Evaluation of Apparent Formation Constants of Host-Guest Inclusion Complexes of Solutes with Soluble Calixarenes Using High Performance Liquid Chromatography

By

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Abstract

By RP-HPLC, soluble and insoluble calixarenes were examined as mobile phase additives using three PAH analytes. Calixarenes are large cyclic molecules and are of interest in chromatography because of their ability to form host-guest complexes with various analytes. Determination of the optimum temperature, concentration, and calixarene size and type (water soluble and insoluble) was used to evaluate apparent formation constants for solutes forming host-guest complexes.

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List of Symbols

SYMBOLS

RP-HPLC	Reversed phase high performance liquid chromatography
psi	Pounds per square inch
C-18	Octadecyl
%	Percent
^{o}C	Degrees Celsius
S	Solute
Cx	Complexing agent
(s)	Stationary phase
(m)	Mobile phase
K_1	Solutes equilibrium between the stationary phase and mobile phase
K_2	Complexation agents equilibrium between mobile and stationary phase
K_3	Equilibrium between unbound solute and solute/agent complex
K_4	Solute agent complex between stationary and mobile phases
R_t	Peak retention time
R_o	Dead time
R_t'	Corrected retention time
k'	Capacity factor
α	Separation factor
N	Theoretical plate number
W_B	Base peak width

$W_{1/2}$	Half-height at peak width
R	Peak resolution
t_{RA} and t_{RB}	Retention time of the analytes
W_A and W_B	Peak widths
TF	Tailing factor
H	Theoretical plate height; Van Demeter equation
A	Eddy diffusion; Van Demeter equation
B	Longitudinal diffusion coefficient; Van Demeter equation
C	Mass transfer effects; Van Demeter equation
ΔH^o	Enthalpy; van't Hoff equation
T	Temperature in Kelvin, van't Hoff equation
ΔS^o	Entropy; van't Hoff equation
R	Universal gas constant; van't Hoff equation
K	Equilibrium constant; van't Hoff equation
φ	Phase volume ratio between the mobile and stationary volumes
K_{assoc}	Association constants
nm	Nanometer; 10^{-9}m
PL	Photoluminescence
DSC	Differential scanning calorimetry
ASIS	Aromatic solvent induced shift
mM	Millimolar;10^{-3} mol/dm^3
UV	Ultraviolet

NMR	Nuclear Magnetic Resonance
β	Beta
γ	Gamma
LC	Liquid chromatography
APT	Absolute pressure transducer
μL	Microliter; one-millionth (10^{-6}) of a liter
ACN	Acetonitrile
THF	Tetrahydrofuran
mL/min	Milliliters per minuet
M	Molarity; moles per liter
[A]	Concentration of solute
K_1	Apparent formation constant; van't Hoff equation
CA	Concentration of calix[n]arene

Chapter I

Introduction

1.1 Reversed Phase High Performance Liquid Chromatography

Reversed phase high performance liquid chromatography (RP-HPLC) is an instrumental technique broadly used for the separation and quantification of analytes. The partitioning of solutes in RP-HPLC occurs between two phases: an insoluble particulate stationary phase and a liquid mobile phase. In RP-HPLC, a polar mobile phase is passed through a less polar stationary phase column at relatively high pressures (up to 5000 psi). The stationary phase is contained in a stainless-steel column packed with small, porous, chemically modified silica particles that reproducibly absorb and retain the analyte solutes preferred for separation.

The chemical modification of the stationary phase gives it the characteristics of a thin liquid coating on porous particles of nearly uniform size having a polarity dictated by the type of chemical modification. In most cases of RP-HPLC, the modification is done to porous silica particles and is the attachment of C-18 alkane-like functional groups; hence the term C-18 bonded phase commonly used to describe such stationary phases. Other bonded phase surface modifications are possible and are also encountered in many circumstances.

If a solute has better solubility in the stationary phase, the elution time is slow; if the mobile phase promotes better solute solubility, it passes through the stationary phase at a faster rate and elutes faster. Each solute gives a peak, which is a concentration distribution profile of solute molecules whose shape is governed by many factors; it appears similar to and is often treated as a Gaussian distribution, although this is not necessarily so. The peak is visible on a chromatogram through use of a detection device placed after the separation column, which is a reference to the solute's retention time within the chromatographic system.

Differences in solute structures or modifications of the mobile or stationary phases that vary the equilibrium distribution between the mobile and stationary phases promote separation of solutes from one another. The partitioning process, shown schematically in Figure 1, contains a series of equilibria; the initial equilibrium, known as the primary equilibrium, involves the analyte partitioning between the mobile and stationary phases. Adding host/guest complexation agents, or other agents that can bind to or modify solute character, to the mobile or stationary phases results in the production of secondary equilibrium processes during the separation. This results in the solute partitioning between the mobile and stationary phases as well as with the complexation or modification agent contained within those phases. The retention times of solutes are changed with changes in temperature because of alterations in the equilibrium constants, which alters the equilibrium process and the resulting observed retention time of the solutes.

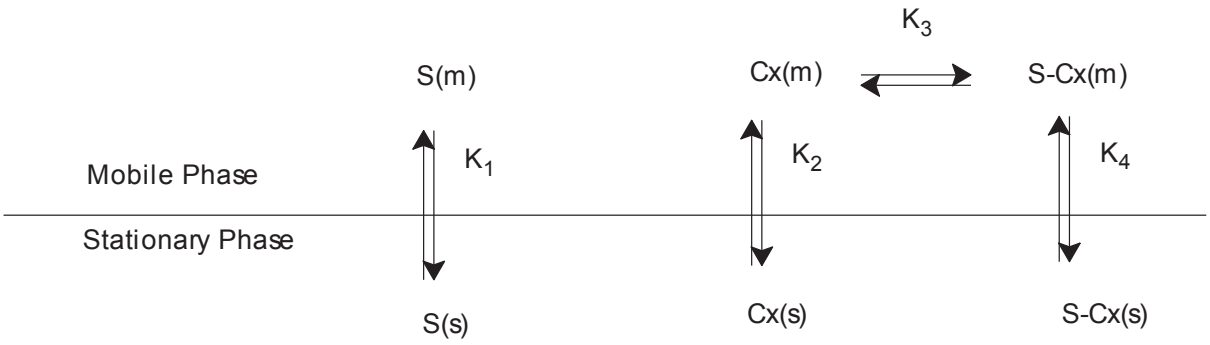


Figure 1: Partitioning Process

S = solute Cx = complexing agent

K_1 = solutes equilibrium between the stationary phase and mobile phase

K_2 = complexation agents equilibrium between mobile and stationary phase

K_3= equilibrium between unbound solute and solute/agent complex

K_4 = solute agent complex between stationary and mobile phases

1.2 Calixarenes

The phenol-derived oligomer commonly called a calixarene, has been of interest since the nineteenth century. The name given to calixarenes was provided through the Greek word *calix* meaning vase and the word *arene* indicating the existence of aryl residues in the macrocyclic array. Depending upon the number of monomer units used to create the calixarene macrocyclic ring (oligomer), a number notation is inserted between the words calix and arene telling how many monomer units are present.

The synthetic reaction that is used to produce calix[n]arenes utilizes formaldehyde and is a base-induced condensation of p-tert-butylphenol as an initial step that forms linear oligomers. A last step occurring in the solvent xylene is used to form the calix[n]arene, which is represented in Figure 2. The calix[n]arene structure consists of phenolic or phenolic-like units linked by methlyene bridges in a repeated manner that result in formation of a cavity-like conformation easily visualized as being cylindrical or cone-like in shape, although other conformations are possible. The calixarene cavity has two main functional components, a non-polar upper rim and a polar lower rim and synthetic yields of 50% or greater with high melting points starting around 240^{o}C. [1]Synthesis of calixarenes has been utilized to create different characteristics for the upper and lower rims. The upper and lower rim functional groups that are attached can be used to vary properties such as solubility, polarity, and chirality,

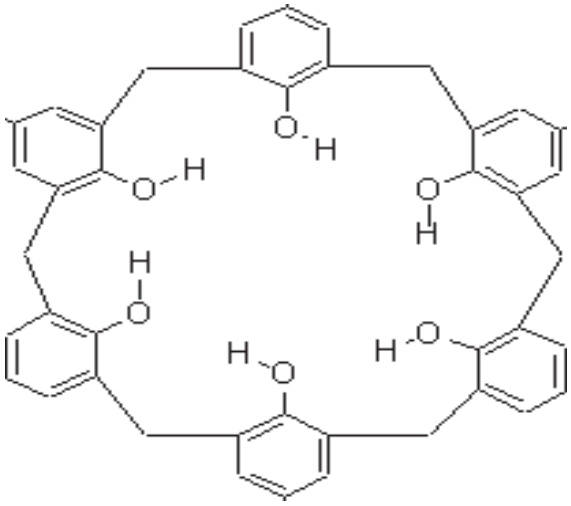


Figure 2: Calix [6] arene

Because calixarenes are linked by methylene bridges, various sizes as well as conformations can be achieved. During the initial step of synthesis, linear oligomers can be used to create cavity sizes as small as four rings or as large as twenty.[2] Examples of different possible conformations are seen in Figure 3. Here it can be seen that calix [4] arene can be formed into a cone, partial cone, 1, 2-alternative and 1, 3-alternative conformations. The most common conformation would be the "up-down" conformation where each phenol unit alternates in position. Each calixarene has the possibility to form a different conformation. The predominant conformation will be dependent upon temperature and functionality of the calixarene. In nonpolar solvents, calix [4] arene prefers the cone conformation, but can produce inversion very rapidly at room temperature. The conformation of calix [8] arene greatly resembles that of calix [4] arene where benzene is used as the smallest aromatic compound and intermolecular hydrogen bonding contributes to the conformational preference. Calix [6] arene takes on a winged or

hinged conformation to ensure the best intermolecular hydrogen bonding. As the ring size increases, the preferred conformations tend to be more planar.

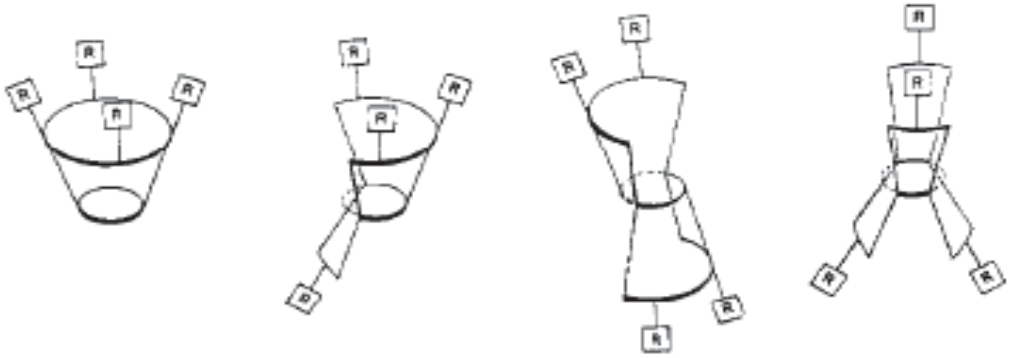


Figure 3: Cone. Partial cone, 1, 2- alternate and 1, 3-alternate conformations for calix [4] arene[3]

One specific trait of calixarenes is their insolubility in water or in solutions of aqueous base. They also have a low solubility in some organic solvents that is attributable to high melting points. This often makes it difficult to characterize, isolate and purify calixarenes. Solvents that can be used to aid solubility include, but are not limited to benzene, chloroform, and pyridine.

In the past twenty years, the process of synthesizing calixarenes has shown great advancement. With the combined effort of synthesis and ability to use chromatography, electrophoresis, NMR, and other types of instrumental analysis, investigations of calixarenes and their host-guest complexes has been vastly increased.

1.3 Water-Soluble Calixarenes

Water-soluble calixarenes were first prepared by Ungaro et al.[4] by using p-tert-calix [4] arene with tert-butyl-α-bromoacetate to form the tetracarboxylic acid of p-tert-calix [4] arene. This tetra ester with a lower rim carboxylic group gave yields of 70%. In the early 1980's sulfonated calixarenes, which were found to be very water soluble, were prepared by Shinkai and

co-workers.[5] With the use of sulfuric acid and calix[n]arene at 100°C, relatively high yield (>70%) of p-sulfonatocalix[n]arene could be achieved.

Modifications to the synthesis have been made to improve removing the tert-butyl groups and replacing the sulfonate groups in a simultaneous step. A picture of the sulfonated calixarene, 4-sulfonic calix [6] arene**,** is shown in Figure 4. With the evolution of water-soluble calixarenes, the problems associated with their insolubility, as well as those associated with their characterization, isolation and purification has been greatly decreased. The ability of water soluble calixarenes to interact with ions and molecules has been an aid in performing catalysis for other calixarene reactions. Their utility for studying host-guest complexation has also caused great interest in these compounds.

