SELECTIVITY AND TEMPERATURE EFFECTS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by

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Introduction

Objectives of This Work

High Performance Liquid Chromatography (HPLC) has experienced an extremely rapid growth rate since the early 1970's and is still growing at a very fast rate as more and more people become acquainted with the technique and its power to separate complex mixtures and perform both qualitative and quantitative analysis on the resulting chromatograms. Much work has been done in the area of HPLC in the past few years, but a problem still exists for both the practicing chromatographer and the novice. This problem is the development of a procedure for a new sample type. The chromatographer may or may not have a variety of columns available to choose from to begin his work. If the columns are not available, he must either purchase or pack his own HPLC column. The problem is, there are a variety of HPLC columns and column packing materials commercially available to choose from, each slightly different in performance. The objectives of this research are threefold:

1. The investigation of temperature on a series of HPLC column packings in both the normal phase and reverse phase mode in order to study its effect on retention, selectivity, and scope using a series of model compounds.

2. The investigation of selectivity on a series of

normal phase HPLC column packings while maintaining constant temperature and mobile phase composition in order to study the effect of surface area, sorbent type (silica vs. alumina), and partially deactivated silica prepared by chemically bonding a hydrocarbon to the silica surface on solute retention time using a series of model compounds.

3. The investigation of selectivity on a series of reverse phase HPLC column packings while maintaining constant temperature and mobile phase composition in order to study the effect of the hydrocarbon chain length of the reverse phase material on solute retention time using a series of model compounds.

The data obtained from the investigations will provide the practicing chromatographer with a better insight into the selection of a particular HPLC column packing to develop his separations and will provide him with useful guidelines as to when temperature control will be important for maintaining his separations or improving them.

Historical

Chromatography had its earliest beginnings in 1903 when a 34 year old Russian botanist, Mikhail Tswett, developed a separation of the pigments in green leaves in a glass column packed with precipitated calcium carbonate and observed colored bands moving down the column as he continued to add fresh solvent. In his lecture (1) and his

paper (2) he referred to the technique as "chromatography", which means "color writing". In his lecture he described the procedure in detail using terminology that is still used today. His first paper on the subject of adsorption chromatography appeared in 1906.

During this same period, David Talbot Day (3,4), an American, was using chromatography for the separation of hydrocarbons from petroleum. Even though the method Tswett and Day developed independently was essentially the same, Tswett is given credit for the development of the technique. Tswett's work is considered to be more significant than Day's because he recognized and correctly interpreted the chromatographic processes and developed the laboratory apparatus necessary to perform the technique. It was Tswett who originated much of the terminology used to describe the basic chromatographic process. Tswett's original technique is still used today for both analytical and preparative separations.

The importance of Tswett's work lies in the fact that not only was he able to resolve complex mixtures into their individual components, but also once separated, the individual components could be isolated without subjecting them to conditions which might alter them. The pure components could then be subjected to further investigations by other techniques.

Little work in liquid chromatography (LC) was done

following Tswett's original work until 1931 when Kuhn and Lederer (5) repeated Tswett's work using calcium carbonate and alumina as adsorbents and separated carotene isomers. After the late 1930's and early 1940's little attention was paid to liquid chromatography due to the advent of thin layer chromatography and gas chromatography in the late 1940's and 1950's. By the late 1960's, the development of gas chromatography began to slow down and liquid chromatography experienced a re-birth. Even though gas chromatography had a wide range of applicability, it was of little use for high molecular weight, non-volatile and/or thermally unstable compounds. It was these types of compounds that were ideally suited to the liquid chromatographic technique.

Liquid chromatography advanced very rapidly during the early 1970's due to improvements in instrumentation and column packing materials (6-8). In many cases, it was the development of highly efficient columns which provided the stimulus for the development of high sensitivity detectors and precise pumping systems that were capable of operating at high pressures.

Classical LC vs. HPLC

Liquid chromatography during the period of 1930-1967 was often characterized by very slow and inefficient separations. A variety of column packing materials were

used (silica or alumina) and in general they were totally porous, irregular in shape, and had a rather large particle diameter (100-200 μm). These materials were packed into glass columns having a large internal diameter (2-8 cm) and a column length to internal diameter ratio varying from 5:1 to 100:1. The mobile phase was passed through the column at very low pressures, employing open columns with no applied pressure. This form of chromatography is the oldest form and is referred to as classical liquid chromatography. The equipment requirements for classical LC are minimal. Samples are introduced on to the column by means of a pipet (1-10 ml). Fixed volumes of the column effluent are collected either manually or by means of an automatic fraction collector and later analysed by ancillary techniques to provide the chromatographer with a histogram of component concentrations. Classical LC was primarily a preparative technique and was unsuitable for quantitative analysis.

From 1967 to the present liquid chromatography has experienced many changes. The separations performed can be characterized as being fast, efficient, and quantitative, and for the first time LC separations began to rival those performed by gas chromatography. The reasons for this improvement in the LC technique are due in part to the advancement of gas chromatographic theory. Gas chromatography (GC) theory predicted that the use of small

particles, uniformly packed into narrow bore columns would result in higher efficiency. Liquid chromatography from 1967 to the present has been referred to as modern liquid chromatography, high efficiency liquid chromatography, high pressure liquid chromatography, and finally high performance liquid chromatography (HPLC). The latter term is the name preferred by most journals. The use of small particles (5-50 μm) required advancements in the instrumentation for HPLC. High pressures (up to 5,000 psig) were required to force the mobile phase through the columns. New injection systems had to be developed such that the samples could be introduced on to the columns at these high pressures, and very sensitive and stable detectors which would provide the chromatographer with a continuous record had to be developed, a chromatogram of the components as they are eluted from the column.

The Development of HPLC Column Packing Materials

1. Adsorption Chromatography

Adsorption chromatography or liquid-solid chromatography normally involves the use of a polar stationary phase and a non-polar mobile phase (normal phase chromatography). The solvent and the solute molecules compete for the active adsorption sites on the stationary phase. Retention of solutes increases as the polarity of

the solute molecules increases or the number of polar functional groups in the molecule increases. Classical LC is primarily a liquid-solid chromatographic technique.

The column packing materials used for classical LC are large (> 100 um), irregularly shaped, totally porous, high surface area (300-600 m^2/g) particles, usually silica or alumina. This type of particle has two serious drawbacks. The first drawback is associated with the irregular shape of the particle and the wide particle size distribution of the materials used. This leads to difficulty in packing a column which will have a homogeneous packed bed. A packed bed which is inhomogeneous will create wide variations in the linear velocity of the mobile phase across the column. It is critical in HPLC to minimize the variation in the linear velocity of the mobile phase due to the slow diffusion of the solute molecules in the liquid mobile phase. This slow diffusion of the solute molecules will contribute significantly to band broadening if the bed is not homogeneously packed. Molecules of solute can not move quickly between zones of faster and slower moving mobile phase. Kirkland (9) has referred to this contribution to band broadening as eddy diffusion and mobile phase mass transfer.

The second serious drawback of the classical LC column packing materials is a result of the totally porous nature of the materials. The problem is associated with

the large particle size and the slow diffusion rates of the solute molecules inside the particle. The materials have deep pores which contain stagnant mobile phase. The slow diffusion of the solute molecules into and out of these deep pools of stagnant mobile phase results in band broadening. This contribution to band broadening has been referred to as stagnant mobile phase mass transfer (liquid-solid chromatography) and stationary phase mass transfer (liquid-liquid chromatography).

New column packing materials designed specifically for HPLC attempt to reduce these contributions to band broadening. The first major advance in solving the problem of eddy diffusion and mobile phase mass transfer was the development of spherical particles. The first spherical material was Porasil, a totally porous, spherical silica particle developed in France by Peichney-Saint-Gobain and commercially available from Waters Associates (10,11). Porasil is a smaller particle than previously used materials having a particle size range from 37-75 μm and is available in a variety of surface areas from 5-480 m^2/g. Porasil reduced the effect of the eddy diffusion and the mobile phase mass transfer contributions to band broadening and, due to its smaller size, it reduced to some extent the stagnant mobile phase mass transfer contribution to band broadening. Porasil is far superior to the classical LC column packing materials and is often the packing material

of choice for preparative work due to its low cost and high sample capacity (high surface area).

There are two ways to reduce the depth of the pools of stagnant mobile phase. The first involves the use of particles having a solid core with a thin, uniform, porous shell. These particles are spherical in shape and the non-porous center eliminates the deep pools of stagnant mobile phase. These particles have been referred to as pellicular, porous layer, superficially porous, and controlled surface porosity supports. Pellicular column packing materials were first used for HPLC by Horn, et al. (12) and were described by Kirkland (13) in detail. The material described by Kirkland was given the trade name Zipax and the particles have a range in size from 25-37 μm and a surface area of less than 1 m^2/g. The thickness of the porous layer is 1-2 μm. The Zipax material and other pellicular materials exhibit high efficiency (minimal band broadening) due to the elimination of the deep pools of stagnant mobile phase; however, they suffer from the disadvantage of having a very low surface area which restricts the amount of sample that can be injected onto the column.

The second approach to minimizing the depth of the pores is to reduce the size of the particle. In this fashion, the particles can be totally porous in order to have high surface area (high capacity) and high efficiency. This type of material was introduced by DuPont in 1972 under the trade

name Zorbax SIL (14) and was a totally porous, spherical material having an average particle size of 5 μm and a surface area of 300 m^2/g. This material overcame most of the problems associated with the older column packing materials. These very small, totally porous packing materials, both spherical and irregular in shape, are available from a variety of manufacturers in pre-packed columns and bulk quantities. These materials are relatively expensive and must be packed by special slurry techniques in order to achieve a homogeneous packed bed. These materials (5 and 10 μm) are the materials of choice at the present time since they possess high capacity, high efficiency, and make it possible to achieve high speed separations.

2. Liquid-Liquid Chromatography

Liquid-liquid or partition chromatography utilizes a solid support onto which is coated a liquid which is immiscible with the mobile phase. The solute molecules distribute themselves between the two immiscible liquid phases according to solubility. The more soluble the solute is in the stationary phase, the longer it is retained. There are certain limitations in the use of a stationary phase which is physically adsorbed on the surface of a solid support. The major limitation is that the stationary phase always has a finite solubility in the mobile phase. In

order to use this technique effectively, it is necessary to use a pre-column (a column prior to the analytical column, packed with a high surface area support with a high loading of the liquid stationary phase) and the mobile phase must be presaturated with the liquid stationary phase. In order to prevent the stationary phase from being stripped off the surface of the solid support, low flow rates have to be used and the column temperature must be controlled. Liquid-liquid chromatography also can not be used with gradient elution techniques due to the problem of solubility of the stationary liquid, and in most cases the technique can not be utilized for preparative work since the effluent is always saturated with the stationary liquid. The solid supports used in liquid-liquid chromatography are the same materials that are used for liquid-solid chromatography. The most popular supports are the pellicular materials due to their ease of packing and their high efficiency.

3. Bonded-Phase Chromatography

Chemically bonded stationary phases eliminated the major practicle problems associated with the use of liquid stationary phases merely adsorbed on the surface of the solid support. The chemically bonded stationary phase packing materials consist of a covalently bonded layer of organic material that has been reacted with the surface of the solid support. These phases have been prepared by a variety of

reactions.

Halasz (15) utilized the free surface hydroxyl groups on totally porous, spherical siliceous particles to prepare a number of packing materials for both gas and liquid chromatography. Silicate esters were formed by reacting alcohols with the active surface hydroxyls. The (Si - O - C) bond formed is hydrolytically and thermally unstable. It is imperative that water be eliminated from the solvents prior to use. The bonded phase is also subject to replacement by lower chain alcohols. This type of bonded phase is sold by Waters Associates under the trade name Durapak (16).

In order to overcome the problems associated with the silicate-ester bonded materials, Able, et al. (17), used hexadecyltrichlorosilane to synthesize chemically bonded gas chromatographic packing materials. A similiar reaction was employed by Stewart and Perry (18) to prepare liquid chromatographic packing materials. The reaction sequence involves the initial hydrolysis of the silica surface to maximize surface hydroxyl groups which can then react with the organotrichlorosilane to form a (Si - O - Si - C) bond which is more thermally and hydrolytically stable than the silicate-ester bond. As a result of this increased stability, aqueous solvents and alcohols can be used without degradation of the chemically bonded stationary phase. Aue, et al. (19), prepared bonded stationary phases which possess a crosslinked structure. The surface of the solid

support is treated with hydrochloric acid and water to hydroxylate the surface. The material is then dried to remove excess water and the support is then treated with an excess of dimethyldichlorosilane (DMCS). The organotrichlorosilane, which is to form the stationary phase, and the DMCS-treated support are added together. The mixture is then polymerized, which results in the stationary phase being covalently bonded to the surface of the solid support via the DMCS. Once the polymerization has been completed, any remaining hydroxyl groups are deactivated with a suitable trimethyl-silating agent and any residual chlorines are replaced by methoxy groups using methanol.

Locke, et al. (20), utilized Grignard and organolithium reactions to synthesize chromatographic packing materials. The solid support is chlorinated with reagents such as $SOCl_2$, $TiCl_4$, or $SiCl_4$ and then reacted with a Grignard reagent or an organo-lithium reagent to produce a stationary phase which is bonded to the solid support through a stable (Si - C) bond.

Chemically bonded stationary phase packing materials have been compared to liquid-liquid stationary phase packing materials and have been found to behave in a similiar fashion; however, there is some confusion as to the mechanism of separations performed on the bonded phase materials. There is some indication that the layer of organic material actually acts as a modified adsorption packing material and

the separations performed on the materials are a result of the partitioning of the solute molecules in the trapped organic component of the mobile phase (21). A wide variety of packing materials are available for bonded-phase chromatography and the reader is directed to the following references for a complete listing and descriptions of the packing materials (22, 23).

Temperature in HPLC

Temperature in LC has been neglected as a column performance parameter for many years. This is quite surprising since almost all of the mechanisms of separation in HPLC involve a process which is temperature dependent. The first reported use of temperature as a chromatographic variable in LC was in 1948 when Le Rosen and Rivet (24) investigated the effect of temperature on the retention times of several compounds eluted on a silica column over the range 10°C to 70°C. They concluded that temperature had little effect on retention time over the range 20°C to 35°C. In 1953, Lie (25) studied the effect of temperature on solute retention time, again using silica gel as the column packing material, over the range -50°C to 200°C and concluded that the retention times of the solutes were affected in different ways. With increasing temperature, some of the retention times increased, some of the retention times decreased, and some of the retention times remained constant.

He also concluded that the effect of temperature on retention time is dependent both in magnitude and direction upon what solvent system was employed for the separation.

Temperature effects in LC were not studied again until 1967 when Locke and Martire (26) studied the effect of temperature on retention times as solutes eluted from a liquid-liquid system. The effect of temperature on liquid-liquid systems was further investigated by Locke (27) the same year. Giddings (28) has shown that the effect of temperature is a complex phenomenon and that it influences all terms in the resolution equation. Ion exchange columns (29) were found to exhibit a temperature dependency of retention times in 1967 and in the same year Horvath (30) studied the effect of temperature on column efficiency and resolution. Hesse and Engelhardt (31), using an alumina column, found that retention time decreases and efficiency increases with increasing temperature. Snyder (32) showed that the increase in efficiency with increasing temperature was probably due to a reduction in the viscosity of the mobile phase thus leading to better mass transport.

Maggs (33, 34) studied the effect of temperature on liquid-solid systems involving solvents containing a polar modifier and came to the same conclusion as Lie and proposed that the moderator distributed itself preferentially in the mobile phase with increasing temperature thereby leading to increased retention due to the exposure of more active

adsorption sites.

The last published work on temperature effects in LC was by Scott and Lawrence (35) in 1969 which dealt with the effect of temperature and modifier on resolution and efficiency. It is interesting to point out that in 1969 Maggs (33) writes, "Recent work has indicated that temperature can no longer be neglected as a column performance parameter in liquid chromatography." It is also interesting to note that currently less than 10% of all liquid chromatographs in use utilize temperature control.

