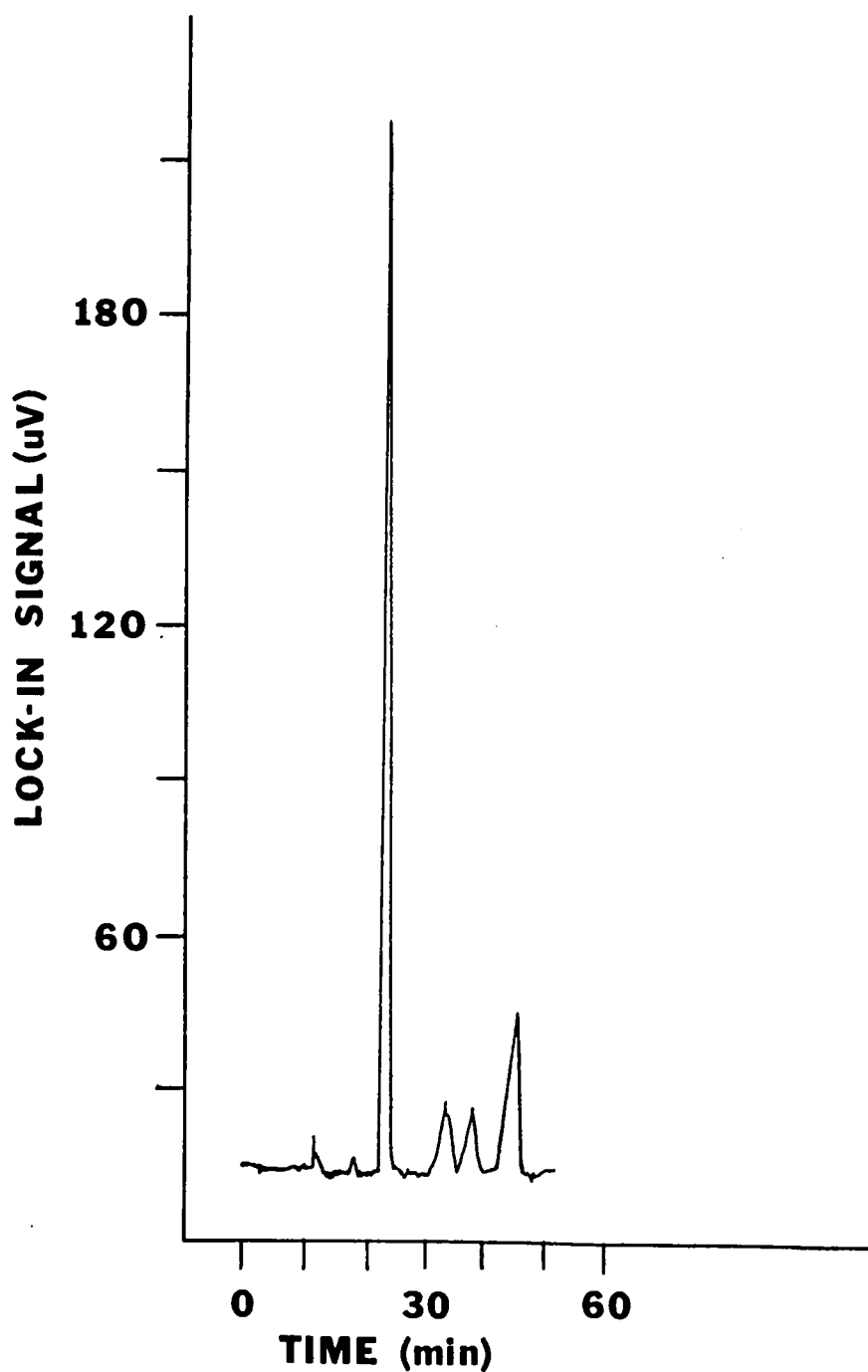


Figure 22. Separation of 1-nitropyrene and dinitropyrene isomers standard mixture. Experimental conditions: column 200 μm i.d. x 90 cm, 5 μm CPS-Hypersil, isocratic elution with 95:5 n-hexane:isopropanol at 1500 psi, detection at 400.0 nm with 1.0 mW average pump laser power. Injected quantities same as in Figure 21.

destruction by high peak laser powers and also the distortion of the probe beam profile by the flow cell.

