

CHIRAL CALIXARENES AS POTENTIAL ENANTIOSPECIFIC STATIONARY
PHASES IN CAPILLARY GAS CHROMATOGRAPHY

by

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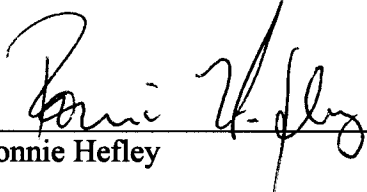
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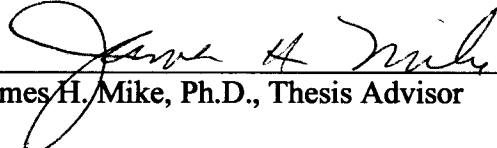
Chiral Calixarenes as Potential Enantiospecific Stationary Phases in
Capillary Gas Chromatography

RONNIE HEFLEY

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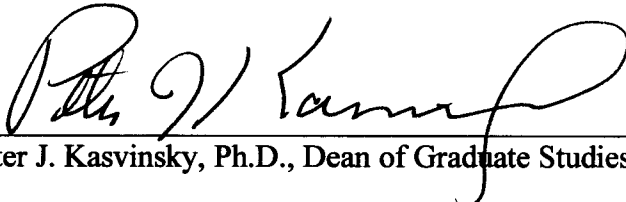
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Abstract

The ability of a chiral calix(4)arene to participate in host-guest interactions could ultimately lead to new types of stationary phases for performing enantiomeric separations in capillary gas chromatography. For this work, chirality was introduced into p-tertbutylcalix(4)arene via addition of L-phenylalanine to the hydroxyl groups located at the bottom of the cup-like structure. The amino-acid derived p-tertbutylcalix(4)arene was absorbed into the polymeric coating of a non-polar siloxane AT-1 stationary phase using evaporation under reduced pressure. Solute retention times were plotted versus the number of alkyl chain carbon atoms in a homologous series of alkyl benzenes. Deviations in the linearity of this plot suggested some type of contribution to solute retention was resulting, possibly from inclusion of the component(s) into the calixarene cavity. Indications of possible host-guest interactions between the amino acid-derived stationary phase with various analytes were apparent from the band broadening due to the slow kinetics, which are typically observed with host-guest interaction. The ability of this newly derived stationary phase to selectively interact with analytes based on their relative polarity was also determined.

Acknowledgements

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List of Abbreviations

AHS	Alcohol Homologous Series
α	Alpha
A	Asymmetry factor
APCI	Atmospheric Pressure Chemical Ionization
amu	Atomic mass unit
β	Beta
k'	Capacity factor
ΔZ	Change in t_r between solutes
CSP	Chiral Stationary Phase
t_0	Dead time (min)
DIPEA	Diisopropylethylamine
DMF	N,N-Dimethylformamide
ECD	Electron Capture Detector
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
F	Flow rate
GC	Gas Chromatography
HATU	O- (7-Azabenzotriazol-1-yl)-N, N, N', N'-hexafluorophosphate
HETP	Height equivalent to a theoretical plate
I.D.	Internal diameter
l	Length of column (m)

List of Abbreviations (cont.)

\bar{u}	Linear velocity
m/z	Mass-to-charge ratio
μL	Microliter
μm	Micrometer
μs	Microsecond
mg	Milligram
mL	Milliliter
min	Minute
mol	Mole
NMR	Nuclear Magnetic Resonance
W	Peak width
psi	Pounds per square inch
r_c	Column radius (cm)
RHS	Reference Homologous Series
R_s	Resolution
t_r	Solute retention time (min)
THF	Tetrahydrofuran
TCD	Thermal Conductivity Detector
TLC	Thin Layer Chromatography
TFA	Trifluoroacetic acid

CHAPTER I

Introduction

A. Gas Chromatography

Chromatography is the general name given to the methods by which two or more compounds in a mixture physically separate by distributing themselves between a stationary and a mobile phase. The stationary phase can be a solid or a liquid supported on a solid and the mobile phase can be either a gas or a liquid, which flows continuously through the stationary phase. Gas chromatography (GC) is a mode of partitioning chromatography where the mobile phase is a gas. GC is the most widely used method for separation of mixtures of gases or for volatile liquids and solids because the separation times are on the order of a matter of minutes, even for very complex mixtures.

B. Detection of Solutes in GC

After GC separates the components of a mixture, they must be detected as they exit the column. The requirements of a GC detector depend on the separation application, which is dependent upon the selectivity of the analysis. For example, one analysis might require a detector that is selective for chlorine-containing compounds. These methods can be either destructive, where the analyte cannot be recovered for further spectroscopic analysis, or it can be nondestructive, where the analyte can be recovered for further spectroscopic analysis.

Thermal-conductivity (TCD) and flame-ionization (FID) detectors are the two most common detectors in GC. A TCD consists of an electrically heated wire or thermistor, where the temperature of the sensing element depends on the thermal

conductivity of the gas flowing around it. When organic molecules displace the carrier gas, the changes in thermal conductivity cause a temperature rise in the element, which is sensed as a change in resistance. A TCD is not as sensitive as other detectors, but is non-specific and nondestructive. A FID consists of a hydrogen/air flame and a collector plate, where ions are collected and produce an electrical signal. When the eluant from the GC passes through the flame, the organic molecules are broken down, producing ions. The ions are collected on an electrode and produce an electric signal. The FID is extremely sensitive, but it destroys the sample. Another widely used type of detector is an electron-capture detector (ECD). The ECD uses a radioactive β^- emitter to ionize some of the carrier gas and produce a current between a pair of electrodes. When organic molecules that contain electronegative functional groups pass through the detector, those functional groups capture some of the electrons and reduce the current measured between the electrodes. The ECD is extremely sensitive, but is limited to the analysis of halogenated compounds. Mass spectrometers (MS) are also widely used as a method of detection for GC. MS use the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules to separate them from each other. MS is therefore useful for quantification of atoms or molecules and also for determining chemical and structural information about the molecules, such as their fragmentation patterns.

C. Peak Broadening and Component Retention

Peak broadening and retention are behavior characteristics unique to each component of a mixture in gas chromatography. The time and manner in which a particular compound elutes is a “fingerprint” characteristic that can be used to help

identify it in a mixture. Resolution and separation of those components are generally controlled by both thermodynamic and kinetic parameters. The thermodynamic and kinetic factors that determine retention times are fundamental properties of a solute relative to the stationary phase. Optimization of these effects results in enhanced chromatographic separations.

The GC oven controls the temperature of the column. Chromatographic separations can be carried out under isothermal conditions, or it is possible to perform temperature gradient separations, where the oven is programmed to change the column temperature at some predictable and reproducible rate ($^{\circ}\text{C}/\text{min}$). A temperature gradient can be especially useful in separating mixtures of substances with widely varying heats of vaporization. The linear velocity (\bar{u}) and the flow rate (F) are two common factors that depend upon the head pressure of the carrier gas. The linear velocity of the carrier gas is described by:

$$\bar{u} = \frac{l}{t_0}$$

The flow rate of mobile phase through the column is described by:

$$F = \frac{\pi (r_c^2) l}{t_0}$$

where: l = column length (cm) and r_c = radius of the column (cm). The dead time (t_0) is the amount of time that it takes for an unretained component to elute from the column and is thus dependent on head pressure of the mobile phase (carrier gas). These factors serve as a reference point for assisting in the determination of the identity of the components in a mixture. With these established parameters in place, the identity of a

compound can be determined by comparison of the experimental retention time versus the literature value for the retention time of that particular component, or versus a standard mixture of various components.

Peak resolution is a function of both the solute distribution coefficient, K_D , between the mobile and stationary phases and the ratio of the volumes, Φ , of mobile and stationary phases in the column. It is possible to experimentally determine the distribution coefficient, but the phase volume ratio is not easily obtainable because of the nature of the stationary phase. Thus, because retention time is a combination of these two factors, a ratio, called the capacity factor (k'), is used to describe chromatographic retention. The capacity factor is defined by:

$$k' = \frac{t_r - t_0}{t_0}$$

where t_r is the component retention time and t_0 is the dead time of the column. Small values for the capacity factor indicate little interaction between component and stationary phase resulting in the elution of solutes close to the unretained peak. Larger values for the capacity factor indicate more interaction between component and stationary phase resulting in longer retention times.

A common thermodynamic description used to characterize the partitioning process of solutes during chromatography can be expressed in terms of the system free energy, where:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT \ln K$$

and:

$$K = \frac{k'}{\Phi}$$

or:

$$\ln K = -\frac{\Delta G^0}{RT} = \ln \frac{k'}{\Phi} = \ln k' - \ln \Phi$$

therefore:

$$\ln k' = -\frac{\Delta G^0}{RT} + \ln \Phi$$

where K is the equilibrium constant, k' is the solute capacity factor, ΔG^0 is the free energy of partitioning, ΔH and ΔS are the enthalpy and entropy of partitioning of the solute from the mobile to the stationary phase, R is the universal gas constant, T is the temperature (in Kelvin), and Φ is the phase volume ratio (i.e., the volume of the stationary and mobile phases relative to one another).

If:

$$\Delta G^0 = n\overline{\Delta G} + b$$

where $\overline{\Delta G}$ is the free energy contribution for each of n carbon atoms in a molecule or chain and b is some constant independent of n , then a plot of $\ln k'$ versus n at a constant T will have a slope of:

$$\frac{d \ln k'}{d n} = -\frac{\overline{\Delta G}}{RT} > 0 \quad \text{if} \quad \overline{\Delta G} < 0 \quad \text{for adsorption.}$$

Therefore, a constant contribution for each of n carbon atoms in a molecule or chain of homologous series would result in a straight line plot.

Gas chromatography is a dynamic process, which includes mass-transport that can be described kinetically. These effects are described using the van Deemter equation.

Peak broadening during a chromatographic separation can be attributed to a number of random molecular processes that can be grouped as either non-column or column effects.

Non-column peak broadening is due to the dispersion of component molecules in the carrier gas in the injector and connections before the column and between the column and detector. Dispersion is most significant in open tubular GC columns, but can be avoided by good installation and sealed connections between injectors, detectors, tubing, and fittings. Minimization of the dead volume will lead to optimized resolution of components.

Column peak broadening for chromatography was shown by van Deemter to be the summation of the result of three molecular diffusion processes. The van Deemter equation expresses this in terms of HETP, the height equivalent to a theoretical plate:

$$HETP = A + \frac{B}{u} + Cu$$

where A , B , and C are constants related to the three major factors affecting the HETP. A represents eddy diffusion and is due to the variety of pathways available between the particles in the column and is independent of the carrier gas velocity. Eddy diffusion is a characteristic of the column packing and can be decreased with smaller and more uniform particles and tighter packing. This results in increased efficiency of the column. Good separation and minimum peak broadening will be achieved using small particles with a narrow size range that are packed uniformly into the column, however, an extremely small support is difficult to pack uniformly. B represents the longitudinal or molecular diffusion of the sample components in the carrier gas due to the concentration gradients inside the column. High flow rates reduce molecular diffusion, which can be changed only by varying the pressure, flow rate, and type of carrier gas. C represents the rate of mass transfer and is due to the amount of time taken for solute equilibrium to be

established between the mobile and stationary phases. Increasing the temperature, which effectively increases the solubility of the vapor components of the sample decreases the rate of mass transfer. Also, by decreasing the flow rate more time is allowed for the solute equilibrium to be established, which results in a decreased rate of mass transfer. All conditions must be adjusted to obtain a balance between molecular diffusion and mass transfer. The three molecular diffusion processes are kept as small as possible to provide the minimum HETP or an increase in efficiency for the sample solute.

With respect to polarity effects, this kinetic dependence results in the response of the FID being proportional to the number of carbon atoms. This response diminishes with increasing substitution by halogens, amines, or hydroxyl groups. Other kinetic interactions that could affect the response of the detector include either some type of chemical reaction occurring between the solute and the stationary phase or an interaction, based on something outside polarity effects occurring between the solute and the stationary phase. A chemical reaction occurring in the column is not desirable because it changes the composition of the eluting component and the chemical behavior of the stationary phase. Some type of host-guest interaction based on an effect outside of those due to polarity could result in a more specific separation without affecting the composition of the eluting component or the chemical behavior of the stationary phase.

Peak resolution is defined simply as the degree of separation between two adjacent peaks. The resolution R_s of a column provides a quantitative measure of its ability to separate two components and is defined as:

$$R_s = \frac{2 [(t_r)_B - (t_r)_A]}{W_A - W_B}$$

where t_r is the retention time for components A and B and W is the width at the base of the peak for components A and B . $R_s < 1$ means the peaks are not resolved; $R_s = 1$ means the peaks are resolved well enough for quantitative analysis, but are not baseline separated; $R_s > 1$ means the peaks are baseline resolved.