Goals in HPLC

When developing liquid chromatographic methods the chromatographer has to decide what is the goal of the separation. Once he has defined the problem then he can begin his selection of the proper mechanism for the separation, ie., liquid-solid, liquid-liquid, bonded-phase, ion-exchange, or exclusion chromatography. After this choice, the chromatographer performs some preliminary separations to insure that his selection was correct. The next step in the development requires that the chromatographer keep in mind the ultimate goal of the separation and while doing so, optimize the separation. The common goals desired from a chromatographic separation are depicted in Figure 1. The speed, resolution, scope, and capacity of the chromatographic system are interrelated, each side of the

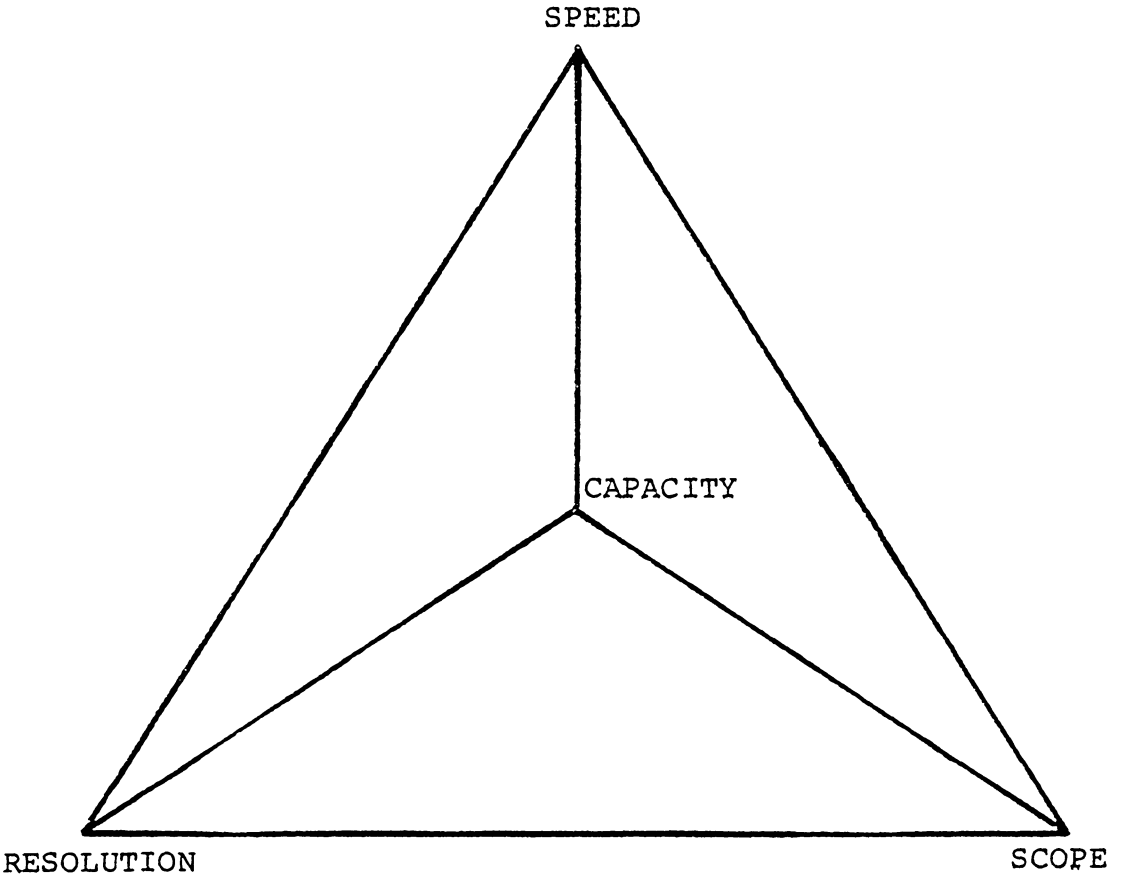

Figure 1. The Chromatography Tetrahedron

tetrahedron being of equal length. In any particular chromatographic system, any one of the desired goals may be improved at the expense of the others.

1. Resolution - The ultimate goal in any chromatographic method is to resolve sample components; therefore, the degree of resolution will be influenced by the other goals.

2. Speed - An important goal for a routine analytical separation is of course speed of analysis.

3. Scope - The scope of the system can be defined as the ability of the column packing material to separate mixtures which cover a wide polarity range. This goal is usually achieved by utilizing gradient elution techniques. The use of gradient elution for quantitative analysis is not practical in many cases due to the expensive equipment required and the time involved to re-equilibrate the system after chromatographing a sample. In cases where time and precision of the analysis are important, the scope of the packing material can become a critical consideration.

4. Capacity - If the goal of the separation is to obtain large quantities of pure materials, usually speed and resolution are sacrificed. The capacity of the system is

determined by the amount of solute that can be placed on the column without causing a loss in resolution. The emphasis of this work is not directed toward the capacity of the chromatographic system. The reader is directed to the references listed for more detailed information concerning preparative liquid chromatography (36 - 37).

Experimental

Equipment and Reagents

The modular liquid chromatograph used for this work consisted of a Spectra-Physics Model 740B Pump (Spectra-Physics Inc., Santa Clara, California) equipped with a closed-loop electronic flow control system and a Spectra-Physics Model 714 Pressure Monitor which digitally displays column pressure to ± 10 psig. The pump is capable of delivering the solvent to the column at pressures up to 7,000 psig at flows from 0 - 4 ml/min. in the analytical mode and from 0 - 20 ml/min. in the preparative mode. When pumping solvents of low viscosity, the standard FR-4S flow restrictor on the Model 740B Pump was replaced with a FR-4H flow restrictor to achieve the most precise flow control. Samples were injected via a Valco Model CV-6-UHPa-N60, 7,000 psig, Sample Injection Valve (Valco Instruments Co., Houston, Texas) equipped with a 10 µl sample loop and a 0 - 10 µl syringe injection kit, consisting of a Model VISF-1 Fill-port Fitting Assembly and a 10 µl specially calibrated syringe, Model VIS-10-7000. A Spectra-Physics Model 748 Column Oven was used throughout the study. The Model 748 Column Oven is a forced air oven which provides precise control of the column temperature over the range from $0^{o}C$ to $100^{o}C$ with a stability of ± $0.1^{o}C$. For the sub-ambient work, coolant (ice water) was pumped through the coolant coil in the oven at a

flow rate of 0.5 liters per minute with a Model MD-15 Magnetic Drive Pump (Fisher Scientific Co., Springfield, New Jersey). The detector used was a Spectra-Physics Model 230 UV Detector capable of operation at 254 nm and 280 nm simultaneously. The output from the detector was connected to a Spectra-Physics Autolab Minigrator Computing Integrator which was used to obtain solvent breakthrough times and retention times of the components of interest. The chromatograms were recorded on a Honeywell Electronik 194 Strip Chart Recorder (Honeywell Process Control Division, Fort Washington, Pennsylvania). All of the solvents used throughout the study were distilled-in-glass grade and were obtained from Burdick and Jackson Laboratories, Muskegon, Michigan. The probe compounds listed in Tables I and II were all of Research Grade (Fisher Scientific Co., Pittsburg, Pennsylvania). The column packing materials listed in Table III were obtained from E. M. Laboratories, Elmsford, New York. Table IV lists the columns used in the evaluations and with the exception of columns 102, 341, and 107 all other columns were slurry packed in our laboratory. Columns 102, 341, and 107 were 250 mm x 4.6 mm i.d. stainless steel, obtained from E. M. Laboratories. All other columns were 250 mm x 3 mm i.d. stainless steel, obtained from Handy and Harman Tube Co., Norristown, Pennsylvania. The columns were fitted with 1/4 in. - 1/16 in. stainless steel column end fittings with replaceable 2 μm stainless steel frits (Spectra-Physics).

Table I: Probe Compounds - Normal Phase

Benzene	Nitrobenzene
Tetrahydronaphthalene	Benzophenone
Naphthalene	m-Dinitrobenzene
Biphenyl	m-Chloroaniline
Anthracene	Phenylether
Phenanthrene	Phenyl-2-propanone
Pyrene	Benzaldehyde
Chrysene	Anthraquinone
Benzo(a)pyrene	Phenol
Triphenylmethane	p-Bromonitrobenzene
Toluene	p-Chloronitrobenzene
Ethylbenzene	p-Chloroaniline
Propylbenzene	Dimethylphthalate
o-Xylene	Azobenzene
m-Xylene	Hydrazobenzene
p-Xylene	m-Toluidine
Diethylbenzene	Diethyl-diphenyl-urea
Chlorobenzene	2-Chloro-5-nitroaniline
Bromobenzene	o-Nitroaniline
Iodobenzene	m-Nitroaniline
Fluorobenzene	p-Nitroaniline
1,2,4-Trichlorobenzene	o-Iodophenol
α,α,α-Trichlorotoluene	o-Nitrophenol
Anisole	p-Nitrophenol
p-Chloroaniline	β-Naphthol
p-Bromoaniline	Acetanilide

Table II: Probe Compounds - Reverse Phase

Benzene	Chlorobenzene
Naphthalene	Iodobenzene
Biphenyl	Fluorobenzene
Anthracene	Nitrobenzene
Phenanthrene	Phenol
Pyrene	Anisol
Chrysene	Benzaldehyde
Benzo(a)pyrene	Acetophenone
Toluene	Phenylether
Ethylbenzene	Benzophenone
Propylbenzene	Diethyl-diphenyl-urea
o-Xylene	Anthraquinone
m-Xylene	Dimethylphthalate
p-Xylene	Diethylphthalate
Diethylbenzene	Dibutylphthalate
Bromobenzene	Acetanilide

Table III: List of HPLC Column Packing Materials Used in This Study

Name	Base Material	Specific Surface Area (BET) m^2/gm
Lichrosorb SI-60	Silica Gel (neutral)	500
Lichrosorb SI-100	Silica Gel (neutral)	300
Lichrosorb ALOX T	Basic Aluminum Oxide 150 (type T)	70
Lichrosorb RP-2	Chemically Modified Silica Gel (Dimethyl-dichlorosilane), Stable Towards Hydrolysis	500
Lichrosorb RP-8	Hydrocarbon Phase Chemically Bonded to Silica Gel (C_8)	500
Lichrosorb RP-18	Hydrocarbon Phase Chemically Bonded to Silica Gel (C_{18})	500

Table IV: List of Columns Used in This Study

Column Number	Packed With
10	Lichrosorb SI-60 10 µm
14	Lichrosorb SI-100 10 µm
28	Lichrosorb ALOX T 10 µm
102	Lichrosorb RP-2 10 µm
341	Lichrosorb RP-8 10 µm
107	Lichrosorb RP-18 10 µm

The high pressure slurry packing apparatus has been described elsewhere (39) and was used by itself and in conjunction with a Model 705 Stirred-Slurry Column Packer (Micromeritics Instrument Corp., Norcross, Georgia) Figure 2. The ultrasonic bath used in the column packing procedure was a Model G-210-80 (Ultrasonic Instruments, Farmingdale, New York).

Procedures

Column Packing

Two different solvent systems were used for packing the columns. For the packing materials that were relatively polar (SI-60, SI-100, ALOX T, and RP-2), the solvent system used was a 50/50 mixture of chloroform and ethanol. For the packing materials that were relatively non-polar (RP-8 and RP-18), the solvent system used was a 75/25 mixture of chloroform and 2,2,4-trimethylpentane. The procedure for packing all of the columns was the same with the exception of the solvent system. The procedure for packing a 250 mm x 3 mm i.d. column with SI-60 was as follows: into a 50 ml beaker was placed 1.5 gm of the packing material. The material was dried in an air oven for 4 hours at 150^{o}C. Upon cooling to room temperature, 30 ml of degassed solvent (50/50 mixture of ethanol and chloroform) was added and the mixture was placed in the sonic bath for 10 minutes. The stirring bar was placed in the packer cup followed by the degassed slurry. The magnetic stirrer was turned on and adjusted to

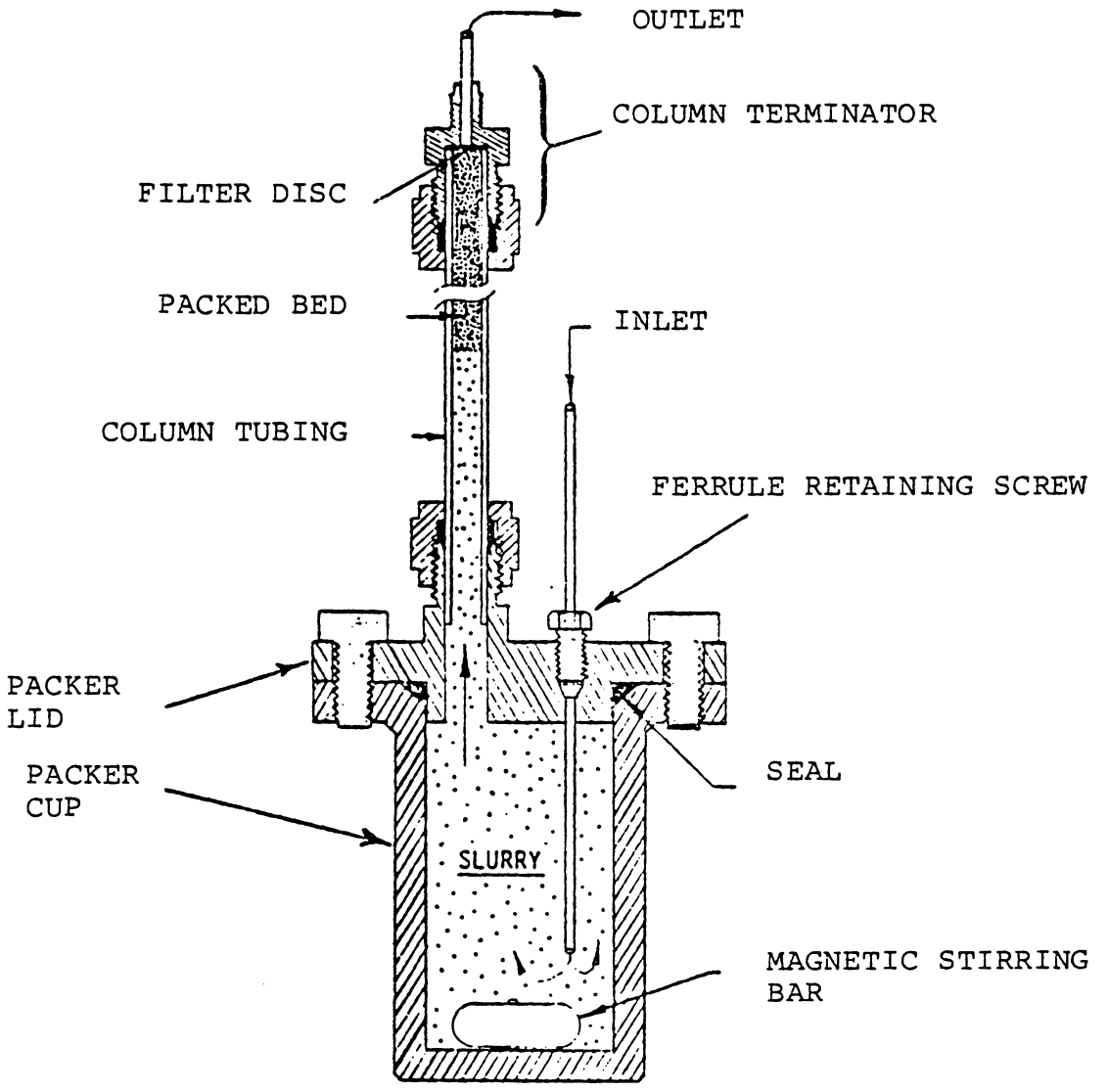

Figure 2. Model 705 Stirred-Slurry Column Packer

insure that the particles were suspended. The stirred-slurry packer was assembled and the pump was started at a pressure of 5,500 psig. The solvent was pumped through the column until 200 ml of effluent had been collected. At this time the pressure was slowly decreased to atmosphere, the column was removed from the stirred-slurry packer and the inlet column end fitting was attached.

Column Activation and Equilibration

All columns were activated in the same manner. After packing, the column was placed into the LC system and 50 ml of the following solvents were passed in series through the column at a flow rate of 2 ml/min.: chloroform, acetonitrile, methanol, acetonitrile, methylene chloride, and hexane. The columns were then equilibrated with the mobile phase over-night at a flow rate of 2 ml/min.

Sample Preparation

Each sample was prepared individually in a 25 ml tightly stoppered glass container to be used throughout the course of the work. The solvent for the samples used in the normal phase work was distilled-in-glass n-pentane and the solvent for the samples used in the reversed phase work was distilled-in-glass acetonitrile.

Data Handling

Retention times of the components of interest were obtained from the print-out of the Minigrator as was the solvent breakthrough time. These times were used to calculate the k' of the compound from the following relationships:

$$K = \beta k'$$

where: K = the partition coefficient = $\frac{\text{gm solute/ml A phase}}{\text{gm solute/ml B phase}}$

β = the phase ratio = ml B phase/ml A phase

k' = the capacity factor = gm in A phase/gm in B phase

$$k' = \frac{t_r - t_m}{t_m}$$

where: t_r = retention time of the compound

t_m = solvent breakthrough time

Column Evaluation

After equilibration of the column with the mobile phase at 25°C, the flow rate was adjusted to give a mobile phase linear velocity of 0.3 cm/sec. After the pressure stabilized, 10 µl of each of the sample solutions were injected and their k' values were calculated. Upon the completion of the series of samples, the temperature was decreased to 20°C and the column was allowed to equilibrate

for one hour or until three repetitive injections gave the same retention time. The series of samples was again injected and the k' values were calculated. This same process was repeated for all temperatures and solvent systems under study.

Results and Discussion

Column Packing

The column packing procedure and equipment used for the in this work was similiar to that developed by Spectra-Physics (39) for Spherisorb. Spherisorb is a very narrow particle size distribution of spherical, micro-porous silica particles designed specifically for HPLC. The only modification to the equipment used was the use of a stirred slurry reservoir in the place of the fixed un-stirred reservoir. The purpose of the stirred slurry reservoir was to maintain a homogeneous slurry of the packing material during the packing procedure. One of the problems encountered when attempting to slurry pack high efficiency columns using very small particles is the particle size distribution of the material being used. If the particle size distribution is wide, and a static reservoir is used, as soon as the particles are placed into the slurry reservoir, the particles will begin to segregate. The wider the particle size distribution, the faster the particles will segregate. According to Stokes Law, spherical particles will fall with increasing velocity until the force of gravity is balanced by the frictional resistance created by the solvent. Several approaches can be taken to overcome the segregation of particles once they have been placed into the slurry

reservoir. The first is of course the most obvious, which is to use particles having a very narrow range of particle size distribution. Although this seems to be the logical approach, it is also the most time consuming and the most expensive approach. The commercially available materials for HPLC specify a particle size distribution and state that the average particle size is, for example 10 μm with a particle size analysis: d_{10} = 8 μm; d_{50} = 10 μm; and d_{90} = 13 μm, meaning that 90% of the particles will pass through a 13 μm screen. The problem is that 10 - 20% of the particles are either very large or very small. In order to obtain the narrowest particle size distribution, it is necessary to re-size the particles by suspending the particles in solution, allowing the bulk of the particles to fall, and then removing the top layer of liquid which contains the very small particles, or fines, and discarding them. the process would then have to be repeated except in this case the upper layer would be retained and the lower layer, containing the very large particles, would be discarded. This technique could result in the loss of up to 50% of the material but would guarantee a very narrow particle size distribution. The problem with this method is that it is very time consuming and could effectively double the cost of already expensive packing materials.