A sheath flow cell is basically comprised of a sample stream which is introduced into the center of a flowing sheath stream. If the linear velocities of the two streams are such that the flow profiles remain laminar, the sheathing stream will not appreciably mix with the sample stream. Proper manipulation of the flow rates of the two streams can provide an extremely small detection volume. The ratio of the volumetric flow rates of the sample and sheathing streams have been investigated by Zarrin and Dovichi (111). They were able to adjust the sample volume of a laser illuminated sheath flow cell over three orders of magnitude. Illuminated volumes ranged from 50 fL to 100 pL.

One advantage of a sheath flow cell results from employing a sheathing solvent which has a refractive index closely matched to that of the sample stream. When the change in refractive index at the interface of the sheathing and sample streams is minimized, specular scatter is greatly reduced. This advantage can enhance the S/N when performing fluorescence measurements (112) and light scattering measurements (113).

The high peak powers of the pump laser employed in this work often caused a hole to be burned through the thin walled 200 μm i.d. fused silica flow cells. The cause of this was either absorption of energy by impurities in the fused silica or by accumulated debris on the flow cell inner walls. It was observed during experiments that the process of flow cell failure often began on the inside of the flow cell wall and

worked its way outward. This was caused by the the deposition of photodecomposed solute or impurities onto the flow cell wall at the point of incidence of the pump laser. This "hot spot" eventually caused a complete fracture of the flow cell. This phenomena has been observed by other investigators in the field (114). The sheath flow cell helps solve this particular problem by providing a sample cell which has walls that are made of a flowing sheathing solvent which constantly wash away any decomposed products. The problem of damaging the thin fused silica flow cell walls of the previously employed flow cell is eliminated when using a sheath flow cell. The heavy quartz walls of the sheath flow cell apparatus do not experience the high power densities that the thin fused silica were exposed to. The spot size of the pump laser, when it intersects the outer sheath flow cell walls, is larger than when it intersects the thin fused silica flow cell walls. Due to the larger spot size the power density is correspondingly lower and the flow cell destruction problem minimized.

Pump beam distortion is caused by the curvature of the 200 μm i.d. flow cell walls. The change in refractive index from air to fused silica to mobile phase to fused silica to air which the probe beam experiences sets up a series of optical elements which serve to distort the tightly focused probe beam. This problem is further complicated by the desire to use as large a Z_1 value as possible. As Z_1 increases so does the spot size of the laser according to equation 15. As Z_1 increases the radius of curvature of the laser beam wavefronts at the

flow cell decreases (the radius is ∞ at ω_0) and this increases the distortion of the beam.

The sheath flow cell shown in Figure 23 was constructed and investigated for use in capillary LC. The small tube at the center of the large square tube was employed so that extremely large volumes of sheathing solvent would not be needed in order to match sheath to sample flow rates.

Initial experiments were conducted using 320 μm i.d. slurry packed fused silica columns to separate NBD derivatized amines. Laser fluorescence detection was performed by using the sheath flow cell. No significant band broadening was experienced when detection accomplished within the fused silica exit capillary was compared to detection within the sheath flow cell. This success prompted attempts to employ the sheath flow cell for photothermal refraction detection in order to address the problems associated with detection in the fused silica flow cell.

Initial investigations into the employment of the sheath flow cell for photothermal refraction were accomplished using a sheath cell that was a simplified version of the one shown in Figure 23. The flow cell was constructed of 6 mm square quartz tubing and the center tube was eliminated. In its place a 200 μm i.d. silica capillary, epoxied into a 22 Ga stainless steel tube, was used to feed in the sample stream. This sheath flow cell was placed into the flow cell holder of Figure 9, p. 47, and the sample stream pumped with a syringe pump.