Peak resolution is strongly affected by peak symmetry. In chromatography, it is assumed that peaks emerge with either a Gaussian or Poisson type concentration profile. The asymmetry factor (A) mathematically describes the symmetry of the peak and is defined by:

$$A = \frac{b}{a}$$

where b is the distance after the peak center and a is the distance before the peak center to the detector response measured at 10% of the total peak height. A value of one would indicate a mathematically symmetric peak; values less than one result from fronting peaks; values larger than one result from tailing peaks. Any host-guest interactions that are additional to bulk absorption result in an increased retention time. The kinetics of this interaction would be slower than the kinetics of the absorption, which is called mixed-mode retention and is characterized by tailing peaks. This would also be indicated by increased values for both the capacity and asymmetry factors.

D. Host-Guest Complexation

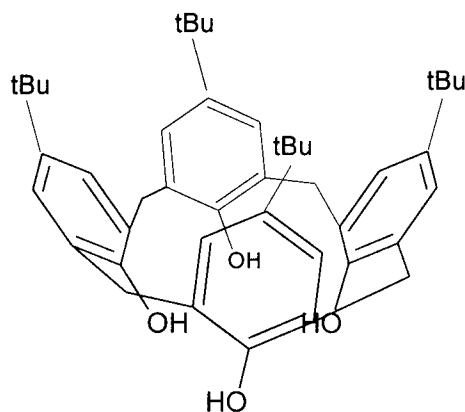
A type of separation that requires a more specific interaction than those due strictly to polarity is the resolution of enantiomers. A racemic mixture is composed of components that differ only with regard to their optical activity. As a result, polarity

effects alone are not enough to separate and resolve this type of mixture. Enantiomers only interact specifically with other enantiomers. Such separations thus require an enantiomeric stationary phase.

In chromatography, host-guest complexation was first performed using cyclodextrins, which have similar structure and inclusion characteristics as calixarenes (1). Studies have indicated that this host-guest complexation results in an enantioselective stationary phase by allowing selected components to fit themselves into the cavity of the cyclodextrin, much like an enzyme and substrate in biochemical systems. This enantioselectivity was demonstrated toward carbohydrates and nitrogen containing compounds and was based on the selectivity of the formation of inclusion complexes between solute and stationary phase.

E. Calixarenes

Recently, calixarenes have drawn attention in the area of chromatographic separations due to the host-guest interactions that this compound has been shown to form. Calixarenes are macrocyclic phenol-formaldehyde polycondensates that possess an interesting basket shaped intramolecular cavity (2) (Figure 1).



p-tert-butylcalix(4)arene

Figure 1. Structure of 4-tert-butylcalix(4)arene

When used as components of stationary phases in chromatography, calixarenes are capable of forming inclusion complexes that affect the retention of solutes as compared to non-derived stationary phases. Because of their similarity to cyclodextrins, which have been shown to resolve enantiomers, chiral calixarenes could potentially be used for enantiomeric separations.

Calixarenes have been regarded as a third generation of host compounds, after cyclodextrins and crown ethers, because they can form inclusion complexes with ionic and neutral molecules. They are also easily modified to give more functionalized host calixarenes, which may have great potential in analytical chemistry (3). Calixarenes act as a platform that can be used to support a variety of functional groups. The resulting calixarene derivative has similar characteristics to the compound from which it was made. The synthesis of chiral calixarenes is of great value for the development of a new class of chiral host molecule (4). This might provide a suitable enantiomerically active stationary phase that could be used for separations of optical isomers, much like the cyclodextrins in current use in GC columns.

In gas chromatography, the stationary phase is most commonly a polymeric liquid coated on the inner surface of a capillary column. Static coating is a desirable method of preparing a derivatized column because of its relative simplicity.

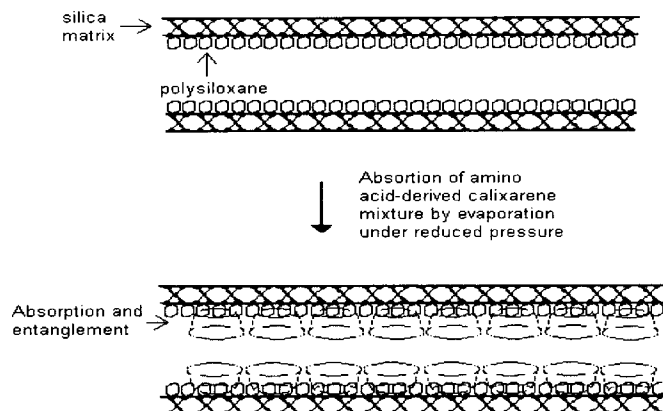


Figure 2. Diagram of absorption process

This method is done by absorption of the stationary phase into the nonpolar wall of a column containing dimethylpolysiloxane (Figure 2). It was hoped that chirality might be introduced into the non-chiral calixarene structure through this synthetic strategy.

For the sake of simplicity, immobilization of the modified calixarene was accomplished by absorption into the silicone polymer coating of an Alltech AT-1 capillary column (Figure 2).

CHAPTER II

Statement of the Problem

Gas chromatography (GC) is the method of choice for separation and analysis of volatile or gaseous mixtures. A problem facing modern chromatographers is the resolution of mixtures containing optical isomers (enantiomers). Chromatographic separation of enantiomers requires the utilization of chiral stationary phases. To prepare these phases, chiral substrates are either attached to the inner wall of a silica capillary column or embedded within the polymeric matrix of an existing capillary column.

Calixarenes, a class of organic, basket shaped molecules, are promising candidates for these types of separations. They are not inherently chiral, however, and must be modified through organic synthetic techniques to make them suitable for potential utilization in chiral separations.

This project involves derivatizing t-butylcalix[4]arene with phenylalanine residues through the hydroxyl groups at the bottom of the cup-like structure. The synthetic method is based on a well characterized addition of alanine to calix(4)arene, but required significant modification for addition of phenylalanine. The phenylalanine-derivatized calixarene was subsequently absorbed into a dimethylpolysiloxane (nonpolar) stationary phase. The gas chromatographic behavior and characterization of the absorbed phenylalanine-calixarene derivatives were determined by analysis of standard GC capillary column test mixtures, two types of homologous series of compounds, isomeric mixtures, and phenolic mixtures. For the homologous series of compounds, plotting solute retention data (corrected retention times or capacity factors) against molecular

descriptors (number of carbon atoms, phenyl rings, or functional groups) helped to provide an indication of host-guest behavior through observations of deviations from linearity and changes in retention behavior.

CHAPTER III

Literature Review

Enantiomeric separations of racemic mixtures are an ever-growing aspect of gas chromatography (GC). The earliest type of enantiospecific stationary phase was based on hydrogen bonding interactions (5). Chiral stationary phases in GC result in enantiomeric separations with a mechanism due to solute-solvent enantioselective hydrogen bonding interactions. Today, enantiomeric separations are still based on these interactions.

Chiral separations have many different applications; for pharmaceuticals, one enantiomer is usually more effective than the other. In a few instances the less effective enantiomer even has toxic properties (6). Derivatized polysiloxanes have shown sufficient enantioselectivity and thermal stability to separate various pharmaceuticals into enantiomeric pairs within a reasonable time (7). Derived polysiloxanes have also been widely used as chiral stationary phases. Polysiloxanes attached with a β -cyclodextrin side chain have shown good film formation, excellent isomer separation and good enantiomeric separation ability. It was also observed that the ability of these derived stationary phases to separate m- and p-chlorotoluenes decreases with a decrease in temperature in the range of 80-110°C (8).

The enantiomeric purity of amino acids and the amount of racemization in peptide synthesis and peptide hydrolysis can easily be determined by the use of capillary GC on several hydrogen-bonding chiral stationary phases (9). In 1966, Gil-Av et al. reported the first successful separation of enantiomers of derivatized amino acids on a chiral amino acid derivative (10). Later, Feibush developed chiral stationary phases containing

diamide moieties, which proved to be even more useful because of their high enantioselectivity and reduced polarity, which lead to reduced retention times (11). A different approach to enantiomeric separations of amino acids derivatives by GC consists of the use of modified cyclodextrins. Following the success of modified cyclodextrins as selective stationary phases in various modes of chromatography, calixarenes are now considered interesting synthetic selectors in GC because of their high thermal stability and their unique cavity-type supramolecular shape (2,12).

A tripeptide derivative provided excellent enantiomer separation of a variety of racemic mixtures, including alcohols, amines, amino acids, carboxylic acids, hydroxyl acids, and amine acids via GC (5). Chiral recognition of this tripeptide derivative was shown to be dependent upon hydrogen bonding between solutes and chiral stationary phases (13). Other chiral stationary phases based on these same principles are being developed and applied in all aspects of chemistry. GC chiral separations of several derivatized trimethylsilyl ethers have shown excellent baseline separations of the enantiomers, highly increased sensitivity of detection, no significant concentration dependence, and decreased analysis time.

Calixarenes possess an interesting basket shaped intramolecular cavity (14). Since they possess a cylindrical architecture similar to that of cyclodextrins, they are expected to form inclusion complexes (15). These compounds have been regarded as the third generation of host compounds since they can complex with ionic and neutral molecules and are easily modified to the more functionalized host calixarenes, which may have great potential in analytical chemistry (16). When used as selective components of stationary phases in capillary GC, calixarenes are capable of forming inclusion

complexes with many metal ions and organic molecules. Formation of inclusion complexes are determined by the overall macrocyclic structure, most importantly by the cavity size, but also by the nature of the functional groups which act as the binding sites (17). Calixarenes assume the role of a platform supporting a variety of ligating groups, resulting in molecules having similar characteristics to those of the compound from which the calixarene derivative was made.

Until September of 1998, there had been no report on the application of calixarenes in GC, and the reasons are due to the high melting point of calixarene derivatives which leads to difficulties in coating them onto the internal wall of the capillary column, thus resulting in poor column efficiency (18). A facile method to resolve this problem was to attach the calixarene molecule directly onto a polymer substrate, such as a polysiloxane. Recently, two materials were reported to have been developed as GC stationary phases, involving a calixarene unit that was directly dissolved into the polysiloxane or attached onto the main chain of the polysiloxane. The results showed a high column efficiency and unique selectivity for aromatic isomers (3,19). In 1977, Chirasil-Val, a chiral polysiloxane with (S)-valine-*tert.*-butylamide anchored to carboxypropyl-modified dimethylpolysiloxane was reported (20). Since then, polysiloxanes have received increasing attention as suitable liquid matrices for anchoring and dispersing chiral moieties (6).

A series of amino acid ester hydrochlorides bonded on a silica calix(4)arene tetraester phase were shown to be retained in order of their hydrophobicity and are shown to selectively include amines in the calixarene cavity. These amino acid-derived calixarenes were reported to encapsulate primary amines and not secondary or tertiary

diamide moieties, which proved to be even more useful because of their high enantioselectivity and reduced polarity, which lead to reduced retention times (11). A different approach to enantiomeric separations of amino acids derivatives by GC consists of the use of modified cyclodextrins. Following the success of modified cyclodextrins as selective stationary phases in various modes of chromatography, calixarenes are now considered interesting synthetic selectors in GC because of their high thermal stability and their unique cavity-type supramolecular shape (2,12).

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Until September of 1998, there had been no report on the application of calixarenes in GC, and the reasons are due to the high melting point of calixarene derivatives which leads to difficulties in coating them onto the internal wall of the capillary column, thus resulting in poor column efficiency (18). A facile method to resolve this problem was to attach the calixarene molecule directly onto a polymer substrate, such as a polysiloxane. Recently, two materials were reported to have been developed as GC stationary phases, involving a calixarene unit that was directly dissolved into the polysiloxane or attached onto the main chain of the polysiloxane. The results showed a high column efficiency and unique selectivity for aromatic isomers (3,19). In 1977, Chirasil-Val, a chiral polysiloxane with (S)-valine-*tert.*-butylamide anchored to carboxypropyl-modified dimethylpolysiloxane was reported (20). Since then, polysiloxanes have received increasing attention as suitable liquid matrices for anchoring and dispersing chiral moieties (6).

A series of amino acid ester hydrochlorides bonded on a silica calix(4)arene tetraester phase were shown to be retained in order of their hydrophobicity and are shown to selectively include amines in the calixarene cavity. These amino acid-derived calixarenes were reported to encapsulate primary amines and not secondary or tertiary

amines in the calixarene cavity, indicating a sterically controlled selectivity (21). Transport efficiency was found to be closely related to the hydrophobicity of the amino acid esters. It was postulated that the ammonium group of the guest is anchored in the carrier cavity, forming an endo-calix complex (22). Since then, amino acids have been effectively attached to the platform of calixarenes and homotrioxa-calix(3)arenes. It is encouraging that the recognition, separation, and analysis of enantiomers with this kind of chiral calixarene has been recently accomplished (23). These amino acid-derived calixarenes are expected to be useful as new recognition sites in the design of enzyme mimics in totally synthetic systems, and it has been suggested that the synthesis of chiral calixarenes would be of great value for the development of a new class of chiral host molecule (4).