The second approach is the use of a slurry solvent which has a density very close to the density of the column

packing material. This will reduce segregation of the particles and is most often referred to as the balanced density slurry packing technique (40,41) and usually involves the use of highly toxic and noxious solvents such as mixtures of tetrabromoethane and tetrachloroethane. This technique when used to pack silica gel columns will result in very efficient columns (38).

A third way of decreasing segregation of the particles while they are in the slurry reservoir is to use a slurry solvent that has a high viscosity. This will increase the frictional resistance the solvent will place on the falling particles. This technique is referred to as the viscous slurry packing technique (42) and will result in very efficient columns if they are packed at extremely high pressures, up to 20,000 psig. The reason for the high pressure during packing is the high resistance to flow through the column generated by the high viscosity of the slurry solvent that is used.

The final factor controlling the segregation of the particles is determined by the nature of the packing material itself, its chemical characteristics, i.e. polarity and charge. A slurry packing method involving the use of ammonia stabilized slurries has been used to prevent agglomeration of the particles by the repulsion of the charged particles (14).

If the slurry is stirred during the packing procedure,

segregation of the particles due to Stokes Law will be minimized and then the requirement for the slurry solvent is that the solvent chosen must wet the column packing materialto prevent agglomeration. A relatively polar slurry solvent should be used with silica particles and a relatively non-polar slurry solvent should be used with the reverse phase column packing materials.

Once a stable slurry has been obtained, the next factor influencing the efficiency of the column is the bed compactness. This is determined by the linear velocity of the particles as the packed bed is formed and is dependent upon the type of pumping system used to force the slurry into the column. If a pneumatic amplifier pump is used, as was the case in this work, the flow rate through the column is determined by the applied pressure to the pump and the flow rate will decrease during the packing procedure as the height of the packed bed increases. A constant flow pumping system can also be used if the pump is capable of pumping at a high flow rate, up to 20 ml/min. The requirement for pressure is that the pressure developed during the packing of the column must be higher than the highest pressure that the column will experience during normal use in order to avoid bed settling during use.

The requirement for column efficiency of the slurry packed columns used in this work was that these columns should be comparable to the columns that are commercially

available. Figure 3 shows a plot of the height equivalent to a theoretical plate (HETP) vs. linear velocity of the mobile phase for one of our columns. This plot shows that this column is indeed efficient and comparable to columns that are commercially available and comparable to columns packed in our laboratory by a different method (38). It should be pointed out at this time that column efficiency alone does not separate compounds, selectivity is also necessary to effect a separation. It is possible to use the most efficient column currently available and not have any separation due to the fact that the distribution coefficients of the compounds of interest are identical with the column and solvent system used.

When slurry packing columns, it is important to take several precautions.

1. The slurry solvent and the driving solvent must be carefully degassed to remove all dissolved gasses which could come out of solution when the pressure is removed at the completion of the packing procedure. If dissolved gasses are present in the newly packed bed, they will come out of solution when the pressure is reduced and can cause voids to develop inside the column. Degassing of the solvent is accomplished by using a vacuum flask connected to a water aspirator. Vigorous shaking or stirring of the solvent while under vacuum aids in removing the dissolved gasses.

2. Once the slurry solvent has been added to the dried

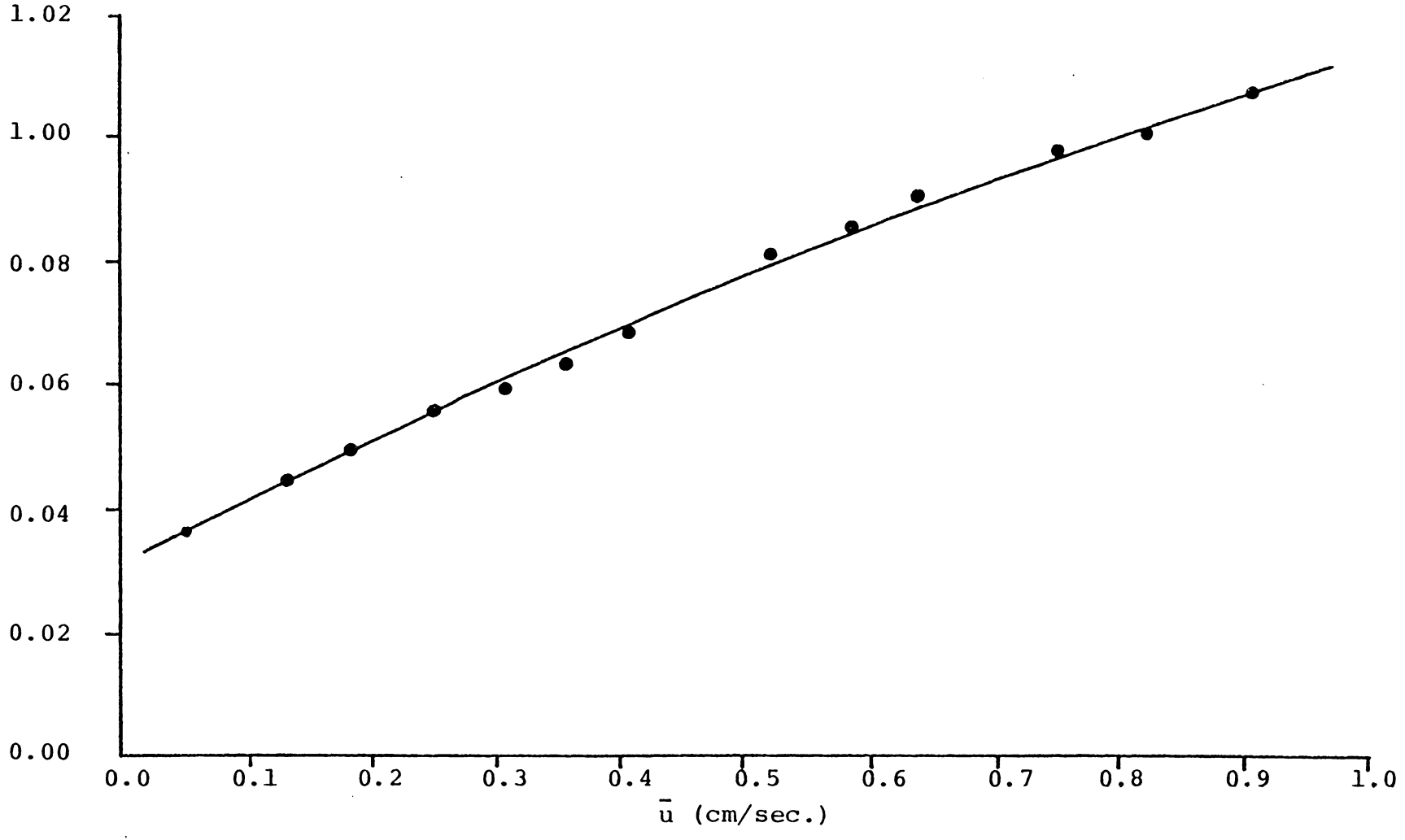

Figure 3. Lichrosorb SI-100, 10 µm Silica, 250 mm x 4.6 mm, 0.01% Acetonitrile in Hexane, 25°C, Anthracene

column packing material, the slurry should be degassed by using an ultrasonic bath. Vigorous shaking of the slurry should be made periodically to insure that the particles are wetted and that the air in the pores is replaced by the slurry solvent. If the particles are not thououghly degassed, voids can develop in the column when the pressure is reduced at the completion of the packing procedure. If the particles are not wetted, agglomeration of the particles can occur and a homogeneous bed will not be formed.

3. At the completion of the column packing procedure it is critical that the pressure to the column be reduced slowly to atmospheric pressure to avoid dissolved gasses or trapped air in the pores of the particles rapidly expanding and creating voids in the column.

4. The column should be removed from the slurry packer carefully to avoid disturbing the inlet to the column. Once the column has been removed, a razor blade should be used to trim the top of the column bed.

5. After the inlet end fitting has been placed on the column and tightened, the column should be labeled and the direction of flow during packing should be indicated. The direction of flow through the column should always be kept the same as the direction of flow during the packing procedure. This is important because the outlet end of the column is usually more compact than the inlet end and reversing the flow direction could cause the bed to shift

and result in voids being developed in the column.

Equipment Evaluation

Before beginning the column evaluation, the system repeatability was checked to determine the errors which might be introduced into the data due to flow rate changes and solvent changes from batch to batch. Table V lists the results of this repeatability study. This study was performed over a period of two days and involved the use of two different batches of solvent. On the second day the system was shut down and restarted twice to test the resetability of flow rate and column temperature. The system was run overnight between the first and second day. The column was column #9, packed with Lichrosorb SI-60, 10 μm silica, 250 mm x 3 mm i.d. The sample chosen for this evaluation was a mixture of benzene, naphthalene and anthracene in n-pentane. The sample solvent, n-pentane, was chosen because it is less polar than the mobile phase, 0.01% acetonitrile in hexane, and repetitive injections of the sample would not change the activity of the column. Another reason for using n-pentane was the fact that as it passed through the UV detector it created a baseline upset which could be detected and its time measured by the Minigrator. The reason for the baseline upset is that the refractive index of n-pentane is different than that of the mobile phase and as the n-pentane passes through the UV cell, the light is

Table V: Equipment Evaluation

Date	Solvent Breakthrough Time (sec.)	Retention Time of Benzene (sec.)	Retention Time of Naphthalene (sec.)	Retention Time of Anthracene (sec.)
12-27	92	153	204	293
	92	153	203	292
	92	153	203	291
12-28	92	153	203	293
	92	153	203	293
	92	153	203	293
	92	152	202	292
Mean	92	153	203	292
Std. Deviation	±0.0	±0.4	±0.6	±0.8
Rel. Std. Deviation	0.0%	0.2%	0.3%	0.3%

scattered and the photocell responds to the change in intensity of the light striking it.

Selectivity Study - Normal Phase Chromatography

Two solvent systems were used to compare the different columns in the normal phase mode; 0.01% acetonitrile in hexane and 80% hexane, 15% dichloromethane, 5% acetonitrile. All columns were equilibrated with the mobile phase overnight at 25°C and a flow rate of 2 ml/min. The samples were chromatographed at 25°C and a mobile phase linear velocity of 0.3 cm/sec. Tables VI and VII list the compounds that were chromatographed and the k' values that were obtained on the different columns. Several observations can be made by examination of Tables VI and VII. As the surface area is decreased from SI-60 to SI-100, we note that the k' values also decrease. This is as one would expect. The lower the surface area, the fewer the number of active sites for adsorption and this decrease in the number of active sites results in the decreased retention of the compounds. If we compare the silica columns with the alumina column, we see that for compounds that can hydrogen bond, their retention is greater on the silica columns. This is due to the higher surface area on the silica columns vs. the alumina column. For compounds that have a high degree of aromaticity, fused ring aromatics, we see that they are more retained on the alumina column than on the silica columns. This indicates

Table VI: k' For Probe Compounds Eluted on 6 Different Columns ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane; Temperature, 25°C.)

Column	SI-60	SI-100	ALOX T	RP-2	RP-8	RP-18
Compound						
Benzene	0.51	0.29	0.17	0.24	0.07	0.09
Tetrahydronaphthalene	0.64	0.35	0.27	0.22	0.03	0.07
Naphthalene	0.88	0.47	0.77	0.31	0.07	0.13
Biphenyl	1.29	0.66	1.09	0.38	0.06	0.10
Anthracene	1.48	0.76	15.42	0.41	0.09	0.24
Phenanthrene	1.61	0.79	8.68	0.44	0.08	0.23
Pyrene	1.67	0.84	18.61	0.46	0.09	0.33
Chrysene	2.71	1.30	----	0.62	0.10	0.50
Benzo(a)pyrene	2.88	1.33	----	0.65	0.13	0.78
Triphenylmethane	3.73	1.71	----	0.63	0.01	0.01
Toluene	0.55	0.30	0.17	0.23	0.04	0.07
Ethylbenzene	0.53	0.29	0.16	0.19	0.03	0.05
Propylbenzene	0.48	0.26	0.15	0.18	0.02	0.02
o-Xylene	0.63	0.35	0.22	0.23	0.03	0.07
m-Xylene	0.58	0.31	0.19	0.22	0.03	0.07
p-Xylene	0.56	0.30	0.18	0.22	0.03	0.07
Diethylbenzene	0.51	0.28	0.15	0.16	0.01	0.01
Chlorobenzene	0.43	0.24	0.19	0.20	0.06	0.09
Bromobenzene	0.46	0.25	0.23	0.20	0.06	0.10
Iodobenzene	0.48	0.26	0.27	0.20	0.06	0.10
Fluorobenzene	0.49	0.28	0.19	0.24	0.06	0.09
1,2,4-Trichlorobenzene	0.33	0.18	0.22	0.15	0.03	0.09
α,α,α-Trifluorotoluene	0.46	0.26	0.19	0.22	0.02	0.03
Anisole	5.61	2.65	1.33	1.41	0.17	0.26
p-Chloroaniline	7.37	3.58	4.60	1.52	0.16	0.32
p-Bromoaniline	7.37	3.59	5.19	1.47	0.16	0.32
Nitrobenzene	9.17	4.26	4.55	2.08	0.28	0.48
Benzophenone	----	----	----	12.28	0.44	1.09
m-Dinitrobenzene	----	----	----	11.61	1.10	2.34
m-Chloroaniline	----	----	----	----	High	High
Phenylether	----	----	1.07	0.57	0.03	0.08
Phenyl-2-propanone	----	----	----	----	1.67	----
Benzaldehyde	----	----	----	8.66	0.87	1.48
Anthraquinone	----	----	----	----	1.11	3.50
Phenol	----	----	----	----	5.59	8.93

Table VII: k' For Probe Compounds Eluted on 6 Different Columns ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile; Temperature, 25°C.)

Column	SI-60	SI-100	ALOX T	RP-2	RP-8	RP-18
Compound						
Benzene	0.23	0.14	0.08	0.17	0.00	0.01
Naphthalene	0.27	0.17	0.09	0.19	0.00	0.01
Biphenyl	0.27	0.19	0.11	0.18	0.00	0.01
Anthracene	0.33	0.21	0.13	0.20	0.00	0.02
Phenanthrene	0.34	0.21	0.14	0.21	0.00	0.02
Pyrene	0.34	0.21	0.13	0.21	0.00	0.06
Chrysene	0.41	0.26	0.15	0.24	0.00	0.08
Benzo(a)pyrene	0.41	0.26	0.18	0.25	0.00	0.14
Triphenylmethane	0.31	0.20	0.13	0.19	0.00	0.00
m-Dinitrobenzene	1.91	1.02	0.80	0.81	0.00	0.00
Anisole	0.39	0.23	0.14	0.25	0.00	0.00
p-Bromonitrobenzene	0.59	0.35	0.24	0.33	0.00	0.06
p-Chloronitrobenzene	0.59	0.34	0.24	0.33	0.00	0.00
Nitrobenzene	0.73	0.41	0.27	0.41	0.00	0.00
Benzophenone	0.71	0.40	0.26	0.39	0.00	0.00
Phenylether	0.30	0.17	0.41	0.18	0.00	0.00
Anthraquinone	0.88	0.49	0.36	0.46	0.00	0.05
Benzaldehyde	0.89	0.49	0.35	0.49	0.00	0.00
m-Chloroaniline	2.91	1.51	2.64	1.33	----	----
p-Chloroaniline	4.11	2.07	3.67	1.79	----	----
Dimethylphthalate	1.97	1.04	0.68	0.86	0.00	0.00
Azobenzene	0.27	0.74	0.11	0.18	0.00	0.00
Hydrazobenzene	0.28	0.19	0.11	0.19	0.00	0.00
m-Toluidine	4.09	1.93	3.37	1.63	0.01	0.00
Diethyl-diphenyl-urea	2.43	1.30	0.38	1.21	0.00	0.08
2-Chloro-5-nitroaniline	2.16	1.15	3.00	0.99	0.00	0.00
o-Nitroaniline	2.47	1.30	3.24	1.12	0.00	0.01
m-Nitroaniline	5.71	2.81	9.00	2.16	0.15	2.31
p-Nitroaniline	8.13	3.93	19.83	2.98	0.07	0.19
Phenol	2.61	1.48	----	1.42	0.07	0.08
o-Iodophenol	1.36	0.92	----	0.73	0.02	0.01
o-Nitrophenol	1.02	0.69	----	0.38	0.02	0.00
β-Naphthol	2.90	1.76	----	1.51	0.06	0.10
p-Nitrophenol	----	----	----	2.38	0.05	0.06
Acetanilide	----	8.97	9.29	6.08	0.65	1.36

that different forces are responsible for retention on silica and alumina. The importance of hydrogen bonding in adsorption on silica has been shown in several studies (43). Hydrogen bonding in adsorption on alumina seems to be less important and induction forces are more important. It is interesting to note the reversal in the elution order of anthracene and phenathrene on silica and alumina. This can be explained by an examination of the nature of the adsorption sites on silica and alumina. The sites on silica responsible for adsorption are surface hydroxyl groups. These sites are composed of free hydroxyls and reactive hydroxyl groups, Figure 4. The free hydroxyl groups are free to rotate on the surface and are distributed evenly over the silica surface and the reactive hydroxyl groups occure in bunches on the silica surface. The alumina surface sites are composed of strong surface fields corresponding to the position of the aluminum ions in the lattice structure, Figure 5. These surface fields are arranged on the surface in linear rows and will preferrentially adsorb linear molecules such as anthracene since the molecule is better able to align itself with the linear row of adsorption sites than the angular phenanthrene molecule.

