SYNTHESIS AND CHARACTERIZATION OF A t-OCTYLCALIX[5]ARENE DERIVATIZED CAPILLARY COLUMN FOR GAS CHROMATOGRAPHY

by

M. KATHLEEN LESLIE CRIPE

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Synthesis and Characterization of a
t-Octylcalix[5]arene Derivatized Capillary
Column for Gas Chromatography

M. KATHLEEN LESLIE CRIPE

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M. Kathleen Leslie Cripe — 6/1/98
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Approvals:

[signature] — 6/1/98
Thesis Advisor — Date

Renee L. Falconer — 5/29/98
Committee Member — Date

Sherri R. Lovelace-Cameron — 6/1/98
Committee Member — Date

[signature] — 6/5/98
Dean of Graduate Studies — Date

ABSTRACT

A fused-silica capillary column was statically coated with the calixarene oligomer p-tert-octyl calix[5]arene (derived from p-tert-butylphenol), using the static method. A thin layer of the calixarene was deposited on the surface of a pure non-polar stationary phase (OV-1) fused-silica capillary column. Retention times were plotted against the number of carbon atoms in a homologous series of alkyl benzenes. Deviations from linearity indicated possible specific interactions. Previous similar studies involving calix[4]arenes showed a direct relationship between steric arrangement of the analyte molecules and their retention behavior suggesting an important contribution of inclusion into calixarene cavities. Investigations into possible host-guest interactions between the newly synthesized stationary phase with various analytes was studied by employing quantitative structure-retention relationships (QSRR). Band broadening (due to slow mass transfer) indicated inclusion complexes were formed. They also show a correlation toward analytes containing π-electron donor aromatic systems. A relationship between the shape of the analyte molecule and it's retention behavior was also determined.

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DEDICATION

This work is dedicated to my husband, Jay, whose never-ending patience, caring, and understanding has allowed me to stay focused throughout these years. Without your understanding and encouragement, this project would not have come to fruition.

To my parents, Jim and Marilyn Leslie, for instilling in me the importance of education and guiding and supporting me towards the achievement of my goals.

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To my brother, Jimmy, whose humor always helped me through the hard times.

I've learned from you all, I admire and love you.

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LIST OF ABBREVIATIONS

α	Alpha
W	Base width of peak
β	Beta
k	Capacity factor
cm	Centimeter
CSP	Chiral Stationary Phase
CD	Cyclodextrin
t_m	Dead time
ECD	Electron Capture Detector
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
γ	Gamma
GC	Gas Chromatography
GLC	Gas-Liquid Cromatography
GSC	Gas-Solid Cromatography
H	Height equivalent to theoretical plate
i.d.	Internal diameter
L	Length of column
u	Linear Flow Rate
μL	Microliter
μm	Micrometer

mg	Milligram
mL	Milliliter
m	Millimeter
min	Minute
π	Pi
PTFE	Polytetrafluroethylene
psi	Pounds per square inch
ptBC4A	p-tert-butylcalix[4]arene
QSRR	Quantitative Structure-Retention Relationship
RHS	Reference Homologous Series
t_r	Retention time
s	Standard deviation
TCD	Thermal Conductivity Detector
TED	Thermionic Emission Detector

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CHAPTER I

INTRODUCTION

A. Theory of Gas Liquid Chromatography

Gas chromatography (GC) was first developed in 1951 by Cremer (1). This technique, like all forms of chromatography, involves separating substances by partitioning between a stationary phase and a mobile phase. GC is characterized by the use of a gaseous mobile phase and a solid or liquid stationary phase. Solid phases include substances such as silica or alumina that are highly surface active. Liquid phases are solid phases that are modified by the liquid. The liquid is retained on the surface of the solid by either adsorption or chemical bonding.

There are two types of columns containing stationary phase supports utilized in gas chromatography: packed columns and open tubular, or capillary, columns. Packed columns are made from metal or glass tubing ranging in lengths from 3-20 ft, and are coiled for compactness. They are typically filled with surface active powders that are coated with a liquid to form the stationary phase. Open tubular columns are made from various lengths of a narrow bore (0.1 to 0.4 mm i.d.) tubing made of fused silica or glass. In capillary columns, a thin layer of a liquid stationary phase is coated on the inside surface, where it is held by adsorption. Capillary columns tend to give more efficient and faster separations than packed columns (2).

There are several advantages associated with the use of gas chromatography versus other types of chromatography. First, the use of low viscosity gases allows for

longer columns, which makes better and more efficient separations attainable. Secondly, inert gases do not interact with the solute, allowing for the separation of volatile substances (3). Finally, numerous detectors are available for fast, and accurate measurements of the sample components.

Common low viscosity mobile phase gases include helium, nitrogen, and argon. The inlet gas pressure needed to sustain a constant flow through the column depends on the dimension of the column and the nature of the stationary phase within the column, as well as the gas viscosity.

The distribution of the component between the mobile phase and the stationary phase determines the retention time, t_r, or the time it takes an individual component to travel through the column. A solute that partitions preferentially into the stationary phase is more retained than one that remains in the mobile phase. The time that it takes for an unretained solute (i.e. one that stays in the mobile phase only) to transverse the column is known as the dead time, t_m.

Liquid samples are commonly injected with a syringe through a rubber septum into a small heated chamber attached at the beginning of the column. The sample must enter the column very quickly and be deposited as a thin "plug" at the top of the column. In order to vaporize the entire sample simultaneously, the temperature of the chamber must exceed the boiling points of all the sample components. If not, the sample may not separate and the column may be blocked or contaminated. If the temperature is too high, however, it may cause significant sample decomposition (4).

By changing the temperature, the flow rate, and or the nature of the stationary phases, the retention times of the sample components may be varied. Unlike liquid

chromatography, the mobile phase in GC has little interaction with the solute. It's function is primarily as a carrier. Separation may be made isothermally or by a particular ramping rate by starting at a lower temperature and raising it to a higher temperature at a specified rate. Figure 1 shows a block diagram of the basic components of a gas chromatograph.

There are a variety of detectors available for GC, including thermal conductivity (TCD), thermionic emission (TED), flame photometric (FPD) and electron capture (ECD) detectors. The simplest, and often least expensive of the available detectors is the flame ionization detector (FID). Figure 2 shows a cross-sectional view of a typical FID. The FID is considered to be a nonselective detector and is capable of detecting a wide variety of substances. The FID works by burning the column effluent in a hydrogen-air flame and measuring the current produced from ions formed during combustion. Two characteristics make this detector useful: excellent sensitivity, and relative insensitivity to small temperature changes of the mobile phase or detector chamber. One drawback to the FID is that it is a destructive technique and cannot be used in situations where the separated components need to be saved (5).

B. Coating Capillary GC Columns

There are three common methods used for coating capillary columns: the dynamic method, the static method, and the wall-immobilized method. The dynamic method involves taking a liquid and forcing it through a capillary, which leaves behind a

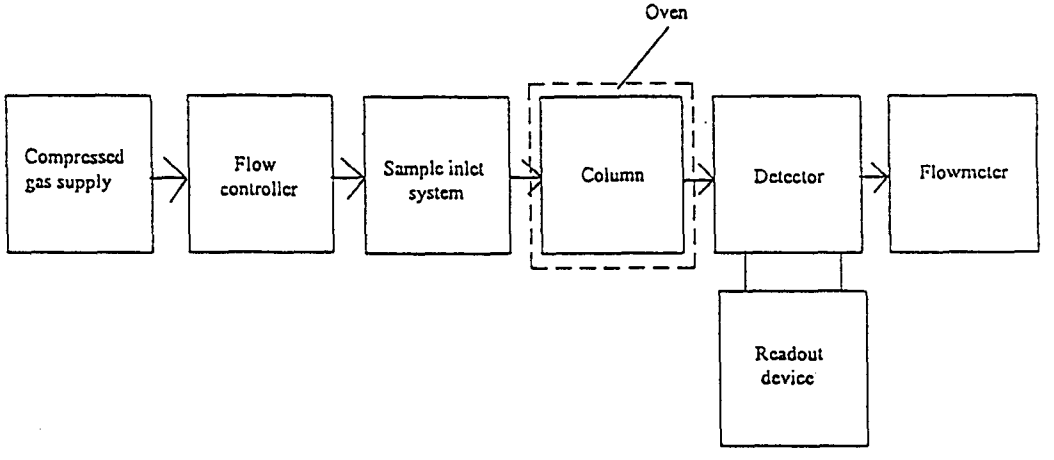


Figure 1. Block diagram of a Gas Chromatograph

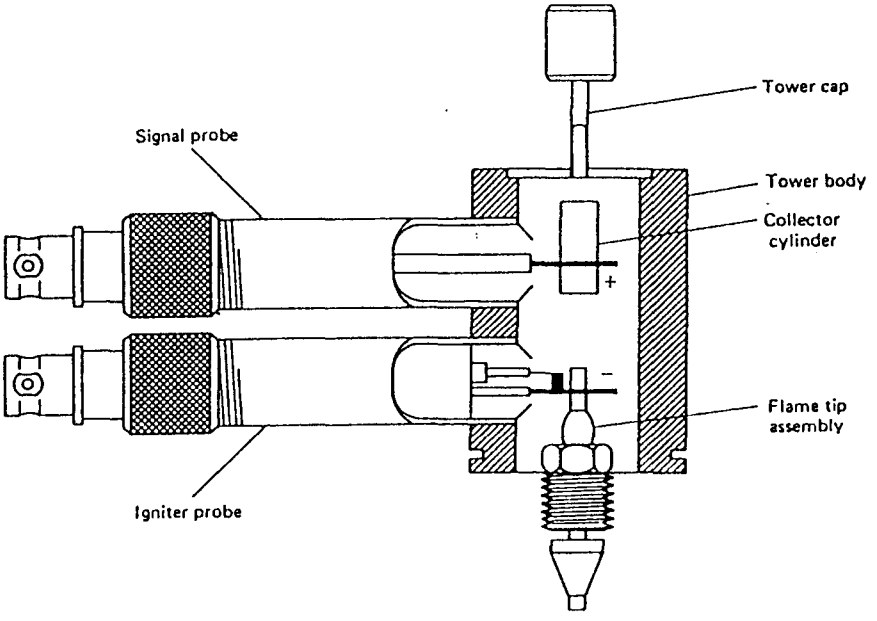


Figure 2. Cross-sectional view of a flame ionization detector (6).

thin film on the capillary wall. A plug of mercury forced through the column will reduce the thickness of the film left behind the coating solution by roughly one half (7). The concentration of the solution is used as the primary parameter for determining the film thickness. This technique typically gives good results for non-polar stationary phases in capillary columns (8).

The wall-immobilized method involves taking the stationary phase and converting it to an alkene derivative followed by permethylation. It is generally coupled with a polymer to produce a new viscous stationary phase that can be coated onto the inside wall of a capillary column. This method results in an extremely rugged and stable column that can be used at much higher temperatures than other types of columns. Once the column has been coated, the stationary phase cannot be removed or stripped from the capillary wall.

These characteristics are good for separation of chiral compounds. Chiral selectivity is achieved by specific host-guest interactions on the stationary phase. In this type of column the chiral selectors are linked, through a single attachment, along the length of an organosilane polymer. This polymer is subsequently immobilized on the capillary wall (9). The major drawback to this method is the special equipment needed to coat the column.

The static method, which was first developed in 1958 (10), involves completely filling a column with a dilute solution of the stationary phase, closing the column at one end, and evaporating the solvent under reduced pressure to leave a coating of the stationary phase inside the column. There are three primary features of static coating. First, the stationary phase used to cover the internal column wall fills the column from

one end to another completely without any breakthrough segments. This provides a straightforward quantitative basis for measurement of the film thickness, provided the concentration of the stationary phase component in the filling solution is known.

Secondly, the solvent is removed under vacuum causing the meniscus to travel from one end to the other. With this process the stationary phase experiences no motion, hence the term, "static". Since the stationary phase material is directly deposited as the final coating it is largely unaffected by gas flow or solvent vapor. Conversely, columns which have been dynamically coated may be severely affected by these influences due to the low viscosity of the stationary phase.

There are several reasons why static coating is often better then dynamic coating. First, static coating can be applied to any stationary phase, provided it can be dissolved in an appropriate solvent. Second, there are no obvious limits to solution viscosity, allowing for great diversity in phase coatings; even high-molecular weight polymers can be used as stationary phases. Third, static coating provides the most uniform coating result, making the film thickness of the coating very predictable. This allows for mass production of columns with known characteristics.

One weakness in the static method is the demanding nature of the technique. However, like any other laboratory technique, once mastered, it becomes easier. In 1968, Bouche and Verzele (11) standardized the method with the following procedure. First, the stationary phase was dissolved in a precisely weighed amount of dichloromethane. A small pressure was applied to the column followed by sealing one end of the column using a solution of sodium silicate. When temperature equilibrium was attained, it was connected to a vacuum line, evacuated, and finally flushed with an inert gas.

Once the column has been coated, it is important to determine certain characteristics of the column. Two standard solutions, the Grob mixture and the Polarity mixture, give excellent information for comparison of various physical properties of the column being tested (12) such as, adsorption of hydroxyl functions, adsorption of aldehyde functions, separation efficiency, acid-base behavior and film thickness. This basic information is needed to evaluate the separation efficiency of a column and to determine specific purposes to which the column may be applied.

C. Calixarenes

Calixarenes are a class of macrocyclic molecules discovered in the 1940's by Nieder and McCoy (13). They are composed of phenolic monomer units and range in size from 4-8 units that are linked by methylene bridges to create basket-shaped molecules (Figure 3). The general formula, calix[n]arene, is used with *n* being the number of monomer units. The basket-shape is thought to give the molecule special inclusion properties due to it's ability to form complexes with certain guest molecules, depending on the substituent on the calix[n]arene.

Calixarene derivatives are formed from the starting p-tertbutylphenol molecule by condensing formaldehyde onto the para-substituted phenol in the presence of a base. The condensation of p-tert-butylphenol and formaldehyde has been shown by Gutsche and coworkers (14) to yield the t-butylcalix[4]arene, the t-butylcalix[6]arene, and the

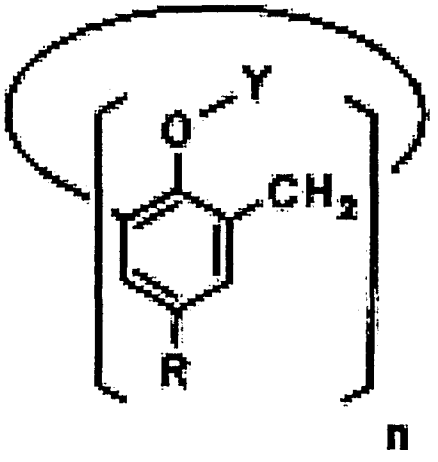


Figure 3. Basic calixarene structure where n= 4,5,6,7 or 8.

t-butylcalix[8]arene. Small amounts of the t-butylcalix[5]arene were also recovered. Figure 4 illustrates the calix[5]arene structure.

Calixarenes have several desired physical properties for use as stationary phases in GC. They tend to have high melting points, low solubility in water, high solubility in most organic solvents such as chloroform, benzene, and toluene, and are stable at a relatively large range of temperatures. They are also relatively nontoxic. Crystalline structures of calixarenes show that they contain strong intramolecular H-bonds. One of the most unique properties of calixarenes is their ability to form inclusion complexes with several organic molecules. The degree of complexation appears to be dependent upon the functional group attached to the calixarene. Molecular complexes with neutral molecules and metallic cations have been found both in solid and liquid states (15).

The first study done on inclusion complexes for chromatography were performed using cyclodextrins, which have a molecular structure and inclusion characteristics similar to the calixarenes (16). Derivatized cyclodextrins were coated directly on to the inside wall of a capillary column using the static method. These experiments showed that the fully pentylated α-cyclodextrin could be used to reverse the retention order of the analytes being separated relative to a standard GC column (21). This was very useful for determining optical impurities since the less concentrated isomer would elute first avoiding the problem of quantifying a shoulder peak.