The fundamental principles and properties of calixarenes and their derivatives have shown that the simultaneous attachment and ligating functions at the upper and lower rims leads to receptors with two different binding sites (ditopic receptors), demonstrating the possibilities offered by calixarenes as basic platforms for GC (24). These new molecular recognition phases based on macrocyclic calixarenes show enormous potential for the tailoring of chromatographic selectivity through calixarene functionality (17).

CHAPTER IV

Materials and Methods

A. Materials

All reagents used in this work were of the highest purity available and used as received. 4-tertButylcalix(4)arene was purchased from Fluka (Milwaukee, WI). Protein sequencing grade TFA was purchased from Aldrich (Milwaukee, WI) and stored under an inert atmosphere. THF was distilled over sodium metal and stored under an inert atmosphere. NaH was a 60% dispersion in mineral oil and was purchased from Aldrich (Milwaukee, WI). Tetramethylammomium hydroxide was a 10 wt % solution in H₂O and was purchased from Aldrich (Milwaukee, WI). L-phenylalanine tert-butylester HCl was purchased from Chem-Impex International (Wood Dale, IL) and was stored at 0°C.

The solvents (HPLC grade) used throughout this work were purchased from Fisher Scientific (Fairlawn, NJ). All gases were obtained from Praxair (Cleveland, OH). Helium and hydrogen used as the carrier and detector gas, respectively, were of Ultra high purity grade. Compressed air was used as an oxidizer for the H₂ that was distributed to the detector.

The AT-1 fused silica capillary column (100% dimethylpolysiloxane) was purchased as a 30m column, but was subsequently cut into 10m lengths. The column had an I.D. of 0.25mm and a film thickness of 0.25µm. The column ends were cut with a ceramic scribe as straight as possible to ensure proper installation and optimum results. The reducing unions and ferrules used were 1/16" – 1/32" and 1/32" to 0.4mm, respectively, both purchased from Valco (Deerfield, IL).

Each of the test mixtures was composed of 10 mg of the corresponding solute dissolved in 100 mL of methanol. All weighings were performed using a Mettler Balance (Mettler Instrument Corporation, Hightstown, NJ). Each test mixture was stored in a sealed container at room temperature when not in use.

B. Methods

The synthesis of the amino acid-derived t-butylcalix(4)arene was based on a well-characterized addition of alanine to calix(4)arene (13). This synthetic method required significant modification for the addition of phenylalanine, however. This addition involved attachment of phenylalanine residues through the hydroxyl groups at the bottom of the calixarene cup-like structure.

The phenylalanine-derived calix(4)arene was subsequently absorbed into a dimethylpolysiloxane (nonpolar) stationary phase via a slightly modified static immobilization method (14). This method involved filling a capillary column with a dilute solution of the phenylalanine derived calix(4)arene, closing one end of the column with a capped reducing union, applying pressure to help drive the calixarene into the polymer matrix, and subsequently evaporating the solvent under reduced pressure. This method left behind a coating of the chiral calix(4)arene on the inside wall of the capillary column.

1. Synthesis of the Chiral Calixarene

The amino acid-derived calix(4)arene was prepared according to a reported procedure (13), with the following modifications (Figure 3). p-Tertbutylcalix(4)arene

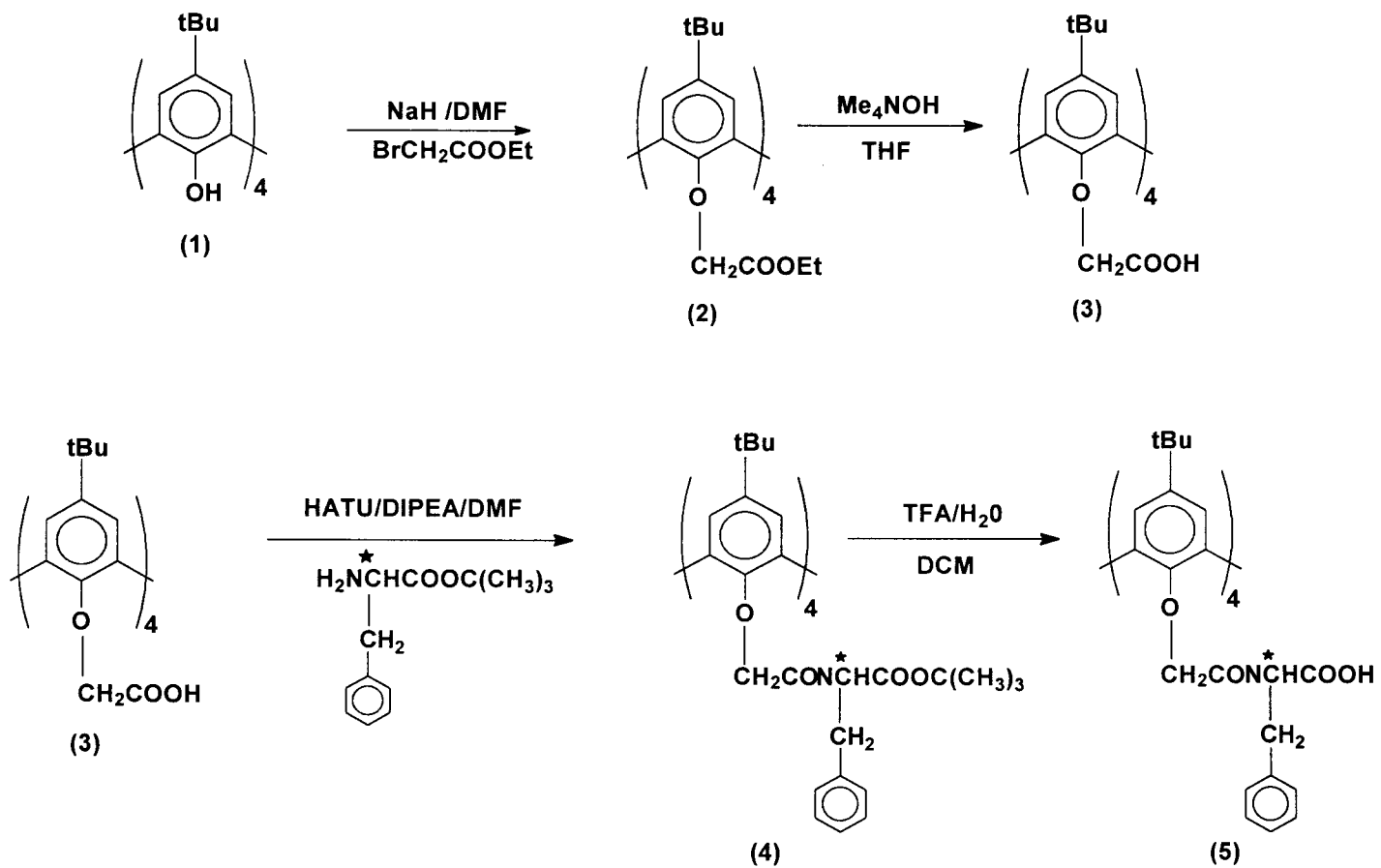


Figure 3. Synthesis of amino acid-derived calix(4)arene

(0.067g) was suspended in dry DMF (2.1mL) and was treated with NaH (0.025g). Ethylbromoacetate (0.060mL) was then added and the mixture was stirred at 80°C for 4 hours. The reaction mixture was allowed to cool and was then treated with a second portion of NaH (0.015g). Ethylbromoacetate (0.030mL) was then added and the mixture was stirred again at 80°C for approximately 20 hours to ensure complete alkylation.

The hydrolysis of the alkylated p-tertbutylcalix(4)arene was performed by dissolving the alkylated p-tertbutylcalix(4)arene (0.998g) in THF (50mL). This solution was added to 10% aqueous tetramethylammonium hydroxide (50mL) and heated at reflux for 24 hours. After cooling, the reaction mixture was acidified with concentrated HCl and stirred overnight at room temperature. The resulting bilayered solution was rotovapped until all of the THF was removed, inducing precipitation of the hydrolyzed product. The hydrolyzed product was filtered, washed with water, and oven dried at 100°C.

The addition of the amino acid to the hydrolyzed product was performed entirely under an atmosphere of argon. All glassware and reagents were dried and additions were done in such a way as to keep water out of the reaction. The hydrolyzed product (1.60g) was dissolved in dry DMF (80mL) with stirring. HATU (2.75g) and DIPEA (2.80mL) were added to this solution at room temperature. The resulting solution was cooled to 0°C and L-phenylalanine t-butyl ester HCl (1.87g) was added and allowed to stir at 0°C for the first hour. This solution was allowed to warm to room temperature and stirred overnight. The product was precipitated out of solution by addition of water and filtered. It was subsequently dried under vacuum (the product should not be heated). The addition product was cleaned by dissolving it into a minimum amount of a 1:1 solution of distilled

THF/ hexanes (reagent grade). This mixture was pushed through a plug of silica to remove impurities. The silica was rinsed several times with the 1:1 solution to ensure optimum retainment of the product. All of these washings were collected in the same receiving flask and were rotovapped until the solvent was removed, inducing precipitation of the addition product. The addition product was allowed to dry by vacuum filtration.

The hydrolysis of the addition product was performed by dissolving the addition product (0.100g) in a solution of TFA containing water (5%) at 0°C. The solution was allowed to warm to room temperature where it was stirred for 1 hour. The status of the reaction was checked by TLC (20% THF/hexanes) to ensure completeness of the reaction. Water was then added to the reaction mixture to precipitate the product. The hydrolyzed amino acid-derived calix(4)arene was filtered and washed with water.

2. Absorption into the Stationary Phase

The absorption of the calixarene derivatives was performed according to literature methods (14) with the following modifications. In a pre-cleaned screw cap with septum vial, approximately 1 % (w/w) of the calixarene derivative was dissolved in methylene chloride. The mixture was shaken to ensure complete dissolution. The septum vial was pierced with a 22-gauge needle and the column was fed through the needle into the solution. The needle was then removed, leaving the column end in solution with an airtight seal around the column through the septum. The body of the column was hung on a metal rod directly above the vial containing solution. A 20 mL plastic syringe fitted with a 22-gauge needle was used to supply the column with a small, but constant amount

of pressure. The syringe was prepared by drilling a hole about halfway down the syringe completely through the barrel and the plunger. To apply pressure onto the solution (pushing it into the column), the plunger was pulled out as far as possible, and then inserted through the septum into the vial. Pressure was applied by pushing in the plunger which was then held in place by placing a small rod through the drilled hole to achieve constant pressure (Figure 4). After several drops of the calixarene solution exited the outlet end of the column, the outlet end of the column was sealed by closing one end of a 1/16" – 1/32" Valco reducing union and attaching the open end to the column with a 1/32" to 0.4mm Valco reducing ferrule.

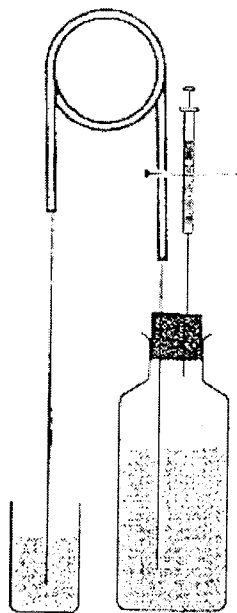


Figure 4. Diagram of column filling apparatus

3. Evacuation of the Solvent

The inlet end of the column was removed from the column filling apparatus and attached to a tank containing ultra high purity N_2 . Pressure was introduced into the

column and gradually increased to 70 psi, where it was held for 20 minutes. This procedure pushed the calixarene derivative into the silicone polymer stationary phase attached to the walls of the column. The pressure was gradually decreased and the inlet end of the column was placed on a vacuum line and the column was placed in a 30°C water bath for 40 hours.

4. Conditioning the Column for Use

The inlet end of the column was then sealed and the column was preconditioned by heating at 160°C for 1 hour followed by another hour at 180°C heating. After preconditioning, the column was mounted on the GC where it was conditioned for 3 hours at 120°C with a carrier gas (He) flow rate of 1.0mL/min. The column was cooled and removed from the GC and stored at room temperature with septa covering both ends.

5. Descriptions of Prepared Stationary Phases

Columns containing tetra(L-phenylalanyl)-4-tetra(tert-Butyl)-1-tetra(carboxymethyl)calix(4)arene tetra(tert-Butyl)ester were prepared along with columns containing tetra(L-phenylalanyl)-4-tetra(tert-Butyl)-1-tetra(carboxymethyl)calix(4)arene. This was done to investigate the differences in the properties of the stationary phases due to drastically different polarities and to also help in the overall characterization of the stationary phases. The underivatized AT-1 stationary phase was used as a reference for comparison of the derived stationary phases.

6. GC Parameters

The GC unit consisted of a Varian (Walnut Creek, CA) 3400 CX Gas Chromatograph with Star Chromatography (Version 4.01) software equipped with a flame ionization detector (FID). The capillary column used as a reference was an Alltech Heliflex AT-1 (Deerfield, IL) 10m x 0.25mm ID x 0.25 μ m. The derivatized stationary phases were absorptions onto this AT-1 column. Table I lists the chromatographic operation conditions used for characterization of the stationary phases.