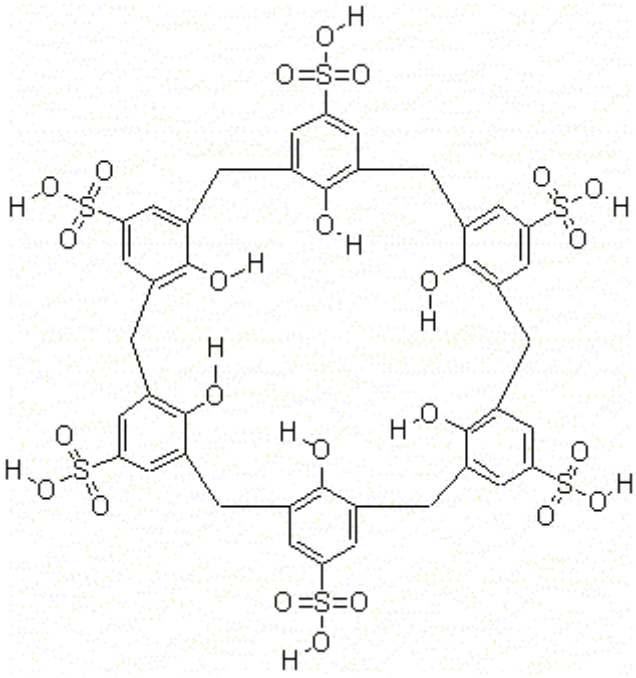


Figure 4: 4-Sulfonic Calix [6] arene

1.4 Mobile Phases in HPLC

Each different chromatographic technique uses mobile phases to enhance or modify analyte separation and hence improve the signal output produced on the chromatogram. Utilization of additives as components of the mobile phase can also be employed to create variations in separation methodologies or procedures to improve chromatography.

In RP-HPLC, gradient mobile phases are commonly used to increase the concentration of an organic modifier in a regular fashion during the chromatographic run. The polarity of the mobile phase solvent in relation to the polarity of the stationary phase controls the separation of solutes with widely varying polarities, partition coefficients, or capacity factors. In RP-HPLC, mobile phases are used to elute solutes in order from most hydrophilic to most hydrophobic; the more soluble the solute (analyte) is in water or in a hydrophilic (water-like) mobile phase, the more it favors the mobile phase and the lower its partition coefficient (i.e., it partitions less into the stationary phase) and, therefore, its capacity factor.

Ion-exchange chromatography focuses on cation or anion separation through covalent surface modification of stationary phases with species containing charges. Separation is achieved through an exchange of ions from the mobile phase with those on the charged stationary phase.

Ion-pair chromatography is applicable to the separation of weakly-ionizable samples (acidic or basic) using reversed-phase (mostly polar) solvents and stationary phases. In ion-pair chromatography, samples interact with ion-pair reagents, which are essentially ion-exchange compounds that are soluble in the mobile phase. The characteristics of the modified mobile phase such as the ionic strength, ion-pairing agent concentration, and pH affect the separation and retention of the weakly-ionized analytes. A water-miscible organic solvent and water are used in this type of chromatography and mobile phase additives are utilized to create solvent

selectivity. With RP-HPLC, a common anionic ion-pairing reagent is trifluoroacetic acid and tetrabutylammonium phosphate is a commonly used as a cationic ion-pairing reagent.[6] Other ion-pairing agents are also available.

Similarities between all mobile phases used in these various techniques are that they should be commercially available, pure as possible, dissolve the sample and allow for easy recovery, not react with the column packing, compatible with the detector, and low in viscosity. In some cases, a volatile mobile phase can be used to help recover a sample analyte after chromatographic separation. Composition of mobile phases affects the lifetime and performance of a HPLC column. The most common mobile phases used are methanol, acetonitrile, methylene chloride, tetrahydrofuran, and water.

1.5 Characterization of Liquid Chromatography

Peak retention, capacity factor, separation factor, number of theoretical plates, resolution, and peak symmetry are ways to characterize liquid chromatography.

The time from injection to the analyte peak elution is the analyte peak retention time, R_t. Time between injections to detection of an unretained analyte is defined as the dead time, R_o. Corrected retention time, R_t', is peak retention time minus the dead time.

$$R_t' = R_t - R_o$$

The capacity factor is the interaction between stationary phase and analyte. The higher the k' value, the more effective the stationary phase is at retaining analyte.

$$k' = \frac{R_t'}{R_o}$$

The ratio of two capacity factors is considered the separation factor (α). The separation factor is a measure of the relative position or spacing between the peak maxima.

$$\alpha = \frac{k_2'}{k_1'}$$

The ability of the analyte to flow through the column with minimum band broadening is calculated as the theoretical plate number, *N*. In the equation, R_t represents peak retention time, W_B is base peak width, $W_{1/2}$ is half-height at peak width. The quality of the column's packed bed is indicated in this equation. The picture in Figure 5 shows the calculation of resolution, theoretical plate count, and peak symmetry.

$$N = \frac{16(Rt)}{W_B}$$

$$= \frac{5.5(R_t)}{W_{1/2}}$$

Separation between two adjacent peaks on a chromatogram is the measurement of peak resolution, R. Resolution of two peaks, represented by A and B, is as follows:

$$R = \frac{2(t_{r_B} - t_{r_A})}{W_A - W_B}$$

The retention time of the analytes are expressed by t_{RA} and t_{RB} and the peak widths at the baseline are W_A and W_B. A resolution that is less than 1.0 cannot be reliably quantified while resolution of 1.5 specifies that the two components are baseline separated.

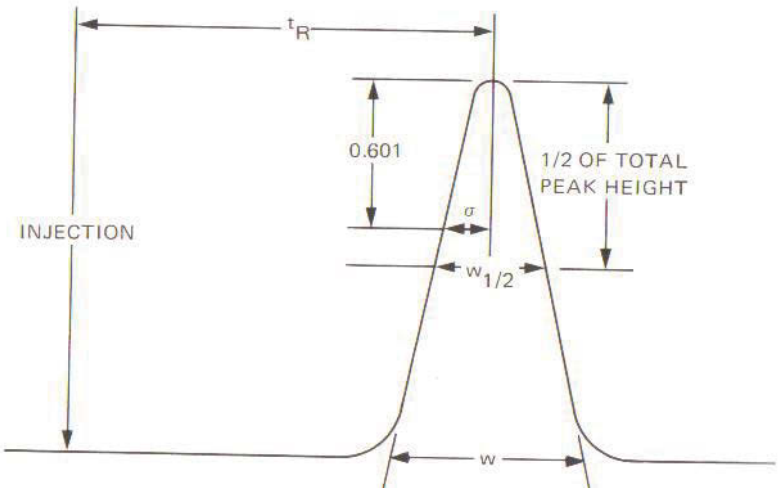


Figure 5: Calculation of Resolution, Theoretical Plate Count, and Peak Symmetry[7]

The peak symmetry determines the amount of peak fronting or peak tailing. A and B represent the distances from beginning of the peak front to the maximum, and B from the end of the peak tail to the maximum; representing 10% of actual peak height. One way of measuring peak tailing or fronting is by calculating the tailing factor. To some extent, most chromatographic peaks tail (TF > 1) where fronting (TF < 1) is less common.

$$Peak\ Symmetry = \frac{B}{A}$$

The Van Deemter equation:

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

is commonly used to describe band-broadening processes that occur during chromatography (column dispersion). In the Van Deemter equation, H signifies the theoretical plate height. The A term, also called eddy diffusion, results from the multiple

possible flow paths that can be taken by the solute molecules through the column packing material. The longitudinal diffusion coefficient, B, is due to the movement of solutes from areas of high concentration to low concentration. The term C refers to a phenomenon known as mass transfer effects. Mass transfer effects can occur when solute molecules move across stationary and mobile phase boundaries (phase transfer) at a rate significantly slower than the solvent flow rate resulting in random band-broadening due to "smearing" of the solute band.

Finally, the linear velocity of the mobile phase through the column is represented by the term, u. Generally, flow-path effects are independent of linear flow, longitudinal diffusion is inversely proportional to linear flow (higher flow means less diffusion and vice versa), and mass transfer effects are linearly proportional (higher flow means more diffusion and vice versa).

1.6 Thermodynamics

Absorption (partitioning) is the primary process normally associated with reversed-phase HPLC. Absorption is a bulk solution property where a solute partitions between two immiscible phases. The term absorption is frequently confused with adsorption, which is a surface phenomenon. Adsorption is the primary mechanism of separation for non-modified silica stationary phases. Sometimes during chromatography, both processes occur simultaneously resulting in peak-tailing being observed on the chromatogram. Absorption mimics the equilibrium process associated with solvent extraction, hence the thermodynamics of the elution process, also associated with the solute(s) retention time, can be described in a fashion similar to that used for solvent extraction.

Partitioning involves an organic stationary phase species that, through chemical bonding, is attached to the surface of packing particles. Adsorption relies on the analyte species being absorbed onto the surface of polar packing.

This expression:

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

which is derived from the van't Hoff equation can be used to probe the relationship of partitioning of solutes between the mobile and stationary phases as the temperature varies.

Because:

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

We can write:

$$ln\ k' = -\frac{\Delta G^o}{RT} + ln\varphi$$

Here ΔH^o represents the enthalpy of the partitioning of the system, T is temperature in Kelvin, ΔS^o is the entropy of the partitioning of the system, R is the universal gas constant, K is the equilibrium constant, k' is the capacity factor, and φ is the phase volume ratio between the mobile and stationary volumes.

1.7 Formation of Host – Guest Complexes

The ability for calixarenes to form host-guest complexes in solid state was established before finalized conformations of calixarene structures were adopted. These "true molecular complexes" have the ability to hold their shape, in or out of solution, in the absence of a guest

and independent of its complexing forces. Variations of the inflexibility of the guest molecule determine the calixarene binding ability. Use of X-ray crystallography aided in determining host-guest complexation.

Endo- and exo-calix complexes are the two main types of complexation observed with calixarene host-guest complexation. In the formation of endo-calix complexes, guest molecules are held within the center, or aromatic portion, of the calixarene. This part of the calixarene molecule does not permit a great amount of flexibility of aromatic guest molecules, which allows the calixarene to have good stability in maintaining the cone conformation. A non-aromatic guest (i.e. acetonitrile) allows flexibility and permits formation of the 1, 3 conformation with the nitrogen of the acetonitrile oriented downward. Formation of an exo-calix complex allows the guest molecule to be bound within either the lower or upper part of the functional groups on the rim of the calixarene.

When water soluble calixarenes are used to form host-guest complexes with metal cations, complexation ability increases as the size of the calixarene cavity increases. The ability of the guest ion to fit precisely within the cavity occurs because of the ability of the calixarene to donate electron pairs. This creates high association constants (K_{assoc}) and favors the formation of stable 1:1 complexes. Seen in Figure 5 are 1:1 (endo-calix) and 2:1 (exo-calix) complexes of calixarenes. Organic amines such as phenol blue, in the presence of p-alkylcalixarenes have been shown to form an endo-calix complex. Phenol Blue is known for its high absorption in water and use as a solvent polarity indicator. The complexation of the two happens because the calixarene provides a microscopic environment that is higher in polarity. It also causes the photon absorption of the phenol blue to decrease in energy, moving from 660 nm to 685 nm. Changing the substituents of the lower rim (i.e. replacing hydrogen with a methyl group) causes downward

shifts at low and high concentrations; the latter is less noticeable. The use of various techniques is required to determine the aggregation characteristics of the calixarene esters. The formation of a 1:1 complex can be observed via shifts in fluorescence maxima (energy shifts) for bulkier lower rim groups.

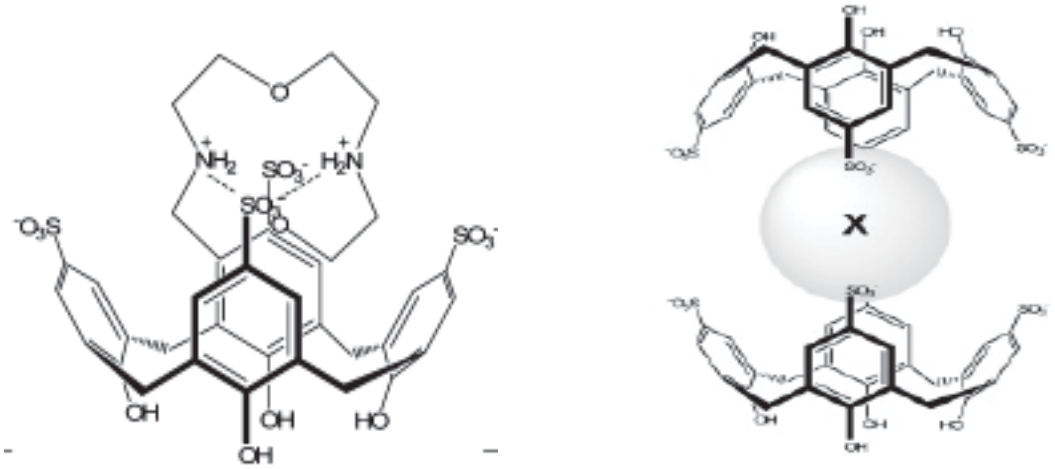


Figure 6: 1:1 complex of p-sulfonatocalix [4] arene and diaza-12-crown-4 and 1:2 complexes of p-sulfonatocalix [4] arene and a molecular capsule formation [8]

Chapter II

Historical

2.1 Functionalization of Calixarenes

Shinkai et al. investigated the possibility that water soluble calixarene cavities can recognize the size of guest molecules.[14] It was determined using IR and NMR that in an aqueous system, these calixarenes at low concentrations can form 1:1 complexes with phenol blue and anthrol blue. The binding constants for the calixarene host/guest complexes are sensitive to the change of cavity size of the calixarene, indicating the presence of size selectivity in host-guest complexation.