Results of several early cyclodextrin studies indicated that the cyclodextrin stationary phases demonstrated enantioselectivity toward carbohydrates and nitrogen containing compounds. When both the stationary phase and solutes were chiral, separation of the solute enantiomers could occur. Both hydrophilic and hydrophobic

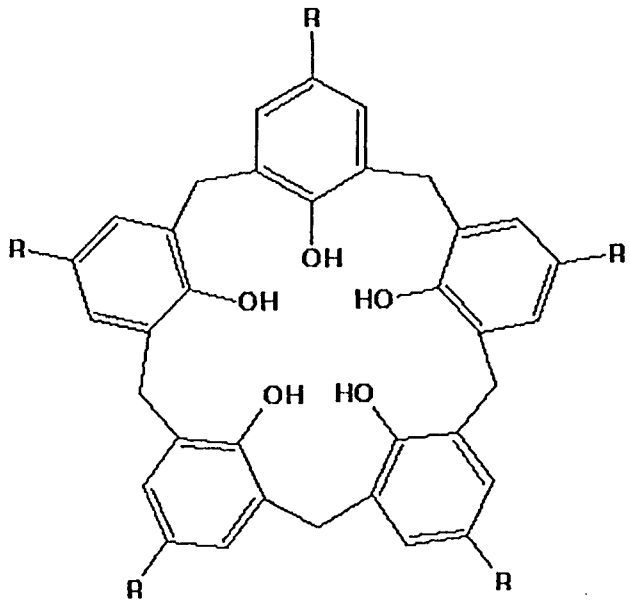


Figure 4. Calix[5]arene structure.

carbohydrate derivatives formed highly selective inclusion complexes. It was also determined that polar solutes showed an opposite enantioselectivity to non-polar alkyl derivatives (17, 18).

The first use of calixarenes in GC capillary columns was in 1982 (19). The static method was employed to coat the column so inclusion properties could be studied based on low temperature deposition of the calixarenes on the surface of a column. Based on this study a direct relationship was determined between the steric arrangement of the analyte molecule and it's retention behavior, pointing to the importance of inclusion into the calixarene cavity. Other studies have since been with calixarenes in GC columns using derivatives of either the calix[4]arene or the calix[6]arene.

D. Host-Guest Interactions

Because of their structure, calixarenes and modified calixarenes present a cavity into which a guest molecule can be included. The dimension of the cavity depends on the functional groups attached to the calixarene. Molecular complexes with neutral molecules and metallic cations have been found both in solid and liquid states.

Crystal structure analysis has been used to show the intramolecular H-bonds and intermolecular interactions involved with metal cations and organic molecules. A detailed description of the dynamic structure of calixarenes appears to be of fundamental importance in order to understand the binding forces by which hosts and guests are held together. One factor is the number of phenolic units present and the nature of the substituents on the ring. Another is the ability of the hydroxyl groups to bind alkali metal

cations and form highly structured complexes. According to Vicens and Bohmer (15), in order for calixarenes to complex, hosts must have binding sites which cooperatively contact the guests. They found the more highly organized hosts and guests are for binding and low solvation prior to their complexation, the more stable will be their complexes.

In order to determine if a host-guest complex formed, quantitative structure-retention relationship (QSRR) methods are employed. QSRR investigates the general selectivity of GC stationary phases towards analytes (21). Plotting the retention data versus various molecular descriptors such as, boiling points, molar volumes, polarizabilities and dipole moments in the underivatized column should give a linear relationship. After the column is derivatized a deviation in linearity indicates the presence of host-guest interactions, due to the fact that the inclusion complex will cause band broadening.

CHAPTER II

LITERATURE REVIEW

Carbohydrates have been repeatedly used as chiral selectors in both liquid and gas chromatography. The class of carbohydrate macrocycles known as cyclodextrins have been widely studied due to their interesting inclusion properties both in the solid state and in aqueous solution (21). Cyclodextrins are cyclic oligosaccharides composed of D(+)-glucopyranose units interconnected by $\alpha-$ (1,4) bonds. It is believed that the cyclodextrin cavity forms inclusion complexes with various types of metallic cations as well as with molecules.

Calixarenes are a class of macrocyclic molecules, discovered in the 1940's (13), made up of phenolic units meta-linked by methylene bridges and possessing basket-shaped cavities. Calixarenes act as cyclodextrin analogues. Most of these compounds are able to form inclusion complexes with several organic guest molecules. According to previous studies (22) their inclusion properties are based on the guest molecules, which enter the cavity and are inferred from crystalline data. They are strongly dependent on the size of the macrocycle and on the nature of the substituent.

Properties of calixarenes have been thoroughly investigated. The outcomes of these studies have led us to the conclusion that calixarenes generally have high melting temperatures, have low solubility in water, but will dissolve in most organic solvents. They are very stable, non-toxic molecules, with strong intramolecular H-bonds. Because of their cyclic structure, they can be modified to present a cavity in

which a substrate molecule can be included. It is believed that this characteristic depends on the functional group attached (15) to the phenolic hydroxyls at the narrow edge of the calixarene.

Because of these properties and characteristics, chemists over the past few years have begun to study cyclodextrins and calixarenes in order to develop various types of stationary phases for both liquid and gas chromatography. The development of gas chromatographic capillary columns involved two basic types, coated varieties and most recently used wall-immobilized types.

The first commercial chiral stationary phase (CSP) for gas chromatography based on cyclodextrins was a polysiloxane-L-valine-tert-butylamide copolymer, which was coated onto glass capillaries. The brand name was Chirasil-Val, and it proved effective for numerous enantiomeric separations. However, limitations included not being widely applicable since most of the separations made were of racemic amino acid derivatives. At the high temperatures needed for GC (~200°C), the column led to racemization of the packing and decomposition and bleeding of the CSP. As a result, enantioselectivity decreased significantly at higher temperatures. For these reasons and others, chemists further studied alternatives for stationary phases. Li (23) subsequently produced a wall-immobilized version of Chirasil-Val.

A number of cyclodextrins have been used in stationary phase coatings for capillary gas-liquid chromatography (GLC). Two different approaches have been studied. The first involves extensive derivatization of the cyclodextrin with one or more hydrophobic and /or polar substituents, producing a gelatinous material, which can be coated directly on the inside wall of the capillary. Konig et al. (24) employed this

method when they prepared several derivatives of the cyclodextrins and coated them, using to the static method, onto Pyrex® glass capillary columns. They found the fully pentylated α-cyclodextrin showed a remarkable enantioselectivity towards the enantiomers of carbohydrate derivatives and some nitrogen compounds. Also, the alkylation of the cyclodextrins appeared to increase their hydrophobicity, enabling their use as liquid phases for capillary GC. The thermal stability of the perpentylated α-cyclodextrin was remarkably with no deterioration of column performance, even at temperatures above 200°C. This study also showed a correlation between the chiral selector and the chiral substrate.

In the 1980's a wide variety of CSP's were developed for liquid chromatography. Some of these CSP's were based on naturally occurring molecules such as proteins and cellulose (25), while others developed by Armstrong and Li (26) used synthetic molecules. One of the advantages of these synthetic molecules was that enantiomeric modification could be used to reverse retention order in order to confirm separations. Their new chiral GC stationary phases consisted of hydrophilic and hydrophobic derivatives of cyclodextrins, which were found to be highly selective forming inclusion complexes with vaporized solutes. This led them to suggest that polarity had an opposite enantioselectivity to nonpolar alkyl derivatives.

The permethyl derivatives of cyclodextrin were made in two steps, after which the capillaries were coated via the static method. A study of permethyl derivatives indicated it was possible to reverse the order in which the analytes eluted, using enantiomeric modification. This was useful when determining optical purities in

which one enantiomer was in excess. It was preferable to have the less concentrated isomer elute first since the other one usually produced a large trailing peak.

The second approach for making chiral capillary columns for gas chromatography involves taking simple methoxy-functionalized cyclodextrins and dissolving them in an appropriate GC liquid stationary phase (i.e. polysiloxane or polyethylene glycol). Schurig and Nowotny (27) demonstrated this type of column by using permethylated β-cyclodextrin to resolve a number of volatile racemic solutes on high resolution glass open-tubular columns. Their coating consisted of the permethylated β-cyclodextrin and a specific amount of OV-1701 in n-pentane and dichloromethane. Their coating proved to have several advantages that included improved chemical and thermal stability, formation of molecular inclusion compounds, and increased hydrophobicity at the entrance of the chiral activity site. It therefore provided a new versatile stationary phase that improved the enantiomeric separation of different classes of compounds.

In the beginning, most of the work on CSP's for GC used amino acids, peptides, and various derivatives. Armstrong, et al. (23) developed a new class of hydrophilic, relatively polar CD derivatives that could be used as selective chiral stationary phases. The permethylation was performed using methyl iodide after dissolution in dichloromethane. Capillaries were coated via the static method. These columns are now commercially available from Advanced Separation Technologies (Whippany, New Jersey). Their work gave the first comparison of α-,β -, and γ - cyclodextrin derivatives as GC stationary phases for resolution of racemic compounds. The study showed that a cyclodextrin, or theoretically any chiral molecule, should be able to

interact enantioselectivity with molecules adsorbed on its exterior surface or at the top or bottom of the torous. When strong inclusion complexes were formed, there often was band broadening due to slow mass transfer on the stationary phases.

Another more recent study done on cyclodextrin derivatives by Bicchi, et al.(28) found a significant difference between using cyclodextrin derivatives alone or diluted in polysiloxane. Diluted phases with different polarities produced different interactions and, as a consequence, had different retention times for components in complex mixtures. This made it possible to identify enantiomers of an optically active compound. They concluded that the difference in retention produced by differing polarity is more significant than that produced by different CD derivatives diluted with the same stationary phase. In addition, the latter can produce inversion of the elution order, while a CD derivative with different polarity phases does not produce this effect.

Although coated columns have been extremely useful as CSP's for GC, when they are subjected to extreme temperatures, extreme flows or to large injection volumes, the integrity of the coating may change and efficiency can decline. In addition, they generally have shorter lifetimes. More recent studies have used wall-immobilized GC capillaries. Benecke and Schomburg (9) prepared the first CD wall-immobilized GC column by immobilizing a polymer using free radical generating agents. Soon after Konig et al. (24) reported alkylated derivatives of β-cyclodextrin, and Schurig et al. (27) dissolved methylated cyclodextrins in silicon oil to produce effective CSP's. Shurig later made a polymer of permethyl- β-cyclodextrin and dimethylpolysiloxane (Chirasil-Dex) which was thermally immobilized.

The wall-immobilized method appears to be favored due to the fact that immobilized chiral stationary phases are often more rugged and stable than the coated columns. This enhances the column lifetime, and the temperature limits on the GC in which it can be run.

Armstrong and associates (23) used the immobilization method in examining the GC enantioseparation of a large number of chiral compounds. Three different wall-immobilized columns were constructed. Their results led them to identify host-guest interactions via complex formation due to chiral selectivities. They concluded that immobilized CD columns appear to be more stable and more useful in resolving larger, bulky chiral compounds and high boiling compounds which contain strong H-bonding groups, such as diols. In a later study Armstrong (29) reported using a simple immobilized cyclodextrin-based stationary phase for fused silica capillaries, which were hardier. At lower temperatures, the lifetime of the coated columns sometimes exceeded that of the bonded-phase columns, but longevity and performance at high temperatures was most effective on the immobilized column.

Since calixarenes are structurally, and functionally analogous to cyclodextrins, it was an obvious progression to study the inclusion properties of calixarenes in gas chromatography. The first experiment of this type was done in 1982 at Charles University in Prague. Mnuk and Feltl (30) studied the inclusion properties of calixarenes, by doing a low temperature deposition of calixarenes onto the surface of a suitable support (19). The stationary phase was prepared by dissolving ptBC4A in dichloromethane and transferring it to a column containing pure silanized Chromosorb W. The retention behavior of a homologous series of toluene, ethylbenzene, n-

propylbenzene, and n-butylbenzene on ptBC4A indicated a strong influence of the phenolic hydroxyls in the calixarenes on the overall interaction mechanism for the sorbates. Quantitative structure-retention relationships (QSRR) were found to be suitable for studying the selective properties of ptBC4A under GC conditions. The QSRR's suggest that the ptBC4A formed inclusion complexes with benzene, lower n-alkyl benzenes, m-xylene, dichloromethane, trichloromethane, methanol, and ethanol. This indicated that the phenolic hydroxyls at the narrow edge of the calix molecule participated strongly in the overall interaction mechanism.

Mnuk and Feltl (30) also did a similar study using calixarenes as selective components of stationary phases in capillary gas chromatography. They studied the selectivity of the basic calixarene series toward alkyl-substituted benzenes, dimethyl cyclohexane isomers, and chloromethanes. A thin layer of a calixarene was deposited onto the surface of a pure fused-silica capillary column. They were able to plot retention data against analyte molecular descriptors such as boiling point, molar volume, polarizability and dipole moment.

Deviations from linearity indicated possible specific interactions. It was concluded there was a direct relationship between steric arrangement of the analyte molecules and their retention behavior was found suggesting an important contribution of inclusion into the calixarene cavities. The highly heterogeneous systems exhibited considerable selectivity, but their efficiency was poor.

CHAPTER III

STATEMENT OF PROBLEM

In gas chromatography, unlike liquid chromatography, the major selectivity for the separation is in the stationary phase. There is always an interest in developing new stationary phases for use in general separation, and for more specific separations such as enantiomers. For compounds that exhibit host-guest interactions, a greater selectivity is often found.

Chiral compounds such as cyclodextrin derivatives have been used as highly selective chiral stationary phases (CSP's). There have been, however, a number of limitations to this variety of stationary phase. First, a single cyclodextrin derivative is often not widely applicable. Secondly, at high temperatures the columns tend to decompose and bleed which leads to loss of enantioselectivity.

Past studies have had a difficult time quantifying the degree of selectivity of the stationary phase towards analytes with widely different chemical properties. This has made for a great need to develop a more stable stationary phase for gas chromatography that could be employed in a variety of separations, at higher temperatures, and have stronger host-guest interactions with a variety of analytes.

Since calixarenes are similar in structure and function to cyclodextrins they have been proposed for use as a stationary phase for chiral separations. They have high

melting points, and are very stable molecules. Because of their cyclic structure they can be modified to present a cavity in which a substrate molecule can be included. The present study entailed doing a low temperature deposition of the calixarene derivative, p-tert-octylcalix[5]arene, onto an OV-1 capillary column. A reference homologous series of toluene, ethylbenzene, n-butylbenzene, and n-propylbenzene was chosen to give evaluations of the analyte-stationary phase interaction by plotting retention data against molecular descriptors. Any deviations from linearity would indicate possible specific interactions. All flow rates and temperatures were held constant before and after derivatization to show that any deviation in linearity was solely due to deposition of the calixarene derivative. The Grob mixture and Polarity mixture were used as a secondary probe for comparison before and after derivatization of the capillary column.

CHAPTER IV

MATERIALS AND METHODS

A. Materials

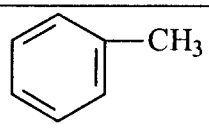
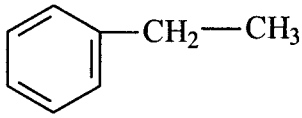
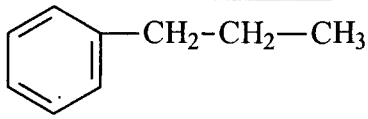
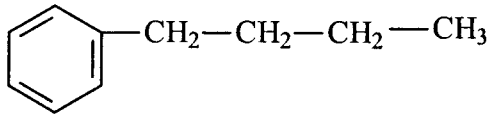
All reagents were analytical grade or of the highest purity available. The p-tert-octylcalix[5]arene, $C_{75}H_{110}O_5$, was purchased through ACROS Organics (Belgium). The Grob mixture and Polarity mixture used for column characterization were pre-mixed solutions purchased from Alltech Associates, Inc. (Deerfield, IL). Also purchased from Alltech were the individual standards for the homologous reference mixture which included: toluene, ethylbenzene, n-propylbenzene and n-butylbenzene, the AT-1 capillary column, and the graphite reducing ferrules.

All solvents were obtained from Fisher Scientific (Fairlawn, NJ). Ultra high purity helium and nitrogen were used for the carrier gas and make-up gas, respectively on the GC. Compressed air and nitrogen were also used. Ultra Pure helium was used as the inert gas to flush the capillary column before and after the vacuum process. All gases were obtained from Airco Products, Inc. (New York, NY). A 5 mL plastic syringe with a 22 gauge 1 1/2 inch disposable needle used in the filling process was purchased from Supelco, Inc. (Bellefont, PA). Sodium silicate solution was obtained from Aldrich Chemical Co. (Milwaukee, WI) for sealing the column ends.

The AT-1 fused silica capillary column was 15 m long and had a film thickness of 0.25 μm and an i.d. of 0.25 mm. Each end of the column was cut flush with a ceramic scriber. The reducing ferrules were composed of 15% Graphite and 85% Vespel®. They accepted columns with and o.d. of 0.5 mm and an i.d. of 0.25 mm. These ferrules had a large end measuring 1/8", which was reduced to 0.4mm at the smaller end.