Table 1. Typical Chromatographic Conditions	
Column:	Alltech AT-1 (catalog number 13753), 10m x 0.25mm ID x 0.25 μ m
Column temperature:	90°C
Sample concentration:	~ 300ng/mL in MeOH
Injection volume:	1 μ L
Detector:	Flame Ionization Detector
Flow rate:	1.0 mL/min with He as carrier gas
Stationary phase A:	1 wt. % unhydrolyzed a.a.-derived product in CH ₂ Cl ₂
Stationary phase B:	1 wt. % hydrolyzed a.a.-derived product in CH ₂ Cl ₂

7. GC Experiments

Chromatographic separations were performed on a series of different solute mixtures. Each mixture of solutes was specifically chosen to determine the physical characteristics of the column. These characteristics included how the stationary phases

behaved with regard to van der Waals interactions (polarity effects), differences in peak shape and separation efficiency (H-bonding effects), indications of intermolecular interactions (π - π interactions), and to determine if host/guest complexation was occurring between the solute and the stationary phase. A reference homologous series of alkyl benzenes (RHS) comprised of toluene, ethyl benzene, propyl benzene, and butyl benzene was separated in order to characterize the π - π interactions and to determine if host-guest complexation occurred between the calixarene and phenyl containing compounds. A homologous series of alcohols (AHS) comprised of 1-propanol, 1-butanol, 1-pentanol, and 1-hexanol was used to look at the effects of H-bonding between components and the stationary phase and to see if host-guest complexation would occur through H-bond interactions at the derivatized bottom of the cup-like structure. A series of phenolic compounds, which contained phenol, resorcinol, and tert-butylphenol, was used to determine the effects of H-bonding and to indicate any π - π interactions and host-guest complexation. Two series of isomeric compounds containing 2-methyl-2-butanol, 3-methyl-2-butanol, and 2-pentanol along with tert-butanol, sec-butanol, and n-butanol were also used to determine the effects of H-bonding. Finally, a mixture containing α - and β -naphthol and (-)-menthol was used to characterize H-bonding effects, π - π interactions, and the effects of polarity upon elution of components between the different stationary phases, and host-guest interactions.

CHAPTER V

Results and Discussion

A. Characterization of the Chiral Phenylalanine-Derived Calix(4)arene

The addition step for the unhydrolyzed product characterized here was described by Pena et al (21) with modification. The modification involved the addition of L-phenylalanine t-butyl ester HCl instead of alanine to the bottom of the calix(4)arene structure. Also, the addition product of this reaction was purified by dissolving it into warm methanol. Precipitation of the product was induced by the addition of water. TLC plates were run in THF and showed only a single product. The ^1H NMR spectrum (Figure 5) in d-chloroform showed only minor impurities. A singlet at 1.1 ppm and a singlet at 1.3 ppm were obtained for protons at the 1 and 9 positions, respectively. Also, singlets indicated protons at position 3 (1.4 ppm), protons at position 2 (6.7 ppm), and protons located at position 8 (7.2 ppm). A quartet located at 3.7 ppm was obtained for protons located at position 6. Multiplets were obtained for protons located at position 7 (3.1 ppm), protons at position 4 (4.5 ppm), and protons at position 5 (7.8 ppm).

The successful synthesis of this product was supported by mass spectral data (Figure 6) obtained from a Bruker Esquire LCMS. Atmospheric pressure chemical ionization (APCI) ionized the compound and the ions were mass-analyzed with an ion trap. A scan range of m/z 200 – 2200 was used with an accumulation time of 426 μs and the polarity set to positive. The $[\text{M}+1]$ peak that was obtained was at m/z 1695, which corresponds to that of the addition product.

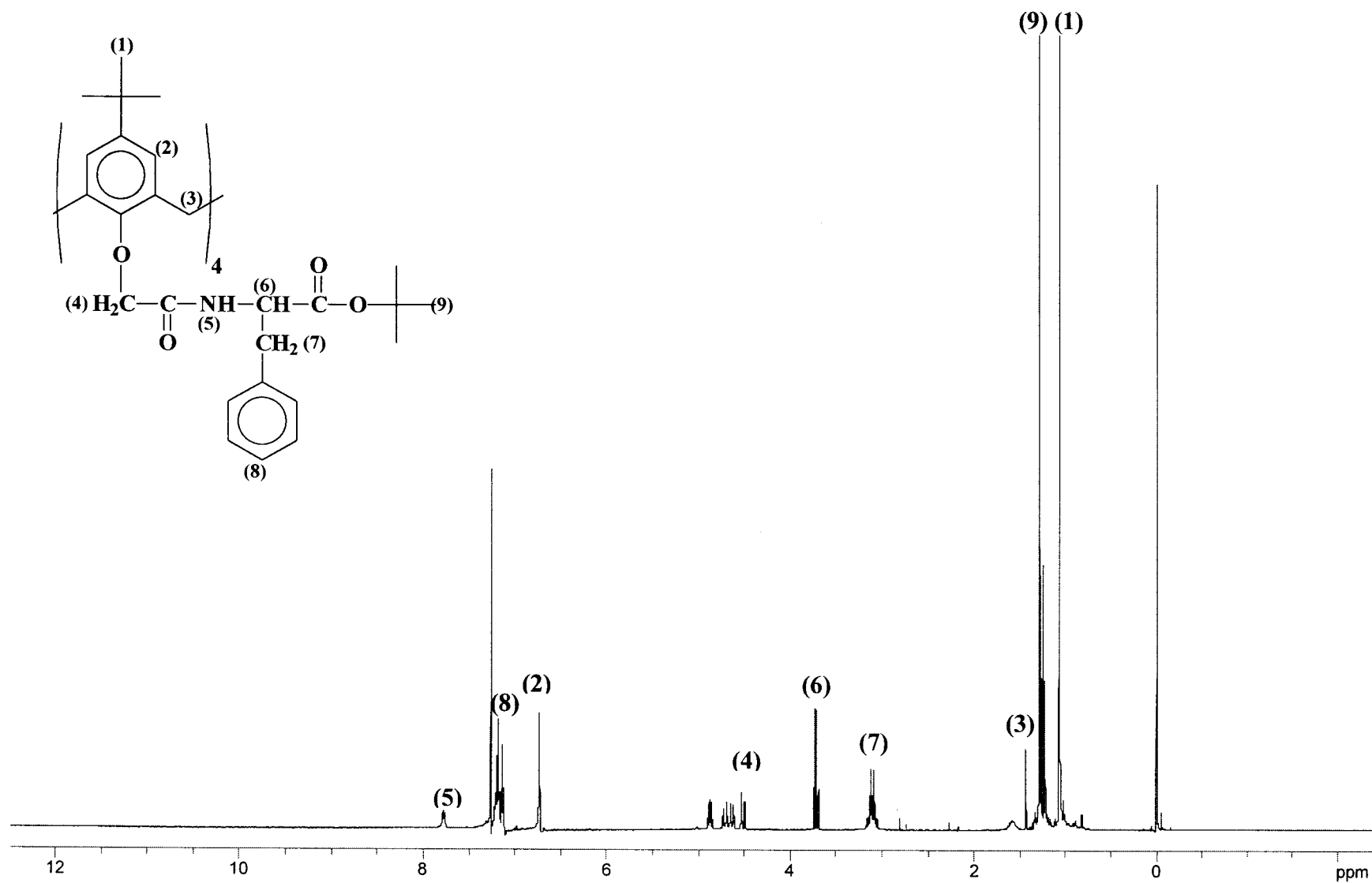


Figure 5. ^1H NMR of unhydrolyzed amino acid-derived calix(4)arene

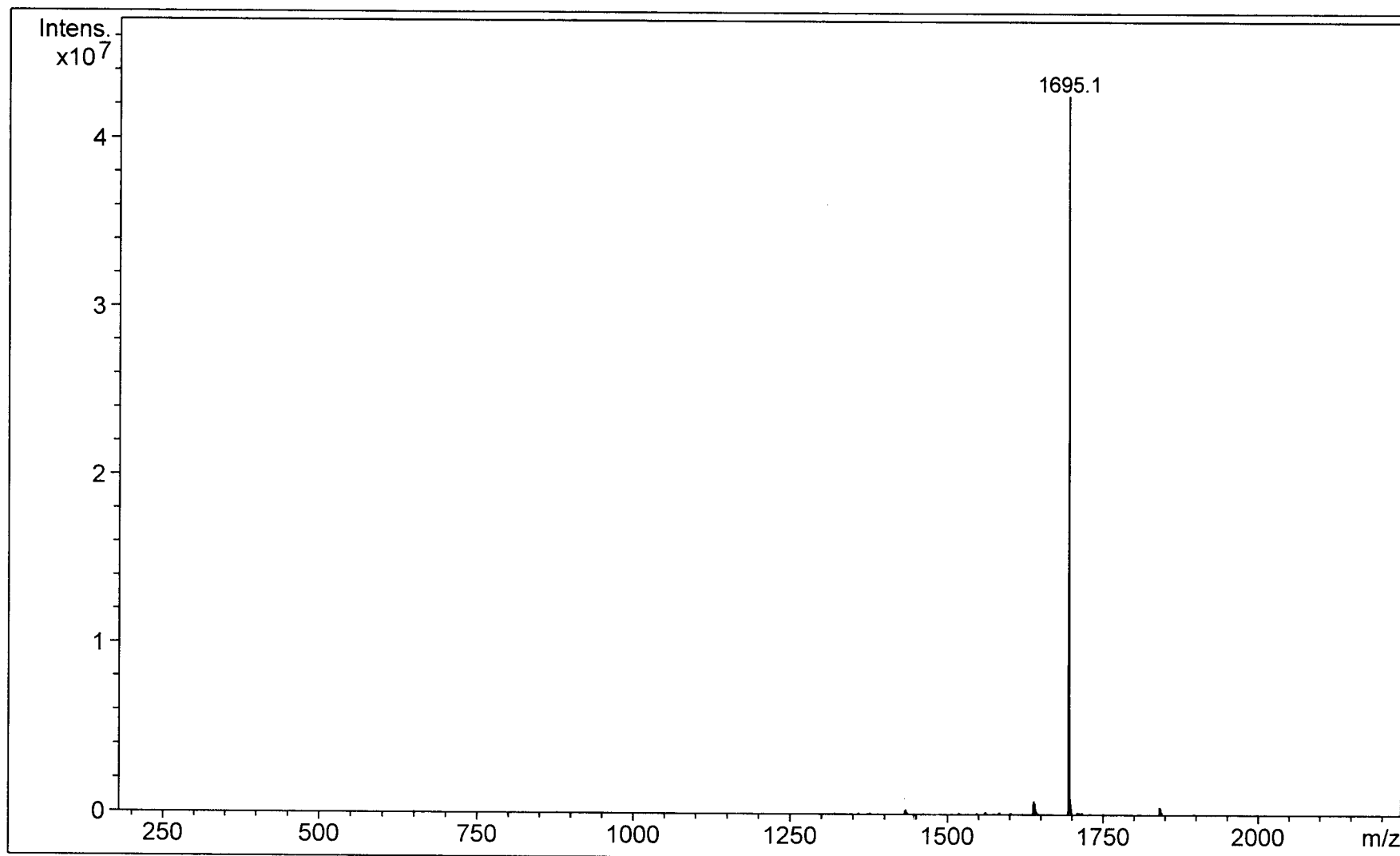


Figure 6. Mass spectrum of unhydrolyzed product

The hydrolysis of the addition step (hydrolyzed product) characterized here was also described by Pena et al (21) with modification. The hydrolysis was performed with TFA and 5% water, without the DCM called for in the literature. TLC plates were run with 20% THF in hexanes and indicated only a single product. The ^1H NMR spectrum (Figure 7) of the product in d-chloroform showed minor impurities. Protons located at positions 1 and 3 resulted in singlets found at 1.0 ppm and 2.5 ppm, respectively. A multiplet was obtained at 3.0 ppm for protons located at position 7, at 4.4 ppm for protons located at position 4, at 4.6 ppm for protons located at position 6, at 6.7 ppm for protons located at position 2, at 7.2 ppm for protons located at position 8, and at 8.5 ppm for protons located at position 5. A broad peak very characteristic of a hydroxyl group was obtained for the protons located at position 9 were found at 5.0 ppm.

The synthesis of this product was supported by APCI mass spectral data (Figure 8). A scan range from m/z 200 – 2200 was used with an accumulation time of 1994 μs and the polarity set to positive. The $[\text{M}+1]$ peak that was obtained was at m/z 1471, which corresponds to that of the final hydrolyzed addition product.