The relationship between water soluble calixarenes having sulfonate groups on the upper and lower rim and their host-guest binding ability was performed by Shinkai et al.[13] Pyrene was used as a guest molecule because of its fluorescence intensity. The increasing concentration of calixarene caused a decrease in intensity and 1:1 complexation. Substitution in the upper rim by alkyl groups resulted in weak, but selective binding sites, while substitution in the lower rim by alkyl groups create strong but non-selective binding sites.

Calix [6] arene hexasulfonate and p-nitrophenol host-guest interaction was studied by Kunsági-Máté et al.[10] The use of photoluminescence (PL), differential scanning calorimetry (DSC), and quantum chemical methods resulted in the characterization of the interaction as a 1:1 complex stoichiometry. Enthalpy-entropy compensation effects from the host-guest binding weakened the host-guest complex in the process. The effect on the enthalpy and entropy were opposite, with enthalpy changing positively and entropy changing negatively.

The reactions of calixarenes and potential guest molecules in solution were reported through the use of aromatic solvent induced shift (ASIS); a process used in ^{1}H NMR.[12] Results showed complex dependence based on the para substituent as well as showing that aliphatic amines have strong interactions with calix [4] arenes in polar solvents. Endo-calix complexes were also apparent in the presence of various amines.

Phosphate buffers, at pH 7 or 8, in the presence of 4-sulphonic calix [6] arene have been determined to have the ability to produce separations between isomeric benzenediols, chlorophenols, and toluidines in capillary electrophoresis.[6] The p-sulphonic calix [6] arene mobile phase additive was used to adjust the selectivity. 3-4mM concentrations of p-sulfonic calix [6] arene assisted with good separations as well as the implication of complexation. The high UV absorption caused further investigation of the buffer to be limited.

Water-soluble calixarene complexation was determined using solid-liquid extraction.[11] With the use of aromatic hydrocarbons in aqueous base as guests, the calculated association constants showed that moderately strong complexes can be formed with carboxyl derivatized calixarenes and complementary size and shape can be distinguished.

2.2 Calixarenes as Mobile Phase Additives in HPLC

Park et al. investigated the retention reduction of isomeric phenols. It was determined that adding para-sulfanto calix [6] arene to either methanol/water or acetonitrile/water mobile phases would cause reduction in retention time and improve the efficiency of separation shown by HPLC.[5] The use of calix [6] arene-p-sulfonate showed that the UV absorption caused could have resulted from the additive not being transparent

in the UV region and a reduction of k values in HPLC retention times due to the complexation between calix [6] arene-p-sulfonate and the phenols.

Complexation and chromatographic properties of nitrophenols and water-soluble calixarenes as mobile phase additives were determined using RP-HPLC by J.S. Millership et al.[4] The retention and separation of nitrophenols were highly influenced by the effects of pH and not by the inclusion complexes. The use of proton NMR and UV spectrometry was used along with HPLC verify this analysis.

For p-sulfoantocalix [4] arene, the host-guest inclusion complexes varied with the retention times of the 10 amino acids at pH 2 and pH 8.[9] Stability constants that formed showed variations through aromatic-aromatic, electrostatic, hydrophobic, and ion paring interactions.

The complexation of water-soluble 4-sulphonic calix[n]arenes (n=4, 6, and 8) was investigated with testosterone in water and buffers.[8] Increasing solubility between 4-sulphonic [6] arene and 4-sulphonic [8] arene was shown to enhance the testosterone complex formation. The stability constants were determined to be low because of the relatively small cavity size of the 4-sulfonic calixarenes, which prohibited entry of the testosterone into the cavity.

The use if calix[n]arenes as mobile phase additives was investigated by C. Lowe.[2] Fluorescence, UV, and visible spectroscopy were used to investigate and compare stationary phases. Solute capacity factors were examined by the presence of the calixarene additive and no calixarene additive within the mobile phase.

M. Sullenberger used modified chiral calixarenes in mobile and stationary phases.[3] Mobile phase studies revealed reductions in retention time and capacity factor while

addition of calixarene in the stationary phase increased retention time and capacity factor. These conditions confirm the formation of host-guest complexes.

2.3 Complex Formation

In the complexation of cyclodextrins with substrates, equations were derived to determine whether a 1:1 or 1:2 stoichiometric relationship existed in the host-guest complex.[20] The determination of binding constants was based on chromatographic retention times, which was dependent on the concentration of the mobile phase additive and stoichiometry of the host-guest complex. Different binding behaviors for the substrates were revealed.

Triterpene acid complexes were investigated by B. Claude et al.[21] The separation of triterpene acid complexes was improved by addition of derivatized β- and γ-cyclodextrins added to the acetonitrile-phosphate buffer mobile phase through reversed-phase liquid chromatography. The values of the apparent formation constants for the host-guest complexes were evaluated using the reciprocal of the retention factor versus the concentration of the cyclodextrin. Strong 1:1 complexes were obtained from decreasing amounts of acetonitrile in the mobile phases.

R. Baudry et al. studied the host-guest stability constants of calixarene complexes with aromatic molecules using HPLC.[7] By adding the calixarene to the mobile phase, the capacity factor of each solute decreased. It was proved that p-tert-butylcalix [8-12] arenes do form host-guest inclusion complexes with the various aromatic compounds tested.

Chapter III

Statement of Problem

3.1 Statement of Problem

Calixarenes are large phenolic cyclic oligomers containing four or more monomer units. Likewise, 4-sulphonic calix[n]arenes are “upper rim” sulphonated water-soluble phenolic cyclic oligomers that contain four or more monomer units. They both contain large hydrophobic cavities, making them of interest in chromatography due to their ability to form host-guest complexes with neutral molecules. It is also a possibility that other conformations of calixarene may accommodate specific conditions or complexes. The formation of such complexes by calixarenes through mobile and stationary additives has been demonstrated in the literature and prior research performed at Youngstown State University.

The importance of this research is to find implications of host guest complexing through the addition of 4-Sulfonic calix[n]arene and 4-tert-butyl calix[n]arene to the appropriate mobile phases. These results will be evaluated in conjunction with mobile phases that contain no calixarene additive. It is also thought that variance in temperature as well as analyte concentrations may play a factor in how host-guest complexes are formed. Through use of Reversed Phase-High Performance Liquid Chromatography (RP-HPLC), chromatographic data will aid in determining the retention of solutes, capacity factors and formations constants. This information will help to create aspects that are significant signs of host guest complexation. Host-guest complexation can be attributed to the decrease in capacity factor of the mobile phases containing additives when compared to those with no additive. The van’t Hoff expression plots associated with these

solutes would be non-linear. This information will be used to determine the best temperature, mobile phase additive and analyte concentration for implication of host guest complexation

Chapter IV

Materials and Apparatus

4.1 Materials

Solvents*	
Methanol, HPLC Grade	Fisher Scientific (Fair Lawn, NJ)
Dichloromethane, HPLC Grade	Fisher Scientific
Tetrahydrofuran, HPLC Grade	Fisher Scientific
Glacial Acetic Acid	Mallinckrodt Chemical Works (Hazelwood, MO)
Deionized water	In-house deionizer
Solutes†	
Benzene≥99%	Aldrich Chemical Company (Milwaukee, WI)
Naphthalene 99%	Aldrich Chemical
Anthracene 97%	Aldrich Chemical
Reversed-phase test mixture-D4	Alltech Associates, Inc. (Deerfield, IL)
Mobile Phase Additives†	
4-Sulphonic calix [n]arene (n=4, 6 and 8) (95, 95and 97%)	Acros Organics USA (Morris Plains,NJ)
4- tert-Butylcalix [n] arene (n=4, 6 and 8) (99, 96 and 97%)	Acros Organics USA
Columns	
Guard column: C18 100A 5 micron 7.5mm x 4.6mm	Alltech Associates, Inc. (Deerfield, IL)
Column: Nucleosil C18 5 micron 250mm x 4.6 mm	Alltech Associates, Inc.
* Each solvent was filtered through a 0.2μ filter before use.	
† All reagents were ACS grade or better	

4.2 Apparatus for HPLC Analysis

A Waters 2695 Chromatographic System was used for chromatographic experiments. It consisted of a LC pump, absolute pressure transducer (APT) including an online degasser, Waters 996 Photodiode Array Detector, auto sampler, column oven, 25 µl injection needle and Millenium[32] software.

4.3 Study of Mobile Phases

The preparation of several mobile phases was used to conduct the study of water soluble and non-water soluble calixarenes. For the water soluble calixarenes, 4-Sulphonic calix [n]arene (n= 4, 6 and 8), a solution of 85% methanol and 15% water was prepared The non-water soluble calixarene, 4-tert-butylcalix[n]arene (n= 4,6 and 8), was prepared by using 90% of a mixture containing 93.5% acetonitrile (ACN), 5% dichloromethane, and 1.5% acetic acid, also described as "Solution A". This was then combined with 10% tetrahydrofuran (THF). All mobile phases contained a concentration of 1.0 $x10^{-4}$ M, 5.0 $x10^{-5}$ M, and 1.0 $x10^{-5}$ M respectively of the 4-Sulphonic calix [n]arene (n= 4, 6 and 8) and 4-tert-butylcalix[n]arene (n= 4,6 and 8) as individual additives. A no calixarene additive mobile phase was also used. All mobile phases were used at three various stationary phase column temperatures (35ºC, 45ºC, and 55ºC). Before all injections, the system was monitored for one hour to ensure that the column temperature (35ºC, 45ºC, and 55ºC) for the series was achieved and equilibrated for the last 25 minutes with the mobile phase intended to separate all analytes. The injection volume used for each sample was 10µL with a 0.8 mL/min flow used for 4-tert-butylcalix [n] arene and 1.0mL/min for 4-sulphonic calix [n]arene.

4.4 HPLC Test Mixture Preparation

Analytes used to analyze the mobile phase additives consisted of a phenyl ring homologous series which included benzene, naphthalene, anthracene and named the BNA series. Each of the analytes were dissolved in acetonitrile for insoluble calixarenes and methanol for water soluble calixarenes at a given concentration of $1x10^{-4}M$.

A reversed-phase test mixture, Reversed-Phase Test Mix-D4, was used periodically to ensure that stability of the stationary phase. The reverse phase mixture consisted of uracil, phenol, N, N-diethyl-m-toluamide and toluene.

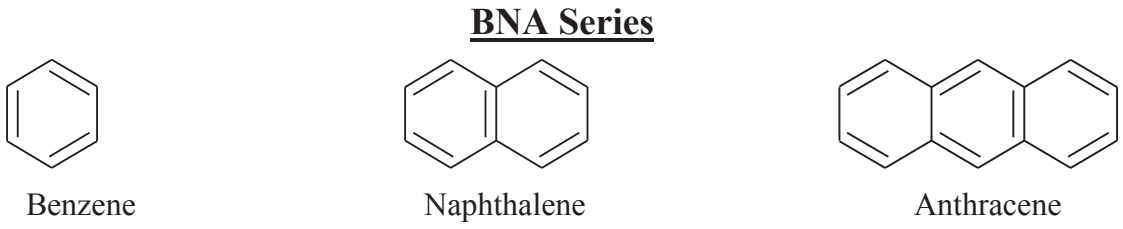


Figure 7: Solutes used for host-guest interactions

Chapter V

Results and Discussion

5.1 HPLC Mobile Phase Characterization

4-tert-butylcalix[n]arene and 4-sulfonic calix[n]arene (n=4, 6, and 8) were used as mobile phase additives. The water insoluble 4-tert-butylcalix[n]arenes were added to a mixture of acetonitrile, dichloromethane, acetic acid and tetrahydrofuran. The 4-sulfonic calix[n]arenes, which are water soluble, were added to a methanol and water mobile phase. As a baseline, the two mobile phases mentioned above were made without the use of calixarenes. The analytes of interest are a group of homologous benzene rings. The analytes' retention time, capacity factor, host-guest complexation, formation constants as well as binding constants were examined against dependence of temperature and additive concentration with the use of RP-HPLC analysis. The retention time for each solute was directly related to the increase of phenyl ring size in the homologous series. These solutes followed a trend of decreasing retention time with increasing temperature and constant retention time with increasing solute concentration**.**

5.2 Result Analysis

A corrected retention time (R_t') was calculated based upon the dead time (R_o) and measured peak retention time (R_t):

$$R'_t = R_t - R_o$$

The capacity factor (k') was calculated by taking the corrected retention time and dividing it by the dead time (R_o):

$$k' = \frac{R'_t}{R_o}$$

In the case of 1:1 complex (host-guest) formation.[7] it can be determined that the relationship between calixarene retention times and concentration in the mobile phase can be expressed as:

$$\frac{1}{k'} = \frac{1}{\varphi K[A]} + \frac{K_1[(CA)]}{\varphi K[A]}$$

where φK[A] is equal to the phase volume ratio (φ) multiplied by the concentration of solute ([A]); K_1 is the apparent formation constant and (CA) is the concentration of calix[n]arene. The following series of equilibria were used to determine the given equilibrium expression:

1. $S + A \xleftrightarrow{K} SA$ $\qquad K = \frac{[SA]}{[S][A]}$
2. $S + CA \xleftrightarrow{K_1} SCA$ $\qquad K_1 = \frac{[SCA]}{[S][CA]}$
3. $S_{tot} = S + SA + SCA$ $\qquad k' = \frac{\varphi[SA]}{[S] + [SCA]}$

By replacing equations 1 and 2:

$$k' = \frac{\varphi K[A][S]}{[S](1 + K_1[CA])}$$

Canceling [S] and solving for 1/k' gives the equation mentioned above.