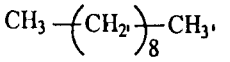
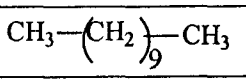
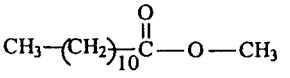
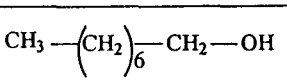
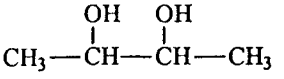
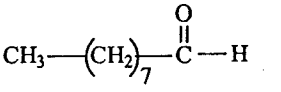
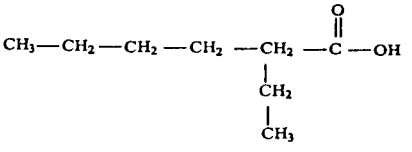
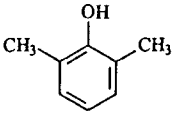
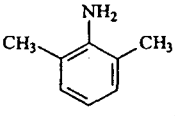
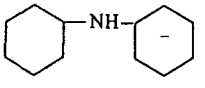
All solutions of the reference homologous series were prepared with methanol as the solvent. A Mettler H20 balance (Mettler Instrument, Princeton, NJ) was used to weigh all chemicals. Each of the homologous series components were placed in 100 mL volumetric flasks and diluted to the mark for individual stock solutions, as outlined in Table 1.

Table 1

toluene	10 mg/mL	
ethylbenzene	10 mg/mL	
n-propylbenzene	10 mg/mL	
n-butylbenzene	10 mg/mL	

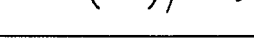
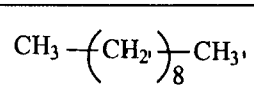
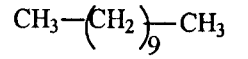
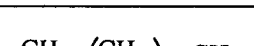
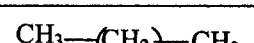
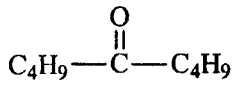
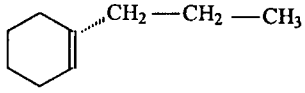
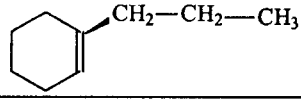
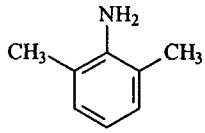
A mixture of all 4 components was prepared by placing 10 mg of each component into a 100 mL volumetric flask and diluting to the mark. All glassware was pre-cleaned with acetone. Stock solutions were stored at 20°C when not in use. The Grob Mixture and the Polarity Mixture were used as purchased. The Grob components, which were all dissolved in dichloromethane, are outlined in Table 2.

Table 2

n-decane	28 mg/mL	
n-undecane	29 mg/mL	
methyl decanoate	42 mg/mL	
1-octanol	35 mg/mL	
2,3-butanediol	53 mg/mL	
n-nonal	40 mg/mL	
2-ethylhexanoic acid	38 mg/mL	
2,6-dimethylphenol	32 mg/mL	
2,6-dimethylaniline	32 mg/mL	
dicyclohexylamine	31 mg/mL	

The Polarity mixture had hexane as the solvent and each component was at a concentration of 4 mg/mL. The individual components of the Polarity mixture are outlined in Table 3.

Table 3

n-nonane	4.0 mg/mL	
n-decane	4.0 mg/mL	
n-undecane	4.0 mg/mL	
n-dodecane	4.0 mg/mL	
n-tetradecane	4.0 mg/mL	
5-nonanone	4.0 mg/mL	
2-prop-1-cyclohex, trans	4.0 mg/mL	
2-prop-1-cyckohex, cis	4.0 mg/mL	
2-6-dimethylaniline	4.0 mg/mL	

B. Methods

The static method, as first outlined by Bouche and Verzele (8) and later modified for silica capillary columns by Grob (24), was used as a basis for preparing the derivatized column, with modifications done as needed.

1. Preparation of the Calixarenes

In a pre-cleaned filling bottle with septum and cap, 0.1300 g of p-octylcalix[5]arene was dissolved in 100 mL of methylene chloride. This mixture was shaken vigorously for approximately two minutes to ensure complete dissolution. The septum was placed in the neck of the filling bottle and the cap tightly crimped onto the bottle. The cap was sealed with parafilm to ensure an airtight fit. Figure 5 depicts the bottle, septa and cap used.

2. Coating the Column

To prepare for filling a 4 cm piece of PTFE tubing (0.5 mm i.d. x 1.5 mm o.d.), purchased from Alltech Associates (Deerfield, IL) was heated using a conventional bunsen burner to expand the orifice sufficiently until one end was able to slide over the capillary column. Upon cooling, a 6 cm section of 1/8" copper tubing was fit snugly over the column. Figures 6 (a) and (b) show the set-up.

The AT-1 column was hung on a metal rod with the inlet end well below the level of the calixarene solution in the filling bottle. The tip of a hypodermic needle was inserted through the septum of the bottle cap. The column was pushed through the needle into the solution. Once inside, the needle was removed to ensure a tight seal around the column. The outlet end of the column with the teflon and copper sleeves was placed into a small beaker (Figure 7).

A 5 mL plastic syringe fitted with a 22 gauge 1.5 inch disposable needle was used to supply the column with a small, but constant amount of pressure (25). The syringe was prepared by using a hot needle to puncture a series of holes approximately 1 cm apart through the barrel and the plunger of the syringe. To achieve the proper amount of

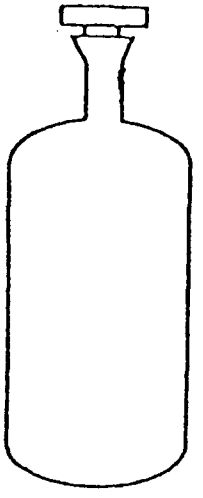


Figure 5. Filling bottle, fitted with rubber septa and cap, which is crimped with an airtight seal.

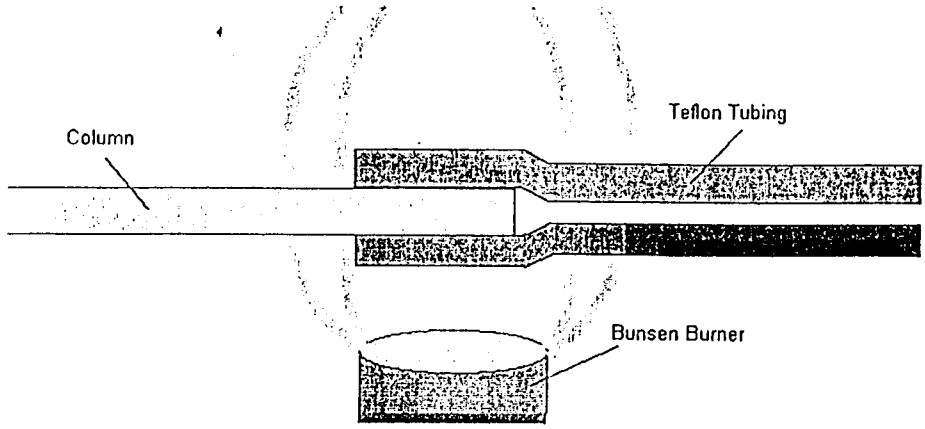


Figure 6 (a). The capillary column is inserted into the teflon tubing while it is heated over the bunsen burner to expand it's orifice.

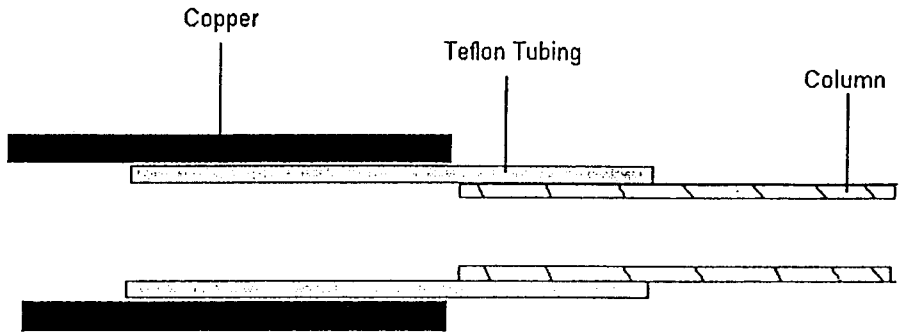


Figure 6 (b). The closure end of the column consists of 1/8" copper tubing which is fit over the capillary column and teflon tubing.

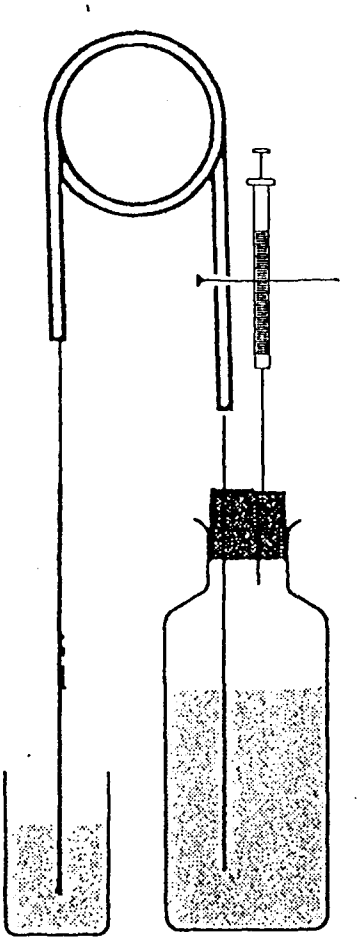


Figure7. Set-up for column filling. The capillary columns hangs on a rod, with the inlet end dipped into the coating solution via the septum. The outlet end with the teflon and copper closure is ready to release the coating solution into a beaker.

pressure, the plunger was pulled out as far as possible, then inserted through the septum of the filling bottle. Pressure was applied by pushing in the plunger. A large, blunted needle, was inserted through both the barrel and plunger through the previously made holes as shown in Figure 8. The syringe was left unattended without any loss of pressure while the column was filling. For complete filling all connections between the Teflon® tubing, the copper sleeve, and the septa and cap on the filling bottle, and all areas surrounding the column had to be airtight.

After several drops of the calixarene coating solution exited the outlet of the column, the copper sleeve was crimped using a Coax crimping tool. The entire process took approximately 20 minutes. The plastic syringe was removed from the filling bottle. The inlet end of the column was removed and immediately pressurized to 20 psi with ultra high purity nitrogen. The pressure was gradually increased to 70 psi and held for twenty minutes.

3. Evacuation of Solvent

Evacuation of the solvent occurred in a constant temperature water bath maintained at 30°C. The elevated temperature was used to speed the evaporation and evacuation process. The filled column (with the outlet end sealed) was submerged in the water bath suspended on a piece of wire support. The open inlet end was recut and placed through a septum and empty bottle similar to that used in the filling process. A small piece of 1/8" copper tubing was placed through the septa and a valve connected to the tubing. At the other end of the valve, rubber tubing was connected to a laboratory vacuum line, controlled by another valve. After adjusting the vacuum the column was evacuated for two hours at 30°C (Figure 9).

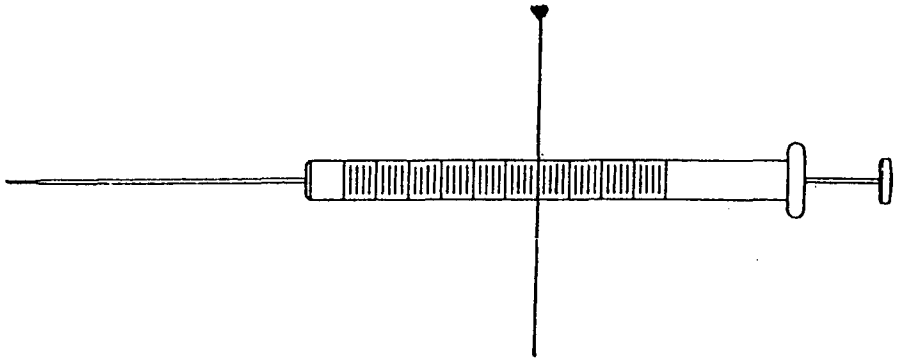


Figure 8. Syringe in the pressure mode. To start, the plunger is pulled back (or removed). When the plunger is pushed in to produce the wanted pressure, the needle is inserted through the holes of both barrel and plunger.

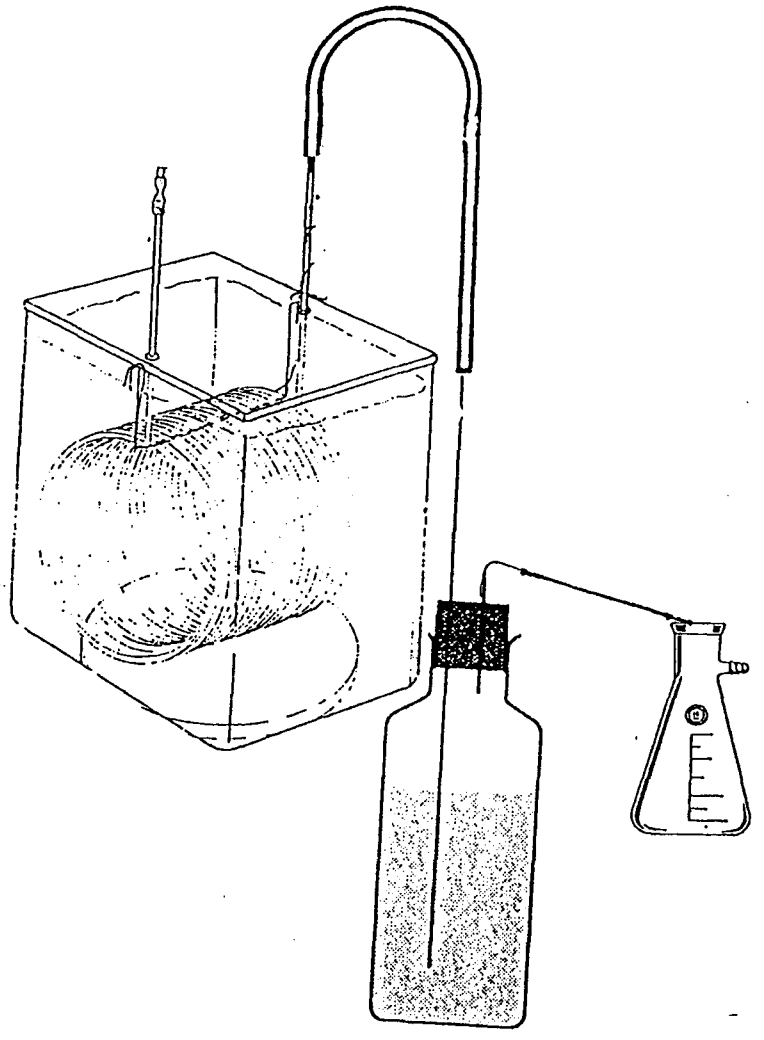


Figure 9. Water-bath set-up needed for evacuation of solvent in the capillary column. Temperature of water bath is set at 300C. The inlet end of the column is placed into a filling bottle via the septa. A small piece of copper-tubing connects the filling bottle to a flow valve which is connected to an erlenmeyer flask which leads to a vacuum line.

After evacuation the column was connected to ultra high purity nitrogen to remove any remaining solution. At this point a few small drops of solution were pushed out. After several minutes of flushing, the column ends were cleaned with methylene chloride, and sealed using a sodium silicate solution.

4. Conditioning the column for use

Keeping both ends of the column sealed, the column was heated in a GC oven at 160°C for one hour followed by another hour at 180°C. After pre-conditioning, both column ends were opened and the column was mounted on the GC where it was conditioned at 120°C for three hours. Helium was used as the carrier gas with a flow rate of 1.0 mL/min.

5. GC Parameters and Setpoints

All measurements were carried out on a Hewlett Packard HP5890 Series II Gas Chromatograph with a flame ionization detector. The chromatograms were recorded on a Hewlett Packard HP 3395 Integrator. The carrier gas volumetric flow rate was 1.00 mL/min at a linear velocity of 20 cm/sec. Methane was injected into the column after it was installed to establish the linear flow rate of the carrier gas and the dead time of the column. The retention time of the methane peak was measured as the dead time, t_m, in seconds. The average linear flow rate was measured by, $u = L/t_m$, where L was the length of the column in cm, u was the average linear flow rate in cm/sec, and t_m the dead time. The column head pressure was set at 5.0 psi and the injector was set on split mode with a ratio of 1:50 for the Grob and Polarity mixtures and 1:150 for the reference homologous series compounds. The capacity factors were calculated by $k = (t_r - t_m)/t_m$, where t_r was the retention time for each individual peak. The column efficiency, H, was calculated by

dividing the length of the column, L, by the total number of theoretical plates, n: $H=L/n$. The number of theoretical plates was calculated after measuring the retention times, t_r, and extrapolating base width of each peak, W, with the following equation: $n= 16(t_r/W)^2$.

Tables 4, 5 and 6 outline the GC parameters for the Grob mixture, Polarity mixture and reference homologous series, respectively, for both the derivatized and underivatized columns.