B. Absorbed Stationary Phases

The data presented in this work was obtained for three types of stationary phases. The columns used contained an AT-1 stationary phase (AT-1), a column with tetra(L-phenylalanyl)-4-tetra(tert-Butyl)-tetra(carboxymethyl)calix(4)arene tetra(tert-Butyl)ester absorbed into an AT-1 siloxane matrix (unhydrolyzed), and a column with

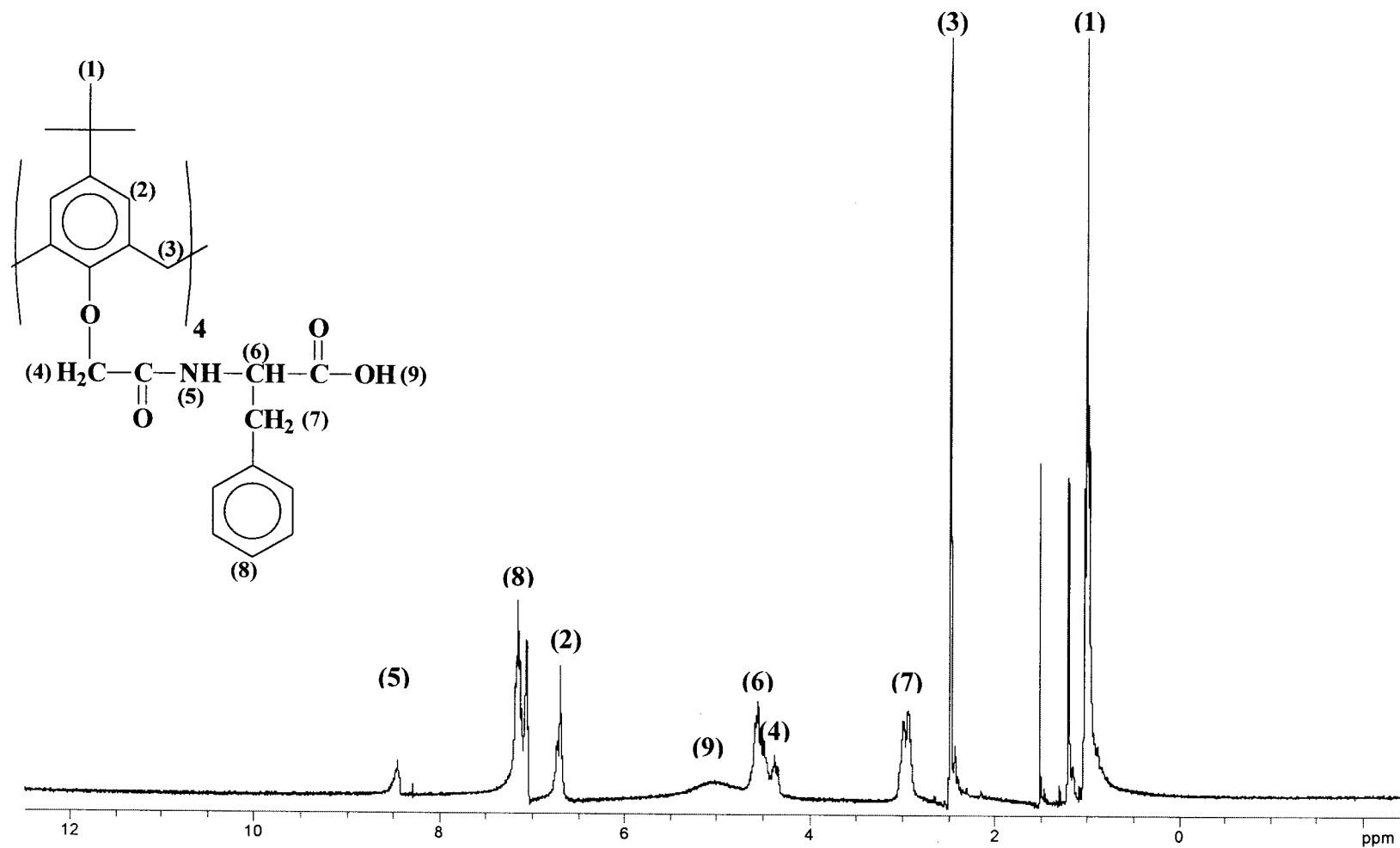


Figure 7. ^1H NMR of hydrolyzed amino acid-derived calix(4)arene

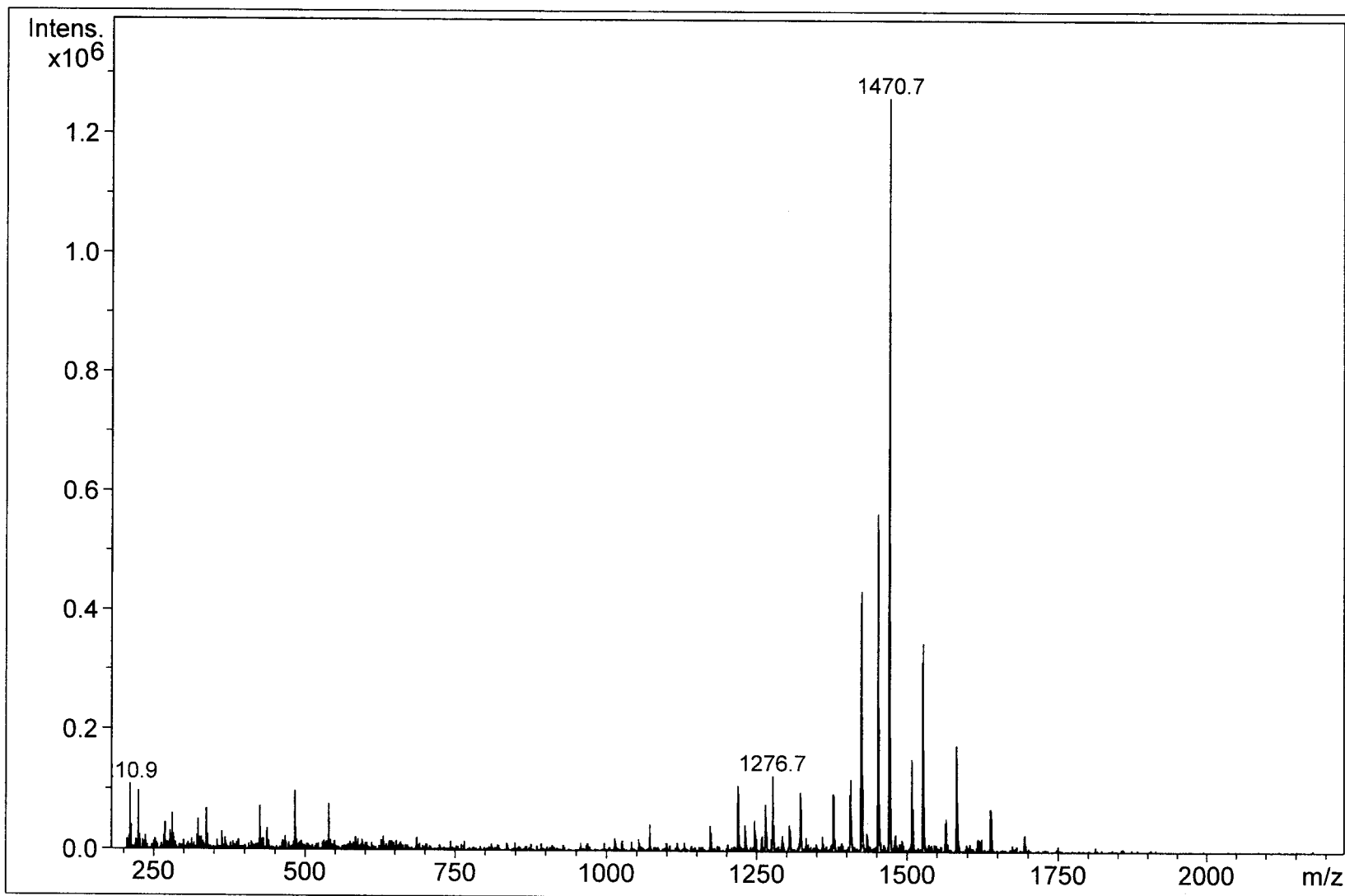


Figure 8. Mass spectrum of hydrolyzed product

tetra(L-phenylalanyl)-4-tetra(tert-Butyl)-tetra(carboxymethyl)calix(4)arene absorbed into an AT-1 siloxane matrix (hydrolyzed).

C. Homologous Series of Alkyl Benzenes

Calixarenes participate in host-guest interactions with molecules that are aromatic or contain aromatic functionality(s). A data summary for the RHS is found in Table II. Comparison of the retention data before and after derivatization with both the hydrolyzed and the unhydrolyzed form of the amino acid-derived calix(4)arene characterized any formation of host-guest interactions between the stationary phase and the RHS. Interactions based solely on polarity resulted in a linear relationship and any deviation from linearity indicated the presence of some π - π interaction (25).

Figure 9 shows a comparison of the retention behavior observed with both the hydrolyzed and the unhydrolyzed stationary phases along with the linear dependency of the retention data for RHS on the underivatized AT-1 stationary phase. There was some evidence of interaction between solute and the unhydrolyzed amino acid-derived stationary phase based on its correlation value of $R=0.9943$, where the correlation for the AT-1 and hydrolyzed amino acid-derived stationary phase were $R=0.9998$ and $R=0.9997$, respectively.

Along with host-guest interaction information, Figure 9 also shows the relative polarities of all stationary phases before and after derivatization. The hydrolyzed product resulted in a stationary phase more polar than the reference AT-1 phase, therefore the retention times associated with it were smaller compared to the AT-1 reference. The

unhydrolyzed stationary phase resulted in a more non-polar stationary phase, therefore the retention times associated with it were larger than the AT-1 reference.

The RHS was also used as a reference to determine the durability of the column. It determined if the behavior of the stationary phases had been altered by either a chemical reaction or degradation from heat. Duplicate trials were performed as the first and last assays on all columns and identical chromatograms were obtained indicating that little or no change had occurred in the chemical characteristics of the stationary phases.

D. Homologous Series of Alcohols

The AHS was separated to help determine if H-bonding was occurring, which would result in extended retention from adsorption of components with hydroxyl functional groups. Figure 10 shows the van't Hoff plot of $\ln k'$ vs. the number of carbons in the AHS where deviation in linearity would indicate the presence of some interaction resulting from H-bonding. The correlation values for the AT-1 ($R=0.99996$), the hydrolyzed ($R=0.99996$), and the unhydrolyzed ($R=0.99998$) stationary phases indicated no significant additional interactions (i.e., due to host-guest formation) between the aliphatic alcohols and either the hydrolyzed or unhydrolyzed stationary phases. Also, the contrast in the differences in retention due to differences in polarity between stationary phases was not as apparent here as with the RHS. Further investigation into H-bonding effects was performed by calculation of the capacity factors (k'), where an increase would indicate some interaction. Table II summarizes the chromatographic data obtained from elution of the AHS. k' between the AT-1 vs. the unhydrolyzed stationary

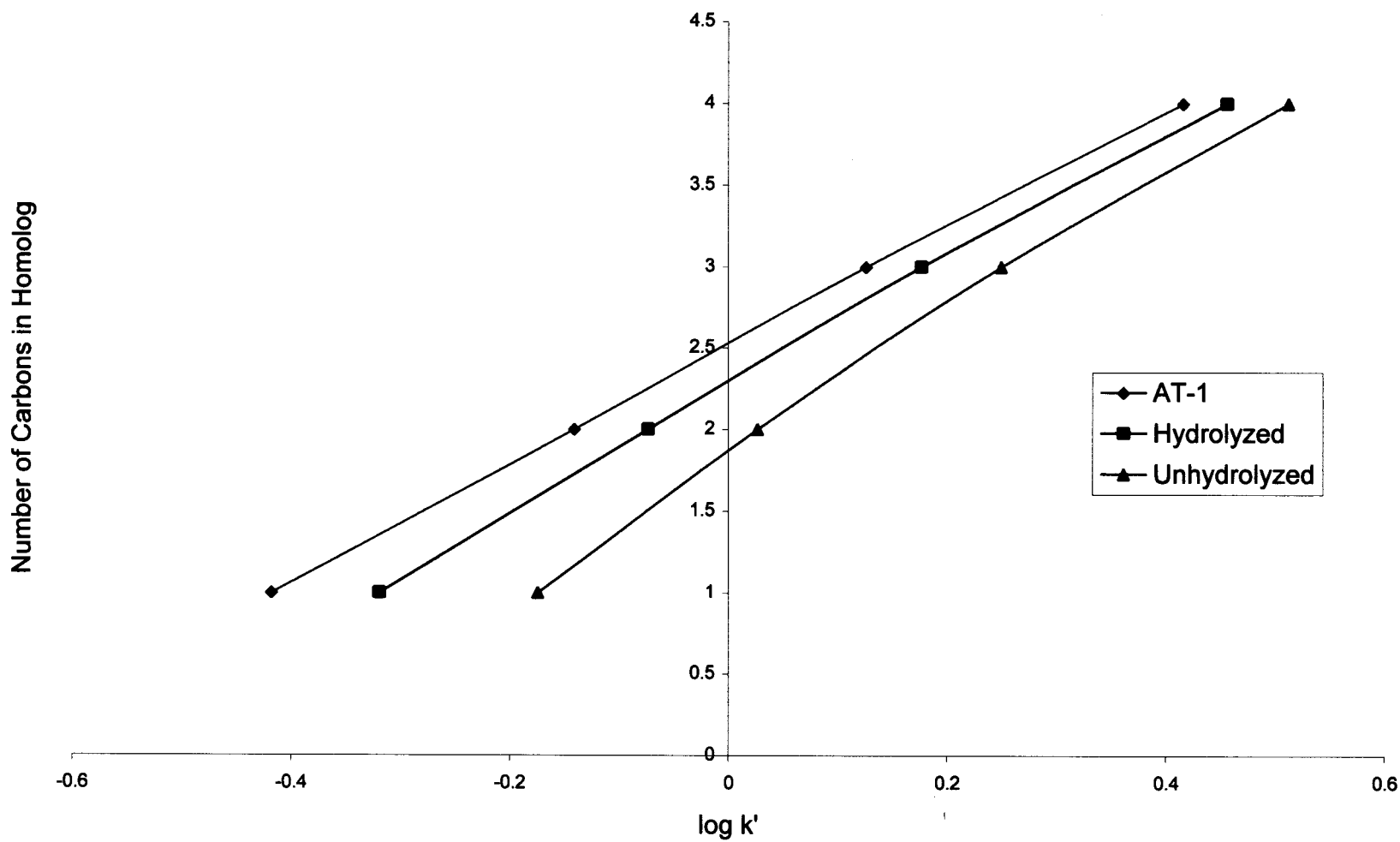


Figure 9. Effects of host/guest complexation upon correlation of reference homologous series at 90°C