Examination of the k' values obtained for the compounds on the RP-2 column and the silica columns, shows that the RP-2 material is acting as a lower area silica. This indicates that the RP-2 material can be used in the normal

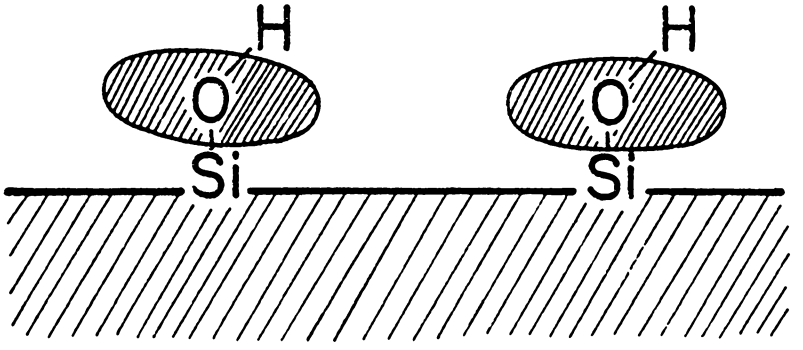

Figure 4. Silica Surface

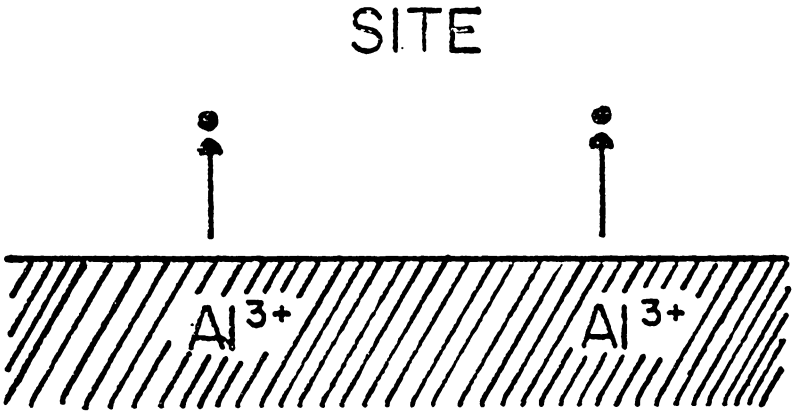

Figure 5. Alumina Surface

phase mode and that it is behaving in the same manner as the un-modified silicas. Examination of the k' values obtained on the RP-8 and the RP-18 columns indicate that there is very little retention and that they are not suitable for use in the normal phase mode.

Temperature Study - Normal Phase Chromatography

Two solvent systems were used to compare the different columns at four different temperatures in the normal phase mode. The two solvent systems were: 0.01% acetonitrile in hexane and 80% hexane, 15% dichloromethane, 5% acetonitrile. The temperatures at which the selected compounds were chromatographed were: 20°C, 30°C, 40°C, and 50°C. Tables VIII - XIX list the compounds that were chromatographed at the different temperatures and the k' values that were obtained on the different columns at the four temperatures. Figures 6 - 8 show graphically the significant data from these tables. After examination of tables VIII - XIX and Figures 6 - 8, several observations can be made. In general, as the temperature is increased, the retention time decreases. This can be explained by the fact that as we increase the temperature, we are increasing the rate of mass transfer between the mobile phase and the stationary phase and at the same time we are increasing the rate of sorption and desorption of the sample molecules. The thermodynamics of the system at higher temperature causes the sample molecules

Table VIII: k' For Probe Compounds Eluted on SI-60 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.51	0.46	0.44	0.44
Naphthalene	0.98	0.78	0.74	0.76
Biphenyl	1.42	1.09	1.05	1.09
Phenanthrene	1.74	1.39	1.31	1.33
Benzo(a)pyrene	3.10	2.52	2.32	2.36
Triphenylmethane	4.06	3.10	2.86	3.10
Anisole	6.24	4.44	4.05	4.16
p-Chloronitrobenzene	8.15	6.07	5.47	5.29
p-Bromonitrobenzene	8.19	6.13	5.52	5.33
Nitrobenzene	10.05	7.50	6.75	6.45

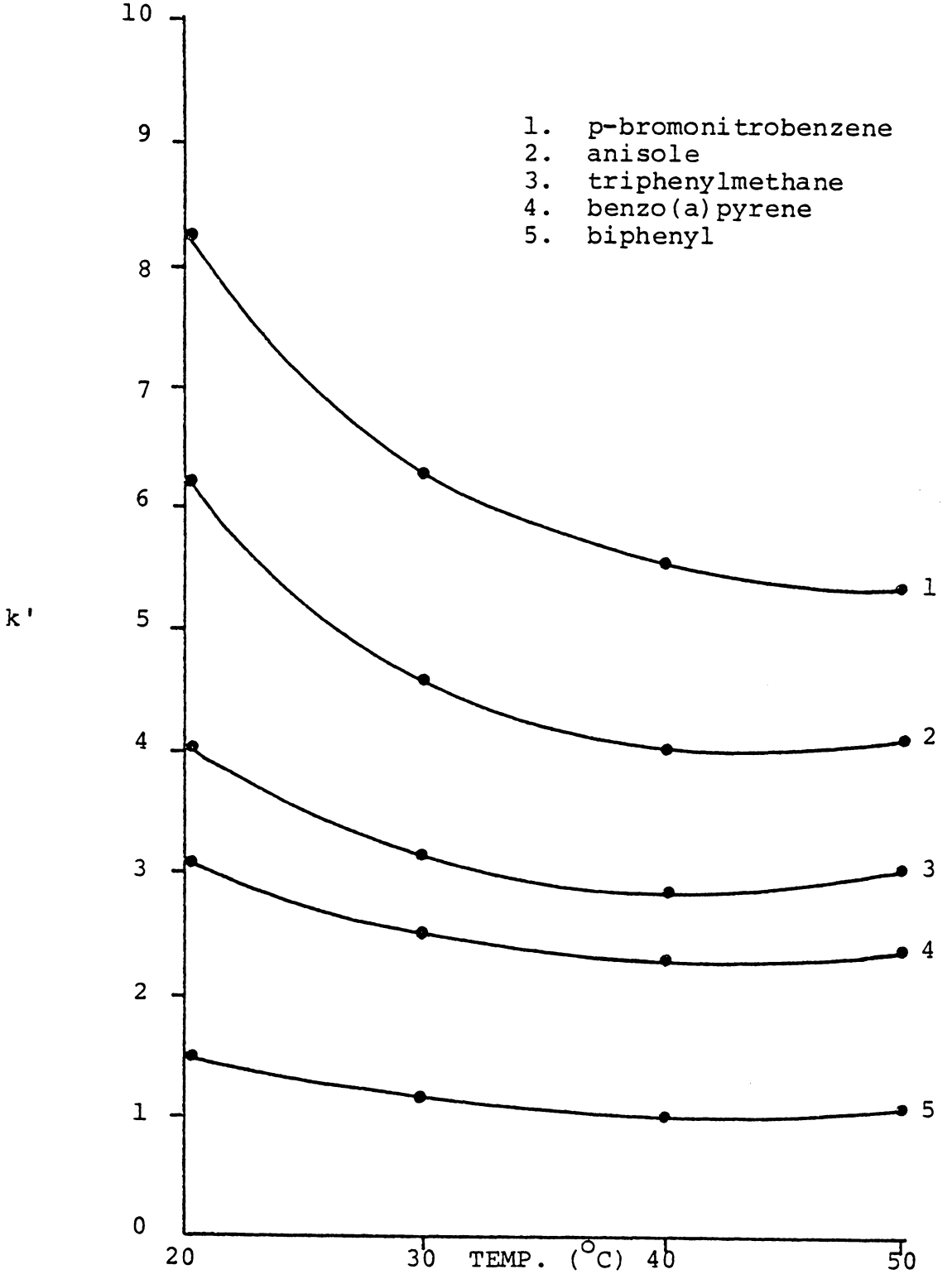

Figure 6. k' vs. Temperature in °C for Probe Compounds Eluted on SI-60 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Table IX: k' For Probe Compounds Eluted on SI-100 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.30	0.26	0.26	0.26
Naphthalene	0.48	0.44	0.43	0.41
Biphenyl	0.67	0.60	0.61	0.59
Phenanthrene	0.83	0.75	0.76	0.71
Benzo(a)pyrene	1.39	1.27	1.26	1.19
Triphenylmethane	1.70	1.60	1.64	1.59
Anisole	2.66	2.43	2.32	2.16
p-Chloronitrobenzene	3.72	3.36	3.08	2.77
p-Bromonitrobenzene	3.74	3.36	3.11	2.79
Nitrobenzene	4.51	4.04	3.66	3.33

Table X: k' For Probe Compounds Eluted on ALOX T ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.15	0.15	0.14	0.16
Naphthalene	0.61	0.53	0.52	0.61
Biphenyl	0.79	0.71	0.67	0.80
Phenanthrene	6.69	4.27	4.02	5.13
Triphenylmethane	----	----	0.92	1.00
Anisole	1.19	0.99	0.98	1.11
p-Chloronitrobenzene	4.17	3.30	3.23	3.59
p-Bromonitrobenzene	4.56	3.58	3.60	4.06
Nitrobenzene	4.03	3.20	3.21	3.61

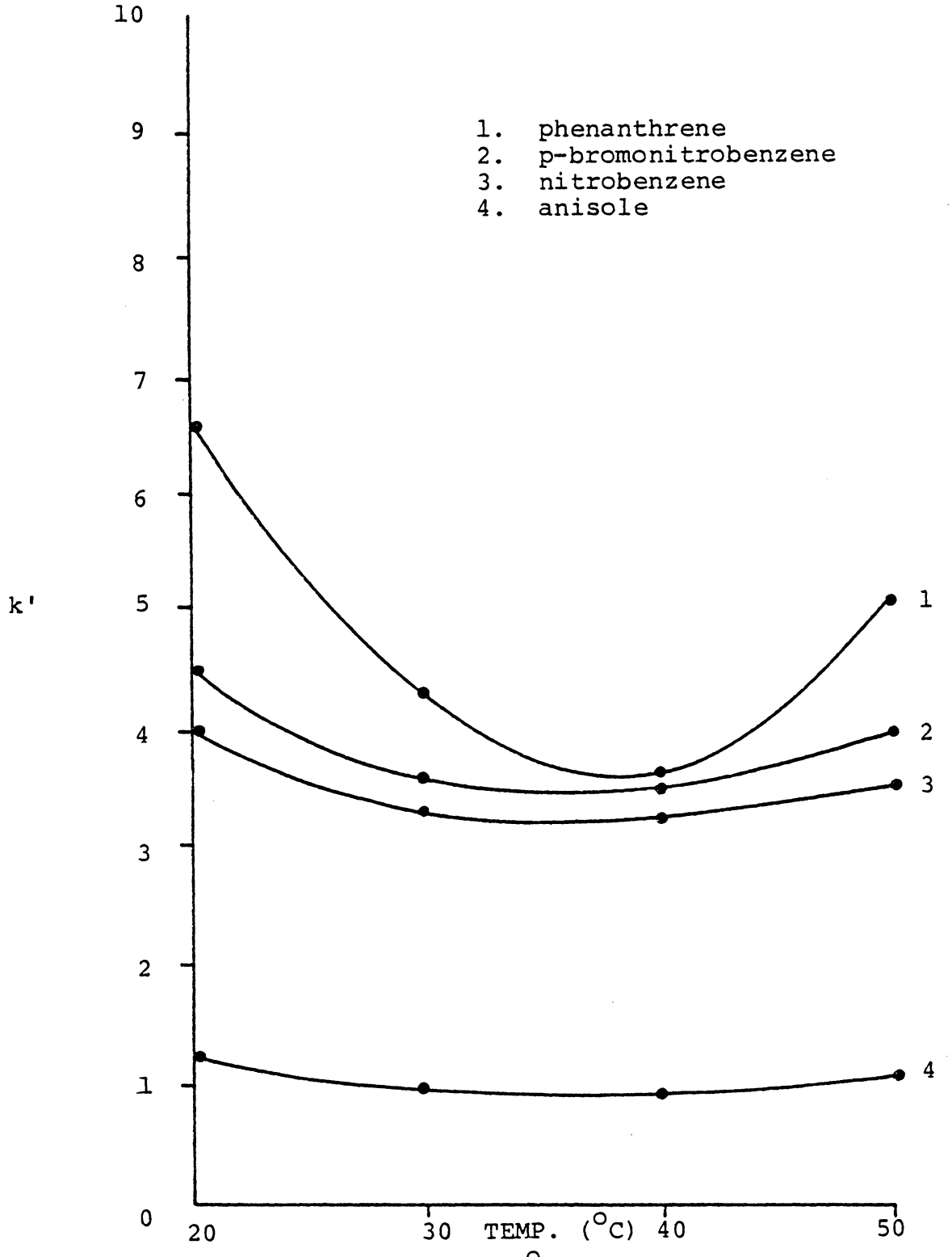

Figure 7. k' vs. Temperature in °C for Probe Compounds Eluted on ALOX T ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Table XI: k' For Probe Compounds Eluted on RP-2 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.23	0.19	0.18	0.20
Naphthalene	0.29	0.25	0.24	0.25
Biphenyl	0.33	0.28	0.27	0.27
Phenanthrene	0.39	0.34	0.34	0.33
Benzo(a)pyrene	0.54	0.50	0.51	0.57
Triphenylmethane	0.46	0.43	0.42	0.41
Anisole	0.95	0.90	0.88	0.85
p-Chloronitrobenzene	1.15	1.06	1.00	0.93
p-Bromonitrobenzene	1.11	1.03	0.98	0.91
Nitrobenzene	1.60	1.48	1.35	1.26
Benzaldehyde	5.64	5.02	4.63	4.16
m-Dinitrobenzene	9.08	7.59	6.70	5.78

Table XII: k' For Probe Compounds Eluted on RP-8 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.07	0.06	0.05	0.05
Naphthalene	0.07	0.07	0.07	0.05
Biphenyl	0.05	0.04	0.03	0.02
Phenanthrene	0.08	0.07	0.06	0.06
Benzo(a)pyrene	0.14	0.11	0.09	0.08
Triphenylmethane	0.01	0.01	0.00	0.00
Anisole	0.17	0.11	0.10	0.10
p-Chloronitrobenzene	0.16	0.12	0.11	0.09
p-Bromonitrobenzene	0.16	0.12	0.10	0.09
Nitrobenzene	0.29	0.22	0.19	0.17
m-Dinitrobenzene	1.11	0.76	0.63	0.57
Phenol	6.71	3.66	2.80	2.23

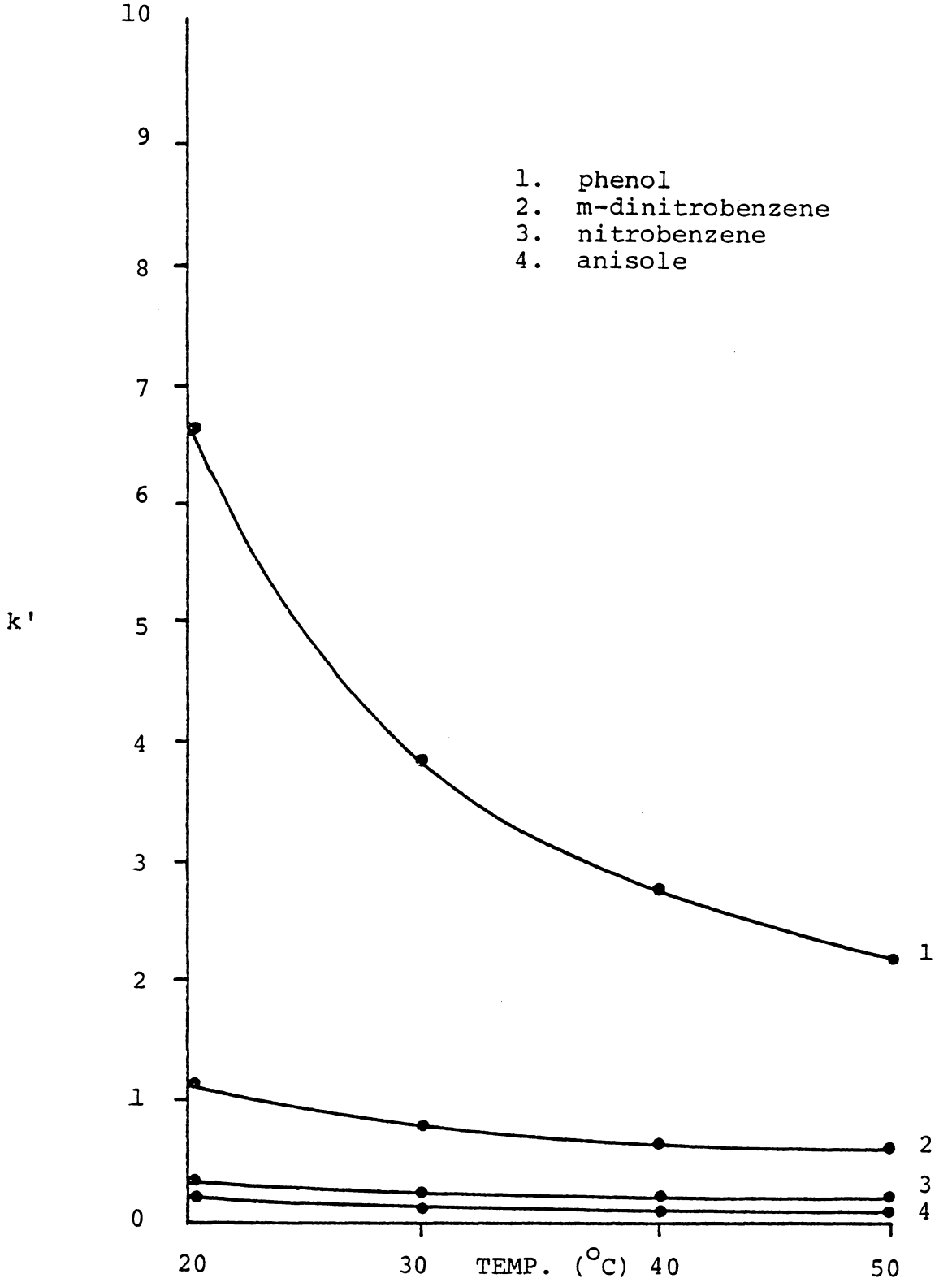

Figure 8. k' vs. Temperature in ^{o}C for Probe Compounds Eluted on RP-8 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Table XIII: k' For Probe Compounds Eluted on RP-18 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 0.01% Acetonitrile in Hexane)