Table 4

Injector Temperature	250°C
Detector Temperature	250°C
Initial Temperature	40°C
Initial Time	0 min
Ramp Rate	6.0°C/min
Final Temperature	190°C
Final Time	0 min

Table 5

Injector Temperature	250°C
Detector Temperature	250°C
Initial Temperature	75°C
Initial Time	4 min
Ramp rate	1.0°C/min
Final Temperature	100°C
Final Time	0 min

Table 6

Injector Temperature	250°C
Detector Temperature	250°C
Initial Temperature	32°C
Initial Time	0 min
Ramp Rate	1.0°C/min
Final Temperature	100°C
Final time	0 min

6. GC Experiments

Before the capillary column was derivatized, several measurements were made using the Grob mixture and the Polarity mixture as test references. The Reference Homologous Series was run as individual components, and as a mixture, with a ramping rate of 1.0°C/min. This reference series was also run isothermally, at 50°C and 60°C. Three to four runs were made with each of the anayltes using a sample injection volume of 1.0 μL, with a Hamilton 10-μL glass syringe. The syringe was cleaned thoroughly with acetone between each run. After derivatization, the experiments were repeated under the same conditions.

CHAPTER V

RESULTS AND DISCUSSION

A. The Making of the Capillary Column

The purpose for developing a new stationary phase based on calixarenes for gas chromatography was to provide insight about host-guest interactions between calixarenes and solutes in the mobile phase. Once the column was derivatized, three solute probe mixtures were injected through the column in order to characterize the derivatization: Grob mixture, Polarity mixture, and a Reference Homologous Series (RHS). Each mixture provided information that described the physical characteristics of the column. Such characteristics included, differences in peak shape, separation efficiency of the column, and indications of intermolecular interaction due to hydrogen bonding, dipolar, or London interactions. Finally, the Reference Homologous Series gave insight into any host-guest interaction that occurred between the calixarenes and any individual components.

Grob, Polarity, and RHS were injected through the column both before and after derivatization. It should be noted that an extreme brittleness of the column was observed after derivatization. The column had to be handled with great care when inserting into the GC and several attempts were necessary before it was installed properly. After all chromatographic runs were done it was noted the column was no longer brittle and appeared to have gone back to it's original flexibility.

B. Timeline of the Column

Over the course of these experiments the capillary column was subjected to periods of high temperature and to long run times. One consequence may have been that the calixarenes did not stay immobilized on the inside surface of the column. The following is the sequence of events, the various temperatures, and the GC conditions during the life of the column throughout this research.

Before Derivatization

1. After initial installation of the capillary column, the carrier gas linear velocity was established at 20 cm/sec. The linear velocity was determined by injecting methane and calculating from the retention time (in seconds) and the length of the column (in centimeters). The split ratio of the injector was set at 1:50. The column was purged with the carrier gas (He) for one hour at room temperature and then conditioned at the maximum isothermal temperature (300°C) for 30 minutes. The column was allowed to cool to 40°C and the first runs were made with the Grob mixture. The parameters were set at: initial temperature 40°C, (0 min hold time), temperature ramp rate 6°C/min, and final temperature 190°C, following the available literature.

2. The Polarity mixture was injected through the column at an initial temperature of 75°C (4 min hold time); temperature ramp rate 5°C/min, final temperature 150°C; injector split ratio 1:50.

3. The RHS was run last through the column under the following conditions: initial temperature, 32°C; temperature ramp rate, 1.0°C/min; final temperature, 100°C. The split ratio was set at 1:150; and the flow rate of carrier gas was 1.00 mL/min. The column head pressure was at 5.0 psi to achieve this flow.

After Derivatization

1. Once both ends of the column were sealed with the sodium silicate solution (after the filling process), the column was heated directly to 160°C, held for one hour and raised to 180°C for one hour. Both ends of the column were opened and the column was mounted on the GC. The column was conditioned at 120°C for three hours with carrier gas at 1.0mL/min flowing thru. Once conditioned, the column was set to the following parameters for injection.

2. The flow rate was measured at 1.00 mL/min, with a head pressure at 7.5 psi, possibly indicating that the column had been derivatized with the calixarenes. The split ratio, initial temperature, temperature ramping rate, and final temperature were the same as previously noted.

3. Next, the column was run isothermally at 50°C with the RHS. All other parameters (flow rate, split ratio and sample size) were kept constant as for before derivatization runs.

4. Then three runs were then made isothermally at 60°C for RHS, keeping all other parameters constant.

5. Following the RHS, the Grob mixture was run at the same parameters as before derivatization.

6. Finally, the Polarity mixture was run as for before derivatization.

Following this series of experiments, it was noted that the isothermal runs at 50°C and 60°C were not done before the column was derivatized. Therefore, to get a comparison of before and after derivatization, a second column of the same type was conditioned and

mounted as the first, and put through the same battery of experiments. This new underivatized column was run isothermally at 50°C, 60°C, and also at 40°C.

7. The derivatized column was once again mounted on the GC. It was noted here that the derivatized column had returned back to it's original flexibility and was easily mounted on the GC without the breakage that had occurred earlier. Isothermal runs at 40°C were attempted with this column. No data was able to be collected due to the fact that the FID detector appeared to be overloaded. The detector was cleaned thoroughly, twice. Several more attempts were made but with no success. It appeared as though the calixarenes that were once deposited as a thin film on the inside wall of the capillary column had bled off due to the high temperatures and the large number of runs to which the column was subjected. Table 7 summarizes the time, temperature, and number of runs the underivatized and derivatized column went through, throughout the course of each experiment.

Table 7

Elapsed Time	Time	Temperature	Experiment	Number of runs
1 hour	1 hour	25°C	Purging Carrier Gas	1
1.5 hours	0.5 hour	300°C	Conditioning	1
3.5 hours	2 hours	40-90°C	Grob Before deriv.	4
5 hours	1.5 hours	75-150°C	Polarity Before der	3
6 hours	1 hour	32-100°C	RHS before der.	3
7 hours	1 hour	160°C	Baking column after derivatization	1
8 hours	1 hour	180°C	Baking column after derivatization	1
11 hours	3 hours	120°C	Conditioning after derivatization	1
12 hours	1 hour	32-120°C	RHS after deriv.	3
13 hours	1 hour	50°C	RHS	3
14 hours	1 hour	60°C	RHS	3
16 hours	2 hours	40-90°C	Grob after deriv.	3
17.5 hours	1.5 hours	75-150°C	Polarity after der.	3
18.5 hours	1 hour	50°C	RHS underivatized	3
19.5 hours	1 hour	60°C	RHS underivatized	3
20.5 hours	1 hour	40°C	RHS underivatized	3
	———	40°C	RHS derivatized	———

C. Mixtures of Alkyl Benzenes

The formation of the host-guest interactions between the calixarene stationary phase and the Reference Homologous Series (RHS) was studied by comparison of the retention data before and after derivatization with t-octylcalix[5]arene. The components of the this series were chosen because calixarenes generally form significant host-guest interaction with aromatic compounds. On a conventional stationary phase where separation occurs only as a function of polarity, it was expected that a plot of log k' vs. carbon number would be linear. Any deviation from linearity after derivatization, would indicate that a host-guest interaction did in fact take place.

Figures 10 and 11 show the chromatograms obtained before and after derivatization when the column was at 60°C. Tables 8 and 9 summarize the average capacity factors calculated from the retention data, before and after derivatization. Table 10 summarizes the capacity factors and their logarithms for both before and after derivatization. Figure 12 shows the linear dependencies of the retention data for RHS, as well as a comparison with the retention behavior observed with the calix[5]arene stationary phase. The conventional stationary phase showed significantly more retention of the RHS.

Since it was apparent that there was some degree of interaction at 60°C, isothermal measurements were made at 50°C. Figures 13 and 14 show the chromatograms of these runs before and after derivatization of the column. As with the 60°C measurements it was obvious that the components were being held longer on the column due to the larger separation of the peaks. In addition, the n-butylbenzene peak was changed significantly, becoming smaller in size as well as showing peak broadening.

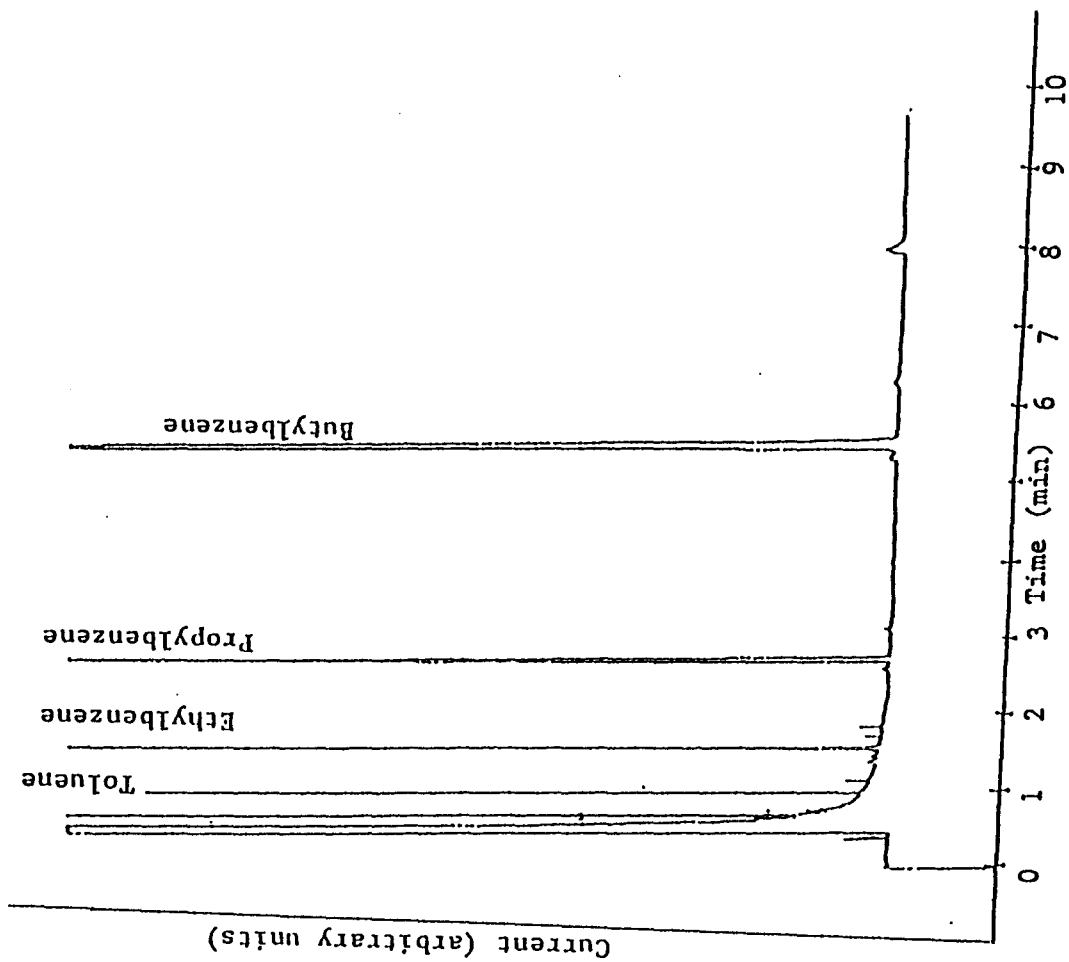


Figure 10. Chromatogram of RHS before column derivatization with column temperatures set at 60°C; split ratio, 1:150.

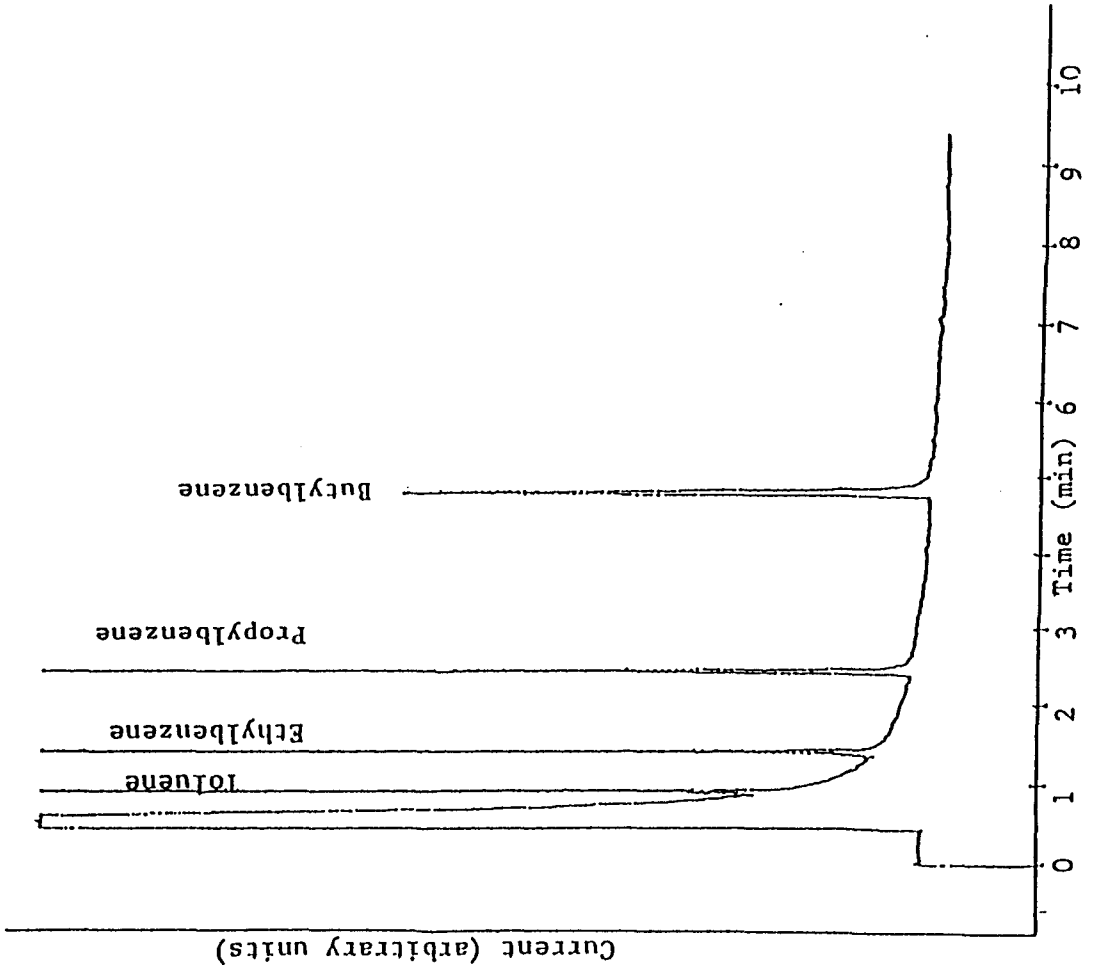


Figure 11. Chromatogram of RHS after column derivatization with column temperature set at 60°C; split ratio, 1:150.