The sheath flow cell did provide an essentially undistorted probe beam image at the detector in the far field. The positional dependence

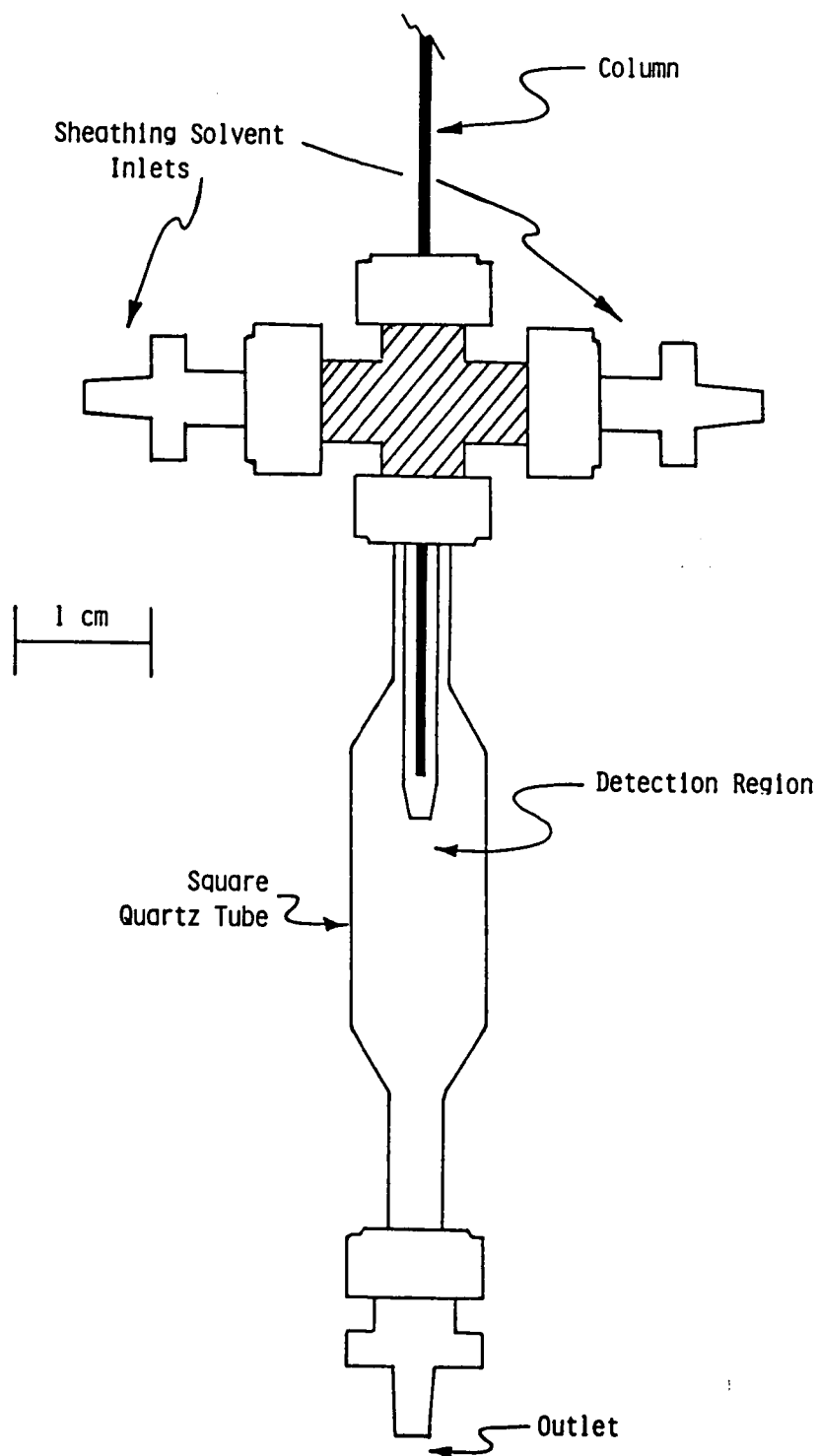


Figure 23. Schematic of sheath flow cell.

of the photothermal refraction signal was again investigated. The results were similar to Figure 14, p. 59, except that the signal was completely antisymmetric about the point of zero signal, much like Figure 6, p. 41. Large increases in Z_1 did not provide the desired increase in signal, but served to strengthen the conclusion that the spot size of the probe laser had exceeded the pump laser spot size and thus a decrease in signal was to be expected.