Temperature / Compound	20°C	30°C	40°C	50°C
Benzene	0.09	0.09	0.09	0.08
Naphthalene	0.14	0.13	0.12	0.11
Biphenyl	0.12	0.09	0.09	0.08
Phenanthrene	0.24	0.21	0.18	0.16
Benzo(a)pyrene	0.90	0.68	0.52	0.42
Triphenylmethane	0.01	0.01	0.01	0.01
Anisole	0.25	0.23	0.21	0.19
p-Chloronitrobenzene	0.32	0.29	0.25	0.22
p-Bromonitrobenzene	0.32	0.29	0.25	0.22
Nitrobenzene	0.49	0.44	0.39	0.34
Benzaldehyde	1.51	1.26	1.05	0.91
m-Dinitrobenzene	2.41	1.97	1.60	1.34
Anthraquinone	3.89	3.09	2.43	2.04
Phenol	9.70	7.14	5.24	4.01

Table XIV: k' For Probe Compounds Eluted on SI-60 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.23	0.22	0.20	0.19
Benzo(a)pyrene	0.42	0.38	0.38	0.37
p-Chloronitrobenzene	0.62	0.55	0.51	0.46
Nitrobenzene	0.74	0.66	0.62	0.57
Anthraquinone	0.88	0.84	0.80	0.77
Benzaldehyde	0.90	0.83	0.79	0.74
m-Dinitrobenzene	2.02	1.77	1.59	1.45
Dimethylphthalate	2.00	1.90	1.86	1.79
o-Iodophenol	1.44	1.26	1.12	0.98
o-Nitrophenol	1.01	0.91	0.87	0.87
Diethyl-diphenyl-urea	2.37	2.44	2.47	2.52
o-Nitroaniline	2.61	2.30	2.08	1.93
Phenol	2.71	2.49	2.33	2.15
m-Chloroaniline	3.07	2.74	2.51	2.26
β-Naphthol	3.00	2.79	2.66	2.48
p-Chloroaniline	4.48	4.04	3.64	3.21
m-Nitroaniline	6.06	5.33	4.81	4.28
p-Nitroaniline	8.67	7.63	6.85	6.14

Table XV: k' For Probe Compounds Eluted on SI-100 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.15	0.14	0.13	0.07
Benzo(a)pyrene	0.27	0.25	0.25	0.21
p-Chloronitrobenzene	0.38	0.33	0.31	0.27
Nitrobenzene	0.45	0.40	0.35	0.33
Anthraquinone	0.52	0.48	0.44	0.43
Benzaldehyde	0.52	0.47	0.44	0.41
m-Dinitrobenzene	1.19	0.97	0.86	0.78
Dimethylphthalate	1.09	1.01	0.97	0.93
o-Iodophenol	0.95	0.85	0.76	0.70
o-Nitrophenol	0.67	0.66	0.69	0.72
Diethyl-diphenyl-urea	1.33	1.35	1.37	1.38
o-Nitroaniline	1.14	1.24	1.12	1.02
Phenol	1.54	1.41	1.33	1.24
m-Chloroaniline	1.64	1.45	1.30	1.17
β-Naphthol	1.81	1.71	1.63	1.57
p-Chloroaniline	2.27	2.01	1.78	1.58
m-Nitroaniline	3.05	2.68	2.38	2.14
p-Nitroaniline	4.27	3.75	3.33	2.98
Acetanilide	9.52	8.85	8.15	7.49

Table XVI: k' For Probe Compounds Eluted on ALOX T ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.08	0.08	0.08	0.07
Benzo(a)pyrene	0.18	0.18	0.18	0.18
p-Chloronitrobenzene	0.24	0.23	0.23	0.21
Nitrobenzene	0.27	0.27	0.25	0.23
Anthraquinone	0.36	0.35	0.36	0.36
Benzaldehyde	0.36	0.35	0.34	0.31
m-Dinitrobenzene	0.82	0.77	0.74	0.69
Dimethylphthalate	0.68	0.68	0.68	0.66
Diethyl-diphenyl-urea	0.39	0.39	0.41	0.42
o-Nitroaniline	3.05	3.19	3.34	3.30
m-Chloroaniline	2.81	2.92	3.16	3.22
p-Chloroaniline	4.90	5.04	5.37	5.53
m-Nitroaniline	8.33	8.82	9.77	10.83
p-Nitroaniline	----	19.01	21.41	----
Acetanilide	8.31	9.35	10.40	11.81
2-Chloro-5-nitroaniline	2.73	2.94	3.21	3.42

Table XVII: k' For Probe Compounds Eluted on RP-2 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.15	0.14	0.14	0.14
Benzo(a)pyrene	0.26	0.22	0.22	0.20
p-Chloronitrobenzene	0.35	0.30	0.28	0.26
Nitrobenzene	0.43	0.37	0.34	0.33
Anthraquinone	0.48	0.43	0.40	0.38
Benzaldehyde	0.51	0.45	0.43	0.38
m-Dinitrobenzene	0.87	0.73	0.64	0.56
Dimethylphthalate	0.90	0.80	0.74	0.69
o-Iodophenol	0.80	0.67	0.59	0.52
o-Nitrophenol	0.41	0.36	0.34	0.30
Diethyl-diphenyl-urea	1.22	1.16	1.14	1.08
o-Nitroaniline	1.20	1.04	0.92	0.82
Phenol	1.50	1.33	1.21	1.09
m-Chloroaniline	1.41	1.22	1.08	0.94
β-Naphthol	1.59	1.43	1.31	1.17
p-Chloroaniline	1.91	1.65	1.45	1.25
m-Nitroaniline	2.31	1.99	1.73	1.51
p-Nitroaniline	3.24	2.76	2.38	2.08
Acetanilide	6.45	5.72	5.09	4.48
2-Chloro-5-nitroaniline	1.05	0.91	0.81	0.71

Table XVIII: k' For Probe Compounds Eluted on RP-8 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.00	0.00	0.00	0.00
Benzo(a)pyrene	0.00	0.00	0.00	0.00
p-Chloronitrobenzene	0.00	0.00	0.00	0.00
Nitrobenzene	0.00	0.00	0.00	0.00
Anthraquinone	0.00	0.00	0.00	0.00
m-Dinitrobenzene	0.00	0.00	0.00	0.00
Dimethylphthalate	0.00	0.00	0.00	0.00
o-Iodophenol	0.02	0.01	0.00	0.00
o-Nitrophenol	0.02	0.01	0.00	0.00
Diethyl-diphenyl-urea	0.00	0.00	0.00	0.01
o-Nitroaniline	0.00	0.00	0.00	0.00
Phenol	0.06	0.03	0.03	0.00
β-Naphthol	0.05	0.02	0.01	0.00
m-Nitroaniline	0.10	0.16	0.14	0.13
p-Nitroaniline	0.06	0.06	0.06	0.05
Acetanilide	0.65	0.67	0.74	0.82
2-Chloro-5-nitroaniline	0.00	0.00	0.00	0.00

Table XIX: k' For Probe Compounds Eluted on RP-18 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 80% Hexane/15% Dichloromethane/5% Acetonitrile)

Temperature	20°C	30°C	40°C	50°C
Compound				
Benzene	0.01	0.01	0.01	0.01
Benzo(a)pyrene	0.14	0.10	0.09	0.07
p-Chloronitrobenzene	0.00	0.00	0.00	0.00
Nitrobenzene	0.00	0.00	0.00	0.00
Anthraquinone	0.03	0.03	0.02	0.01
Benzaldehyde	0.00	0.00	0.00	0.00
m-Dinitrobenzene	0.00	0.00	0.00	0.00
Dimethylphthalate	0.00	0.00	0.00	0.00
O-Iodophenol	0.01	0.01	0.01	0.00
o-Nitrophenol	0.00	0.00	0.00	0.00
Diethyl-diphenyl-urea	0.07	0.08	0.09	0.12
o-Nitroaniline	0.00	0.01	0.01	0.01
Phenol	0.09	0.08	0.08	0.07
β-Naphthol	0.10	0.08	0.08	0.06
m-Nitroaniline	2.00	1.46	1.24	1.25
p-Nitroaniline	0.17	0.15	0.15	0.15
Acetanilide	1.31	1.39	1.53	1.76

to favor the mobile phase rather than the stationary phase. The decrease in retention as the temperature is increased is more pronounced for those compounds that have a high k' value at the low temperature. In general the retention is significantly reduced for those compounds that have a k' above 2 at 20°C as we increase the temperature of the column. It is interesting to note that in some cases the slopes of the lines for the different compounds are not the same as the temperature is increased. This indicates that the selectivity of the column is changing as the temperature is increased. Several of the plots of k' vs. temperature pass through a minimum as the temperature is increased. A good example of this phenomenon is Figure 7, the plot of k' vs. temperature for the alumína column. A possible explanation for an increase in k' was proposed by Maggs (34) in an article in which he showed that for mixed solvent systems in which a moderator is present in low concentrations, as the temperature is raised, the moderator can be made to distribute itself more in the mobile phase and by doing so, the polarity of the stationary phase is increased, that is, more active sites are exposed and this results in increased retention as the temperature is increased. The effect is most pronounced on the alumina column due to the nature of the active adsorption sites, point surface sites vs. area surface sites on silica. The results of Maggs were confirmed by Scott (35) in a later article. It is interesting to note that minimums have never

been reported in the literature. The importance of this phenomenon lies in the fact that temperature can be used to increase the resolution for a pair of solutes by merely increasing or decreasing the temperature of the column. The most important observation that can be made at this point is that the scope of the column packing material is temperature dependent. In general as the column temperature is increased, k' decreases and it is possible to elute a wider of range of sample polarities using the same solvent system, but at a different temperature.

It is also important to note that in the temperature range from 20°C to 30°C the slopes of the plots are the greatest, indicating that for reproducibility of retention times, it is essential to control the temperature of the chromatographic system.

Selectivity Study - Reversed Phase Chromatography

Two solvent systems were used to compare the different columns in the reverse phase mode: (1) 50% water, 50% methanol; and (2) 25% water, 75% methanol. All columns were equilibrated with the mobile phase overnight at 25°C and a flow rate of 2 ml/min. The samples were chromatographed at 25°C and a mobile phase linear velocity of 0.3 cm/sec. Tables XX and XXI list the compounds that were chromatographed and the k' values that were obtained on the different columns. Several observations can be made by examination of Tables XX and XXI.

Table XX: k' For Probe Compounds Eluted on 3 Different Columns ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 50% Water/ 50% Methanol; Temperature, 25°C)

Column	RP-2	RP-8	RP-18
Compound			
Benzene	1.99	3.78	5.87
Toluene	3.31	7.46	12.99
Fluorobenzene	2.34	4.12	6.24
Chlorobenzene	3.57	7.86	----
Bromobenzene	3.95	9.48	----
Iodobenzene	4.75	12.67	----
Nitrobenzene	1.76	2.63	3.55
Phenol	0.76	1.06	1.17
Anisole	2.04	3.63	5.72
Benzaldehyde	1.20	1.59	2.08
Acetophenone	1.41	1.99	2.64
Acetanilide	0.72	0.92	1.04
Dimethylphthalate	1.81	2.08	2.56

Table XXI: k' For Probe Compounds Eluted on 3 Different Columns ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 25% Water/75% Methanol; Temperature, 25°C)

Column	RP-2	RP-8	RP-18
Compound			
Benzene	0.49	0.79	1.22
Naphthalene	0.69	1.47	2.99
Biphenyl	0.92	2.12	4.70
Anthracene	0.99	2.98	9.65
Phenanthrene	0.93	2.77	8.00
Pyrene	1.11	4.17	14.98
Chrysene	1.31	5.37	----
Benzo(a)pyrene	1.59	8.81	----
Toluene	0.65	1.14	1.99
Ethylbenzene	0.81	1.59	2.91
Propylbenzene	1.07	2.34	4.63
o-Xylene	0.78	1.59	3.10
m-Xylene	0.82	1.70	3.33
p-Xylene	0.82	1.69	3.39
Diethylbenzene	1.30	3.24	6.78
Bromobenzene	0.66	1.24	2.19
Chlorobenzene	0.63	1.11	1.88
Iodobenzene	0.69	1.43	2.71
Fluorobenzene	0.51	0.75	1.12
Nitrobenzene	0.40	0.55	0.76
Phenol	0.21	0.29	0.33
Anisole	0.45	0.71	1.14
Benzaldehyde	0.31	0.41	0.53
Acetophenone	0.33	0.45	0.59
Phenylether	0.94	1.96	3.69
Benzophenone	0.64	1.06	1.60
Diethyl-diphenyl-urea	1.07	1.89	2.84
Anthraquinone	0.68	1.73	3.49
Dimethylphthalate	0.33	0.38	0.44
Diethylphthalate	0.50	0.69	0.88
Dibutylphthalate	1.33	2.87	4.73
Acetanilide	0.21	0.26	0.29

As the chain length of the chemically bonded phase increases, the retention of the compounds also increases. Majors and Hopper (44) also observed that as the hydrocarbon chain length of the bonded phase increases, the k' values increased; however, they did not attempt to explain this increase. Gilpin (45) also came to the same conclusion and again no attempt was made to explain the increased retention. Knox and Pryde (46) suggest that the nonaqueous modifier in the mobile phase was extracted by the non-polar bonded phase and that retention was due to a partitioning of the solutes between the mobile phase and the trapped liquid modifier. Telepchak (47) has proposed that retention in the reverse phase mode is due to an adsorption mechanism. Grushka (48) proposed that both mechanisms may be responsible for retention in reverse phase chromatography. The data obtained in this study is consistant with the results that were obtained by the other workers in the field. The increase in retention with increasing hydrocarbon chain length of the bonded phase can be explained in two ways. If retention is governed by an adsorption mechanism, as the amount of hydrocarbon bonded to the silica surface increases (increased chain length) more adsorption sites are available; therefore, increased retention. If retention is based upon partitioning of the solute between the sorbed modifier and the mobile phase, as the amount of hydrocarbon bonded to the surface increases (increased hydrocarbon chain length) more surface is available for the

modifier and more modifier is sorbed thereby increasing the retention due to the greater amount of the less polar liquid on the surface vs. the more polar mobile phase.

Examination of Table XXI shows that as the number of aromatic rings in a compound increases, the retention also increases. Sleight (49) and Locke (50) have proposed that the increase in retention is due to the decrease in solubility of the compound in the mobile phase as the degree of aromaticity increases and as the aliphatic chain length is increased.

Temperature Study - Reversed Phase Chromatography

Two solvent systems were used to compare the different columns at seven different temperatures in the reverse phase mode. The solvent systems were: (1) 50% water, 50% methanol; and 25% water, 75% methanol. The temperatures at which the selected compounds were chromatographed were: 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C. Tables XXII through XXVII list the compounds that were chromatographed at the different temperatures and the k' values that were obtained on the different columns along with the pressure drop across the columns. Figure 9 shows graphically the significant data from these tables. After examination of Tables XXII - XXVII several observations can be made. In general as the temperature is increased, the retention of the compounds decreases and the amount of decrease is a function of k'. The higher the k' at 20°C, the greater the decrease in retention as the temperature

Table XXII: k' For Probe Compounds Eluted on RP-2 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 50% Water/50% Methanol)

Temperature	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Compound							
Benzene	2.13	1.89	1.70	1.54	1.41	1.24	1.14
Toluene	3.58	3.13	2.75	2.48	2.20	1.98	1.77
Ethylbenzene	6.04	5.30	4.57	4.05	3.57	3.07	2.71
Propylbenzene	11.29	9.65	8.17	7.01	6.01	5.08	4.42
o-Xylene	5.42	4.77	4.16	3.67	3.22	2.78	2.49
m-Xylene	5.95	5.19	4.53	3.99	3.46	3.01	2.69
p-Xylene	6.14	5.31	4.63	4.07	3.52	3.06	2.75
Bromobenzene	4.31	3.71	3.25	2.86	2.49	2.18	1.93
Chlorobenzene	3.83	3.35	2.99	2.60	2.30	2.04	1.80
Iodobenzene	5.14	4.45	3.87	3.34	2.93	2.55	2.23
Fluorobenzene	2.48	2.24	2.01	1.78	1.60	1.43	1.29
Nitrobenzene	1.92	1.65	1.47	1.29	1.16	1.05	0.93
Phenol	0.84	0.73	0.66	0.60	0.53	0.48	0.43
Anisol	2.20	1.90	1.72	1.52	1.37	1.24	1.11

Table XXII: Continued

Temperature / Compound	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Benzaldehyde	1.29	1.12	1.01	0.88	0.82	0.73	0.67
Acetophenone	1.51	1.33	1.20	1.07	0.96	0.88	0.81
Dimethylphthalate	1.95	1.67	1.51	1.30	1.17	1.04	0.93
Diethylphthalate	4.47	3.86	3.36	2.90	2.53	2.23	1.96
Acetanilide	0.81	0.69	0.64	0.55	0.51	0.46	0.41