Concentration vs. 1/k' was plotted and the ratio of the slope divided by the intercept was used to determine the K_1 value. The linearity of the plotted values was used in an attempt to determine the type of complexation.

As previously mentioned in chapter one, a form of the van't Hoff equation can be used to examine the relationship across differences in temperature. It can also be used to

determine the enthalpy for the host-guest formation process. By using this form of the van't Hoff equation:

$$\ln k = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \Phi$$

a plot of ln k vs. 1/T can be used to determine if ΔH^o varies with temperature. If the slope of the plot is set equal to $\frac{-\Delta H^\circ}{R}$, ΔH can be readily determined algebraically. The plot can display a straight or curved line. A straight line represents no variation in ΔH; a curved line represents ΔH variation with temperature, but ΔH can be evaluated for any particular point on the curve. Temperature–dependent phase ratio changes are thought to be responsible for curved plots. For each series, an evaluation of temperature-phase ratio changes was also determined.

After chromatographic information was established by adding 4-Sulfonic calix[n]arene and 4-tert-butyl calix[n]arene to the appropriate mobile phases finding implications of host guest complexation consisted of a few steps. The initial step was to show non-linearity in van't Hoff expression plots. One collection of van't Hoff results are plotted by homologous series (number of rings) vs. the natural logarithm of the capacity factor. The next group of van't Hoff plots used was the inverse of temperature vs. the natural logarithm of the capacity factor.

The last aspect suggesting host-guest complexation would be the decrease of capacity factor in mobile phases containing additives compared to the no additive mobile phase. This data allows for each individual analyte to be looked at separately from its homologous series.

Benzene Napthalene Anthracene (BNA) Series

5.3a 90% "Solution A" 10% THF mobile phase-no additive

The mobile phase used in this analysis was a mixture of 90%: 93.5% Acetonitrile, 5.5% Dichloromethane, 1.5% Acetic Acid identified as "Solution A". It was then added to 10% Tetrahydrofuran .[7]

The elution order of each solute based on the variation of three concentrations ($1x10^{-5}$M, $5x10^{-5}$M, and $1x10^{-4}$M) with controlled temperature (45°C) is seen in Figure 8. Benzene could not be examined in this series possibly for two reasons: the elution of benzene occurred before or near the dead volume time or the concentration used was lower than what could be quantified.

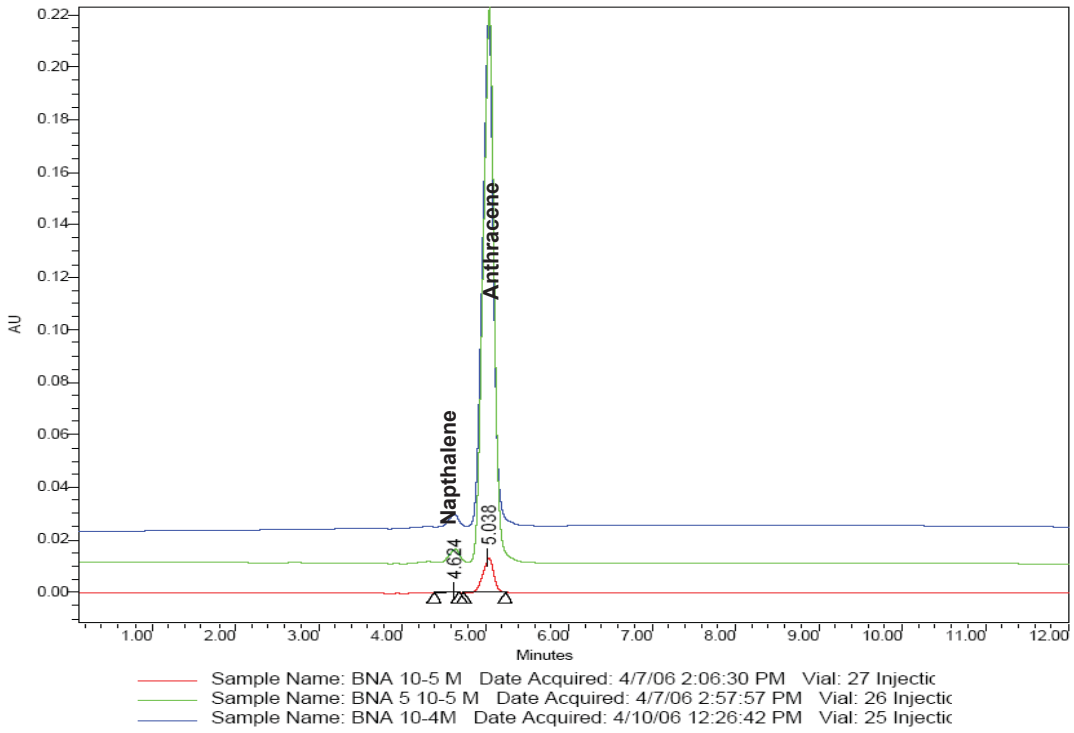


Figure 8: 90% "Solution A" 10% THF mobile phase with three concentrations of BNA solutes at 45°C

5.3b 4-tert butyl calix[n]arene mobile phases

The mobile phase used in this analysis was a mixture of 90%: 93.5% Acetonitrile, 5.5% Dichloromethane, 1.5% Acetic Acid identified as "Solution A". It was then added to 10% Tetrahydrofuran. The addition of 4-tert-butyl calix[n]arenes (n=4, 6, and 8) was introduced to the mobile phases at $1x10^{-5}$, $5x10^{-5}$ and $1x10^{-4}$ concentrations prior to analysis. The chromatograms resulting from the 4-tert-butyl calix[n]arene mobile phase were used to compare to those with no mobile phase additive. Figures 9-11 displays chromatograms based on controlled temperature at 45.0°C.

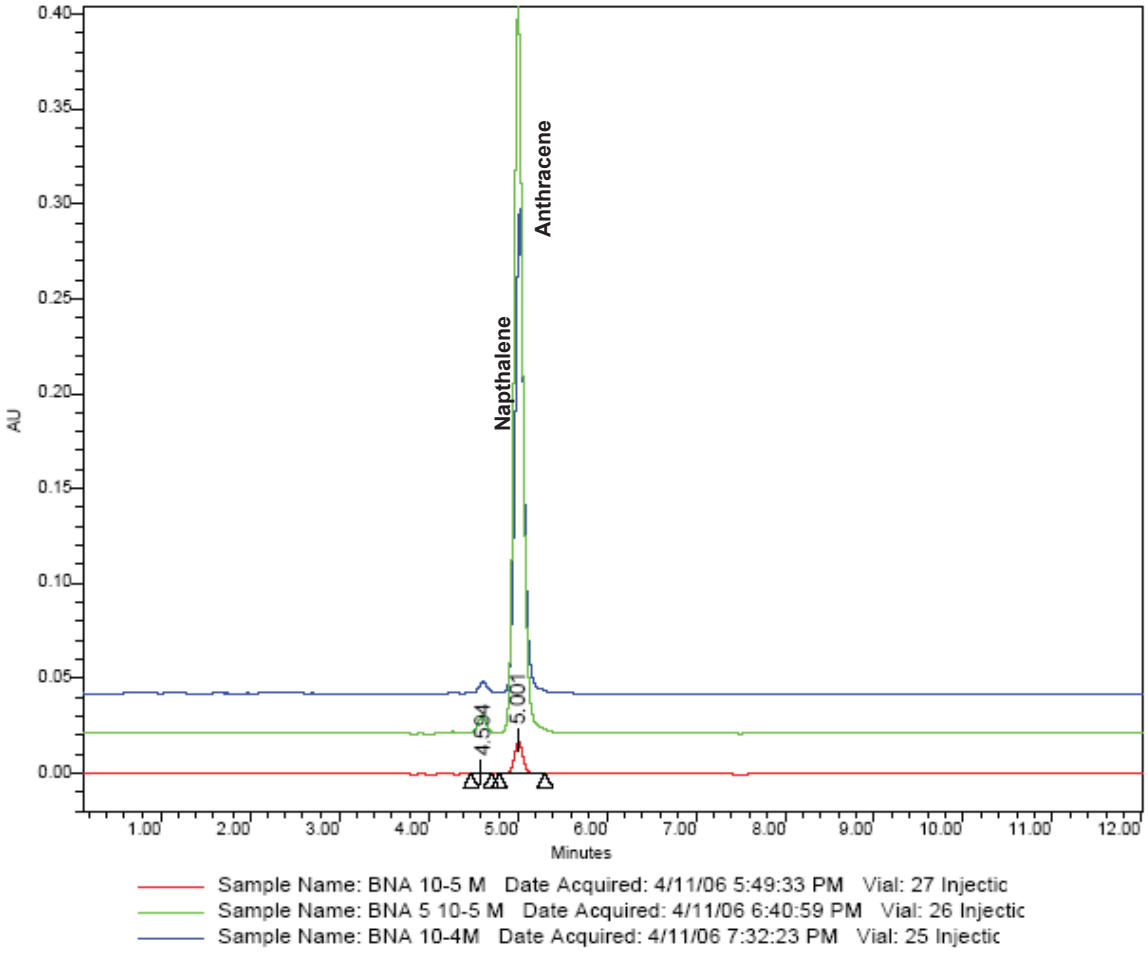


Figure 9: 4-tert butyl calix[4]arene mobile phase with three concentrations of BNA solutes at 45°C

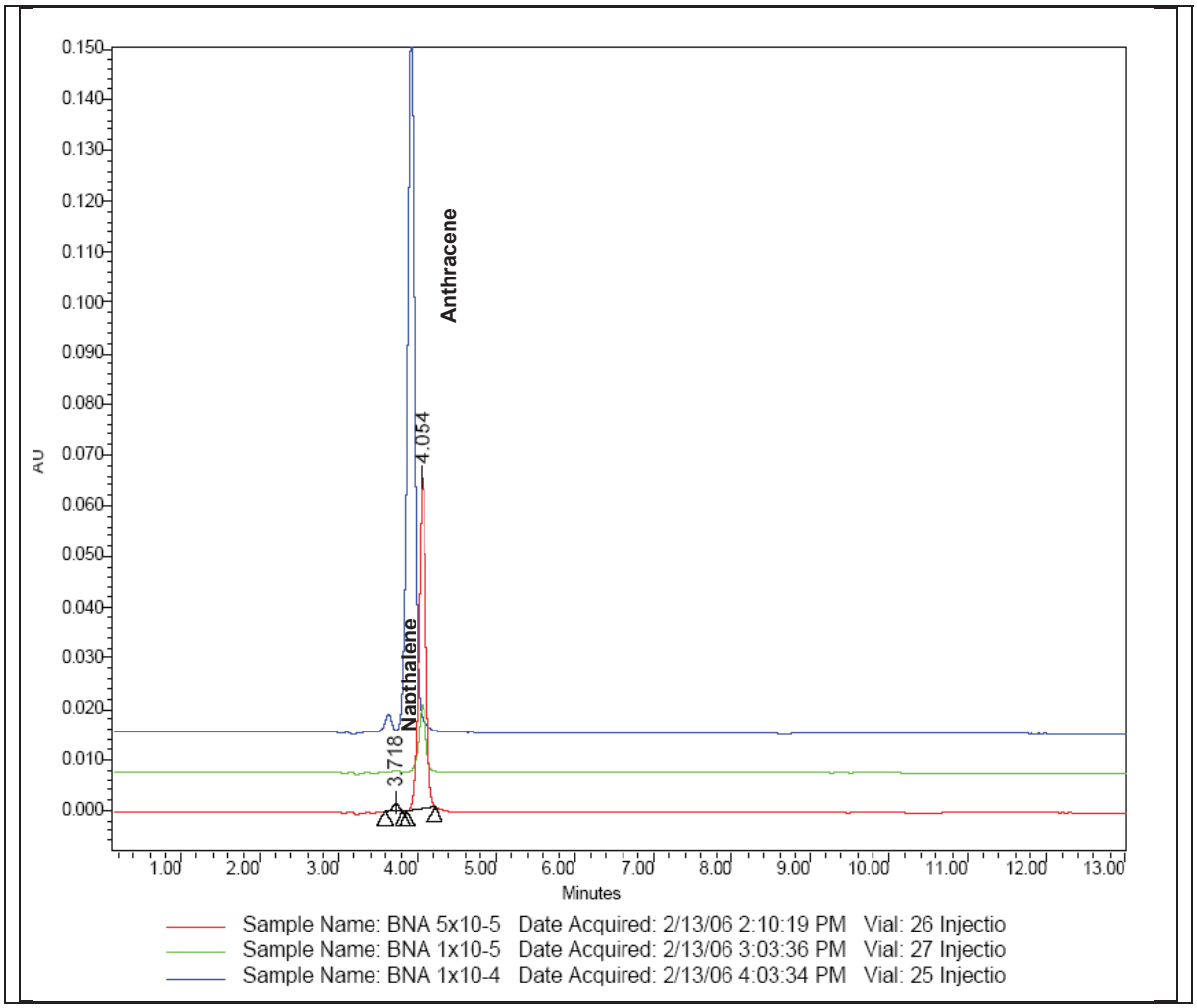


Figure 10: 4-tert-butyl calix[6]arene mobile phase with three concentrations of BNA solutes at 45°C