Table 8

Isothermal @ 60 degrees C					
Before Derivatization					
		Retention Times			
	Run 1	Run 2	Run 3	Average	s
Dead Time	0.750	0.700	0.800	0.750	0.050
Toluene	1.278	1.278	1.276	1.277	0.001
Ethyl Benzene	2.011	2.008	2.013	2.011	0.003
Propyl Benzene	3.495	3.486	3.495	3.492	0.005
Butyl Benzene	6.979	6.961	6.970	6.970	0.009
		Capacity Factors			
Toluene	0.704	0.826	0.595	0.708	0.115
Ethyl Benzene	1.681	1.869	1.516	1.689	0.176
Propyl Benzene	3.660	3.980	3.369	3.670	0.306
Butyl Benzene	8.305	8.944	7.713	8.321	0.616

Table 9

Isothermal @ 60 °C					
After Derivatization					
		Retention Times			
	Run 1	Run 2	Run 3	Average	s
Dead Time	0.8	0.65	0.65	0.700	0.087
Toluene	1.038	1.026	1.021	1.028	0.009
Ethyl Benzene	1.617	1.6	1.591	1.603	0.013
Propyl Benzene	2.785	2.758	2.74	2.761	0.023
Butyl Benzene	5.54	5.484	5.445	5.490	0.048
		Capacity Factors			
Toluene	0.298	0.578	0.571	0.482	0.160
Ethyl Benzene	1.021	1.462	1.448	1.310	0.250
Propyl Benzene	2.481	3.243	3.215	2.980	0.432
Butyl Benzene	5.925	7.437	7.377	6.913	0.856

Table 10

		k' (before)	k' (after)	Number	log k' (bef)	log k' (aft)
Toluene	1	0.708	0.482	1	-0.150	-0.317
Ethyl Benzene	2	1.689	1.310	2	0.228	0.117
Propyl Benzene	3	3.670	2.980	3	0.565	0.474
Butyl Benzene	4	8.321	6.913	4	0.920	0.840

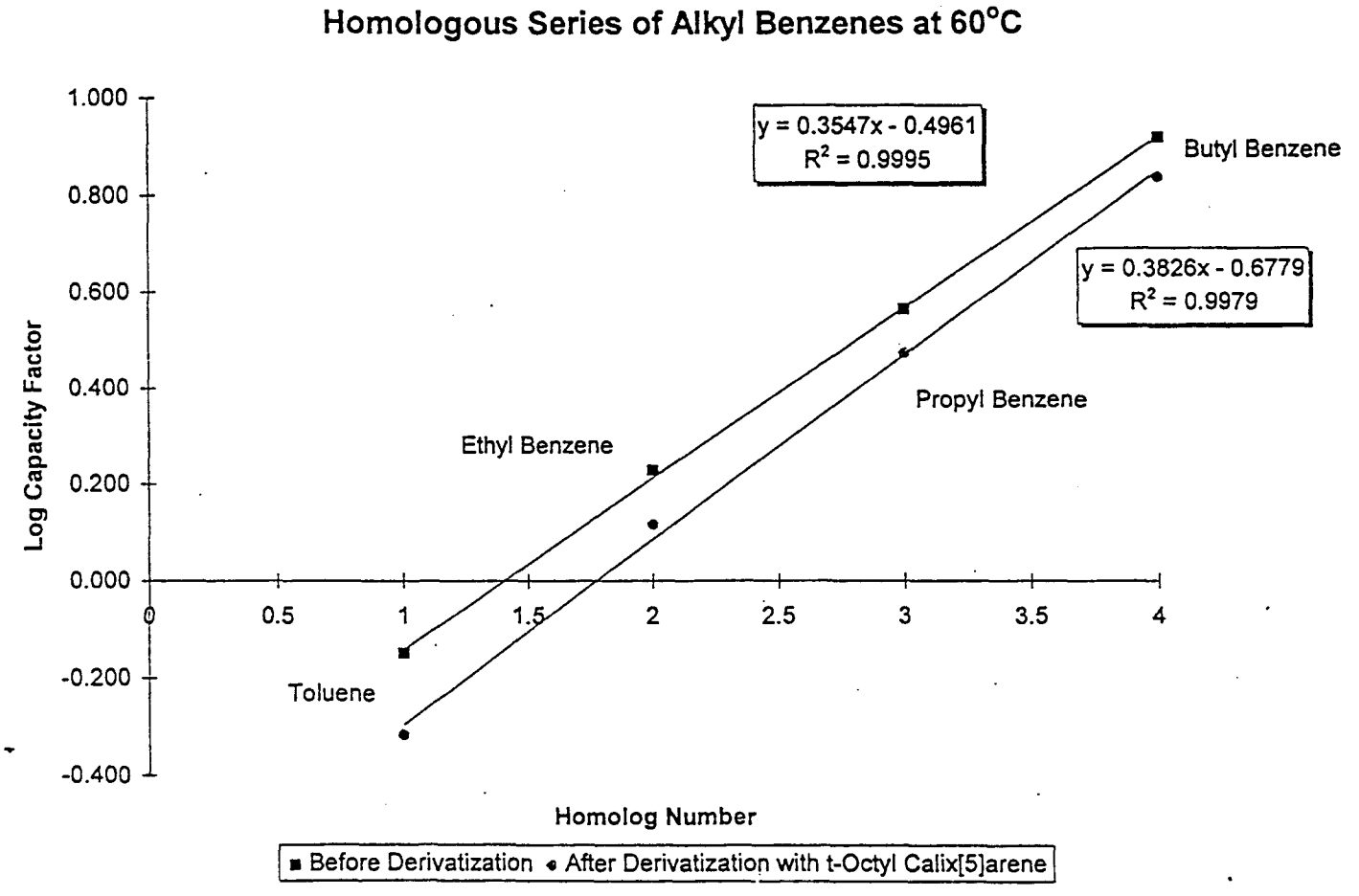

Homologous Series of Alkyl Benzenes at 60°C
y = 0.3547x - 0.4961
R² = 0.9995
y = 0.3826x - 0.6779
R² = 0.9979
Butyl Benzene
Propyl Benzene
Ethyl Benzene
Toluene
Log Capacity Factor
1.000
0.800
0.600
0.400
0.200
0.000
-0.200
-0.400
0
0.5
1
1.5
2
2.5
3
3.5
4
Homolog Number
Before Derivatization
After Derivatization with t-Octyl Calix[5]arene

Figure 12.

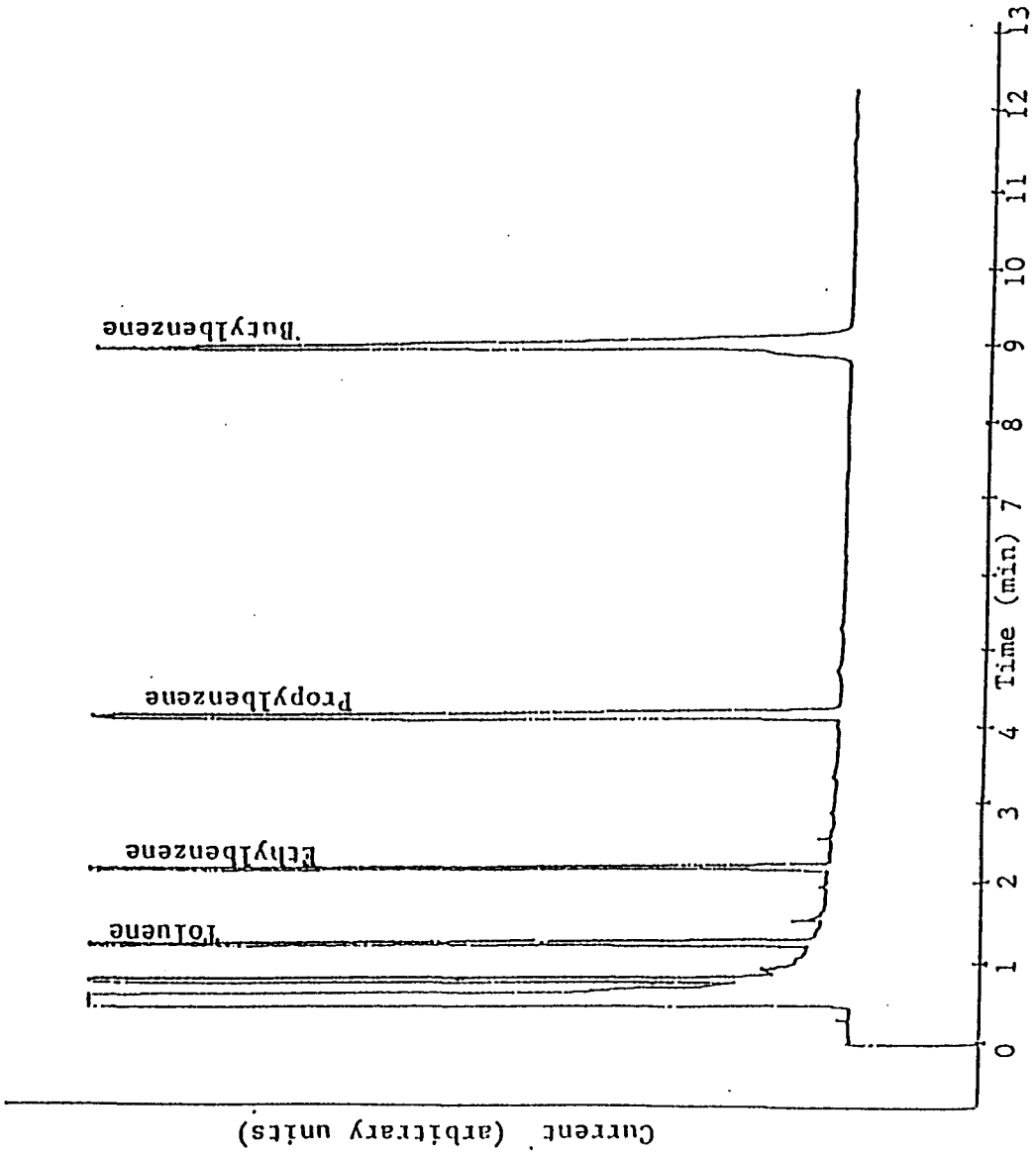


Figure 13. Chromatogram of RHS before column derivatization. Column temperature, 50°C; split ratio, 1:150; sample size, 1μL.

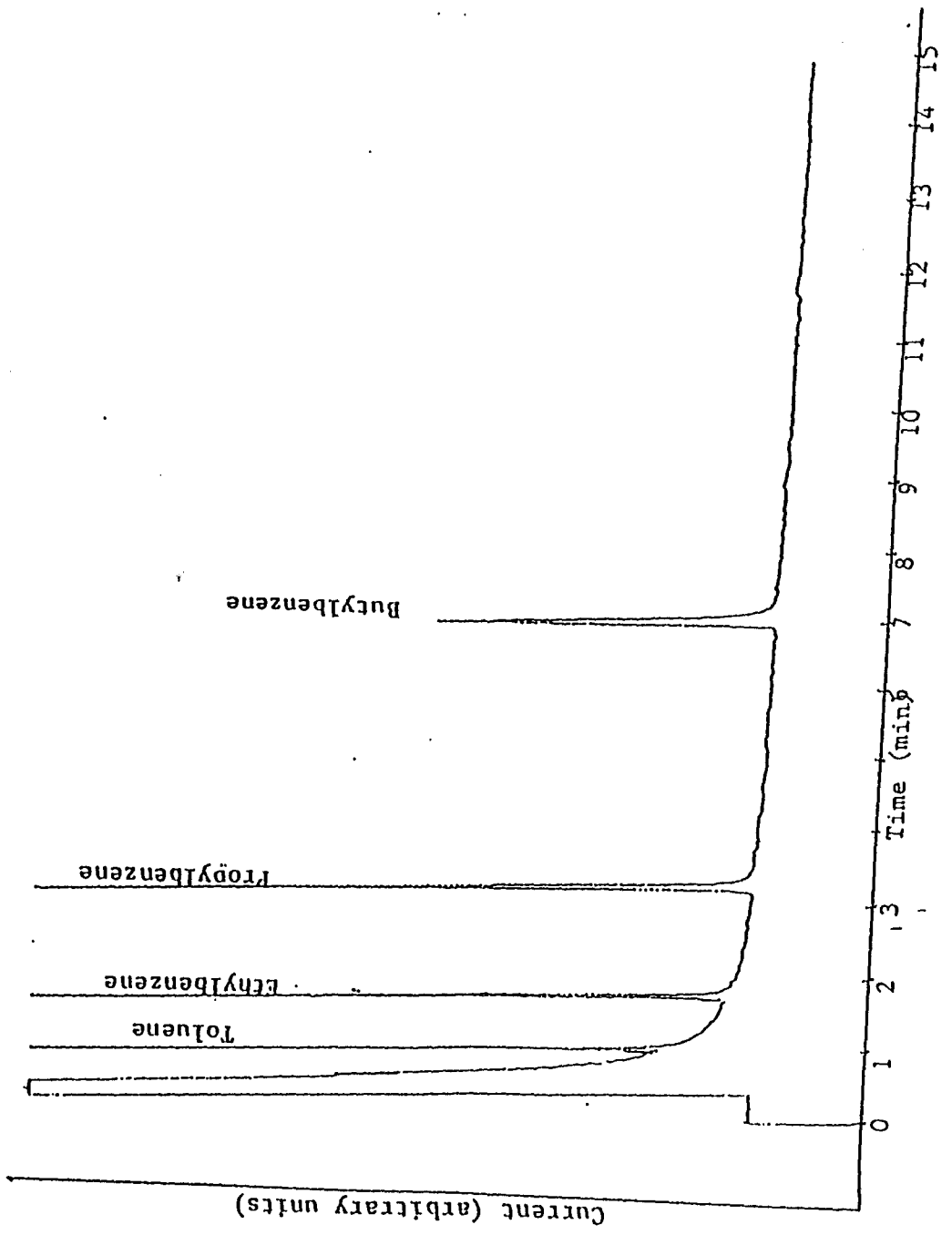


Figure 14. Chromatogram of RHS after derivatization. Column temperature, 50°C; split ratio, 1:150; sample size 1μL.

Tables 11 and 12 summarize the retention times and average capacity factors measured for these runs, before and after derivatization. Table 13 shows the average capacity factors and their logarithms, before and after derivatization. Figure 15 shows the comparison before and after derivatization at 50°C. There is a clear indication of host-guest interactions occurring due to the nonlinearity of the components, especially toluene, ethylbenzene, and n-propylbenzene. Further indications are that there is also a relationship between the shape of the analyte molecule and it's retention behavior.

When plots of the alkylbenzenes on the underivatized and derivatized column were compared in Figures 16 and 17 there was clear indication that there was a relationship between the temperature of the runs and their thermodynamic properties as indicated by the change in slopes before and after derivatization. As the temperature got lower, it was suspected that the effect would be magnified, again indicating a definite host-guest interaction.

D. Polarity Mixture

The Polarity mixture was used in this research as a diagnostic tool in determining if the polarity of the stationary phase was significant altered by the t-octylcalix[5]arene. The polarity of the stationary phase will produce a particular elution order and retention time of the components, therefore comparison of the elution order and retention time between the two columns gave some indication of the polarity of the column.

All components in this mixture were dissolved in hexane at a concentration of 4 mg/mL. Once the column had been coated with the calixarene derivative it appeared that the new derivatized column was significantly more polar than it had been underivatized.

Table 11

Isothermal @ 50 °C					
Before Derivatization					
		Retention Times			
	Run 1	Run 2	Run 3	Average	s
Dead Time	0.800	0.750	0.750	0.767	0.029
Toluene	1.540	1.552	1.553	1.548	0.007
Ethyl Benzene	2.660	2.654	2.659	2.658	0.003
Propyl Benzene	5.034	5.020	5.031	5.028	0.007
Butyl Benzene	10.885	10.860	10.875	10.873	0.013
		Capacity Factors			
Toluene	0.925	1.069	1.071	1.022	0.084
Ethyl Benzene	2.325	2.539	2.545	2.470	0.125
Propyl Benzene	5.293	5.693	5.708	5.565	0.236
Butyl Benzene	12.606	13.480	13.500	13.195	0.510

Table 12

Isothermal @ 50 °C					
After Derivatization					
		Retention Times			
	Run 1	Run 2	Run 3	Average	s
Dead Time	0.55	0.6	0.55	0.567	0.029
Toluene	1.246	1.243	1.237	1.242	0.005
Ethyl Benzene	3.147	2.125	2.128	2.467	0.589
Propyl Benzene	4.028	3.992	3.991	4.004	0.021
Butyl Benzene	8.666	8.598	8.582	8.615	0.045
Toluene	1.265	1.072	1.249	1.195	0.107
Ethyl Benzene	4.722	2.542	2.869	3.378	1.176
Propyl Benzene	6.324	5.653	6.256	6.078	0.369
Butyl Benzene	14.756	13.330	14.604	14.230	0.783

Table 13

		k' (before)	k' (after)		log k' (bef)	log k' (aft)
Toluene	1	1.022	1.195	1	0.009	0.078
Ethyl Benzene	2	2.470	3.378	2	0.393	0.529
Propyl Benzene	3	5.565	6.078	3	0.745	0.784
Butyl Benzene	4	13.195	14.230	4	1.120	1.153