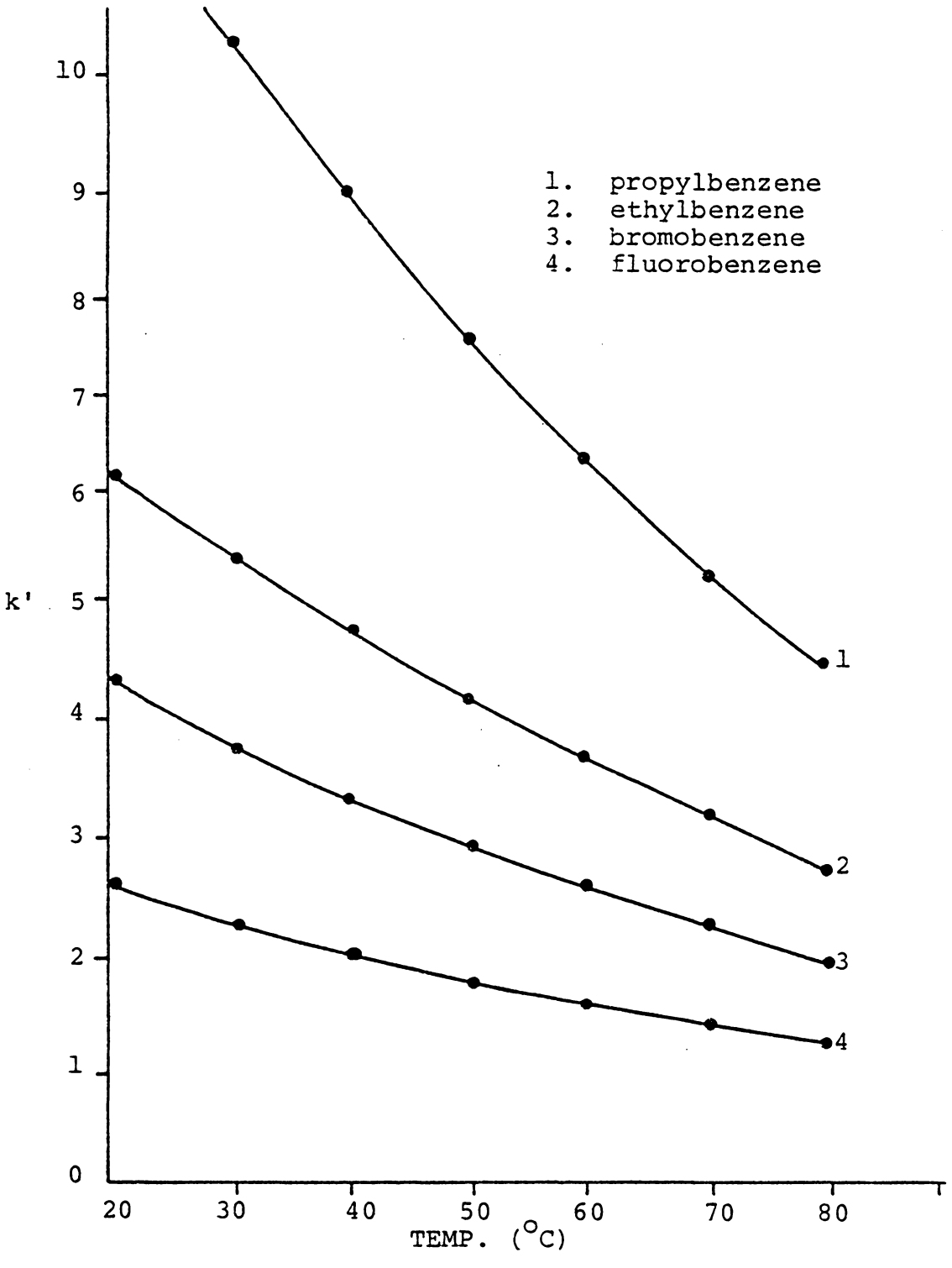

Figure 9. k' vs. Temperature in ^{o}C for Probe Compounds Eluted on RP-2 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 50% Water/50% Methanol)

Table XXIII: k' For Probe Compounds Eluted on RP-8 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 50% Water/50% Methanol)

Temperature	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Compound							
Benzene	4.02	3.48	3.14	2.78	2.40	2.15	1.88
Toluene	8.02	6.76	5.93	5.11	4.39	3.84	3.31
Ethylbenzene	----	----	----	----	----	----	5.48
Propylbenzene	----	----	----	----	----	----	9.87
o-Xylene	----	----	----	8.51	----	----	5.08
m-Xylene	----	----	----	----	----	----	5.59
p-Xylene	----	----	----	----	----	----	5.64
Bromobenzene	10.31	8.58	7.30	6.27	5.25	4.48	3.80
Chlorobenzene	8.59	7.25	6.22	5.35	4.52	3.89	3.30
Iodobenzene	14.05	11.59	9.83	8.12	6.77	5.66	4.76
Fluorobenzene	4.40	3.86	3.45	2.98	2.59	2.29	2.01
Nitrobenzene	2.82	2.46	2.17	1.86	1.60	1.40	1.22
Phenol	1.13	1.01	0.92	0.81	0.70	0.61	0.54
Anisole	3.90	3.37	2.99	2.60	2.24	1.95	1.70

Table XXIII: Continued

Temperature / Compound	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Benzaldehyde	1.69	1.49	1.35	1.20	1.05	0.93	0.82
Acetophenone	2.11	1.84	1.67	1.48	1.28	1.14	1.04
Acetanilide	0.98	0.86	0.77	0.69	0.58	0.52	0.46
Dimethylphthalate	2.23	1.93	1.75	1.54	1.33	1.17	1.04

Table XXIV: k' For Probe Compounds Eluted on RP-18 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 50% Water/50% Methanol)

Temperature	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Compound							
Benzene	6.29	5.57	4.97	4.36	3.80	3.46	3.00
Toluene	14.05	12.11	10.51	8.93	7.71	6.80	5.72
Bromobenzene	----	15.36	----	10.96	9.33	8.11	6.69
Chlorobenzene	----	12.32	10.69	9.01	7.76	6.77	5.63
Iodobenzene	----	21.65	----	15.12	12.77	10.85	8.83
Fluorobenzene	6.69	5.79	5.21	4.51	4.01	3.55	3.03
Nitrobenzene	3.84	3.27	2.87	2.44	2.15	1.87	1.61
Phenol	1.28	1.09	0.99	0.85	0.76	0.68	0.60
Anisole	6.21	5.31	4.63	3.95	3.47	3.01	2.60
Benzaldehyde	2.24	1.93	1.72	1.49	1.33	1.20	1.07
Acetophenone	2.87	2.47	2.19	1.88	1.68	1.49	1.29
Acetanilide	1.13	0.97	0.85	0.72	0.64	0.56	0.49
Dimethylphthalate	2.76	2.36	2.11	1.81	1.60	1.41	1.25

Table XXV: k' For Probe Compounds Eluted on RP-2 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 25% Water/75% Methanol)

Temperature	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Compound							
Benzene	0.54	0.48	0.45	0.43	0.39	0.39	0.36
Naphthalene	0.74	0.65	0.61	0.58	0.54	0.51	0.48
Biphenyl	0.98	0.87	0.79	0.73	0.68	0.64	0.59
Anthracene	1.06	0.94	0.85	0.81	0.74	0.69	0.64
Phenanthrene	1.00	0.88	0.82	0.76	0.74	0.69	0.64
Pyrene	1.18	1.05	0.98	0.89	0.82	0.77	0.71
Chrysene	1.39	1.23	1.13	1.05	0.95	0.88	0.81
Benzo(a)pyrene	1.68	1.49	1.38	1.25	1.13	1.05	0.95
Toluene	0.67	0.61	0.56	0.52	0.50	0.47	0.45
Ethylbenzene	0.85	0.77	0.71	0.65	0.62	0.58	0.54
Propylbenzene	1.12	1.00	0.91	0.86	0.79	0.73	0.66
o-Xylene	0.82	0.74	0.70	0.65	0.60	0.57	0.52
m-Xylene	0.86	0.77	0.73	0.67	0.62	0.59	0.54
p-Xylene	0.86	0.78	0.74	0.69	0.63	0.59	0.55
Diethylbenzene	1.36	1.23	1.13	1.04	0.95	0.87	0.79

Table XXV: Continued

Temperature / Compound	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Bromobenzene	0.68	0.62	0.58	0.55	0.50	0.48	0.45
Chlorobenzene	0.66	0.59	0.56	0.52	0.51	0.46	0.43
Iodobenzene	0.72	0.65	0.62	0.57	0.54	0.49	0.47
Fluorobenzene	0.52	0.49	0.46	0.44	0.41	0.39	0.36
Nitrobenzene	0.44	0.39	0.37	0.35	0.32	0.30	0.28
Phenol	0.24	0.21	0.20	0.18	0.18	0.17	0.16
Anisole	0.48	0.44	0.43	0.39	0.37	0.35	0.34
Benzaldehyde	0.34	0.31	0.29	0.27	0.26	0.24	0.23
Acetophenone	0.35	0.33	0.31	0.30	0.29	0.25	0.24
Phenylether	1.00	0.90	0.83	0.77	0.71	0.65	0.59
Benzophenone	0.67	0.61	0.58	0.54	0.51	0.46	0.44
Diethyl-diphenyl-urea	1.12	1.02	0.93	0.87	0.80	0.76	0.69
Anthraquinone	0.72	0.64	0.59	0.56	0.52	0.48	0.43
Dimethylphthalate	0.34	0.32	0.30	0.29	0.28	0.27	0.23
Diethylphthalate	0.52	0.49	0.45	0.43	0.40	0.38	0.35

Table XXVI: k' For Probe Compounds Eluted on RP-8 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 25% Water/75% Methanol)

Temperature	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Compound							
Benzene	0.80	0.76	0.72	0.66	0.60	0.55	0.52
Naphthalene	1.53	1.39	1.25	1.10	0.99	0.89	0.81
Biphenyl	2.22	1.98	1.76	1.52	1.36	1.19	1.05
Anthracene	3.18	2.74	2.37	2.02	1.75	1.51	1.31
Phenanthrene	2.94	2.53	2.22	1.89	1.63	1.41	1.25
Pyrene	4.53	3.77	3.24	2.71	2.32	1.93	1.66
Chrysene	5.92	4.84	4.05	3.34	2.73	2.31	1.98
Benzo(a)pyrene	10.12	7.79	6.32	5.04	4.00	3.34	2.77
Toluene	1.22	1.12	1.02	0.93	0.83	0.76	0.69
Ethylbenzene	1.69	1.53	1.40	1.26	1.11	0.99	0.90
Propylbenzene	2.51	2.22	1.98	1.77	1.54	1.38	1.22
o-Xylene	1.67	1.52	1.37	1.24	1.08	0.99	0.89
m-Xylene	1.81	1.64	1.48	1.33	1.17	1.05	0.95
p-Xylene	1.81	1.63	1.47	1.33	1.17	1.05	0.95
Diethylbenzene	3.48	3.04	2.66	2.35	2.04	1.80	1.61

Table XXVI: Continued

Temperature / Compound	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Bromobenzene	1.29	1.18	1.07	0,98	0,88	0.79	0.69
Chlorobenzene	1.17	1.06	0.98	0.89	0,79	0.73	0.66
Iodobenzene	1.53	1.39	1.24	1.13	1,00	0.92	0.82
Fluorobenzene	0.79	0.72	0.66	0.63	0.57	0,54	0.48
Nitrobenzene	0.59	0,54	0.58	0.45	0.39	0.37	0.34
Phenol	0.31	0.29	0.26	0,26	0,21	0.22	0.19
Anisole	0.76	0.70	0.64	0.58	0,52	0,49	0.45
Benzaldehyde	0.44	0.41	0.36	0,33	0,31	0.30	0.25
Acetophenone	0.48	0.45	0.40	0.37	0,33	0.32	0.29
Phenylether	2.11	1.83	1.61	1.43	1.23	1,12	1,01
Benzophenone	1.12	0.99	0.88	0,79	0.69	0,66	0.58
Diethyl-diphenyl-urea	1.99	1.75	1.57	1,40	1,23	1,13	1.02
Anthraquinone	1.93	1.57	1.32	1.12	0,95	0.84	0.74
Dimethylphthalate	0.39	0.37	0.35	0,30	0,28	0.26	0.26
Diethylphthalate	0.73	0.65	0,59	0,55	0,51	0.47	0,44

Table XXVII: k' For Probe Compounds Eluted on RP-18 ($\bar{u}$ = 0.3 cm/sec.; Mobile Phase, 25% Water/75% Methanol)

Temperature	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Compound							
Benzene	1.25	1.20	1.12	1.03	0.96	0.93	0.84
Naphthalene	3.11	2.81	2.51	2.18	1.95	1.78	1.56
Biphenyl	5.04	4.36	3.76	3.26	2.84	2.53	2.20
Anthracene	10.96	8.62	7.04	5.73	4.79	4.09	3.44
Phenanthrene	8.85	7.25	6.12	5.00	4.26	3.66	3.11
Pyrene	17.18	13.28	10.76	8.53	6.96	5.86	4.85
Chrysene	----	----	----	13.69	10.76	8.66	6.95
Benzo(a)pyrene	----	----	----	25.90	----	----	11.75
Toluene	2.10	1.93	1.75	1.58	1.46	1.35	1.23
Ethylbenzene	3.10	2.80	2.53	2.23	2.05	1.88	1.66
Propylbenzene	4.94	4.40	3.88	3.36	3.01	2.70	2.38
o-Xylene	3.23	2.93	2.60	2.32	2.09	1.90	1.69
m-Xylene	3.48	3.15	2.79	2.49	2.24	2.03	1.80
p-Xylene	3.56	3.22	2.89	2.52	2.26	2.00	1.83
Diethylbenzene	7.16	6.28	5.54	4.76	4.18	3.62	3.31

Table XXVII: Continued

Temperature / Compound	20°C	30°C	40°C	50°C	60°C	70°C	80°C
Bromobenzene	2.25	2.06	1.89	1.70	1.56	1.41	1.29
Chlorobenzene	1.93	1.78	1.63	1.48	1.36	1.24	1.16
Iodobenzene	2.83	2.56	2.32	2.08	1.88	1.68	1.54
Fluorobenzene	1.14	1.07	1.00	0.94	0.89	0.81	0.78
Nitrobenzene	0.79	0.72	0.65	0.61	0.56	0.52	0.49
Phenol	0.34	0.31	0.28	0.28	0.26	0.24	0.24
Anisole	1.18	1.07	0.99	0.90	0.85	0.77	0.73
Benzaldehyde	0.56	0.51	0.47	0.43	0.42	0.39	0.38
Acetophenone	0.62	0.56	0.51	0.48	0.46	0.42	0.41
Phenylether	3.94	3.44	3.03	2.67	2.36	2.08	1.86
Benzophenone	1.71	1.52	1.35	1.23	1.13	1.01	0.93
Diethyl-diphenyl-urea	3.01	2.68	2.38	2.13	1.93	1.70	1.55
Anthraquinone	3.91	3.11	2.54	2.13	1.80	1.49	1.35
Dimethylphthalate	0.46	0.43	0.39	0.37	0.36	0.34	0.33
Diethylphthalate	0.92	0.84	0.77	0.71	0.69	0.62	0.59

is increased. It is important to note that the slopes of the plots show that over the temperature range from 20°C to 30°C the retention decreases most rapidly with increasing temperature. It is important to control the temperature in reverse phase chromatography in order to obtain repeatable retention times especially when chromatographing compounds with k' values above 2.

The solvents used in reverse phase chromatography are usually relatively viscous compared to the solvent systems used in normal phase chromatography and as a result, relatively high pressures are required when operating the column at ambient temperature. An increase of 25°C results in a significant reduction in the pressure, almost 50%. This reduction in pressure can be important especially when high flow rates are required to reduce the time of an analysis. High flow rates at ambient temperatures with viscous solvents can generate pressures in excess of 5,000 psig and place undue stress on the pumping systems that are currently available. The increase in temperature not only reduces the pressure drop across the column, but also reduces the time for an analysis.

Optimization of Separations

It is possible to reduce the time for an analysis by: (1) increasing the flow rate; (2) using a stronger solvent system; (3) using a column with a lower surface area (normal phase chromatography); and (4) using a column with a shorter

hydrocarbon chain length of bonded phase (reverse phase chromatography); or by (5) increasing the temperature of the column.

Flow Rate

Increasing the flow rate is one of the easiest ways of reducing the time for an analysis. Increasing the flow rate does not seriously affect the efficiency of the column (see Figure 3) and it does not affect the resolution significantly. Increasing the flow rate does increase the pressure drop across the column and increases the amount of mobile phase used. When only a few separations are to be performed, increasing the flow rate is the fastest and easiest way to reduce the time for an analysis assuming that the pumping system is capable of pumping at a high flow rate and is able to withstand the increased pressure.

Stronger Solvent System

Changing from a weak solvent system to a stronger solvent system will reduce the time for a separation by altering the partitioning of the solute molecules between the stationary phase and the mobile phase. A stronger solvent system will reduce the time spent in the stationary phase for all molecules. Changing the mobile phase will result in a change in resolution and a decrease in the k' values of all sample components. A change in the mobile phase may result in the early

components being eluted at the solvent front (no retention) and may cause close eluting peaks to co-elute (no resolution). A change in the mobile phase in indicated when there is good retention of early eluting compounds (k' greater than 2) and good resolution between all sample components (resolution greater than 2).

Using a Column With Lower Surface Area

When a column that has a lower surface area is used, the effect is the same as using a stronger solvent system except that the solvent being pumped through the system does not have to be changed. If there is sufficient retention and resolution, this is a faster way to obtain a reduction in the time for analysis than changing the solvent system.

Using a Column With a Shorter Hydrocarbon Chain Length Bonded Phase

When a column that has a shorter hydrocarbon chain length is used, the effect is the same as when using a column that has a lower surface area.