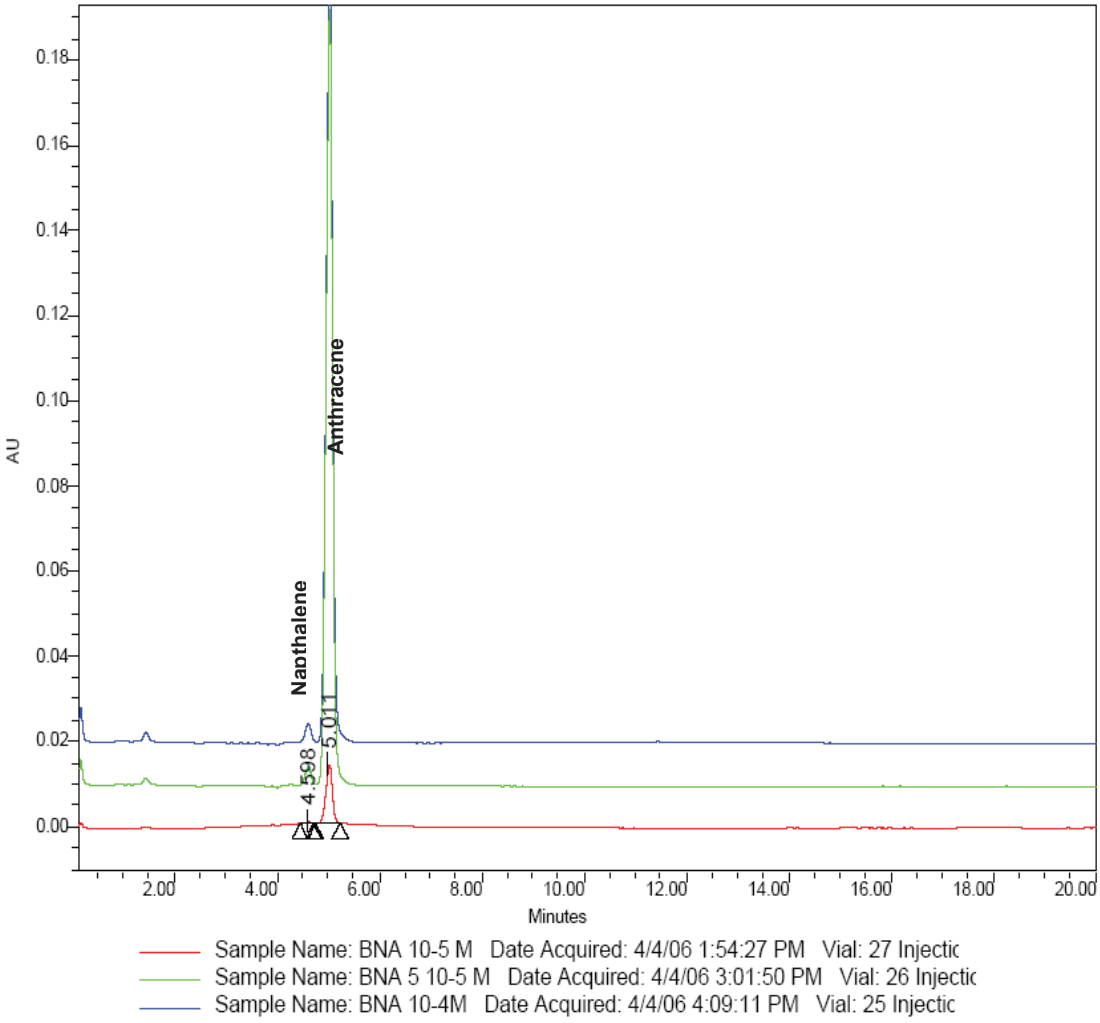


Figure 11: 4-tert-butyl calix[8]arene mobile phase with $1x10^{-5}$M, $5x10^{-5}$M and $1x10^{-4}$M BNA solutes at 45°C

For the BNA Insoluble series, the nature of this group was found to be all linear when plotted using van' Hoff expressions carbon rings vs. ln k' which may be attributed to the retention of only naphthalene and anthracene for most of the conditions used to create data. The linearity would thus signify no host-guest complexation.

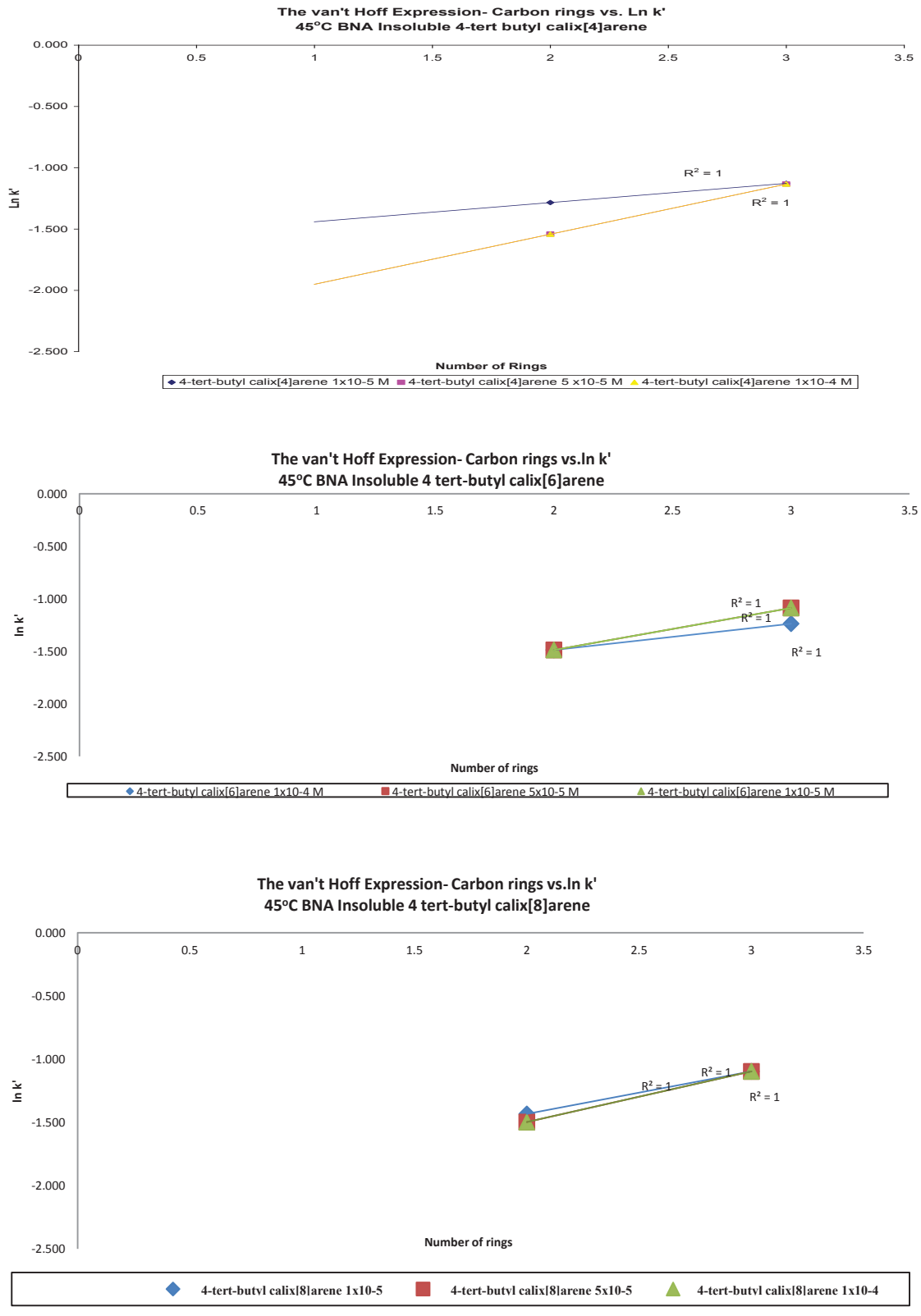


Figure 12: Carbon rings vs. ln k' at 45°C for 4 tert-butyl calix[n]arenes

4-tert-butyl calix[4]arene had the greatest suggestion of host-guest complexation with temperature dependence for the van't off expressios ln k' vs. 1/K. This is apparent in Figure 13 for naphthalene and anthracene at all concentrations; exceptions to this are 4-tert butyl calix[6]arene when used with $1x10^{-4}$M anthracene. All other mobile phases did not create host- guest complexation.

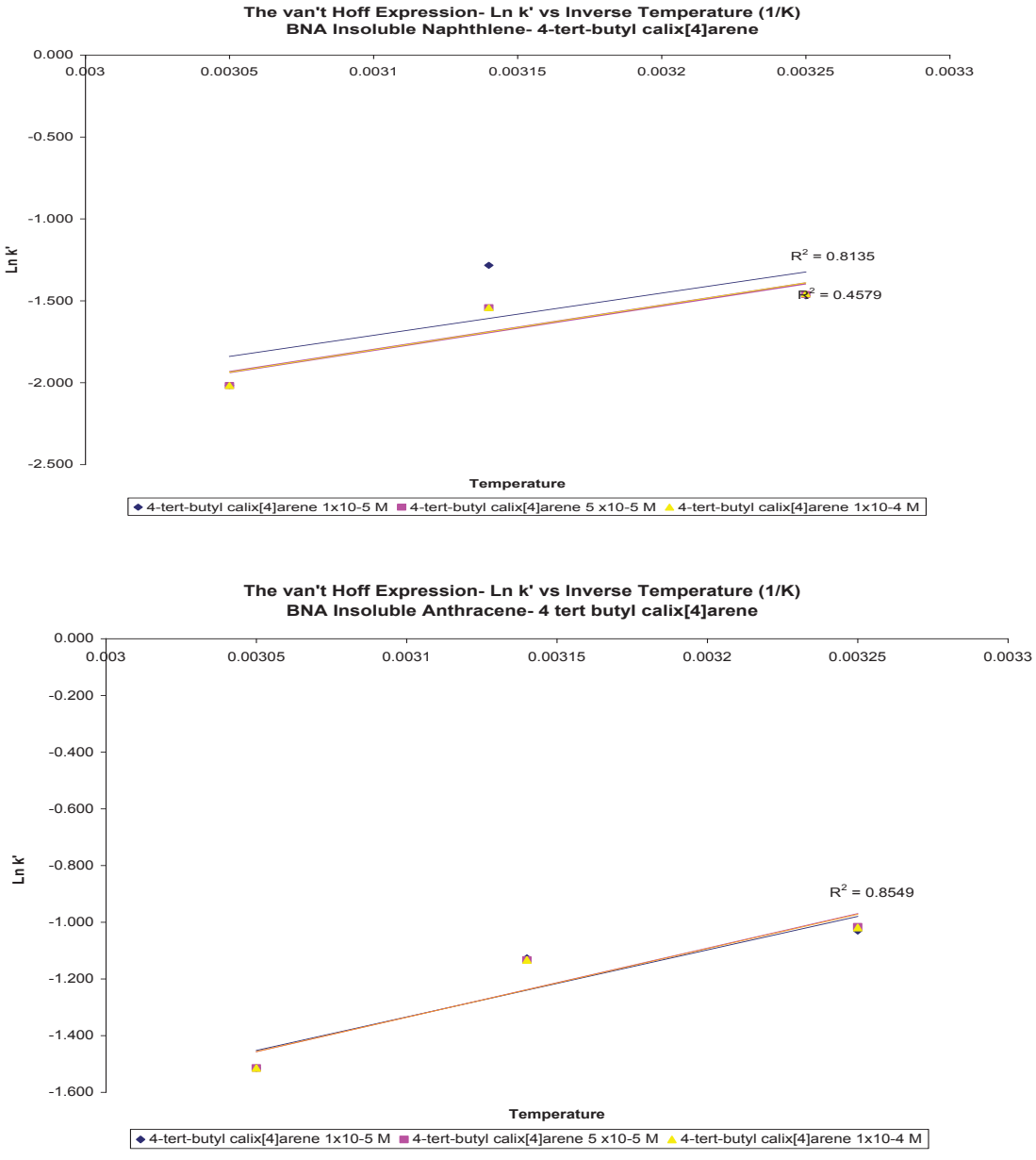


Figure 13: Van't Hoff plots: ln k' vs. 1/K for Napthalene and Anthracene with 4 tert-butyl calix[4]arene additive

The comparison of mobile phase additives and non- additive capacity factors categorized by temperature show that the highest possibility of host- guest complexation would result from the addition of 4-tert-butyl calix [4] arene. Its decrease of capacity factor, shown in figure 14 is greater with the increase of temperature. It can also be stated that majority of the mobile phases are more attracted to the smaller homologous ring (naphthalene) thus, more suited for optimal host-guest compelxation. Anthracene at 45^{o}C with the use of 4-tert- butyl calix[6] arene, however, has an apparent preference for the larger homogolus ring.