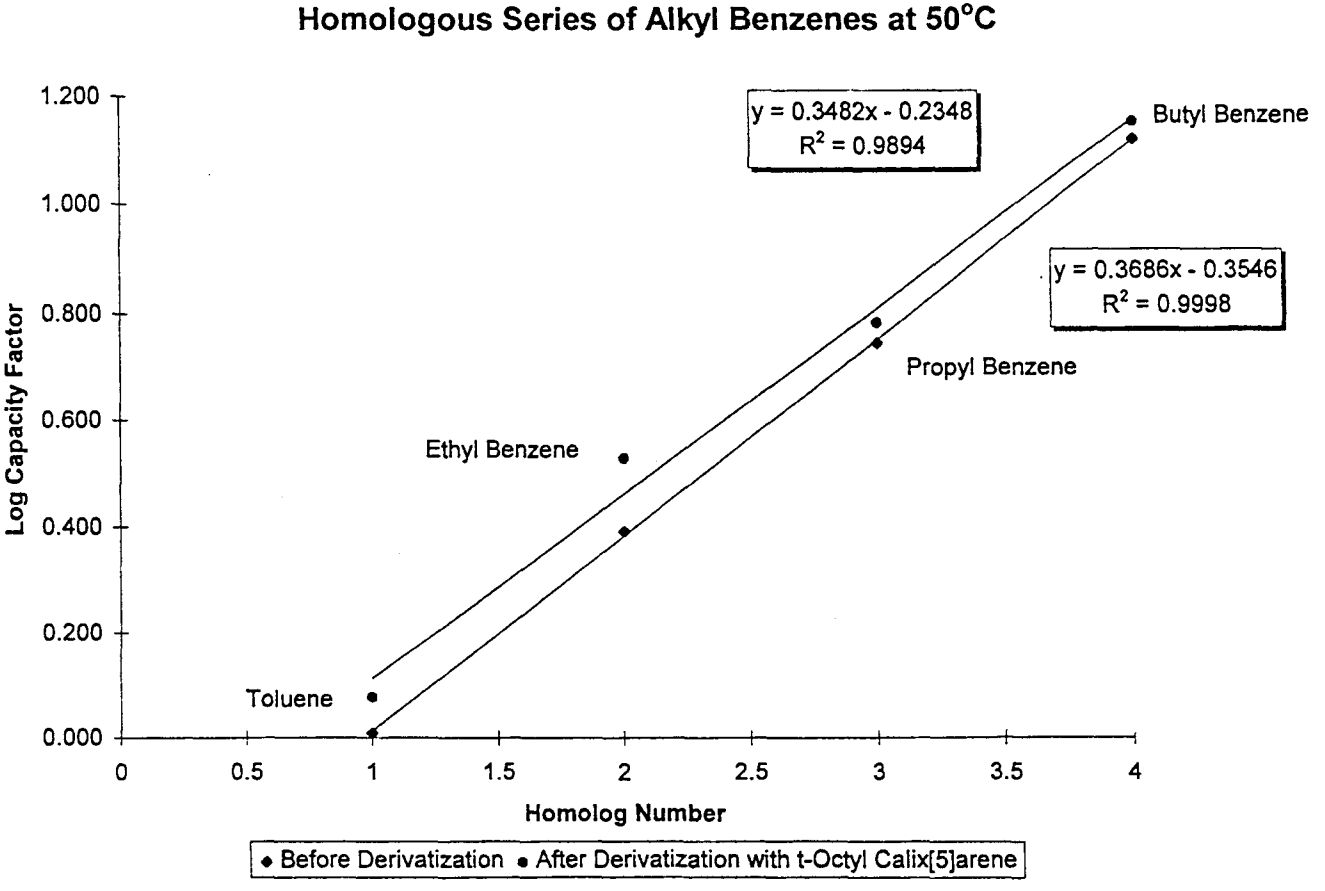

Homologous Series of Alkyl Benzenes at 50°C
y = 0.3482x - 0.2348
R² = 0.9894
y = 0.3686x - 0.3546
R² = 0.9998
Butyl Benzene
Propyl Benzene
Ethyl Benzene
Toluene
Log Capacity Factor
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0
0.5
1
1.5
2
2.5
3
3.5
4
Homolog Number
Before Derivatization
After Derivatization with t-Octyl Calix[5]arene

Figure 15.

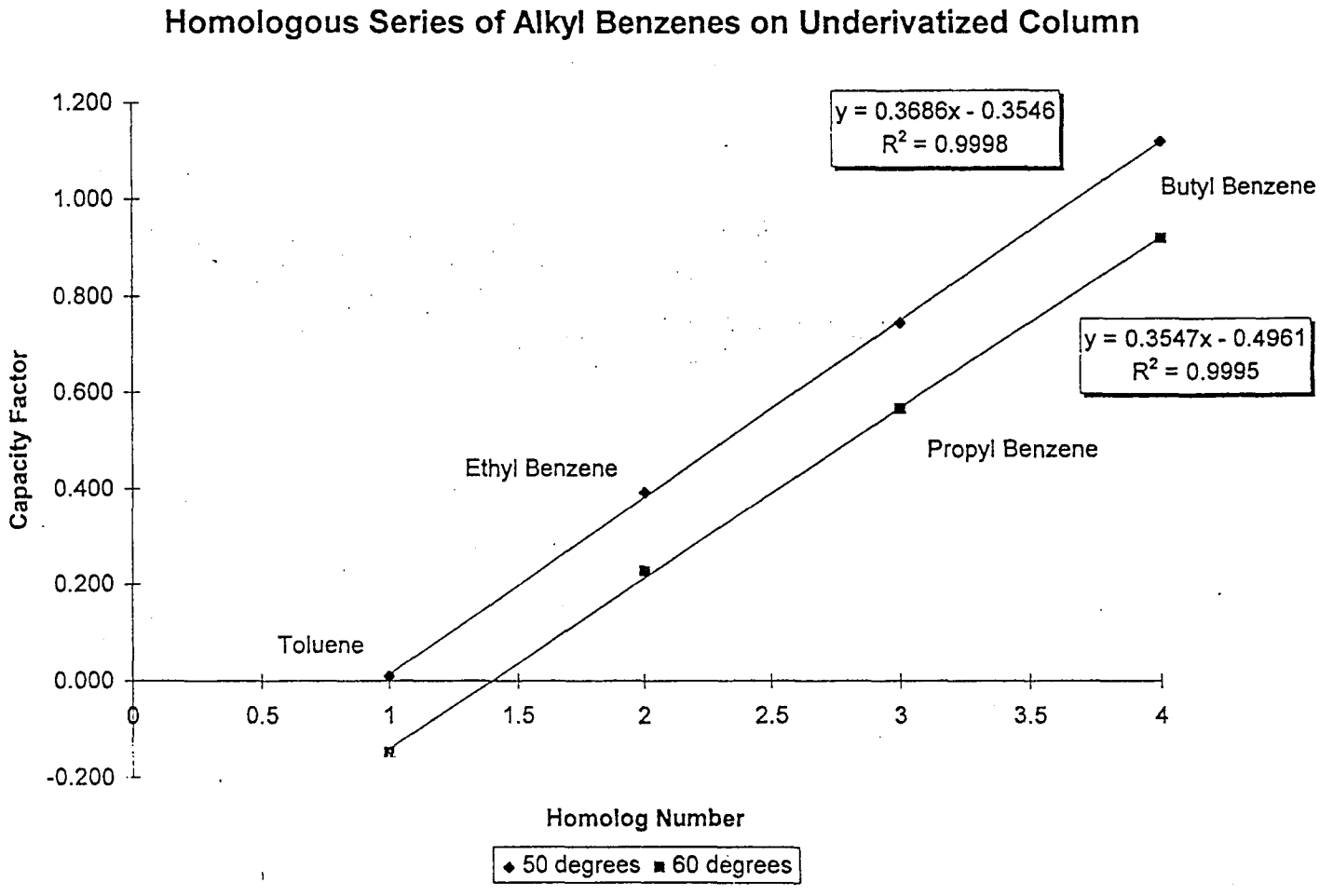

Homologous Series of Alkyl Benzenes on Underivatized Column
y = 0.3686x - 0.3546
R² = 0.9998
y = 0.3547x - 0.4961
R² = 0.9995
Butyl Benzene
Propyl Benzene
Ethyl Benzene
Toluene
Capacity Factor
1.200
1.000
0.800
0.600
0.400
0.200
0.000
-0.200
0
0.5
1
1.5
2
2.5
3
3.5
4
Homolog Number
◆ 50 degrees ■ 60 degrees

Figure 16.

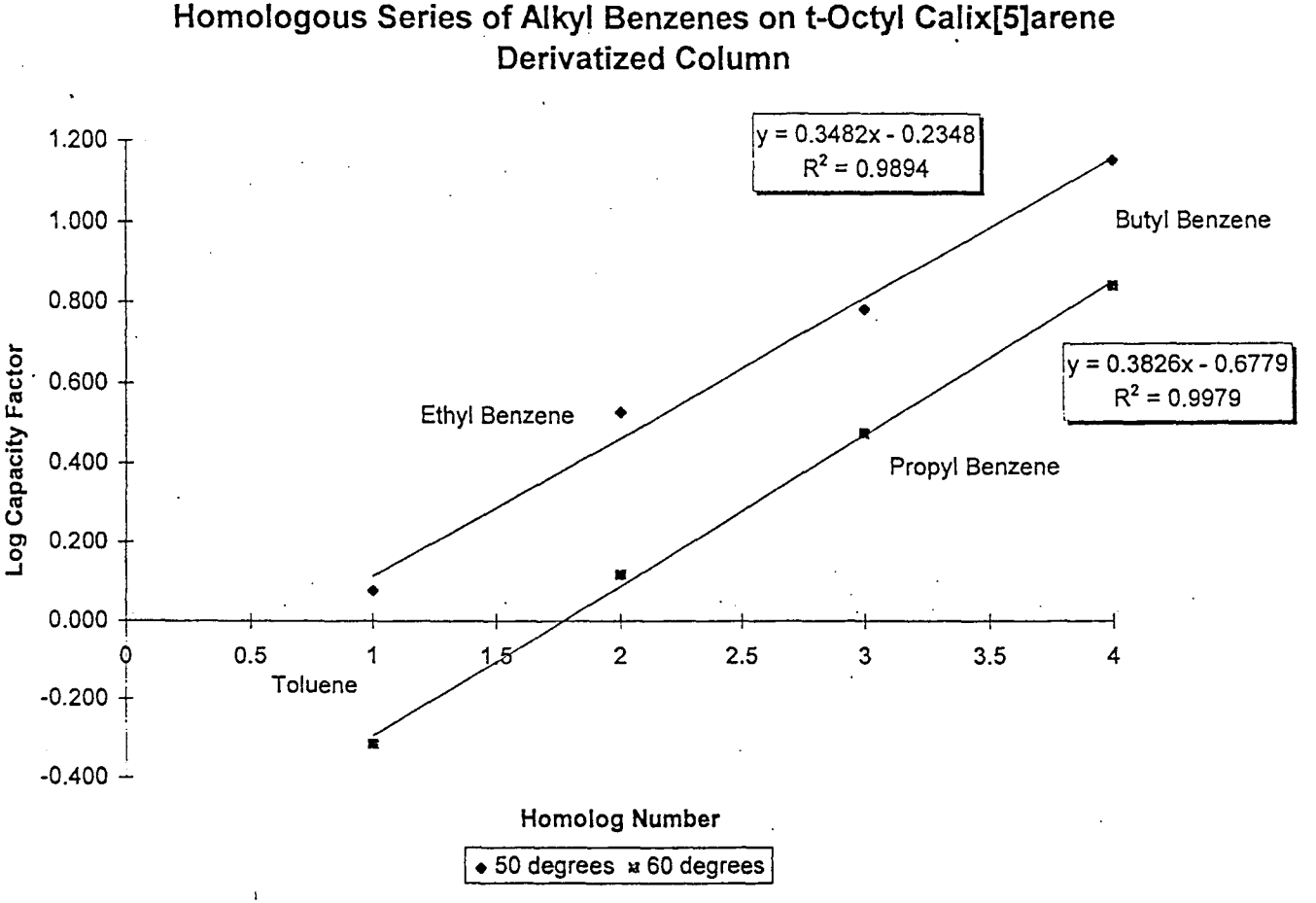

Homologous Series of Alkyl Benzenes on t-Octyl Calix[5]arene Derivatized Column
y = 0.3482x - 0.2348
R2 = 0.9894
Butyl Benzene
y = 0.3826x - 0.6779
R2 = 0.9979
Ethyl Benzene
Propyl Benzene
Toluene
Log Capacity Factor
1.200
1.000
0.800
0.600
0.400
0.200
0.000
-0.200
-0.400
0
0.5
1
1.5
2
2.5
3
3.5
4
Homolog Number
50 degrees
60 degrees

Figure 17.

Figure 18 and 19 show the separation achieved before and after derivatization. Table 14 summarizes the retention times and average capacity factors before derivation, while Table 15 provides the same information after the column had been derivatized. An interesting bar graph depicting the before and after derivatization versus capacity factors is shown in Figure 20. As indicated, the capacity factors for n-nonane, n-decane, and somewhat for n-undecane were higher before derivatization than after. The remaining components had the reverse effect, with there capacity factors being higher after derivatization.

E. Grob Mixture

In 1978 (25), Grob, et al. published an article indicating a single test mixture could be used in gas chromatography to evaluate capillary columns. Each component in the test mixture served as a diagnostic tool to evaluate various specifications such as, separation efficiency, film thickness, acid/base characteristics, and adsorption activity of the column and the system. This test was designed to evaluate a column's characteristics to provide a means of comparison as the column changed, or in this research, as the column was derivatized.

All components in this mixture were dissolved in dichloromethane at the following concentrations: n-decane (28 mg/mL), n-undecane (29 mg/mL), methyl undecaneoate (42 mg/mL), 2,3-butanediol (53 mg/mL), dicyclohexylamine (31 mg/mL), 2-ethylhexanoic acid (38 mg/mL), nonal (40 mg/mL), 1-octanol (35 mg/mL), 2,6-dimethylanaline (32 mg/mL), 2,6-dimethylphenol (32 mg/mL), methyl dodecanoate (41 mg/mL), methyl decanoate (42 mg/mL). The hydrocarbons, n-decane and n-undecane

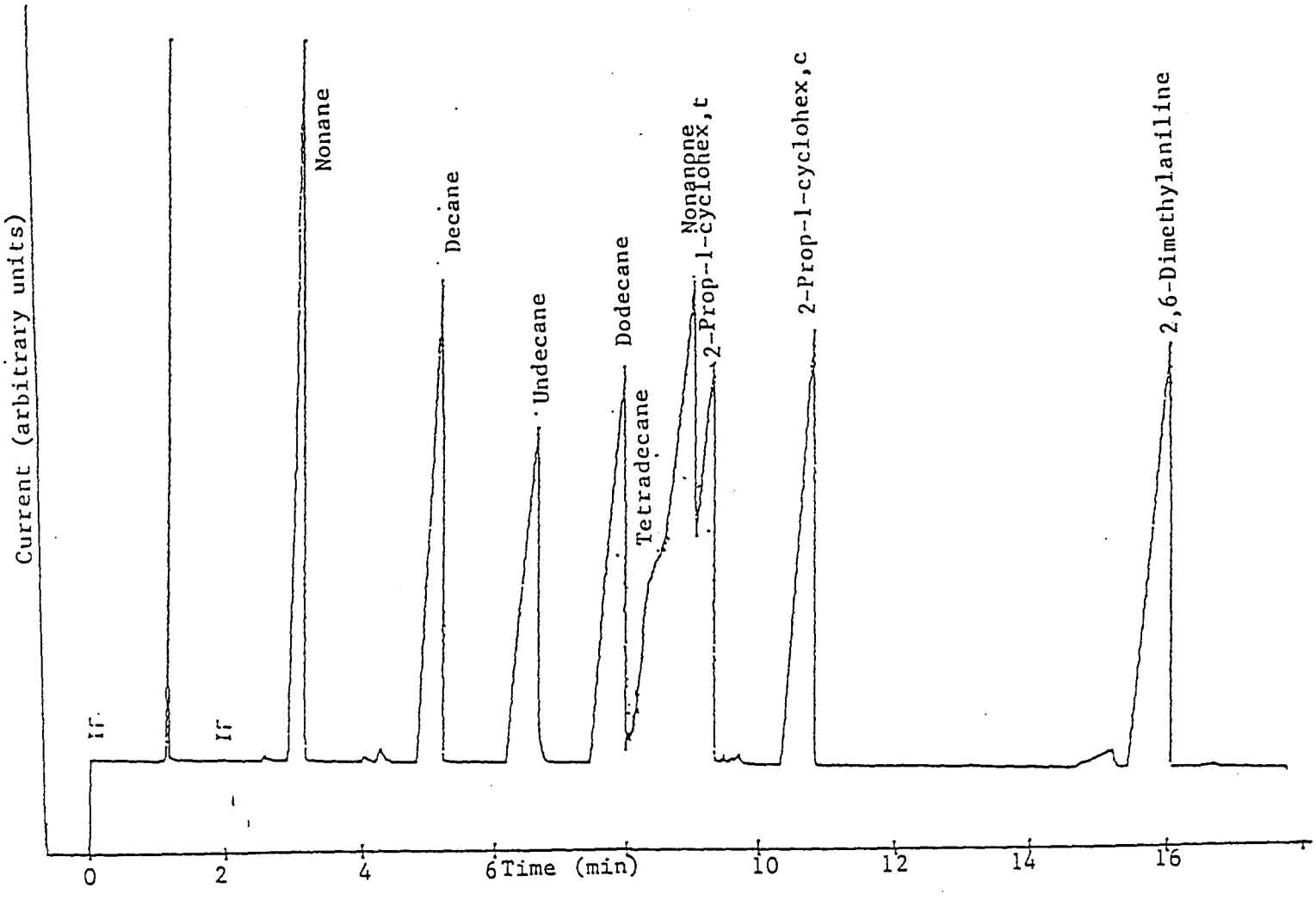


Figure 18. Separation of Polarity mixture before column derivatization. Initial temperature of column, 75°C (4 min hold time); rate, 5°C/min; final temperature, 150°C; split ratio, 1:50; flow rate, 1mL/min.