Increasing the Temperature

An increase in the column temperature will reduce the time for analysis; however, an increase in temperature does not drastically affect the retention of early eluting peaks. The effect is shown in Figure 10. This is a plot of log k' vs.

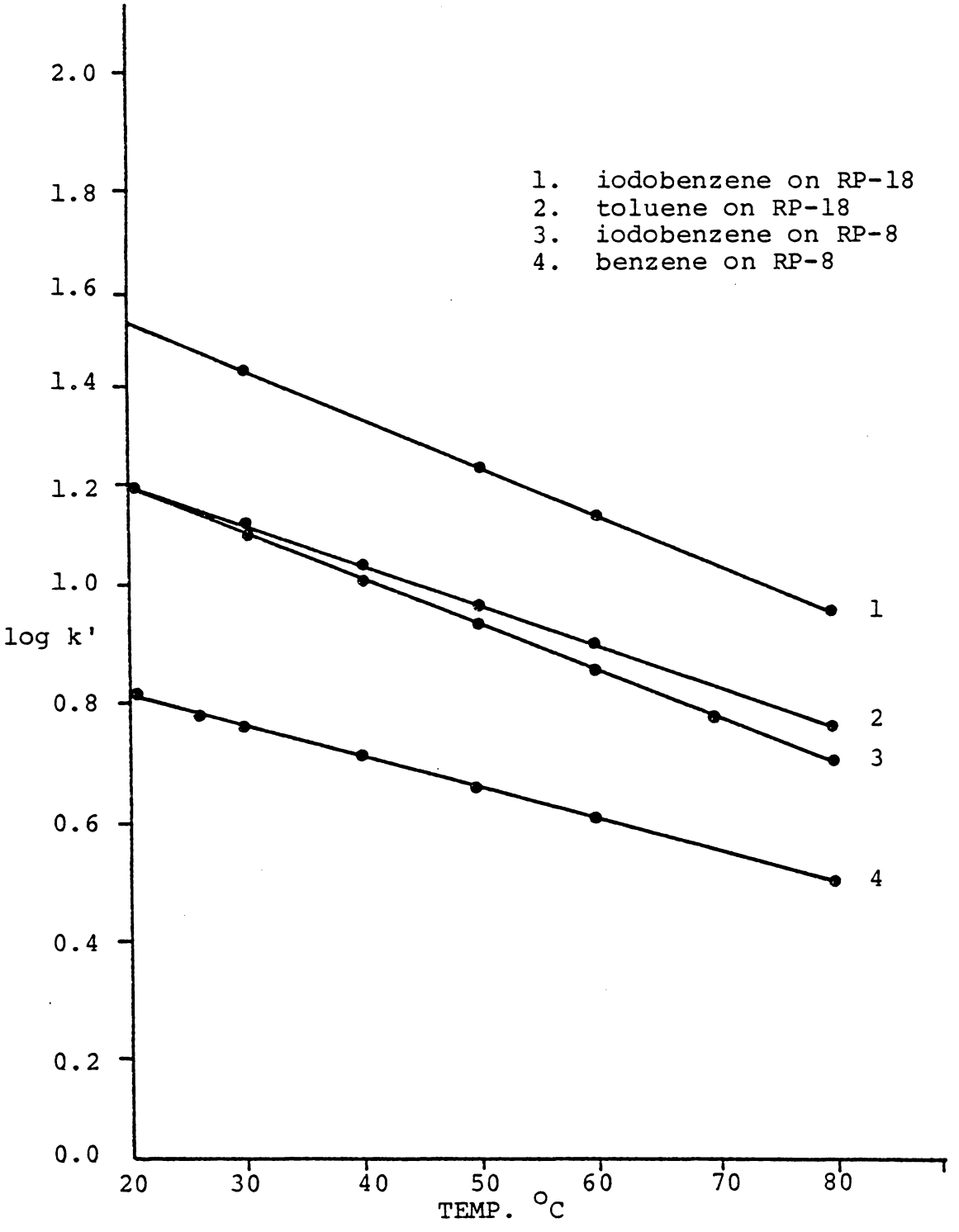

Figure 10. log k' vs. Temperature in °C for Probe Compounds Eluted on RP-8 and RP-18.

temperature. It is noted that the plots are straight lines with differing slopes. This indicates that the selectivity is also changing slightly as the temperature is increased. Figures 11 - 13 show the effect of temperature in reducing the time for an analysis. The time scale for each is 0 to 16 minutes. Note that as the temperature is increased the scope of the column also increases (more compounds can be eluted in the same amount of time). Figures 14,15 show the effect of hydrocarbon chain length. It should be noted that the change from an RP-18 column to an RP-8 column has a more pronounced effect on retention than the increase in temperature on the RP-18 column.

Scope of the Materials

The scope of the column packing materials used in this work was determined by creating a plot of k' for a series of compounds vs. the k' for the same series of compounds chromatographed on a standard column. The column chosen for the standard in the normal phase mode was SI-100 and the column chosen for the standard in the reverse phase mode was RP-8. Figures 16,17 show the results. Examination of the plots show that in the normal phase mode, SI-60 has the steepest slope indicating that it has the narrowest scope. That is, if a compound more polar than nitrobenzene was injected under the same conditions, it would have a large k', 10 or greater; however, if the same compound was injected on the RP-2 column

1. benzene
2. naphthalene
3. biphenyl
4. phenanthrene
5. anthracene

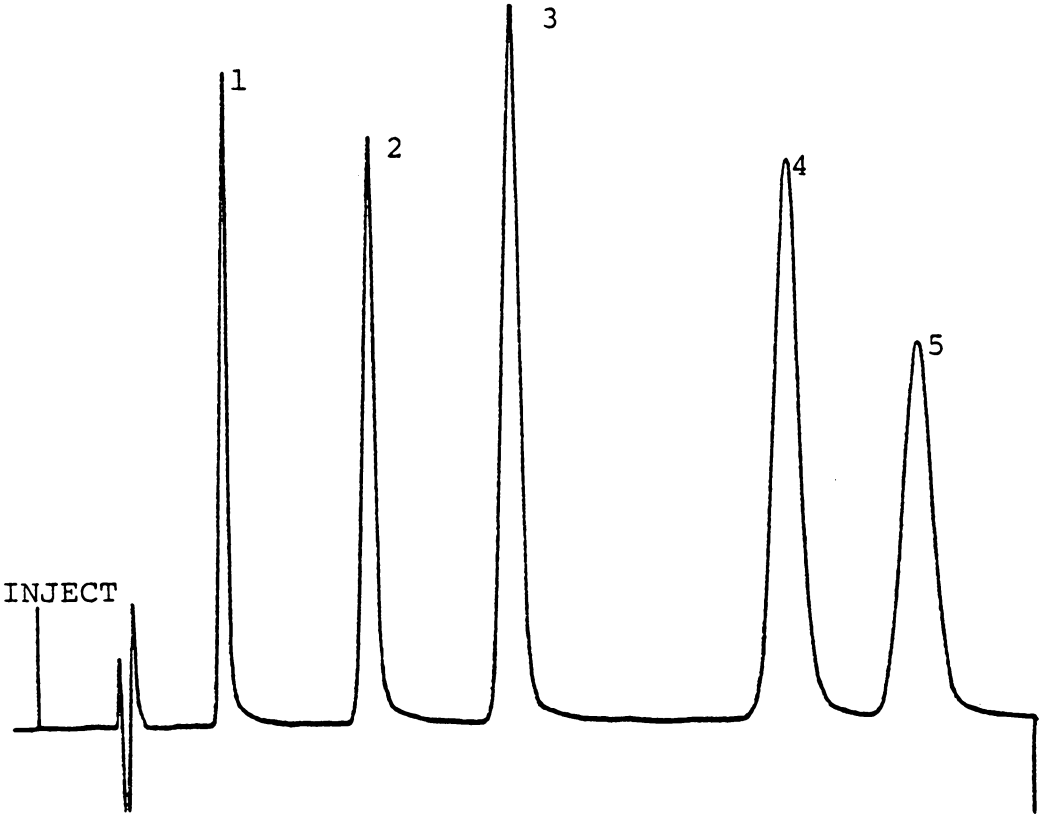

Figure 11. Chromatogram of Benzene, Naphthalene, Biphenyl, Phenanthrene, and Anthracene. Column: RP-18, #107; Mobile Phase: 75/25, Methanol/Water; Flow Rate: 2 ml/min.; Pressure: 1120 psig.; Temperature: 25°C.

1. benzene
2. naphthalene
3. biphenyl
4. phenanthrene
5. anthracene
6. pyrene

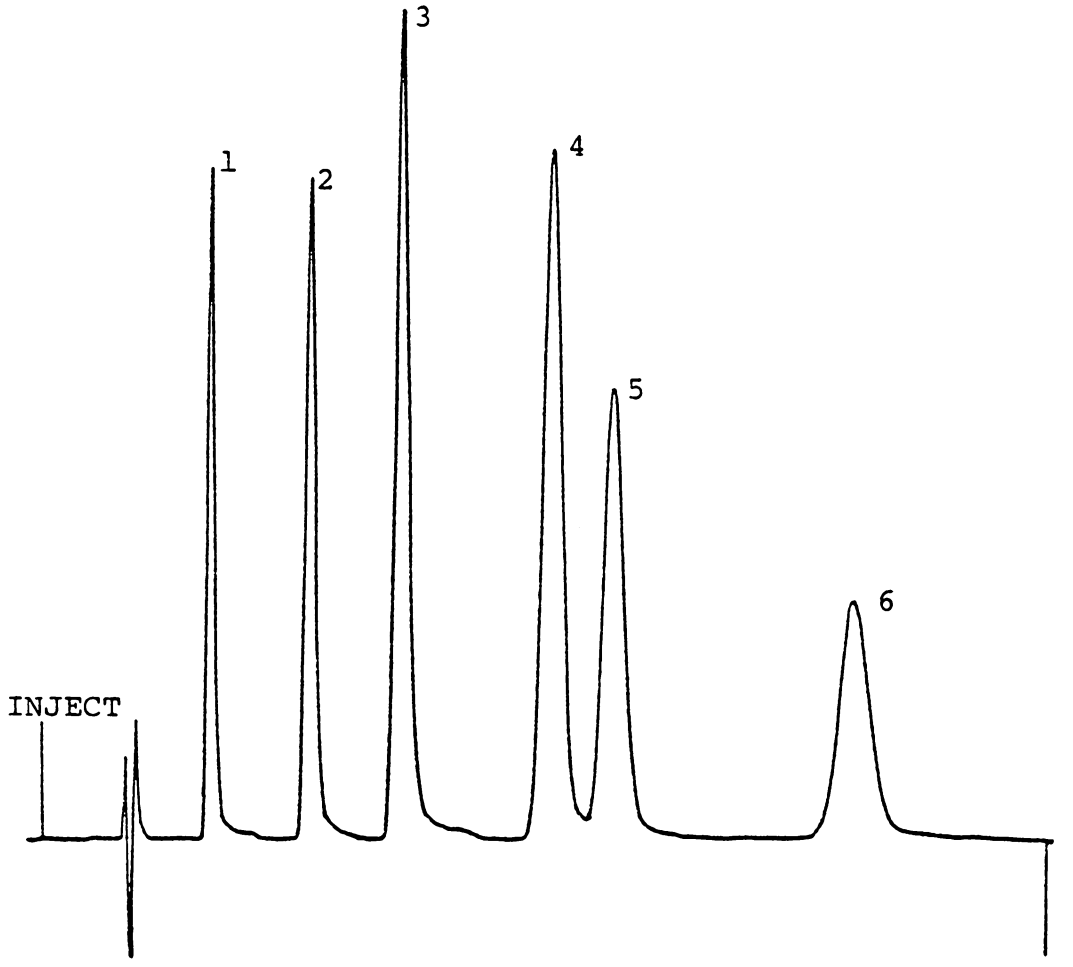

Figure 12. Chromatogram of Benzene, Naphthalene, Biphenyl, Phenanthrene, Anthracene, and Pyrene. Column: RP-18, #107; Mobile Phase; 75/25, Methanol/ Water; Flow Rate: 2 ml/min.; Pressure: 760 psig.; Temperature: 50°C.

1. benzene
2. naphthalene
3. biphenyl
4. phenanthrene
5. anthracene
6. pyrene
7. chrysene

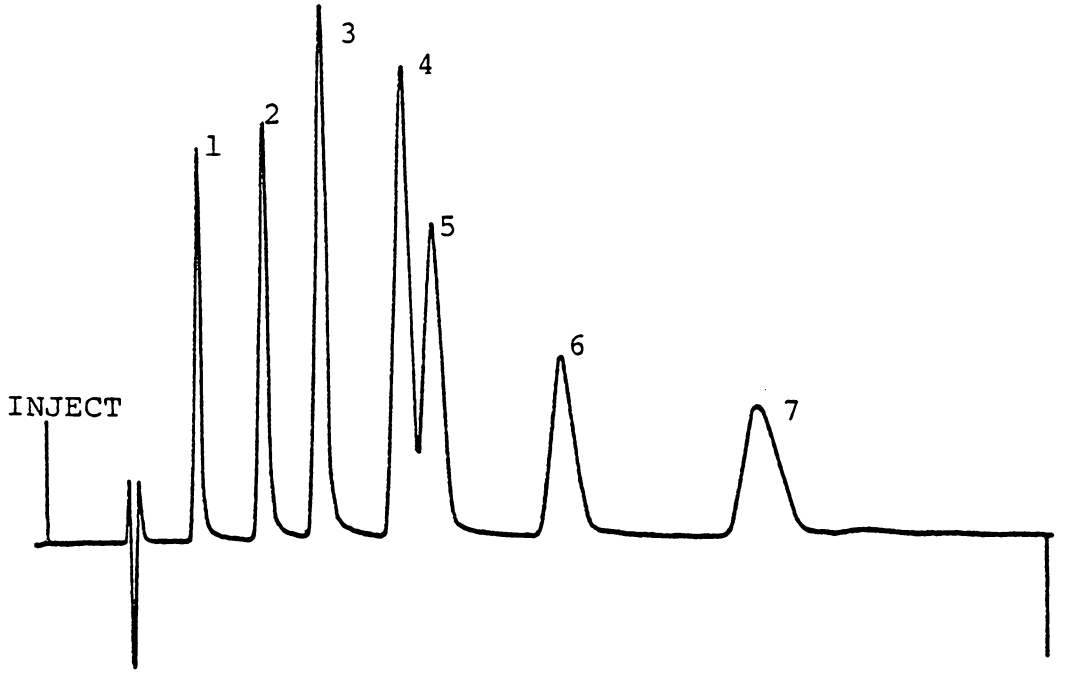

Figure 13. Chromatogram of Benzene, Naphthalene, Biphenyl Phenanthrene, Anthracene, Pyrene, and Chrysene. Column: RP-18, #107; Mobile Phase: 75/25, Methanol/Water; Flow Rate: 2 ml/min.; Pressure: 540 psig.; Temperature: 75^{o}C.

1. benzene
2. naphthalene
3. biphenyl
4. phenanthrene
5. anthracene
6. pyrene
7. chrysene
8. benzo(a)pyrene

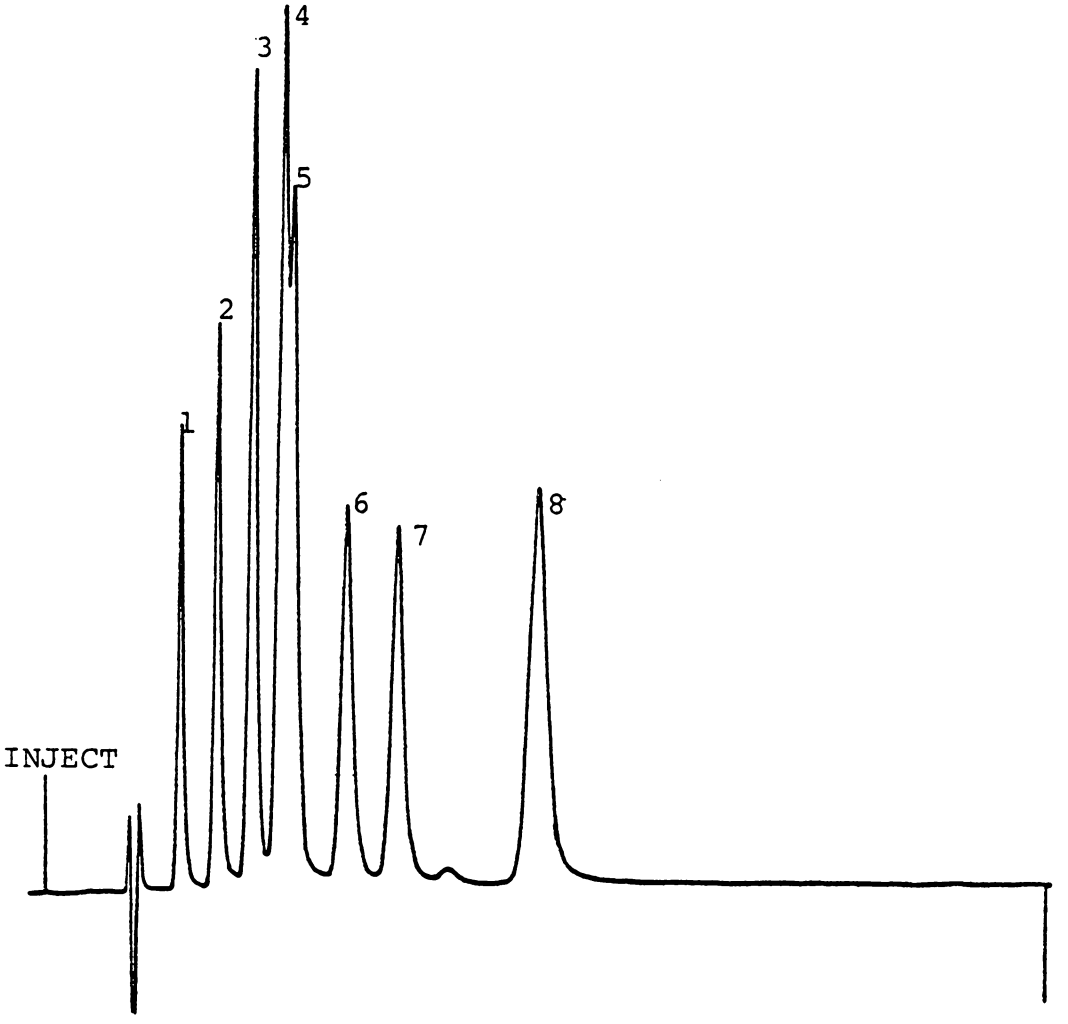

Figure 14. Chromatogram of Benzene, Naphthalene, Biphenyl, Phenanthrene, Anthracene, Pyrene, Chrysene, and Benzo(a)pyrene. Column: RP-8, #341; Mobile Phase: 75/25, Methanol/Water; Flow Rate: 2 ml/min.; Pressure 720 psig.; Temperature: 50°C.