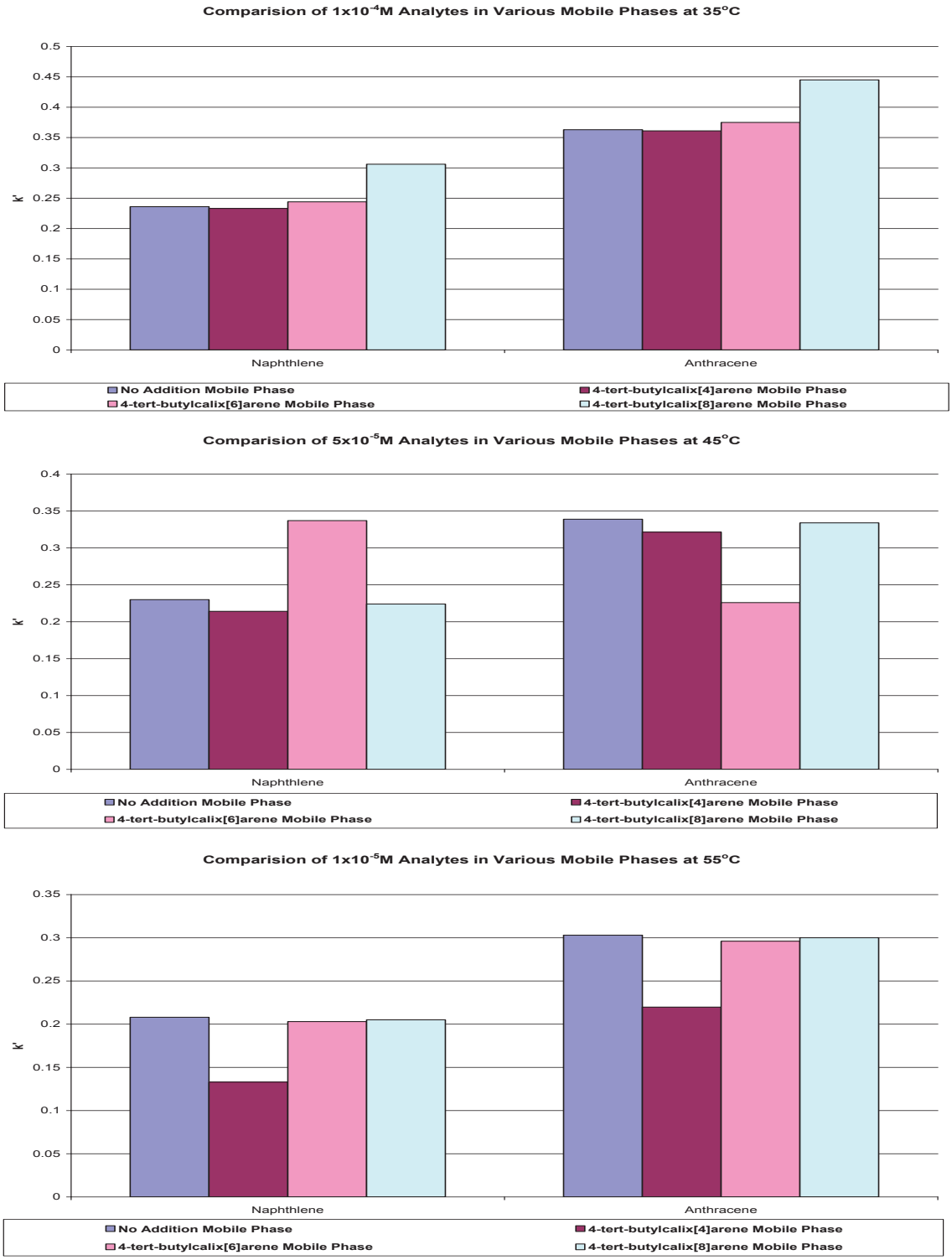


Figure 14: Capacity factor comparisons of 4-tert-butyl calix[n]arene mobile phases

5.3c 85% Methanol 15% Water mobile phase

By the use of several different mobile phases, separation of three PAH's occurred. The first mobile phase used in this analysis was a mixture of 85% Methanol and 15% Water

A chromatogram based on the variation of three concentrations ($1x10^{-5}$M, $5x10^{5}$M, and $1x10^{-4}$M) with controlled temperature (45.0°C) is seen in Figure 15. The detection of benzene at $1x10^{-5}$M was not obtainable in most of chromatograms.

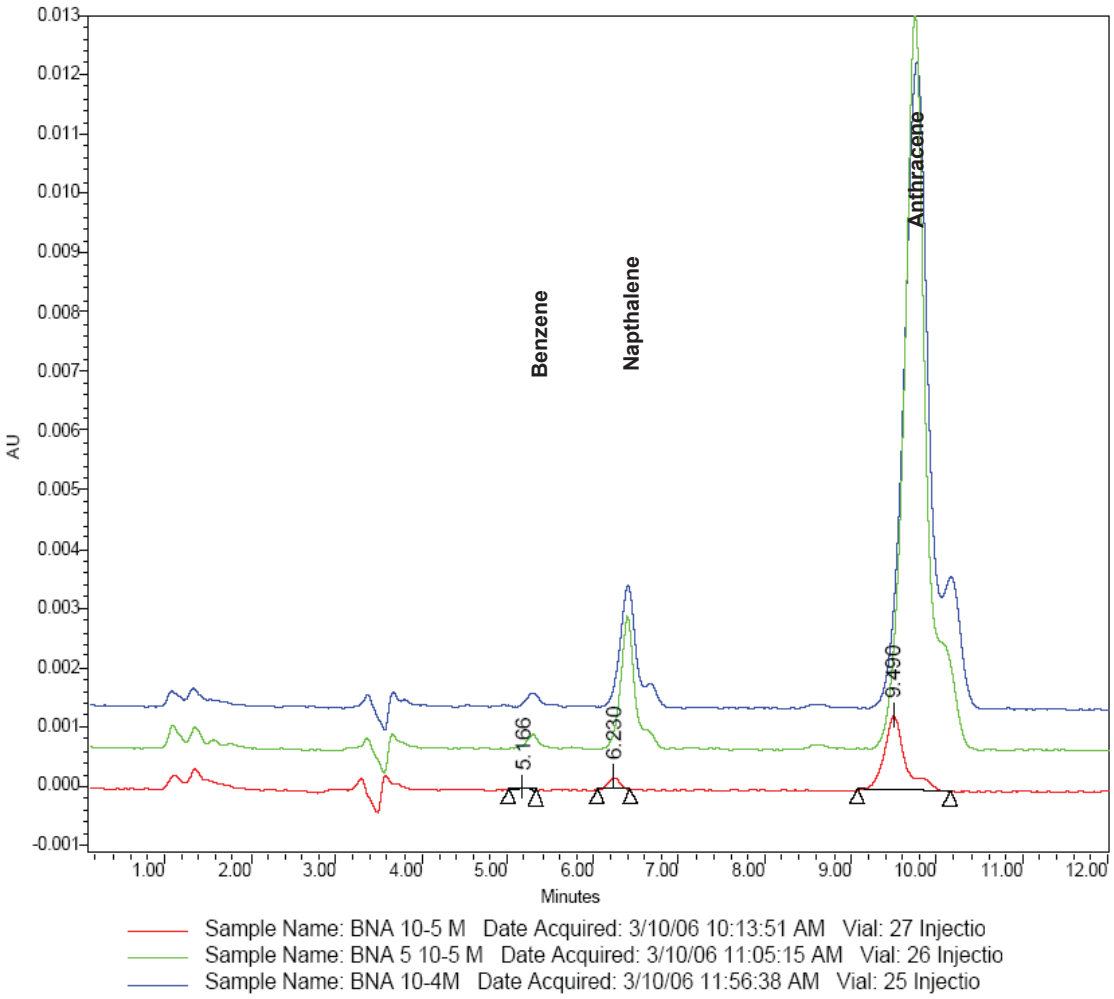


Figure 15: 85% Methanol 15% Water mobile phase with three concentrations BNA solutes at 45°C

5.3d 4-Sulfonic calix[n]arene mobile phases

In this analysis of the BNA Series, a $1x10^{-5}$M, $5x10^{-5}$M and $1x10^{-4}$M additive of 4-Sulfonic calix[n]arenes (n=4, 6, and 8) was used with a mixture of 85% Methanol and 15% Water to potentially create mobile phases that would suggest host-guest complexation. Comparison to the non- additive mobile phase will also be performed. Figures 16-18 shows chromatography with controlled temperature at 45.0°C.

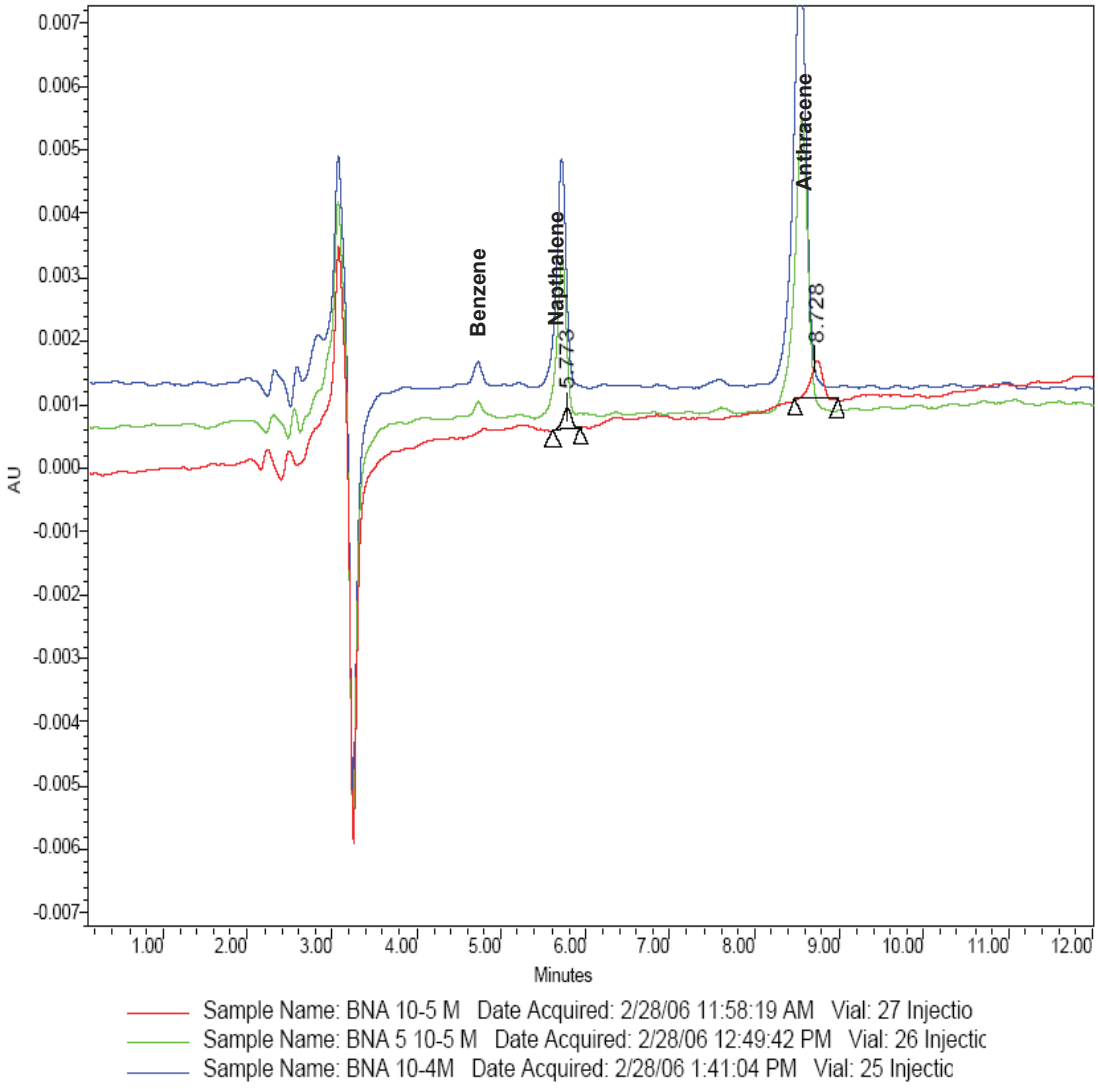


Figure 16: 4-Sulfonic calix[4]arene with three concentrations BNA solutes at 45°C