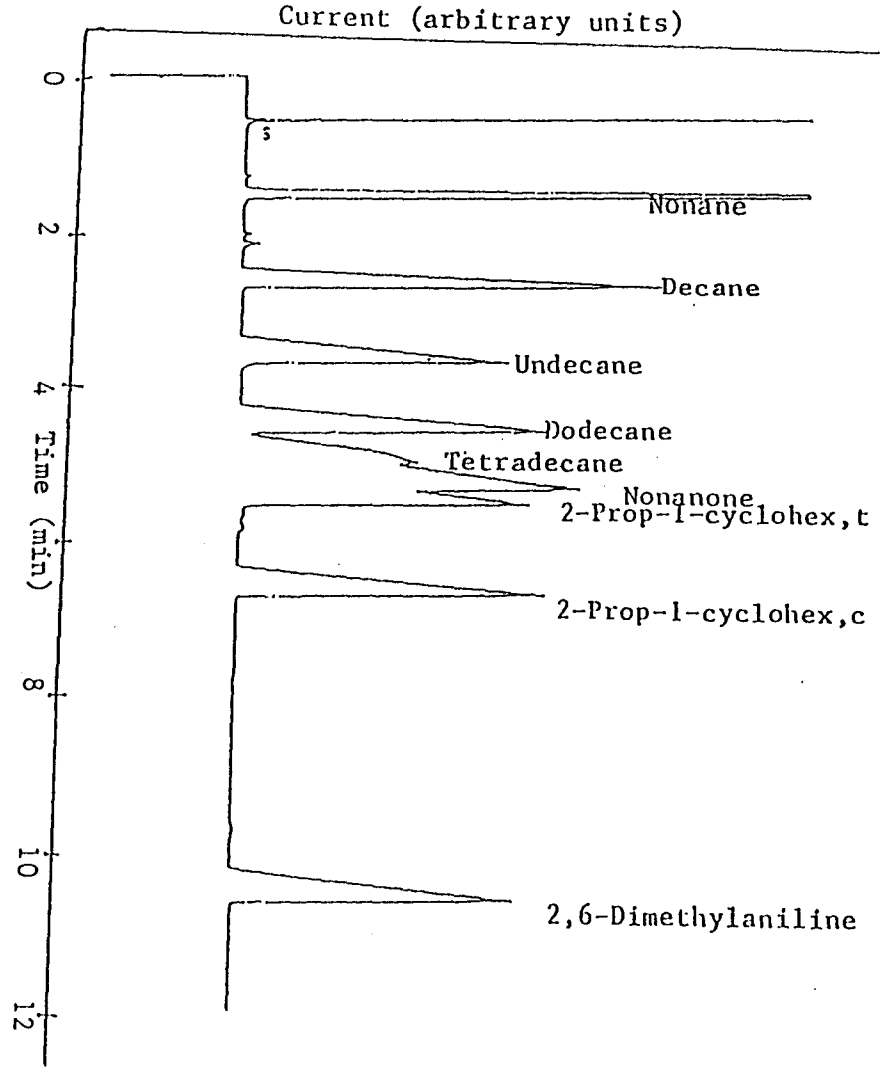


Figure 19. Separation of Polarity mixture after column derivatization. Same GC parameters as figure 18.

Table 14

Polarity Mixture		Retention Times					Capacity Factors				
Before Derivation		1st run	2nd run	3rd run	Average	Stan.Dev.	1st run	2nd run	3rd run	Average	Stan.Dev.
n-Nonane		3.16	3.35	3.332	3.281	0.105	1.75	1.75	1.83	1.78	0.0462
n-Decane		5.405	5.637	5.613	5.552	0.1276	3.59	3.75	3.75	3.7	0.0924
n-Undecane		7.225	7.26	7.24	7.242	0.0176	4.96	5.13	5.08	5.06	0.0874
n-Dodecane		8.618	8.654	8.637	8.636	0.018	6.13	6.21	6.21	6.1	0.0462
n-Tetradecane		8..677	8.702	8.69	8.69	0.0125	6.23	6.25	6.24	6.24	0.01
5-Nonanone		9.728	9.825	9.757	9.77	0.0494	7.08	7.25	7.17	7.17	0.085
2-Prop-1-cyclohex,t		10.06	10.148	10.092	10.1	0.0445	7.42	7.58	7.5	7.5	0.08
2-Prop-1-1cyclohex,c		11.743	11.796	11.76	11.77	0.0271	8.79	8.83	8.8	8.81	0.0208
2,6-Dimethylaniline		17.48	17.513	17.487	17.493	0.0174	14	13.7	13.5	13.73	0.252

Table 15

Polarity Mixture		Retention Times					Capacity Factors				
After Derivatization		1st run	2nd run	3rd run	Average	Stan.Dev.	1st run	2nd run	3rd run	Average	Stan.Dev.
n-Nonane		2.264	2.24	2.249	2.251	0.0121	1.664	1.635	1.499	1.599	0.088
n-Decane		3.944	3.877	3.904	3.912	0.0293	3.64	3.573	3.338	3.517	0.159
n-Undecane		5.414	5.334	5.361	5.369	0.0407	5.369	5.275	4.967	5.204	0.21
n-Dodecane		6.72	6.623	6.657	6.667	0.0492	6.906	6.792	6.397	6.698	0.267
n-Tetradecane		7.295	7.168	7.238	7.234	0.0636	7.582	7.433	7.042	7.352	0.279
5-Nonanone		7.806	7.582	7.653	7.68	0.1144	8.184	7.92	7.503	7.869	0.343
2-Prop-1-cyclohex,t		8.087	7.917	7.974	7.993	0.0865	8.514	8.314	7.86	8.229	0.335
2-Prop-1-1cyclohex,c		9.762	9.651	9.685	9.699	0.0453	10.485	10.354	9.76	10.2	0.386
2,6-Dimethylaniline		15.366	15.268	15.297	15.31	0.0503	17.078	16.962	15.997	16.679	0.593

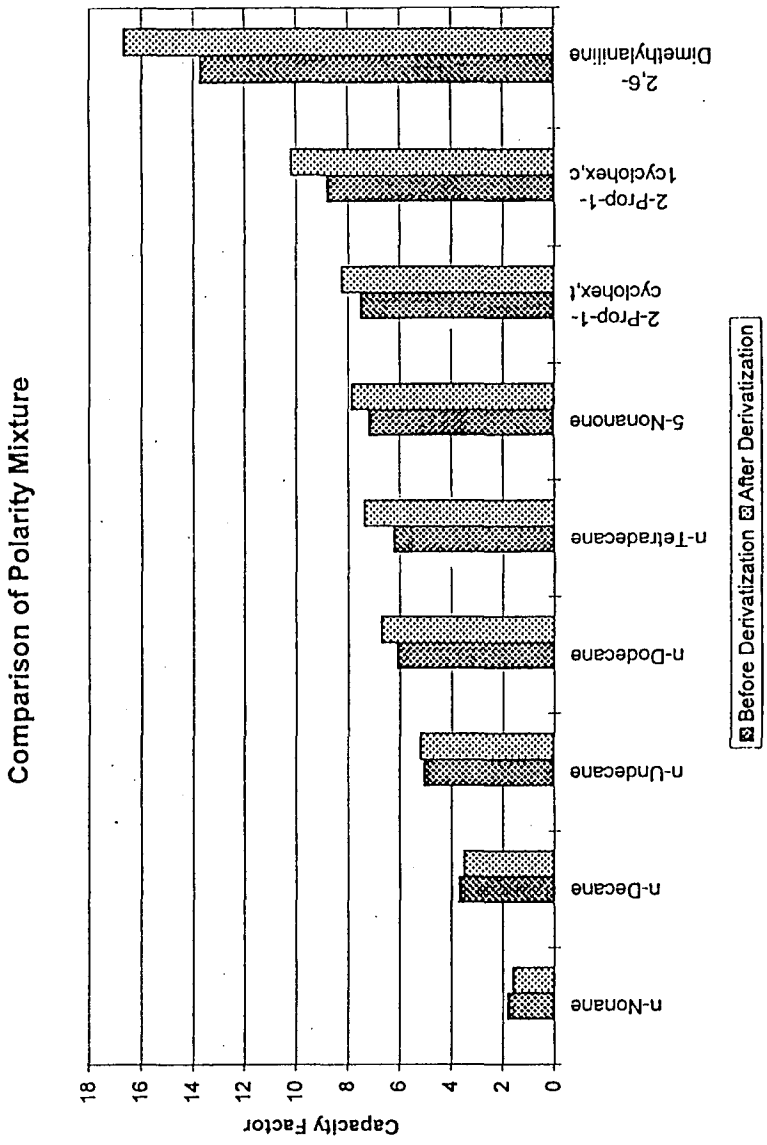

Comparison of Polarity Mixture
Capacity Factor
0
2
4
6
8
10
12
14
16
18
n-Nonane
n-Decane
n-Undecane
n-Dodecane
n-Tetradecane
5-Nonanone
2-Prop-1-cyclohex,t
2-Prop-1-cyclohex,c
2,6-Dimethylaniline
Before Derivatization
After Derivatization

Figure 20.

serve as references for peak shape. They should always retain their symmetry and sharpness, any variation would indicate a poorly installed column. The fatty acids and methyl esters of the mixture, methyl decanoate, methyl dodecanoate and methyl undecanoate, were used to determine the separation efficiency of the column. The alcohols, 1-octanol and 2,3-butanediol, were added due to the fact that hydrogen bonding is often responsible for the adsorption of compounds with hydroxyl functional groups. Silicon stationary phases that have been oxidized will also show tailing of alcohols. Reduced peak height of the aldehyde n-nonanal, would indicate adsorption independent of hydrogen bonding. The last four components of the mixture, the acids and bases provide information on the acidic nature of the column, if any, and the presence of hydrogen bonding or basic sites on the column. These effects could be seen in reduced peak heights or poor peak shape.

Figures 21 and 22 show the separation of the Grob components before and after derivation respectively. One large significant difference is seen for the 2,3-butanediol. Before derivatization it was clearly the first to be eluted with a retention time of 2.892, but after derivatization the peak height and width is significantly lower with a retention time of 2.290. In addition, 2-Ethylhexanoic acid and 2,6-Dimethylaniline appeared to have combined indicated by the trailing peak noted on Figure 22 after derivatization. The separation efficiency for methyl decanoate and methyl dodecanoate increased while the methyl undecanoate decreased as noted in Table 16. Finally, there was a definite increase in resolution of dicyclohexylamine from methyl undecanoate from before and after derivatization. The retention times and capacity factors for the Grob mixture before derivatization are outlined in Table 17. Table 18 includes the same information for the

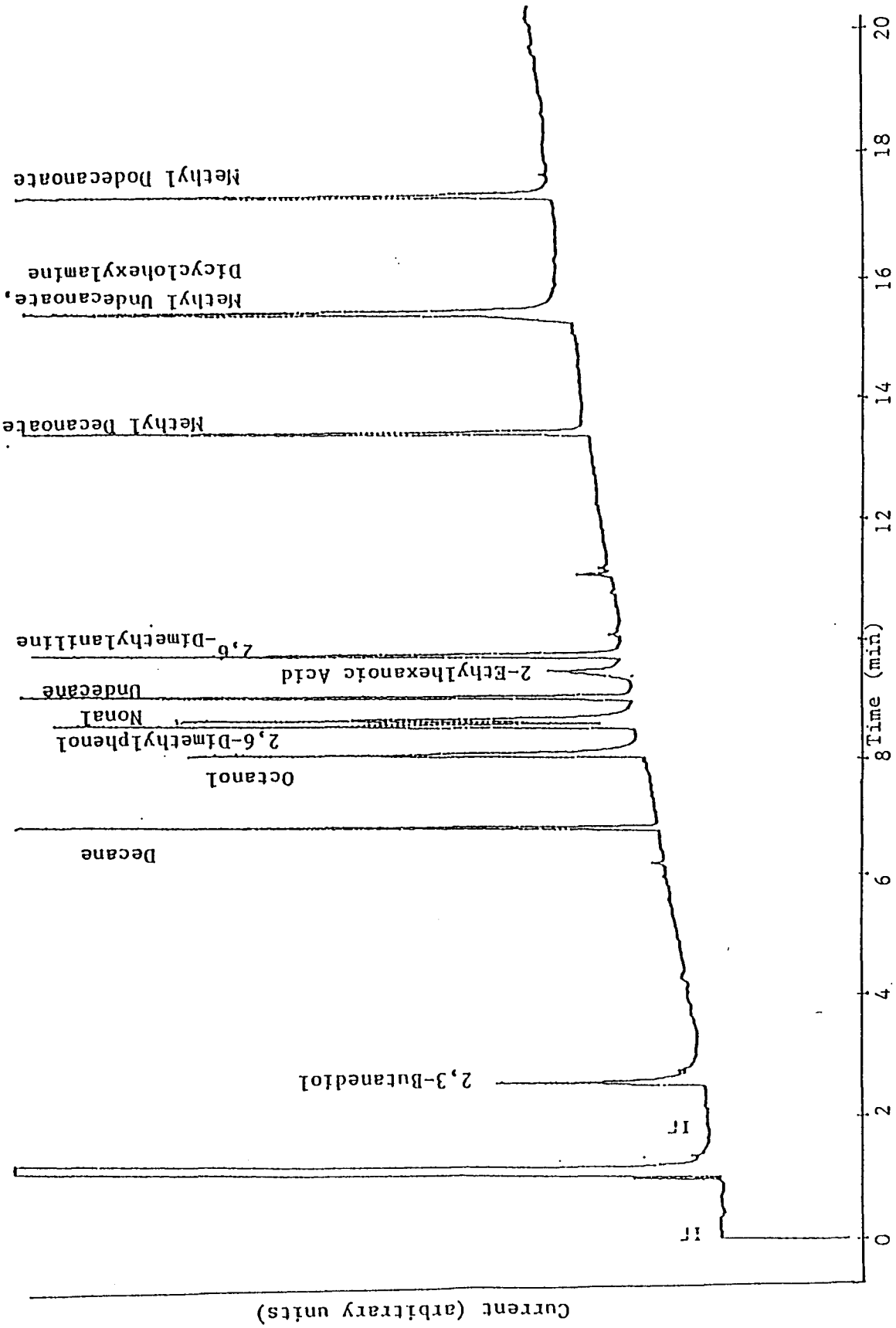


Figure 21. Chromatogram of Grob mixture before column derivatization. The mixture was run according to literature: initial temperature, 40°C (0°C hold time); ramp, 6°C/min; final temperature, 190°C; split ratio, 50:1; flow rate of He (carrier gas), 1mL/min.