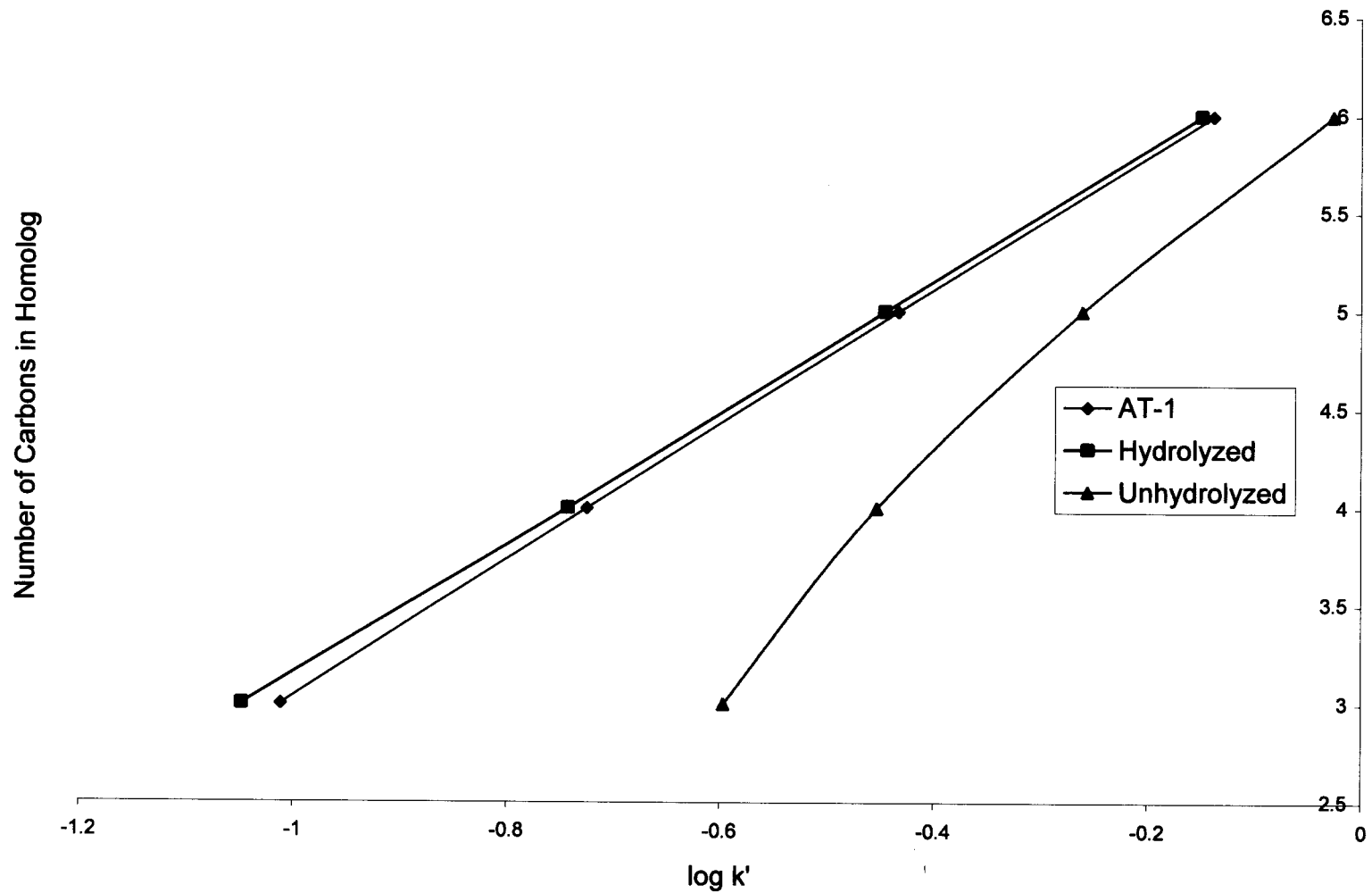


Figure 10. Effects of host/guest complexation upon correlation of alcohol homologous series at 90°C

Compound	# of carbons	t_r^* (AT-1)	t_r^* (Hydrol.)	t_r^* (Unhydrol.)	K (AT-1)	K (Hydrol.)	K (Unhydrol.)
toluene	7	0.1974	0.1811	0.3486	0.38812 ± 1.36%	0.3811 ± 1.13%	0.49800 ± 0.269%
ethyl benzene	8	0.3704	0.3405	0.5970	0.72827 ± 0.565%	0.71654 ± 0.418%	0.85286 ± 0.182%
propyl benzene	9	0.6831	0.6291	1.0460	1.3431 ± 0.280%	1.32386 ± 0.264%	1.49429 ± 0.128%
butyl benzene	10	1.3298	1.2247	1.9752	2.61463 ± 0.100%	2.57723 ± 0.127%	2.82171 ± 0.087%
1-propanol	3	0.3014	0.2361	0.4438	0.60975 ± 1.11%	0.48431 ± 1.03%	0.71008 ± 0.826%
1-butanol	4	0.6532	0.5537	0.9331	1.32146 ± 1.08%	1.13579 ± 0.857%	1.49296 ± 0.638%
1-pentanol	5	1.5856	1.3977	2.2210	3.20777 ± 0.668%	2.86708 ± 0.745%	3.55360 ± 0.564%
1-hexanol	6	4.0405	3.6078	5.5751	8.17419 ± 0.781%	7.40062 ± 0.775%	8.92016 ± 0.492%
2-methyl-2-butanol	5	0.4958	0.4328	0.8222	1.01411 ± 0.485%	0.90639 ± 0.866%	1.32849 ± 0.590%
3-methyl-2-butanol	5	0.7103	0.6267	1.1268	1.45285 ± 0.524%	1.31246 ± 0.942%	1.82065 ± 0.559%
2-pentanol	5	0.8375	0.7402	1.3044	1.71303 ± 0.588%	1.55016 ± 0.996%	2.10761 ± 0.533%

t_r^* is corrected component retention time (min)

Table 2. Component Retention Data

phases shows an increase which suggests an increase of interaction due to polarity effects. The slight curvature of this plot may suggest a hint of host-guest interaction, but it is much smaller than that seen with the RHS. These differences in retention are due mostly to polarity differences.

E. Phenolic Mixture

This phenolic mixture was used to determine the effects of H-bonding and to indicate any host-guest formation and/or π - π interactions. Figure 11 shows the elution of this mixture on all stationary phases at 80°C. All components eluted symmetrically from the AT-1 within approximately 12 minutes. On the hydrolyzed and unhydrolyzed stationary phases, phenol tailed significantly strongly indicating a host-guest interaction. The resorcinol never eluted from either of the derived stationary phases, also indicating a very strong interaction. The tert-butylphenol did not tail, the only differences upon elution being due to the polarity differences that resulted from the derivatization of stationary phases, not from host-guest interaction. This chromatogram gave an indication of the relative component polarity necessary for interaction and the size of the inner cavity created by derivatization of the amino acid-derived calix(4)arene.

F. Isomeric Mixtures

The isomeric mixtures were separated to help determine the effects of various isomeric positions of the hydroxyl group upon that component's ability to H-bond with the stationary phase. Figure 12 depicts a chromatogram of tert-, sec-, and n-butanol, which showed no selectivity toward any of the isomers. The differences in retention

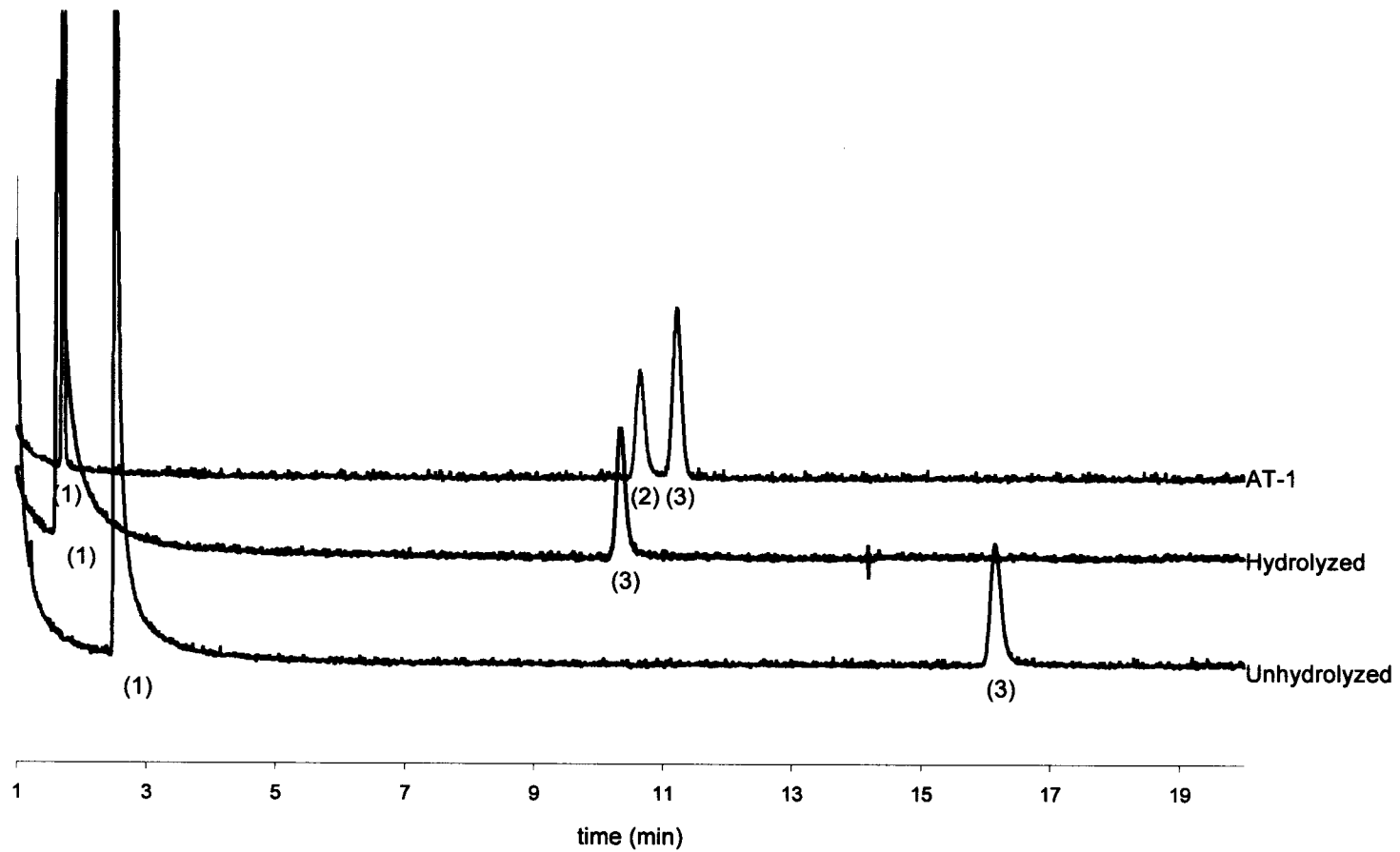


Figure 11. Retention of phenol (1), resorcinol (2), & tert-butylphenol (3) at 80°C