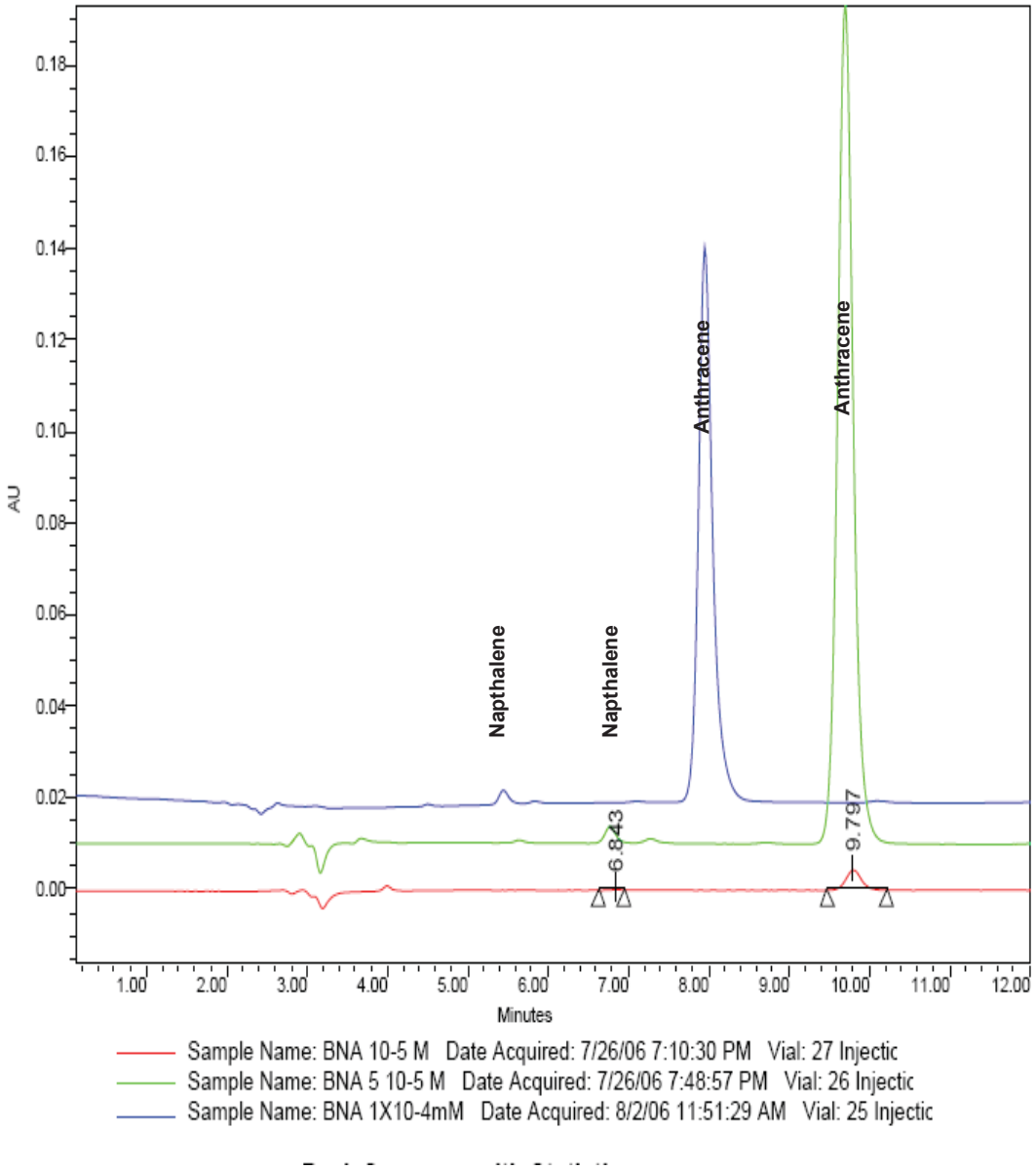


Figure 17: 4-Sulfonic calix[6]arene with three concentrations BNA solutes at 45°C

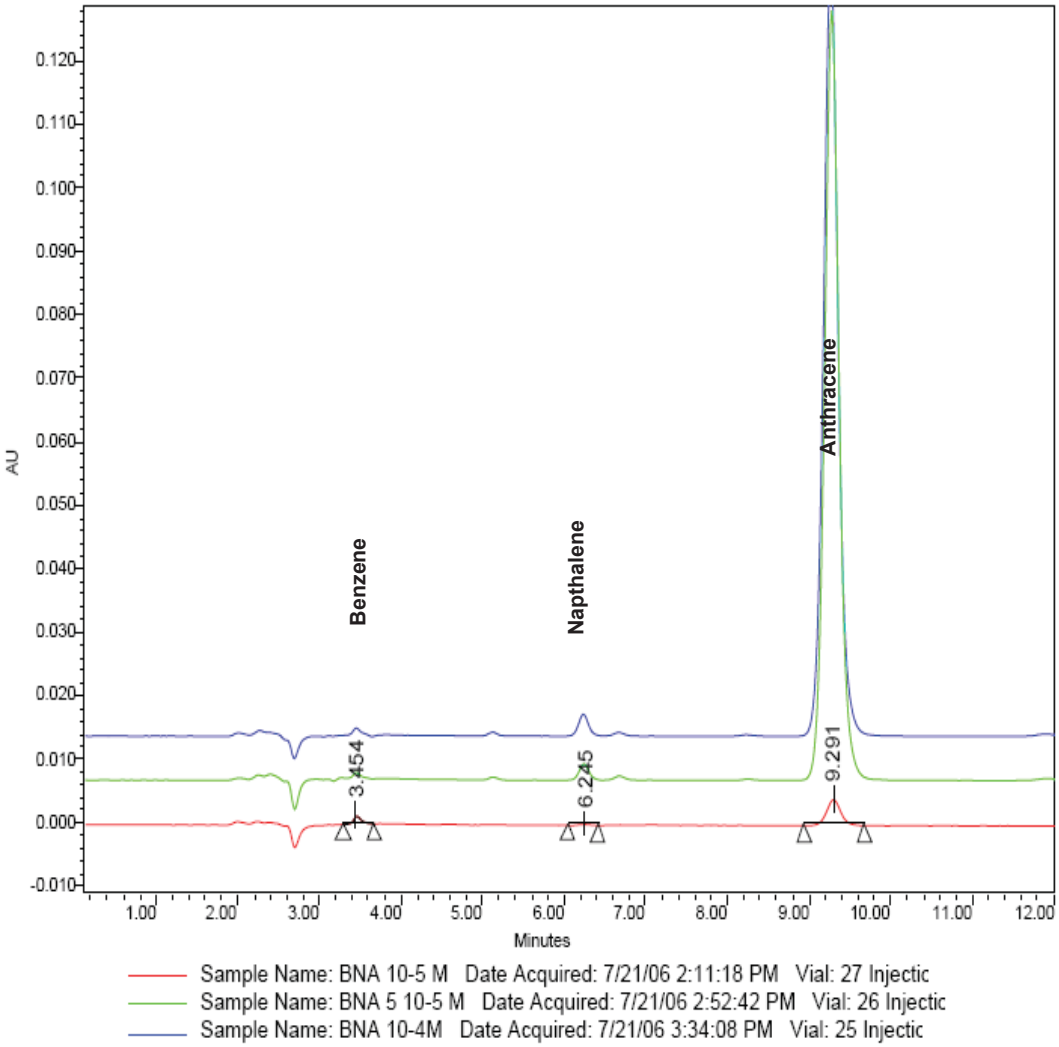


Figure 18: 4-Sulfonic calix[8]arene with three concentrations BNA solutes at 45ºC

As seen in Figure 19, the 45ºC BNA Soluble 4-Sulfonic calix[6]arene concentration of $5x10^{-5}$M and 35ºC BNA Soluble 4-Sulfonic calix[4]arene $1x10^{-4}$M are the only two non- linear plots for the entire series. All other mobile phases signify no host-guest complexes.

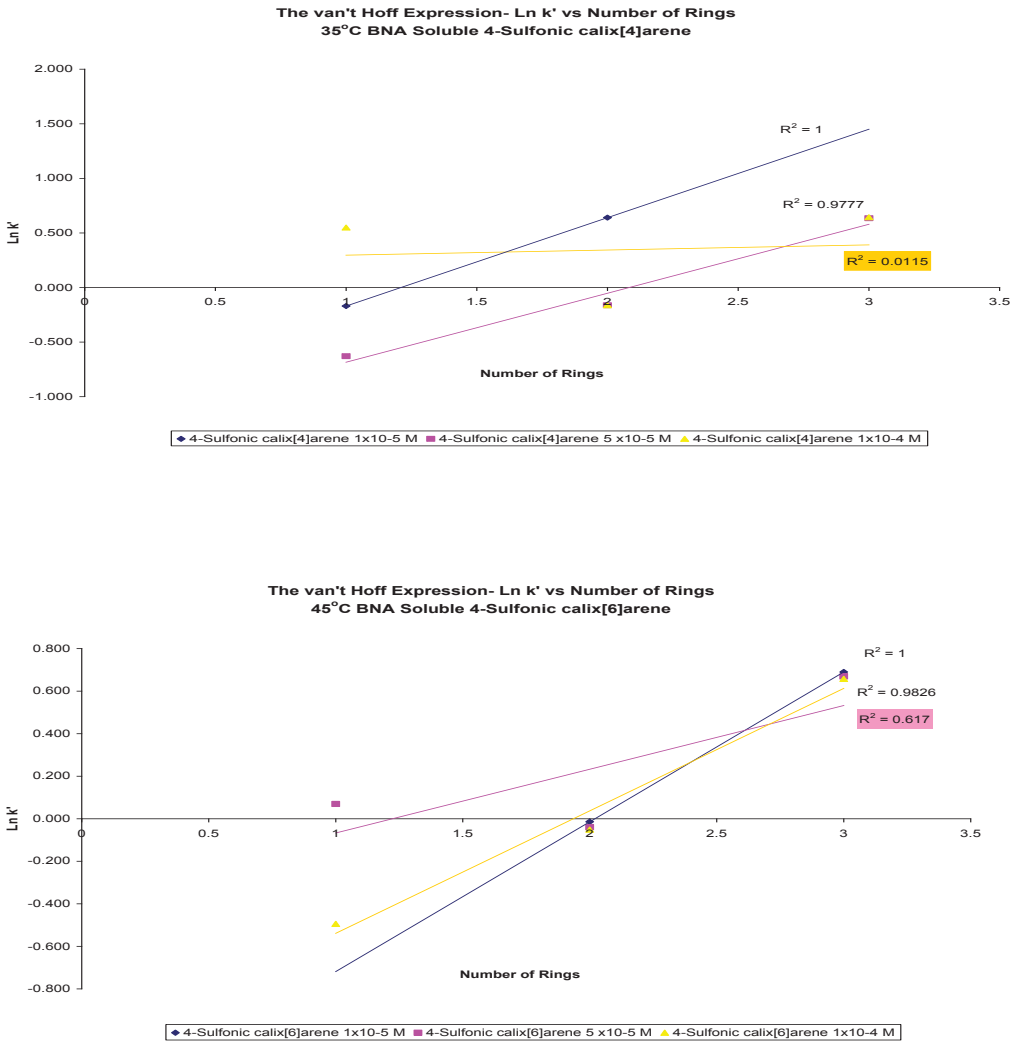
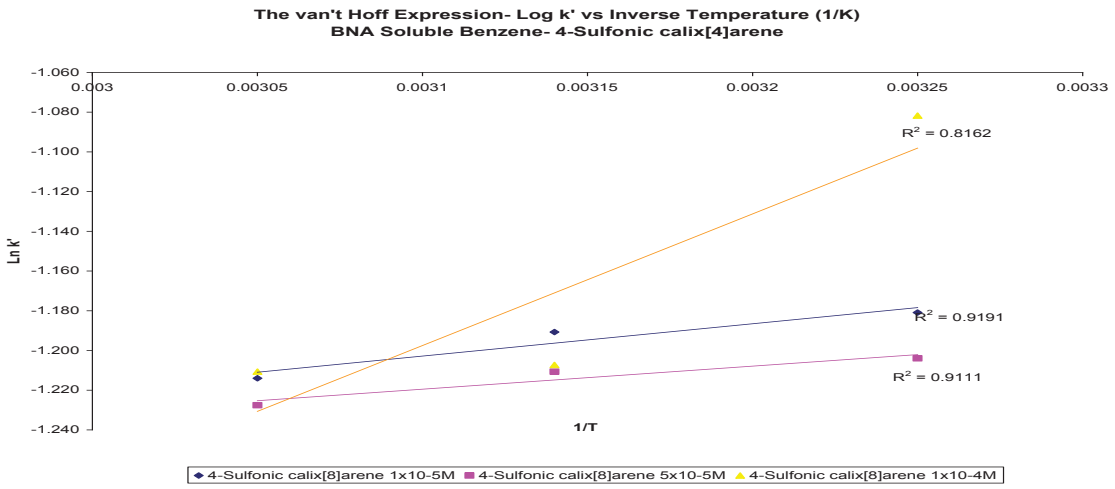
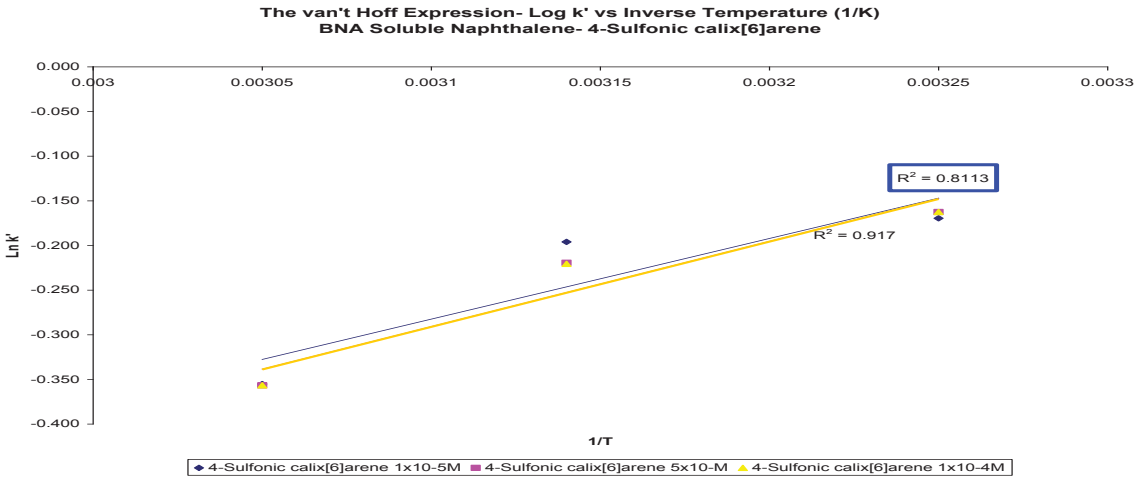