Increases in power were expected to enhance the signal level as had been shown in Figure 13, p. 58. The advantage of the sheath flow cell was to have been the elimination of flow cell destruction problems. The sheath flow cell did solve the cell destruction problems, but at the same time it introduced a new problem. This problem was boiling of the sheathing solvent. The use of average powers of 4 to 10 mW corresponds to peak power densities of 0.87 to 2 GW/cm² using a 50 μ m pump laser spot size at 23 Hz with a pump laser pulse width of 10 nsec. Power densities of this magnitude caused boiling at the beam intersection which caused an unconquerable noise problem. When a small bubble passed across the detector aperture, the lock-in amplifier often provided a noise spike which was greater in magnitude than the expected signal. Methanol, carbon tetrachloride, hexane, acetonitrile and water were studied as potential sheathing solvents, however, all had a finite tolerance for power densities in the range mentioned. Filtration of the solvents with a 0.45 μ m pore size ultrafilter helped the situation, but did not eliminate the boiling problem.

Although the boiling was a major problem, some enhancement of signal level over that obtained in a regular flow cell was observed.

This enhancement was typically never greater than a factor of two and the advantage that this offered over using a regular flow cell was debatable when one considers the extra problems involved with employing a sheath flow cell. The need for a precision syringe pump for the sheathing solvent and high quality, filtered sheathing solvents make the use of a sheath flow cell a rather unattractive alternative. This is not to say that the use of a sheath flow cell is not possible. Miniaturation of the cell and ultrafiltration of the sheathing solvents may lead to a reduced problem with the solvent boiling and may solve the problem of probe beam distortion which plagues the use of capillary flow cells.

CHAPTER 4

THE FUTURE OF LASER BASED PHOTOTHERMAL DETECTION

A sensitive, tunable, low volume absorbance based detector compatible with capillary LC separation techniques has been developed. The obvious advantage of this detector is its capability to address the requirements of sensitivity and low volume dictated by the use of capillary LC. This detector, with further optimization of signal measurement and more stable pump and probe lasers, could be the "universal" detector that capillary LC needs to become a more widely employed chemical analysis technique. Commercial availability of open capillary LC with a sensitive absorbance detector would provide chromatographers with a tool with which virtually any separation could be accomplished. Unfortunately, the cost of a detector of this type is prohibitive as far as the commercial market would be concerned. The cost for the excimer pumped dye laser based photothermal refraction detector developed in this dissertation is approximately \$65,000. This only includes the initial purchase of hardware to run the detector and does not consider the high maintenance costs associated with pulsed laser systems.

This research has helped pioneer the way toward using laser based methods for capillary LC detection. Although the resulting detector is expensive, reductions in cost for this type of detector, or one similar to it, are under consideration. In order to realize this cost reduction, research into a lower cost pulsed or CW tunable dye laser system is necessary. Coupling a lower cost pump laser with one of the more

stable HeNe lasers which are available could cut the cost of the laser portion of the detector substantially. The ultimate solution to this problem would involve the development of an inexpensive (less than \$5,000) multigas filled CW laser which was tunable from 200-800 nm and could provide a power output of up to 10W. Coupling this laser with one of the single-laser/dual-beam configurations proposed by Yang and coworkers (115,116) for thermal lens detection could potentially solve the cost problem. It is unfortunate that the development of this laser is a very nontrivial process.

As problems of this type are considered, it is the destiny of researchers in this field to solve them in a methodical manner and provide the commercial sector with the knowledge and products to help serve society.