1. benzene
2. naphthalene
3. biphenyl
4. phenanthrene
5. anthracene
6. pyrene
7. chrysene
8. benzo(a)pyrene

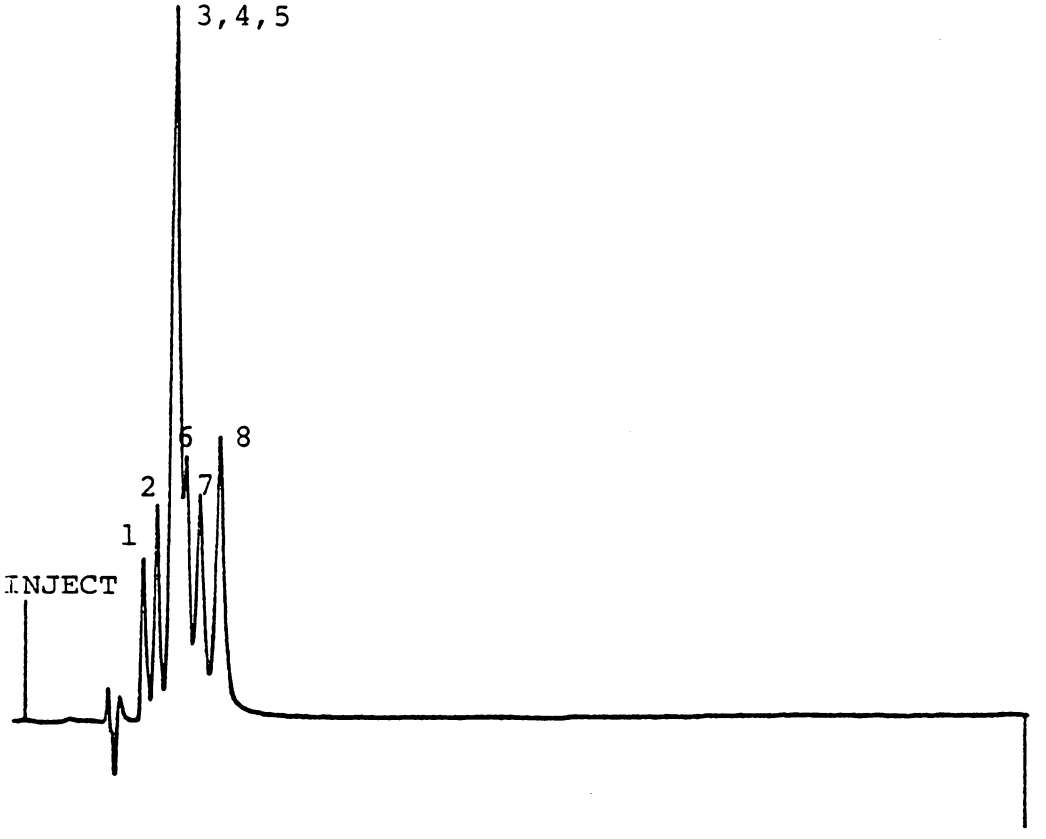

Figure 15. Chromatogram of Benzene, Naphthalene, Biphenyl, Phenanthrene, Anthracene, Pyrene, Chrysene, and Benzo(a)pyrene. Column: RP-2, #102; Mobile Phase: 75/25, Methanol/Water; Flow Rate: 2 ml/min.; Pressure: 840 psig.; Temperature: 50°C.

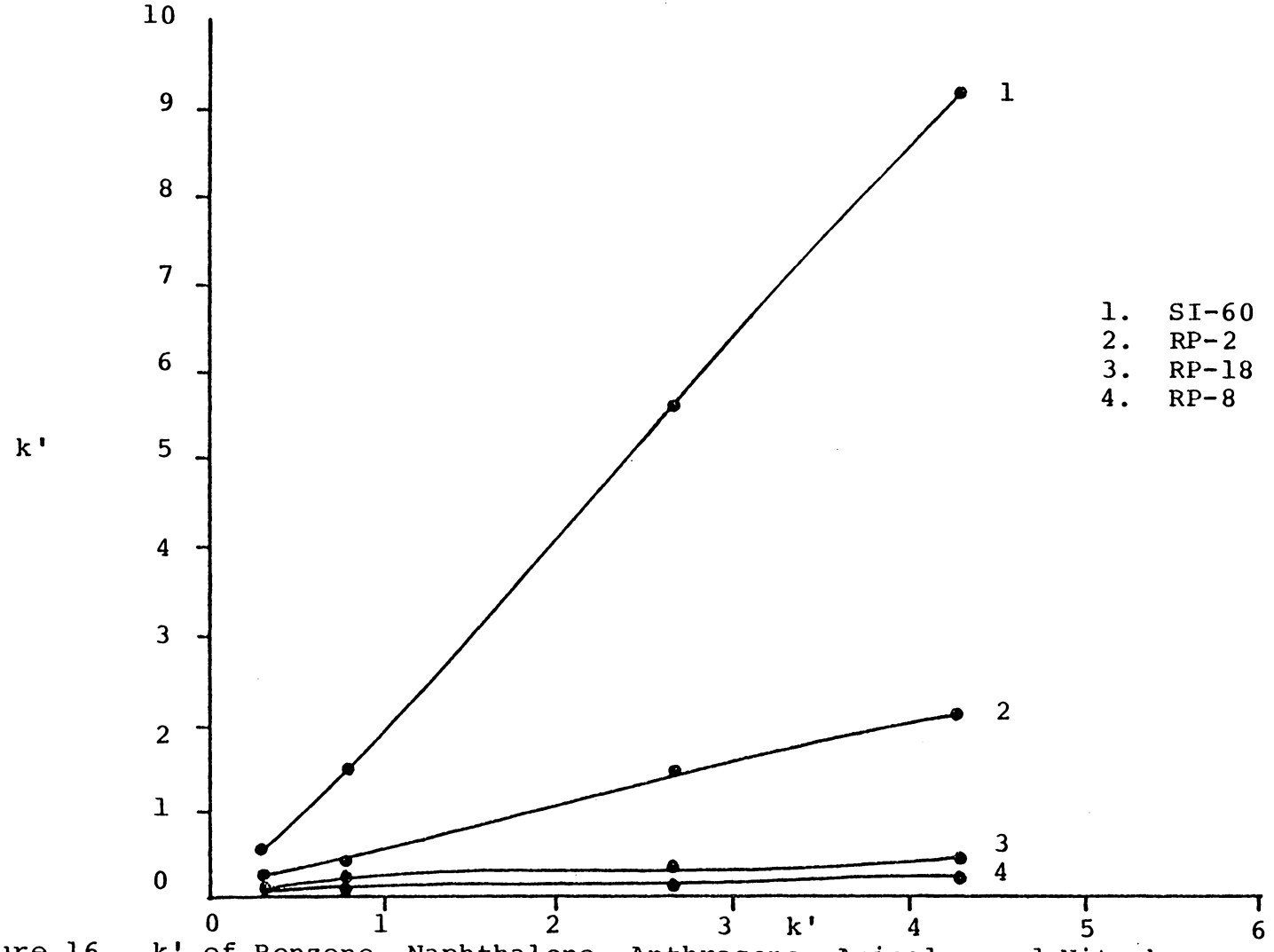

Figure 16. k' of Benzene, Naphthalene, Anthracene, Anisole, and Nitrobenzene on SI-60, RP-2, RP-8, and RP-18 vs. k' of the same compounds eluted on SI-100, Mobile Phase: 0.01% Acetonitrile in Hexane, Temperature: 25°C.

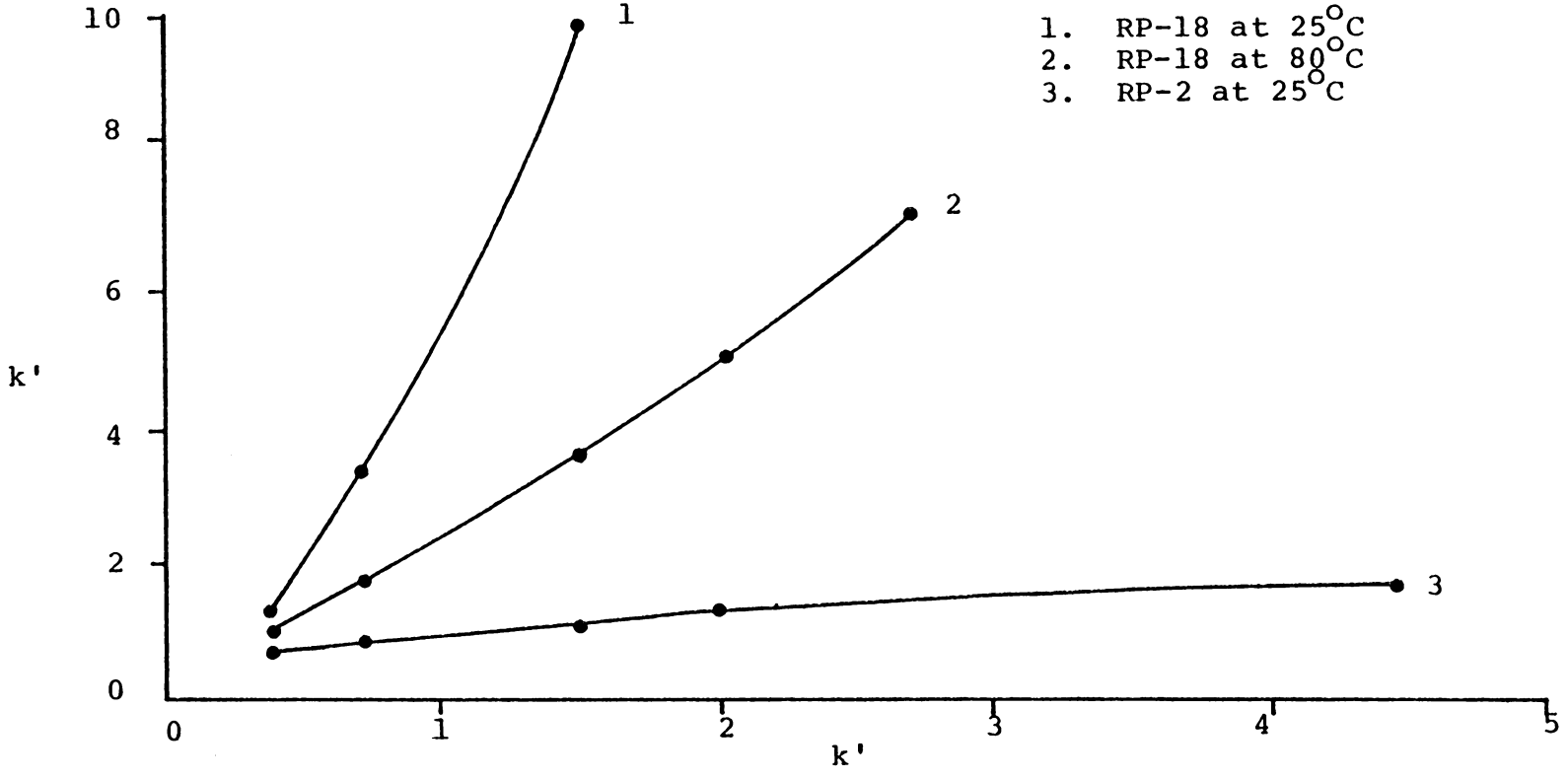

Figure 17. k' of Benzene, Naphthalene, Anthracene, Pyrene, Chrysene, and Benzo(a)pyrene on RP-2, RP-18 at 25°C and RP-18 at 80°C vs. k' of the same compounds Eluted on RP-8, Mobile Phase: 75/25 Methanol/Water, Temperature: 25°C.

it would have a k' value of only 2 or greater and if it were injected on the SI-100 column, it would have a k' value of 5 or greater.

In the reverse phase mode, it should be noted that the RP-18 column has the steepest slope indicating that it has the narrowest scope and RP-2, having the lowest slope, has the widest scope. It should also be noted that going from 25°C to 80°C on the RP-18 column, the slope is lower indicating that as the column temperature is increased, the scope of the column is also increased.

Conclusions

A Study was made involving the use of a series of HPLC columns packed with materials that differed in their surface area (same base material), their composition (silica vs. alumina vs. chemically modified silica), their hydrocarbon chain length (RP-2 vs. RP-8 vs. RP-18). These columns were evaluated in both the normal phase mode and the reverse phase mode. From this study it is concluded that:

1. RP-8 and RP-18 are not useful in the normal phase mode when using solvent systems that are more polar than hexane with a few percent of a more polar solvent such as dichloromethane.

2. RP-2 is a versatile column packing material which is useful in both the normal phase mode and the reverse phase mode. In the normal phase mode it behaves as a low surface area adsorption column and of the three adsorbents, (SI-60, SI-100 and RP-2), it has the widest scope. In the reverse phase mode it also has the widest scope of the three reverse phase columns studied (RP-2, RP-8 and RP-18).

3. Temperature effects in the normal phase mode evidently involve a complex mechanism for separation. The minima that were observed on ALOX T in the plots of k' vs. temperature

all occurred at 35°C. Minima have not been observed by any other workers. We propose that the minima may indicate a change in mechanism. Below 35°C the mechanism may involve both partition and adsorption and above 35°C adsorption may dominate. More work is indicated in this area to explain this phenomenon.

4. Retention on RP-8 and RP-18 in the reversed phase mode may involve partition rather than adsorption. The partition may be between the mobile phase and the small amount of modifier trapped in the bonded phase. More work is indicated in this area to explain this phenomenon.

5. Temperature is an important column performance parameter and should not be overlooked in the optimization and control of separations. It is also critical to control "ambient" temperature when performing quantitative analysis by HPLC when using high surface area adsorbents and bonded phases with long hydrocarbon chain lengths due to the fact that minor temperature changes when using these materials result in significant changes in retention with compounds that elute with a k'=2 or above.

6. Temperature affects selectivity in both normal phase and reversed phase LC and should be useful in the optimization of complex separation problems.

7. Temperature in the reverse phase mode has a significant effect on the viscosity of the mobile phase and allows the chromatographer to work at higher flow rates at higher temperatures without extremely high back pressure.

8. Closely related materials should be separated on the highest surface area material available (normal phase) or on the bonded phase having the longest hydrocarbon chain length (reverse phase).

9. Complex mixtures containing components that vary greatly in their polarity or solubility should be separated on a very low surface area material or a chemically modified silica packing material (normal phase) or a bonded phase having a short hydrocarbon chain length (reverse phase).

10. The use of higher temperature in general increases the scope of the packing material, allowing the chromatographer to expand the useful range of the column packing material when performing isocratic separations.

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SELECTIVITY AND TEMPERATURE EFFECTS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by

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(ABSTRACT)

A study of six liquid chromatographic column packing materials was conducted. The materials investigated were: SI-60, SI-100, ALOX T, RP-2, RP-8, and RP-18 (E. M. Laboratories, Elmsford, N.Y.). The column packing materials had an average particle diameter of 10 μm and were slurry packed into stainless steel columns that were 3 mm i.d. and 250 mm in length.

Two series of probe compounds were utilized to investigate the differences in selectivity exhibited by the column packing materials in the normal phase mode and in the reversed phase mode. A column oven which controlled temperature to within $\pm$ 0.2 ^{o}C over the range of 0^{o}C to 100^{o}C was utilized throughout the work. Four different mobile phases were used, two for the normal phase work and two for the reversed phase work.

The effect of temperature was investigated in both the normal phase mode (20^{o}C - 50^{o}C) and in the reversed phase mode (20^{o}C - 80^{o}C).

The results of the selectivity study indicate that the column temperature can have a pronounced effect in normal phase liquid chromatography. As temperature increased, k'

values went through a minimum in some cases, indicating a change in retention mechanism. As the temperature is increased, the retention of the compounds may be increased, decreased, or remain constant. RP-2 is a versatile column packing material which is useful in both the normal phase mode and the reversed phase mode. In the normal phase mode it behaves as a low surface area adsorption column and of the three adsorbents, (SI-60, SI-100, and RP-2), it has the widest scope. In the reversed phase mode it also has the widest scope of the three reverse phase columns studied (RP-2, RP-8, and RP-18). In reversed phase liquid chromatography, increases in retention as temperature was increased were not observed and plots of log k' vs. temperature in ^{o}C resulted in straight lines with very similiar slopes. Column temperature is however an important operating parameter which is useful in optimizing separations.

Temperature was found to have little effect on the efficiency of the columns but was found to have a significant effect on the scope of the column packing materials in both the normal phase mode and in the reversed phase mode. When utilizing high surface area column packing materials or column packing materials with a high percentage of hydrocarbon bonded phase, temperature control is essential in order to obtain the best reproducibility.