Figure 19: Carbon rings vs. ln k' at 45°C for 4-Sulfonic calix[n]arenes

When looking at the Van't Hoff plots: ln k' vs. 1/K, this series of analytes showed only one concentration for each analyte that suggest host-guest complexation: benzene with $1x10^{-4}$M 4-Sulfonic calix [4]arene, naphthalene with $1x10^{-5}$M 4-Sulfonic calix[6]arene, and anthracene with $5x10^{-5}$M 4-Sulfonic calix[8]arene. Figure 20 displays the linearity represented for this series.





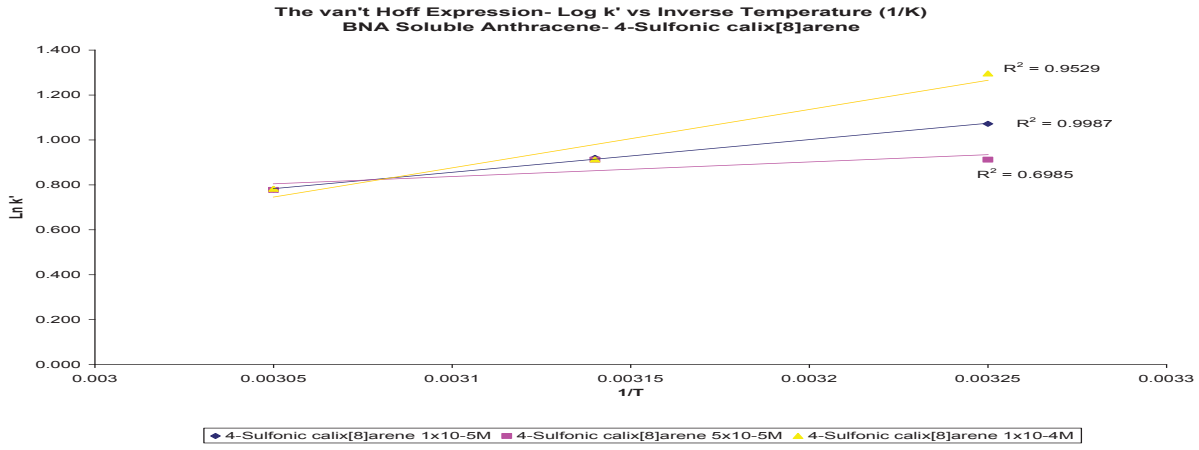
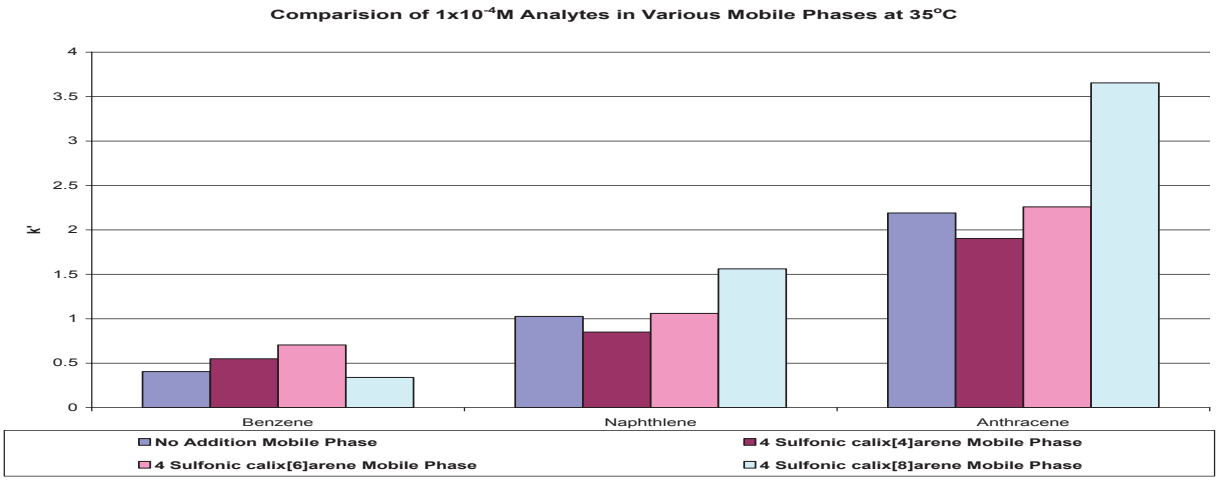
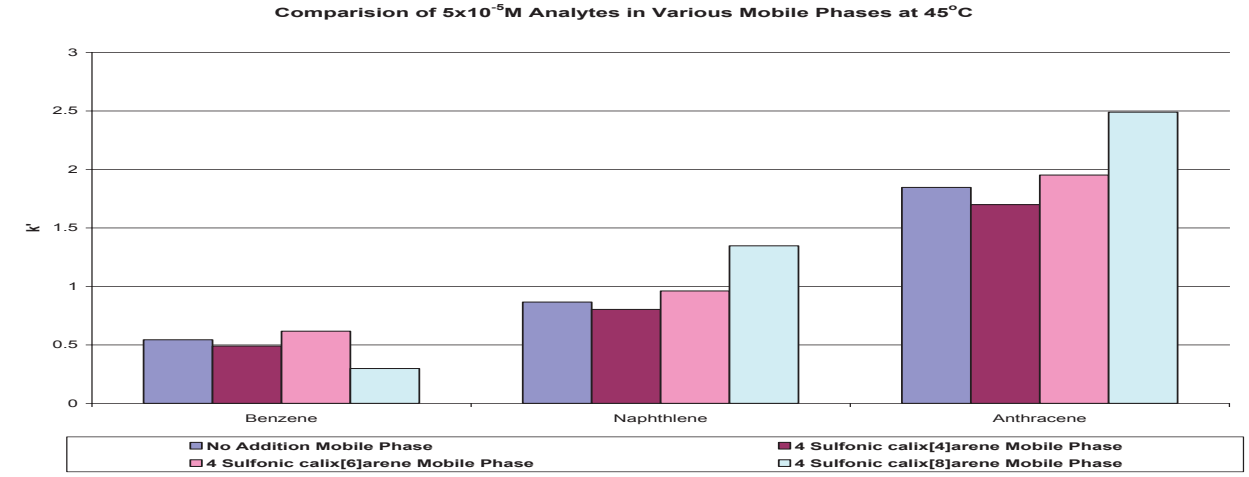


Figure 20: Van't Hoff plots: ln k' vs. 1/K for 4-Sulfonic calix[n]arene additives

Indicated by Figure 21, 4- Sulfonic calix[4]arene has the lowest capacity factor for all analytes when referenced to the no addition mobile phase. It is also apparent that utilizing 55^{o}C contains the largest difference in capacity factor for the entire series. The 4-Sulfonic calix[8]arene additive only had host guest complexation when used with benzene.





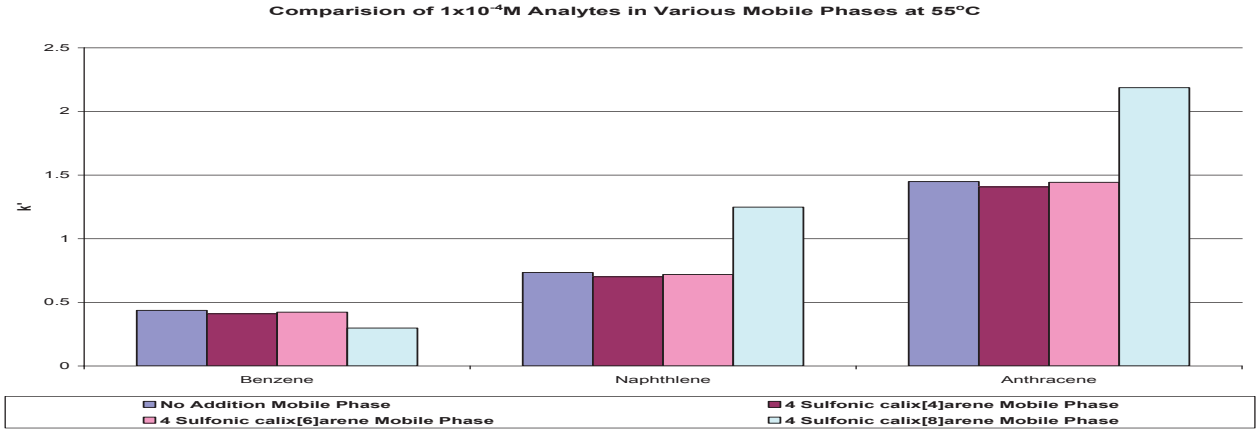


Figure 21: Capacity factor comparisons of 4- Sulfonic calix[n]arene mobile phases

Chapter VI

Conclusion and Future Work

In this research, there were a number of different ways used to determine whether host-guest interactions existed between calixarenes and PAH's. The most efficient determination was the comparison of calixarene mobile phase capacity factors to non-calixarene mobile phase capacity factors. When the additives that suggested host-guest interactions were introduced into the mobile phases, the chromatograms showed a reduction of retention times and capacity factors for each solute. This was more apparent with naphthalene in both the soluble and insoluble calixarenes than any other analyte.

Temperature variation was also found to play an important role in the effects of host-guest complexation. Mobile phases that were temperature controlled 55^{o}C obtained lowered capacity factors than those at 35^{o}C and 45^{o}C. The difference in concentration was also investigated between the ranges of $1x10^{-5}$M to $1x10^{-4}$M. The change in concentration did not affect the elution order of the analytes however; the solutes at $1x10^{-5}$M in some series were not quantifiable. Solutes at $1x10^{-4}$M were found to be the optimal concentration for host-guest complexation.

Future work that may confirm this investigation would be to include a validated HPLC method of investigating naphthalene with analytes at a concentration of $1x10^{-4}$M and 55^{o}C. The use of mass spectroscopy or LC/MS would be also help to indicate any morphological changes in reference to a 1:1 or 2:1 host-guest calixarene complexation. It would also be interesting to see the host-guest interactions for positional isomers of naphthalene or the other analytes used in this research.

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