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APPENDIXES

APPENDIX A

PREPARATION OF OPEN CAPILLARY BONDED PHASE LC COLUMNS

The reverse phase bonded open capillary columns were prepared by a procedure optimized by Vargo (117) and summarized herein. The first step in the preparation the capillaries after drawing is to heat the capillaries in a muffle furnace at 600°C for 24 hours to promote a phase separation. In order to reduce the problem of the capillaries warping at high temperature the column bundle is tied tightly with several wire bands.

After cooling, the inside of the column has to be etched. This process increases the number of surface silanol groups. The surface silanol groups are readily reacted with silylating reagents to form the desired bonded phase. Etching is accomplished by passing a phosphate buffer solution (pH = 7, 0.05 M) through the column. The column is immersed in a 3M HCl bath maintained at 85°C. A carbon electrode is placed in the HCl bath and another in a beaker that contains phosphate buffer as illustrated in Figure 24. The exit of the column is placed in a beaker full of phosphate buffer. A 3kV electrical potential is then applied across the column wall for 24 hours. After 24 hours the column ends are exchanged and the etching is resumed for 24 hours. After etching is completed the column is rinsed with 2 ml of pH 2.00 buffer, 1 ml distilled water, 1 ml methanol and 1 ml of toluene.

The column is now ready for silylation. A 10% w/w solution of the desired silylation reagent in toluene is pumped through the column for

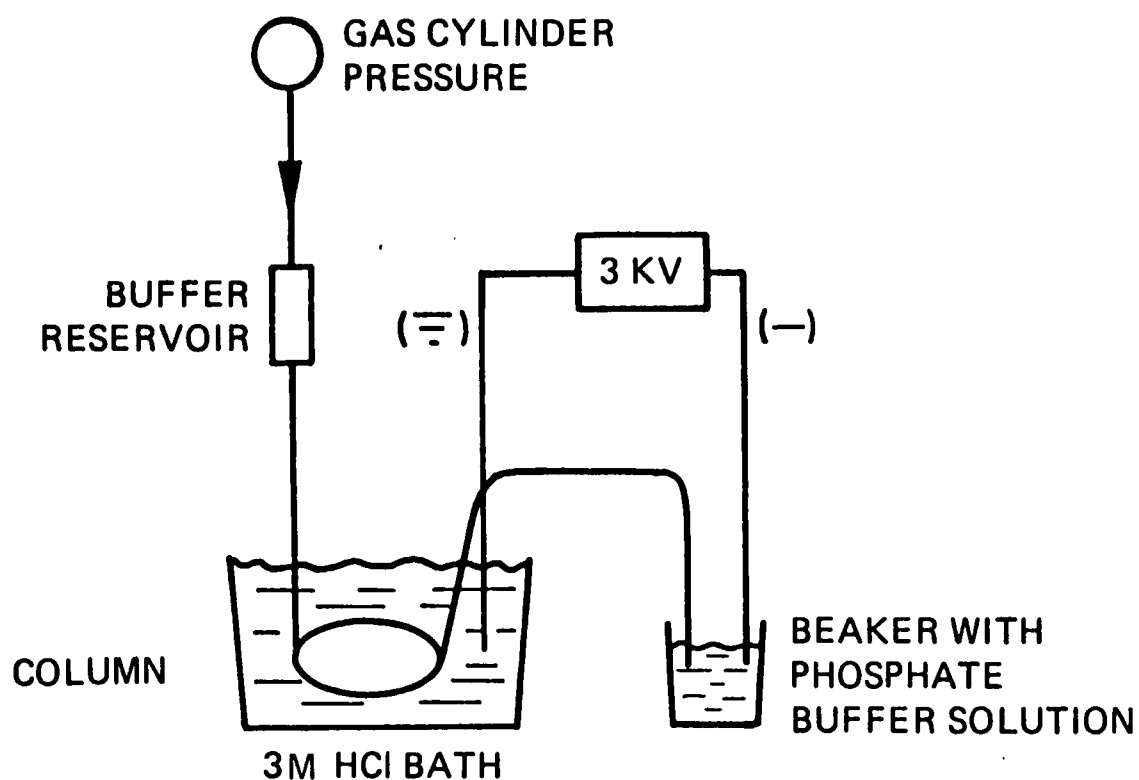


Figure 24. Schematic instrumental diagram for the electro-etching procedure for borosilicate glass.