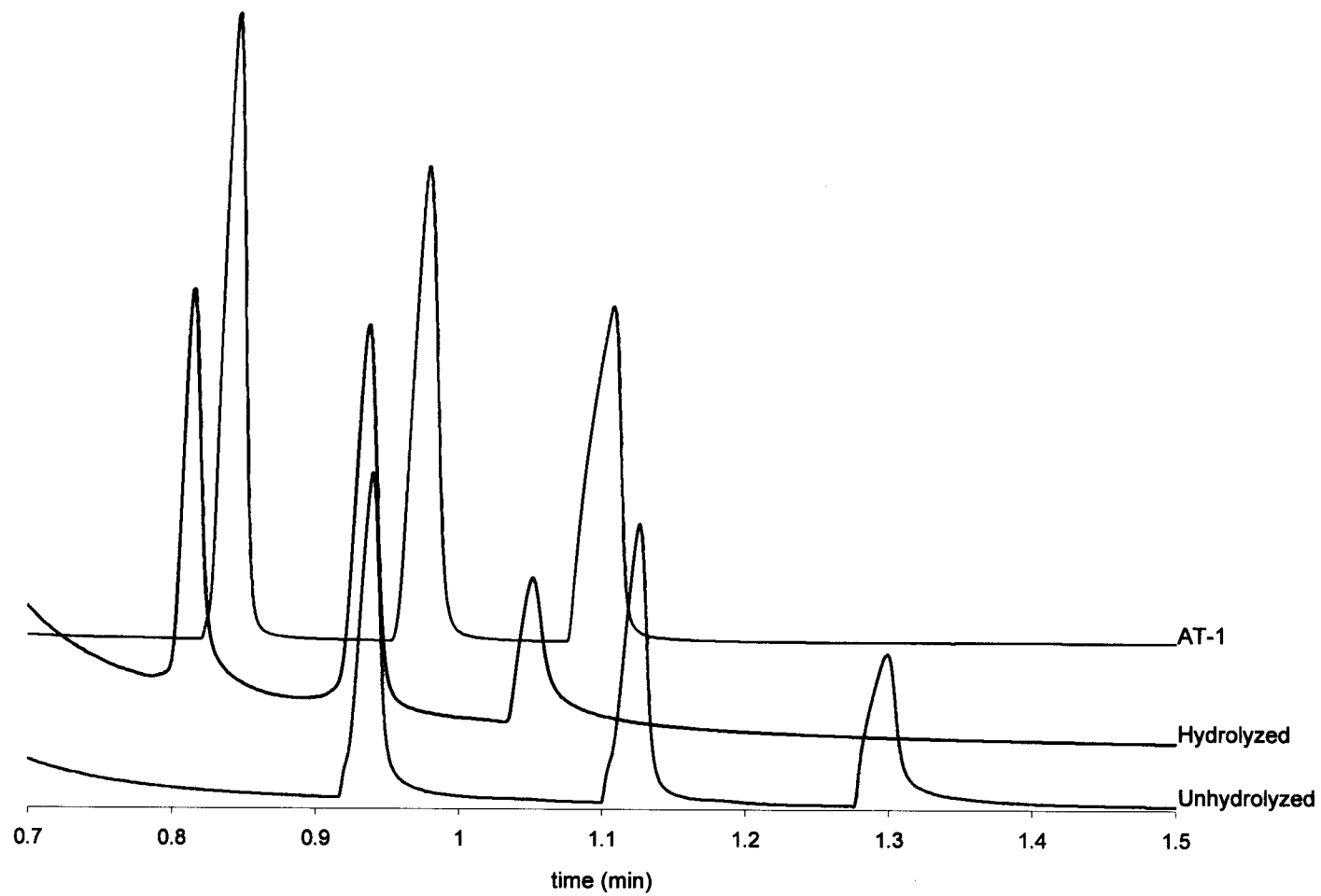


Figure 12. Retention of tert-butanol, sec-butanol, and n-butanol (respectively) at 35°C

were primarily due to differences in the polarities of the stationary phases. The tailing of the components as eluted from the derived stationary phases could possibly indicate a small amount of host/guest complexation. Table III summarizes the asymmetry factors for this isomeric mixture and shows an increase in A for each component when compared to that for the AT-1 Reference.

Compound	t_r	k'	Asymetry Factor
α -naphthol (AT-1)	22.6357	44.3258 \pm 0.014%	1.10
α -naphthol (Hydrolyzed)	20.4467	39.4725 \pm 0.020%	3.67
α -naphthol (Unhydrolyzed)	29.3901	40.5230 \pm 0.147%	1.83
β -naphthol (AT-1)	23.6944	46.9256 \pm 0.054%	1.30
β -naphthol (Hydrolyzed)	21.6217	41.7983 \pm 0.308%	17.0
β -naphthol (Unhydrolyzed)	30.7508	42.4456 \pm 0.047%	5.18
n-butanol (AT-1)	0.6565	1.33598 \pm 0.826%	0.75
n-butanol (Hydrolyzed)	0.5625	1.15101 \pm 0.266%	2.42
n-butanol (Unhydrolyzed)	0.9755	1.55681 \pm 0.644%	1.14
sec-butanol (AT-1)	0.5278	1.07407 \pm 0.836%	0.68
sec-butanol (Hydrolyzed)	0.4487	0.91815 \pm 0.208%	0.71
sec-butanol (Unhydrolyzed)	0.8027	1.28104 \pm 0.669%	0.63
tert-butanol (AT-1)	0.3957	0.80525 \pm 0.933%	0.43
tert-butanol (Hydrolyzed)	0.3268	0.66871 \pm 0.232%	1.07
tert-butanol (Unhydrolyzed)	0.6160	0.98308 \pm 0.742%	0.64

Table 3. Asymmetry Factors

Figure 13 shows the chromatogram of (-)-menthol, which is a large alcohol. This component eluted as symmetrical peaks indicating little interaction due to H-bonding.

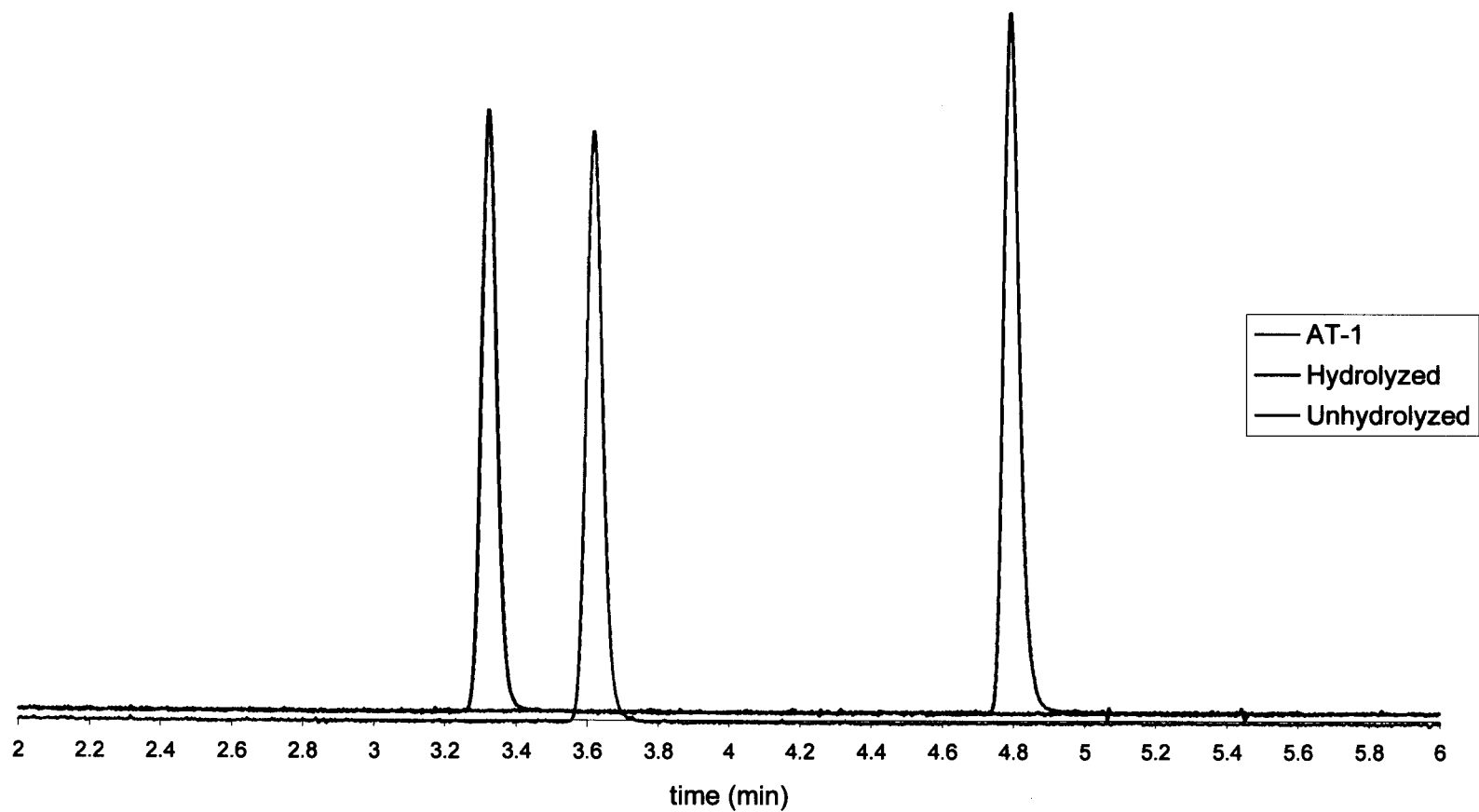


Figure 13. Retention of (-)-menthol at 90°C

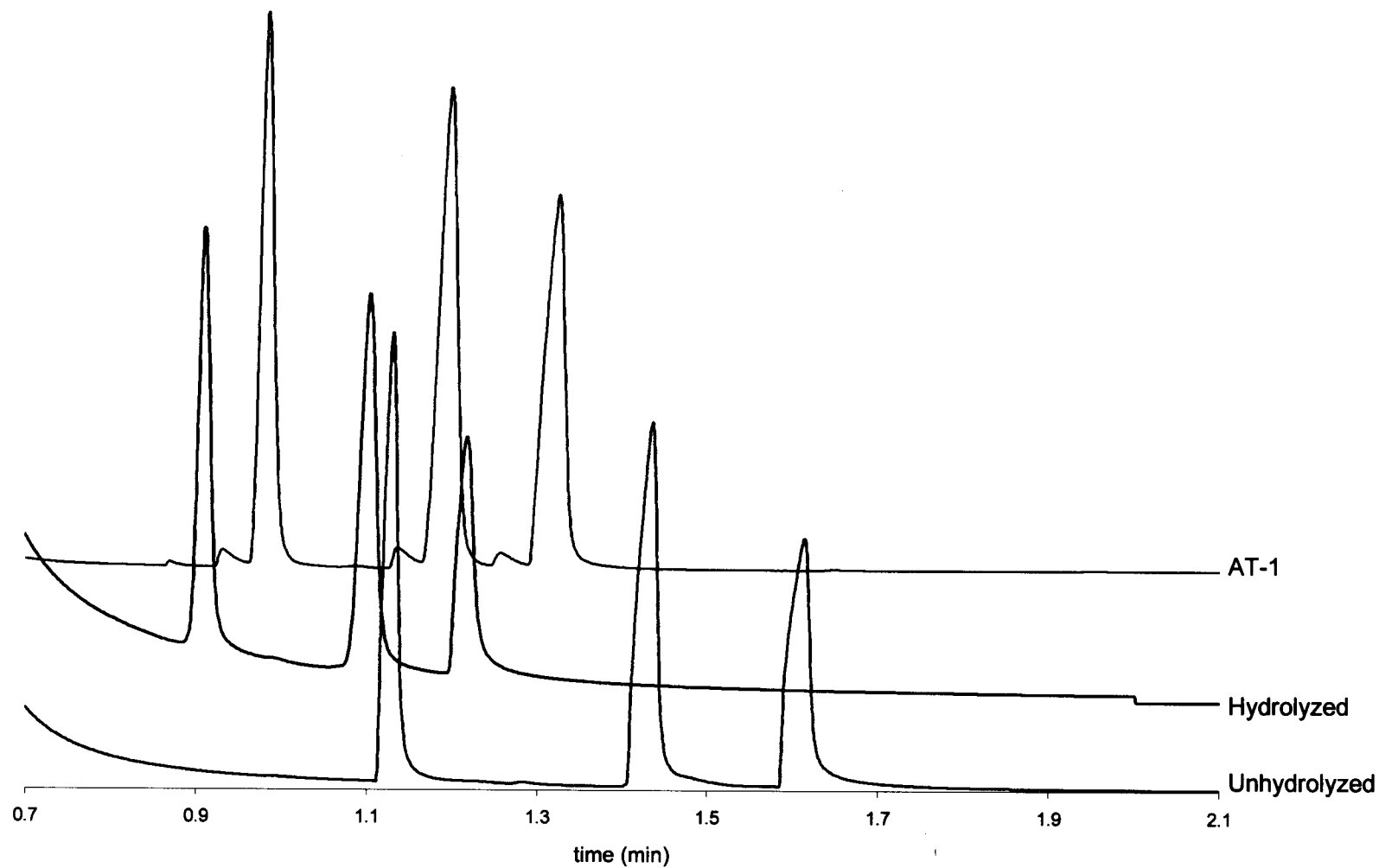


Figure 14. Retention of 2-methyl-2-butanol, 3-methyl-2-butanol, and 2-pentanol (respectively) at 35°C

Eluting another isomeric mixture containing 2-methyl-2-butanol, 3-methyl-2-butanol, and 2-pentanol allowed further investigations into any H-bonding effects. Figure 9 shows the chromatogram of this series and depicts no selectivity of isomers with the only differences upon elution being the differences in polarity. The capacity factors (k') for this series were calculated, where an increase indicated an interaction. Table II summarizes the component retention data obtained from elution of this series, which shows an increase in k' between the AT-1 vs. the unhydrolyzed stationary phase. This suggests an increase in interaction of solutes and the unhydrolyzed stationary phase.