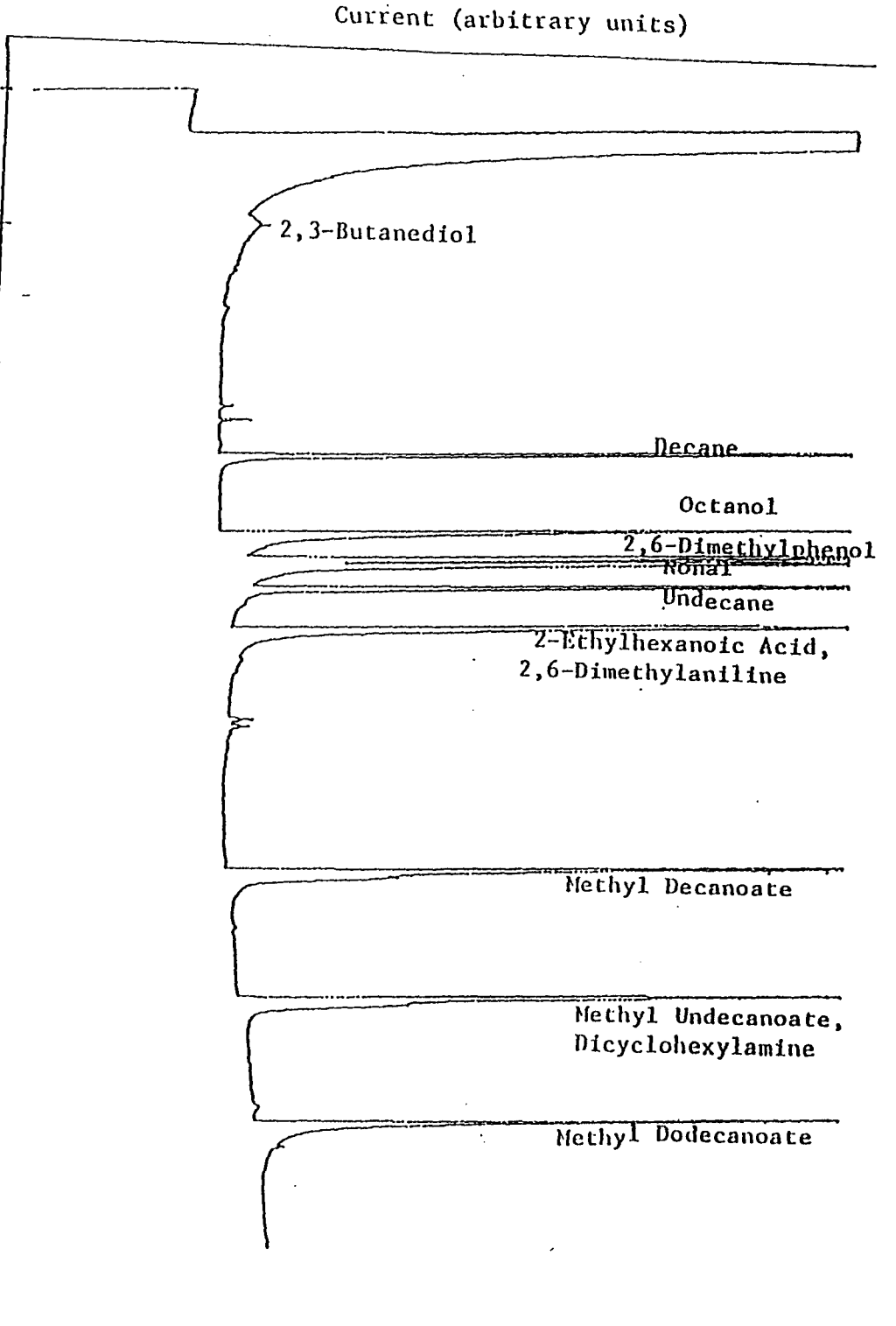


Figure 22. Chromatogram of Grob mixture after column derivatization. Same GC parameters as figure 21.

Table 16

Grob Mixture	Before Derivation				After Derivation			
Components	tr	W	n	H	tr	W	n	H
2,3-Butanediol	2.892	0.3	1486.8	0.01	2.29	0.7	171.24	0.088
n-Decane	7.725	0.2	23870.3	0.00063	6.294	0.15	28170.3	0.00053
1-Octanol	9.145	0.25	21409.5	0.0007	7.68	0.2	23592.3	0.00064
2,6-Dimethylphenol	9.707	0.15	67005.1	0.0002	8.153	0.2	26588.6	0.00056
1-Nonanal	9.813	0.1	154072	9.7E-05	8.26	0.15	48517.4	0.00031
n-Undecane	10.249	0.1	168067	8.9E-05	8.68	0.13	71330.1	0.00021
2-Ethylhexanoic Aci	10.763	0.3	20594.2	0.00073	9.383	0.25	22538.4	0.00067
2,6-Dimethylaniline	11.035	0.2	48708.5	0.00031	9.383	0.25	22538.4	0.00067
Methyl Decanoate	15.336	0.18	116115	0.00013	13.636	0.3	33056.1	0.00045
Methyl Undecanoate	17.615	0.45	24516.6	0.00061	15.872	0.3	44785.8	0.00034
Dicyclohexylamine	17.616	0.45	24516.6	0.00061	15.872	0.3	44785.8	0.00034
Methyl Dodecanoate	19.781	0.15	278249	5.4E-05	18.001	0.25	82953.2	0.00018

Table 17

Grob Mix.	Retention Times					Capacity Factors					
Before derivation	1st run	2nd run	3rd run	Average	Stan.Dev.	1st run	2nd run	3rd run	4th run	Average	Stan.Dev.
2,3-Butanediol	2.89	2.894	2.904	2.892	0.0103	2.1	1.9	2.1	2	2.025	0.0957
n-Decane	7.726	7.731	7.727	7.725	0.0059	6.75	6.75	6.8	6.65	6.738	0.0629
1-Octanol	9.15	9.149	9.15	9.145	0.00984	8.25	8.1	8.2	8.1	8.163	0.075
2,6-Dimethylphenol	9.711	9.713	9.709	9.707	0.00816	8.75	8.7	8.8	8.7	8.738	0.0479
1-Nonal	9.816	9.817	9.815	9.813	0.00556	8.9	8.9	8.95	8.85	8.9	0.0408
n-Undecane	10.253	10.254	10.25	10.249	0.0064	9.3	9.3	9.35	9.3	9.313	0.025
2-Ethylhexanoic Acid	10.745	10.778	10.798	10.763	0.0312	9.85	9.8	9.9	9.65	9.8	0.108
2,6-Dimethylaniline	11.04	11.04	11.038	11.035	0.00822	10.15	10.1	10.2	10.1	10.138	0.0415
Methyl Decanoate	15.341	15.34	15.336	15.336	0.00685	14.4	14.45	14.45	14.35	14.413	0.0479
Methyl Undeacanoate	17.62	17.619	17.616	17.615	0.00688	16.7	16.7	16.72	16.62	16.685	0.0443
Dicyclohexyamine	17.62	17.619	17.616	17.616	0.00688	16.7	16.7	16.72	16.62	16.685	0.0443
Methyl Dodecanoate	19.787	19.785	19.78	19.781	0.00759	18.9	18.85	18.85	18.8	18.85	0.0408

Table 18

Grob Mix.	Retention Times					Capacity Factors				
After derivation	1st run	2nd run	3rd run	Average	Stan.Dev.	1st run	2nd run	3rd run	Average	Stan.Dev.
2,3-Butanediol	2.3	2.25	2.32	2.29	0.036	1.3	1.5	1.32	1.37	0.1101
n-Decane	6.35	6.27	6.262	6.294	0.0486	5.35	5.967	5.294	5.537	0.373
1-Octanol	7.75	7.655	7.635	7.68	0.0614	6.75	7.506	6.68	6.979	0.458
2,6-Dimethylphenol	8.212	8.128	8.118	8.153	0.0516	7.212	8.031	7.153	7.465	0.491
1-Nonal	8.322	8.235	8.223	8.26	0.054	7.322	8.15	7.26	7.577	0.497
n-Undecane	8.741	8.654	8.645	8.68	0.053	7.741	0.616	7.68	5.346	0.524
2-Ethylhexanoic Acid	9.45	9.35	9.343	9.383	0.0582	8.45	9.397	8.383	8.743	0.567
2,6-Dimethylaniline	9.45	9.357	9.343	9.383	0.0582	8.45	9.397	8.383	8.743	0.567
Methyl Decanoate	13.706	13.606	13.595	13.636	0.0612	12.706	14.118	12.636	13.153	0.836
Methyl Undeacanoate	15.945	15.839	15.831	15.872	0.0636	14.945	16.599	14.872	15.472	0.977
Dicyclohexyamine	15.945	15.839	15.831	15.872	0.0636	14.945	16.599	14.872	15.472	0.977
Methyl Dodecanoate	18.076	17.966	17.96	18.001	0.0653	17.076	18.956	17.001	17.678	1.108

Grob mixture, after derivatization. Figure 23 diagrams before and after derivatization with a comparison of the Grob mixture. One interesting point is that all components of the Grob mixture have lower capacity factors after derivatization than before derivatization. This is exactly opposite from what was observed in the Polarity mixture. This is likely due to differences in the separation conditions, although it may also have been indication of column degradation. It may indicate some involvement including the π-π electron interactions with the solute molecule in the cavity of the calixarene.

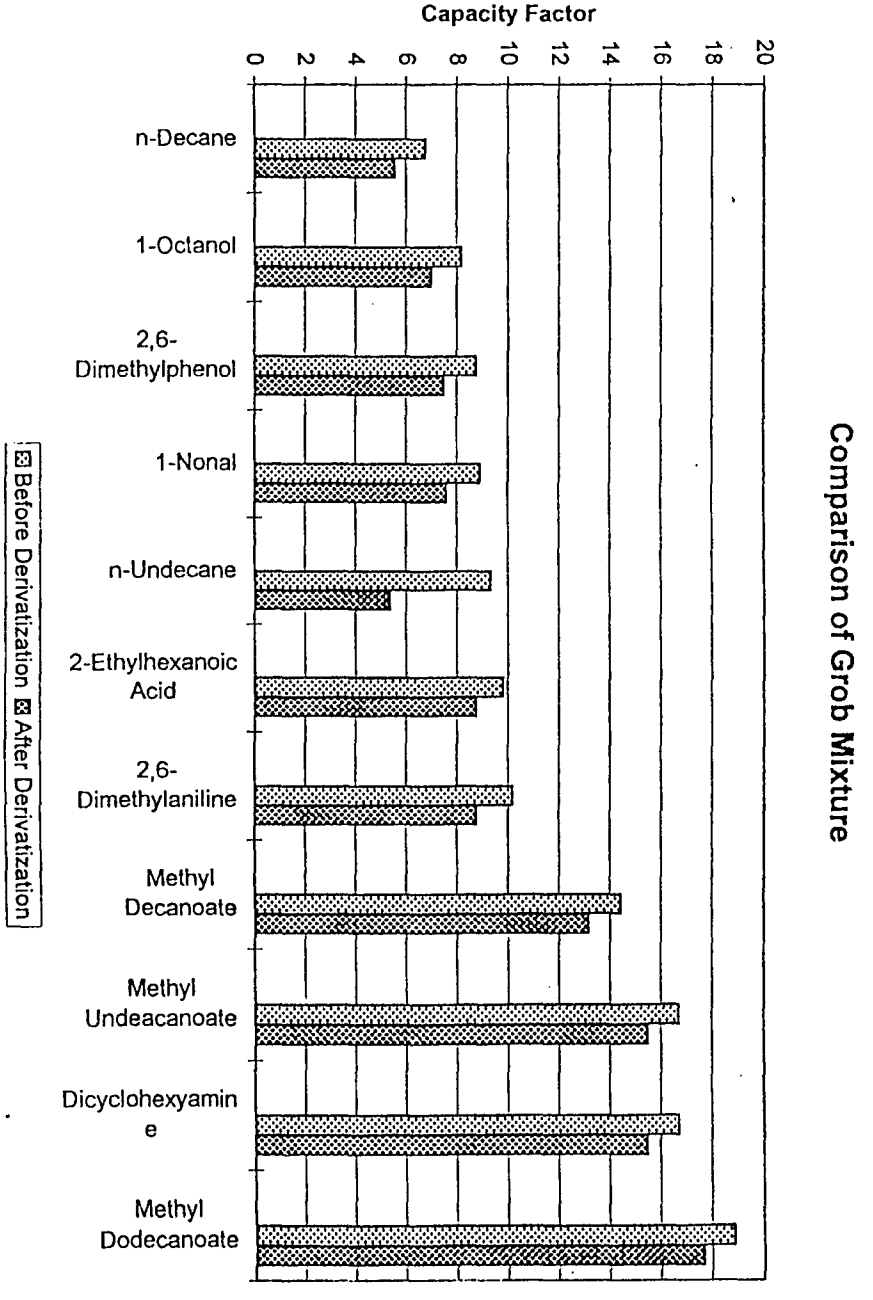

Comparison of Grob Mixture
Capacity Factor
0
2
4
6
8
10
12
14
16
18
20
n-Decane
1-Octanol
2,6-Dimethylphenol
1-Nonal
n-Undecane
2-Ethylhexanoic Acid
2,6-Dimethylaniline
Methyl Decanoate
Methyl Undeacanoate
Dicyclohexyamine
Methyl Dodecanoate
Before Derivatization
After Derivatization

Figure 23.

CHAPTER VI

CONCLUSION

This research has shown that it is possible to statically coat a capillary column for GC by derivatizing the column with t-octylcalix[5]arene. The change in physical properties of the column indicate the column was successfully derivatized. This is illustrated by the change in brittleness of the column before and after derivatization and also the change in increase of column head pressure needed to maintain a flow rate of 1.0 mL/min through the column.

From the data, it is apparent that there is a direct relationship between the log of the capacity factor and the heat of vaporization for all components in the RHS. This is proven through the fact that as the temperature decreased, the capacity factor increased in a linear relationship. Since the logarithm of heat of vaporization is in a direct proportion to the number of carbon atoms, this leads to the logarithm of capacity factor also being in a direct relationship with the number of carbon atoms. All other variables staying constant, this confirms that there must be other forces acting which must include host-guest interactions due to their non-linear plots after derivatization. While there was no retention reversal order with the components of the polarity mixture, there was a significant increase in the polarity of the column as noted by the decrease in retention times before and after derivatization. The efficiency of the column before and after derivatization was shown to increase with methyl decanoate and methyl dodecanoate while the methyl undecanoate decreased, also indicating a host-guest interaction.

A focus of future investigation should involve lower temperatures, such as, 40^0C to better see separations of the components in the RHS. The lifetime of the column might be extended if the evacuation of the solvent step, was extended to overnight. In addition to the RHS that was used in this research, it would be interesting to measure a different series and note if the column would behave in the same fashion after derivatization.

REFERENCES

1. Grob, R.L. In Modern Practice of Gas Chromatography, 2nd ed.; John Wiley & Sons: New York, **1985**: p. 3.

2. Day, R.A.; Underwood, A.L.; Quantitative Anaylsis, 6th ed.; Prentice Hall; Englewood Cliffs, New Jersey, **1991**.

3. Skoog, D.; West, D.; Holler, F.; Fundamentals of Analytical Chemistry, 6th ed.; Saunders College Publishing; New York; **1992**.

4. Christian, G.D.; O'Reilly, J.E.; Instrumental Analysis, 2nd ed.; Prentice Hall; Englewood Cliffs, New Jersey; **1986**.

5. Kennedy, J.H.; Analytical Chemistry: Principles, 2nd ed.; Saunders College Publishing; New York; **1990**.

6. Hargis, L.G.; Analytical Chemistry; Prentice Hall; Englewood Cliffs, New Jersey, **1988**.

7. Grob, K.; Making and Manipulating Capillary Columns for Gas Chromatography, Heidelberg, New York, **1986**, Ch. 3.

8. Frank, H.; Nicholson, G.J.; Bayer, E.; *J. Chromatogr. Sci.*, 15, **1977**, 174.

9. Benecke, I.; Schomburh, G.; *J. High. Resolut. Chromatogr. Chromatogr. Commun.* 8, **1985**, 191.

10. Golay, M.J.; Gas Chromatography, D.H. Desty, ed., Butterworths. London, **1958**, p. 35.

11. Bouche, J.; Verzele, M.; *J. Gas Chromatogr.*, 6, **1968**, 501-505.

12. Grob, Jr., K.; Grob, G.; Grob, K.; J. *of Chromato*gr., 156, **1978**, 1-20.

13. Nieder, J.B.; McCoy, J.S.; *Journal of American Society*, 65, **1943**, 629.

14. Gutsche, C.D.; *Acc. Chem. Res.*, 16, **1983**, 161.

15. Asfari, Z.; Vicens; *Calixarenes.*

16. Gutsche, C.D.; Dhawan, B.; No, K.H.; and Muyhukrishnan, R.; *J. Am. Chem. Soc.*; 103, **1981**, 3782.

17. Schulze, J.; Konig, W.A.; *J. Chromatography*, 355,**1986**, 165.

18. Weller, O.; Schukze, J.; Konig, W.A.; *J. Chromatography*, 403, **1987**, 263.

19. Mnuk, P.; Feltl, L.; *J. of Chromatogr. A*, 696, **1995**, 101-112.

20. Kaliszan, R.; Quantitative Structure-Chromatographic Retention Relationship, Wiley, New York, **1987**.

21. Smolkova-Kevlemansova, E.; *J. of Chromatogr.*,251, **1982**, 17-34.

22. Mangia, A.; *Anayltical Letters*, 16 (A13), **1983**, 1027-1036.

23. Armstrong, D.W.; Li, W.; Chang, C.D.; *Analytical Chemistry*, 62, **1990**, 914-923.

24. Konig, W.; Lutz, S.; *J.of Chromatogr.*, 447, **1988**, 193-197.

25. Linder, K.R.; Mannschrek, A.; *J. Chromatography*, 193, **1980**, 308-310.

26. Armstrong, D.W.; Li, W.; *Anayltical Chemistry*, 62, **1990**, 214-217.

27. Schurig, V.; Nowotny, H.P.; *J. of Chromatogr.*, 441, **1988**, 155-163.

28. Bicchi, C.; D'Amato, A.; Manzin, V.; *J. of Chromatogr. A*, 666, **1994**, 137-146.

29. Armstrong, D.W.; Tang, Y.; Ward, T.; Nichols, M.; *Anayltical Chemistry*, 65, **1993**, 1114-1117.

30. Mnuk, P.; Feltl, L.; *J. of Chromatogr. A*, 732, **1996**, 63-74.