1-2 hours and then the column ends are flame sealed and the column heated at 150° C for one hour. After cooling the column is rinsed with toluene, methylene chloride, acetonitrile and finally equilibrated with mobile phase. The column is now ready for chromatographic application.

APPENDIX B

PREPARATION OF SLURRY PACKED FUSED SILICA CAPILLARY
COLUMNS FOR LIQUID CHROMATOGRAPHY

Typically 200 μm i.d. columns were slurry packed and used for separations. This is not to say that the following construction and packing procedure will not work for columns with other dimensions. The fused silica employed was manufactured by Scientific Glass Engineering Inc, Victoria, Australia (200 μm i.d. x 300 μm o.d. part number 062475 and 50 μm i.d. x 200 μm o.d. part number 062463).

After deciding what length of column is desired, the column must be cut to the proper length plus 10 cm. The extra 10 cm is for the addition of a packing fitting. This fitting is used so that the column can be attached to the high pressure packing reservoir. The fitting consists of a piece of stainless steel 0.020" i.d. x 0.062" o.d., 5 cm long (Upchurch Scientific, Oak Harbor, WA part number U-101) with a 1/16" stainless steel ferrule (Upchurch part number U-321) seated to the same depth as the union of the packing reservoir. The fused silica column is carefully epoxied to the column fitting with Devcon Five Minute Epoxy (Devcon Corp., Danvers, MA) by applying a covering of epoxy onto the fused silica and carefully twisting the stainless steel fitting over the epoxy covered area ensuring that a good seal is accomplished. This is especially important at the entrance end of the column. Allow this to dry overnight and rinse the column with filtered acetone the next day.

After rinsing the column with acetone it should be blown dry with nitrogen. The process of inserting a frit to hold the packing in place is now undertaken. The first step involves preparing the end of the column so that a frit can be cut with the column end. The column is cut with a Newport (Fountain Valley, CA) model FP-1 optical fiber cutter made of carbide steel. This cut must be made so that the column end has a small jagged edge on it. The fused silica, with the polyimide coating intact, is drawn across the fiber cutter. After scoring the piece is bent and broken off gently. It takes practice to learn to cut the fused silica so that it has a clean or jagged break. It is very difficult to cut cleanly fused silica from which the polyimide coating has been removed. The terminal end of the column requires a jagged edge so that the column can be used as a frit cutter (much the same as a cookie cutter). The frit material used was teflon frit with 5 μm pore size (Alltech Associates, Deerfield, IL part number 7171). The frit material was cut from a bulk sheet with a single edge razor blade. The thickness was approximately 100 μm as measured with a micrometer and the pieces were typically 5 mm x 5 mm. This small piece is placed in the palm of one hand and the tip of the column firmly twisted with the other hand so as to cut out a frit. After each attempt the frit should be observed under the high power (60X) of a dissecting microscope. If it is uniform and fairly tight fitting then it should be pushed into the column with a length of 50 μm i.d. x 200 μm o.d. fused silica from which the polyimide coating has been removed. If the frit is an obvious failure it must be removed and another attempt at cutting one made. Removal is easily