G. Mixture of α - and β -Naphthol

A mixture of α -, β -naphthol was chosen to determine if the components were participating in any type of π - π interactions, if any H-bonding was occurring, and the effects of size and polarity of the component upon host-guest formation. Figure 15 shows the chromatogram of the naphthols, in which the relative polarities of the stationary phases can be seen. This data demonstrates the ability of the stationary phases to selectively interact with a particular component based upon its size and relative polarity.

Table III gives the asymmetry factors that were determined for the naphthols. The increasing values for the asymmetry factors for these components strongly suggest interactions between some solutes and both amino acid-derived calix(4)arene stationary phases are occurring. This data also indicated that this newly derived stationary phase is capable of selectively participating in the host-guest interactions with a solute based on relative size and polarity, as the elution of naphthol resulted in

significant tailing of components, while the elution of (-)-menthol resulted in symmetrical peaks.

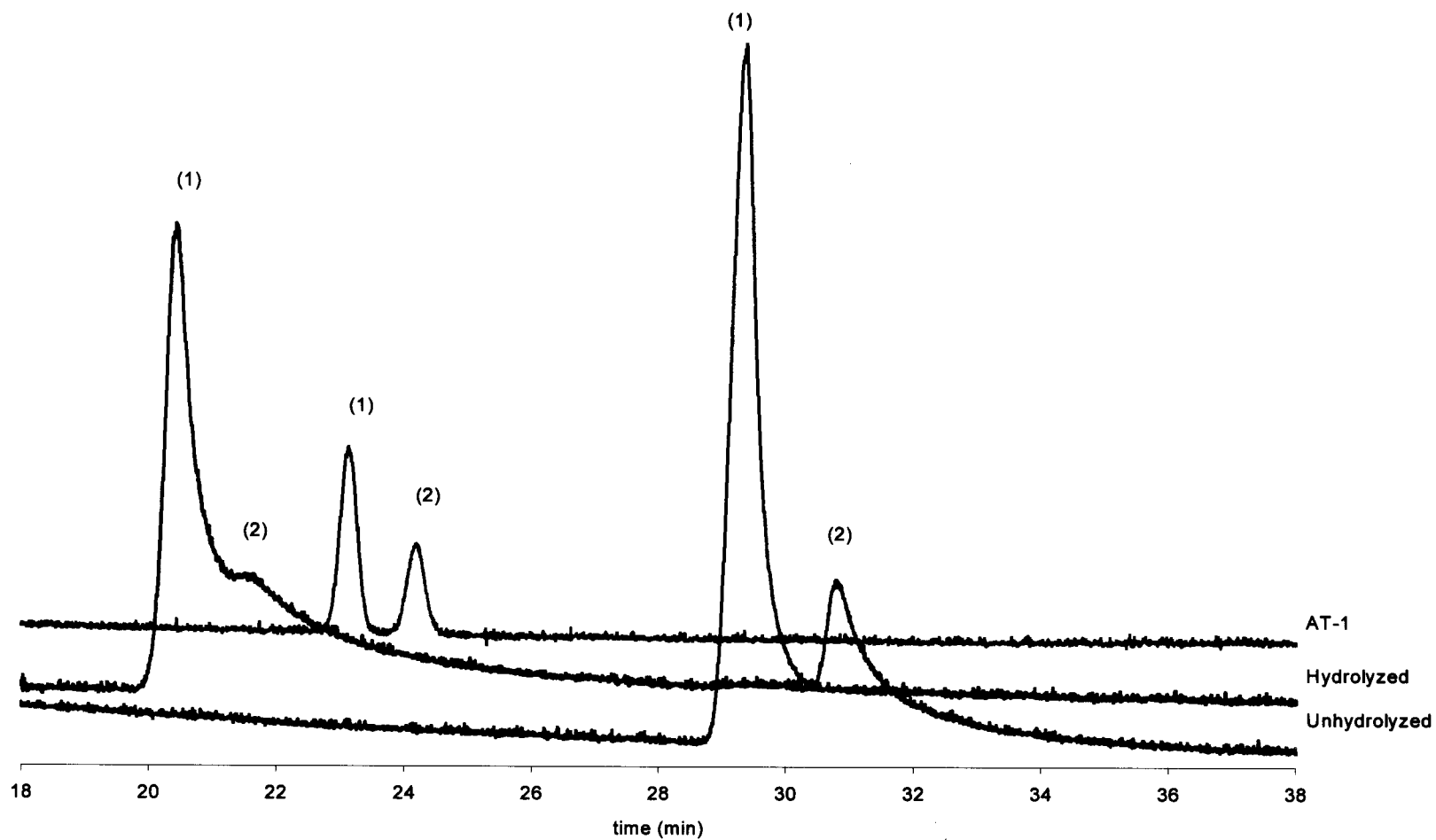


Figure 15. Retention of α -naphthol (1) and β -naphthol (2) at 90°C

CHAPTER VI

Conclusions

This research has demonstrated the successful synthesis of L-phenylalanine derived 4-t-butylcalix(4)arene and its ability to participate in host-guest interactions with particular solutes in gas chromatography. The derivatized chiral calix(4)arene was absorbed onto a non-polar stationary phase, and its behavior with respect to polarity and component size dependence is described in this work.

The synthesis of the L-phenylalanine derived 4-t-butylcalix(4)arene was successful, as supported by mass spectral and ^1H NMR data along with the retention behavior of the solutes on the derivatized stationary phases. The increase in the capacity factor (k') and the increased values for the asymmetry factors strongly suggest host/guest interactions between some solutes and the immobilized calixarene. The differences in the relative polarities of the stationary phases are reflected in the retention times of the solutes. The hydrolyzed derivative increases the polarity and the unhydrolyzed derivative decreases the polarity with respect to the underived AT-1 stationary phase. The asymmetry factors for the chromatographic peaks of the (-)-menthol vs. that for naphthol show that this chiral calix(4)arene can specify the host/guest interactions in which it participates. The host/guest interactions of the L-phenylalanine derived 4-t-butylcalix(4)arene may lead to the additional chromatographic dependence from which enantiomeric separations can be performed.

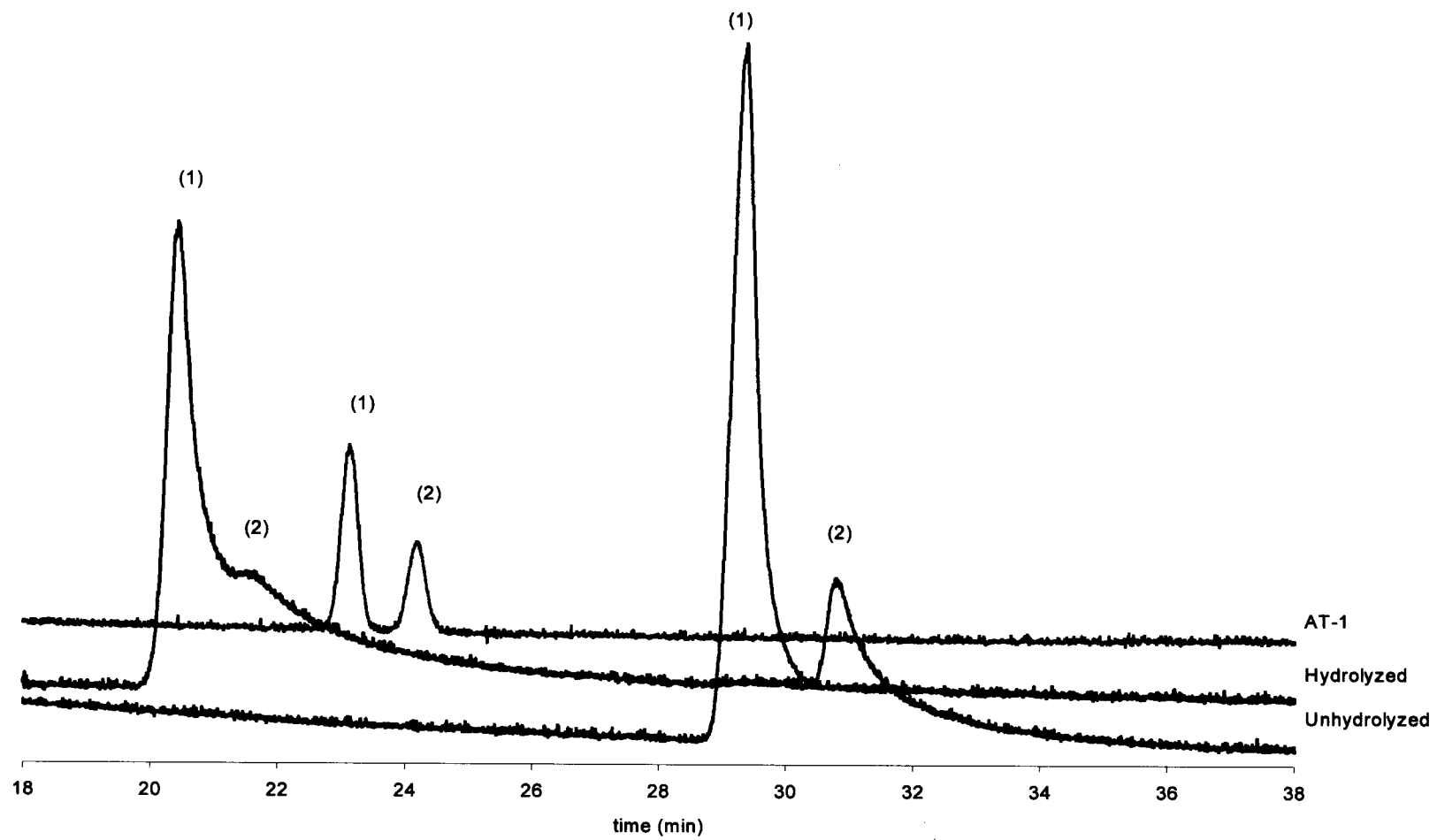


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CHAPTER VII

Future Work

A focus of future work should involve investigating the upper temperature limits of the derived stationary phases. All effects occurring from these columns seemed to be enhanced with temperature, so simply being performed at higher temperatures could potentially optimize some effects.

A study involving the refinement of the synthetic procedure that was roughly mapped out in this research should be performed. Purification of this novel chiral calixarene step-by-step gives the researcher insight into the relative polarity of this compound, along with a higher percent recovery of final product. A better understanding of the relative polarity of this compound and the stationary phase that results from it will draw a clearer picture of the types of components this stationary phase would prefer interaction with.

Exploring the behavior of other test mixtures, (i.e., aceto- through benzonitrile) to give more insight into the size and polarity of the cavity will help determine the types of compounds these columns could potentially show enantiospecificity towards. Calculation and comparison of the binding coefficients of the RHS between columns could provide interesting results.

Future investigations should also include making a chemical attachment of the chiral compound to the inner wall of the column and determining the temperature limits and effects of this adsorption. Also, investigations should be made into the application of the synthetic methods described in this work to the addition of other chiral amino acids to

calix(4)arene to determine the effects of other amino acid-derived calix(4)arenes to host-guest complexation.

Additional investigations should be performed for determining the effects of the thickness of the coating of chiral calixarene deposited on the column after the absorption process. Simply dissolving more of the amino acid-derived calixarene in DCM before introducing the solution into the column could easily increase the concentration of the coating. This increase in coating thickness should result in an increase in the amount of host-guest interaction between solute and stationary phase, which would be reflected in increased values for the asymmetry factors for the injected components. Optimizing these interactions will hopefully lead to a better understanding of the enantiomeric separations in which this type of stationary phase can be applied. Also, an increase in the asymmetry factors of the components upon increased concentration of the coating in the column would provide further evidence of host-guest complexation.

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