accomplished by gently flicking the end of the column and expelling the frit. A frit which appears viable should be pushed into the column about 15-20 mm. Now a terminal frit retainer must be epoxied into the end of the column to hold the frit in place. The terminal frit retainer consists of a 6-8 mm piece of 50 μ m i.d. x 200 μ m o.d. fused silica. Both ends of the frit retainer must be cut as square as possible without jagged ends so that any areas which may disrupt the flow profile are minimized. This requires a lot of practice at cutting with the fiber cutter and close observation of the cut end with the microscope. When 3 or 4 retainers have been cut, the polyimide has to be removed. In order to accomplish this, the frit retainers are placed in a crucible and the crucible is placed in a 600°C furnace for 20 minutes. This will remove the polyimide coating and any residue from the cutting process. From this point on the retainers should be handled with a small forceps so that any dirt or grease which may affect the strength of the epoxy bond will be minimized. The frit retainers are hard to handle and disappear rapidly if dropped. One of the frit retainers is epoxied into the terminal end of the column. The frit retainer should extend approximately 2.5-3 mm into the column. Devcon epoxy is used to hold the retainer in place. The column is positioned under the microscope in the viewing field and held in place with a piece of tape. The frit retainer is inserted to the proper depth and supported so that the two tubes are concentric and not tilted. Fresh epoxy is prepared and a small amount is applied to the joint of the column and frit retainer. Capillary action will pull the epoxy into the column to effectively seal the frit

retainer into the end of the column. Care must be taken to only feed enough epoxy into the joint so that the frit retainer is held in place and not clogged in the process. Care must also be taken not to get any epoxy onto the frit retainer except at the column retainer junction. If this happens it may be impossible to attach a flow cell to the column after it is packed. Do not handle the column for at least 30 minutes after the retainer has been epoxied. Do not attempt to pack the column until the joint has dried overnight.

The apparatus employed to slurry pack the fused silica columns consists of a Haskel (Burbank, CA) model 27502 pneumatic amplifier pump and a slurry reservoir. The slurry reservoir is constructed from a piece of 30 cm 1/8" i.d. stainless steel tubing with 1/8" column end fittings (Alltech part number 200611). The reservoir is attached to the column via narrow bore 1/16" stainless steel tubing. The columns attach to the reservoir via a 1/16" stainless steel union (Upchurch part number U-437).

The slurry packing procedure is adapted from pioneering work by Gluckman and coworkers (107). The stationary phase was slurry packed at a slurry ratio of between 6 and 8 using acetone as the slurry and packing solvent. Slurry ratio is defined as the ratio of solvent volume (μL) to weight of packing (mg). The packing was suspended in a slurry by ultrasonification and stirring. Before introducing the slurry into the column, the column is filled with the packing solvent. The slurry is drawn up into the packing reservoir with a syringe. The reservoir is quickly reattached to the column and pump. Packing is initiated at 3000

psi and quickly increased to 5000 psi. The reservoir is tapped with a wrench during the packing procedure in order to reduce chance of the reservoir clogging. The column is usually packed within 15 minutes. The solvent flow is stopped after one hour and the pressure within the column is allowed to equilibrate with the atmosphere. Once the column has depressurized, the flow cell can be epoxied into place, once again taking care not to clog the exit of the retainer at the end of the column.

The injection procedure employed a 1 cm length of 50 μ m i.d. fused silica. One to two mm of the polyimide was removed for the end of this piece and both ends were smooth and square cut. In order to make an injection, the short piece was filled with sample by sonicating it in the sample for a couple of minutes. The entrance end of the column was removed for the pump and while viewing under the microscope, the short sample container inserted into the end of the column. The 1-2 mm portion without polyimide coating will fit into the column end. The polyimide coating will stop the rest of the piece from going far enough into the column to disrupt the packing. The column is again carefully attached to the pumping source and the separation initiated. In order to make it easier to attach the column to the pump, a short length of 0.25" empty analytical scale column was used as a pumping reservoir. The capillary column with the injection tube easily fit into the reservoir without fear of breaking off the injection tube.

VITA

Charles Nicholas Kettler was born in St. Louis, Missouri, on November 22, 1952. He graduated from Kirkwood High School, Kirkwood, Missouri, in June 1971. He enlisted in the United States Air Force in February 1972 and was honorably discharged in December 1976. His undergraduate degree in chemistry was obtained from the University of Arizona in December 1980. Graduate study was undertaken in 1981 at the University of Tennessee, Knoxville, with the Doctor of Philosophy degree being awarded in March 1987.