EFFECT OF COLUMN TEMPERATURE ON ELUTION ORDER IN GAS CHROMATOGRAPHY
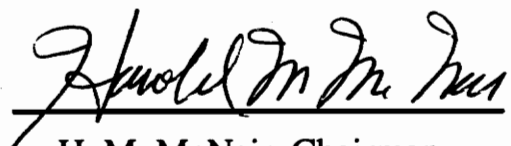
By

Kien (Josette) Chhiev Heng

Thesis submitted to the Graduate Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of
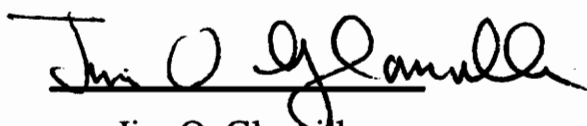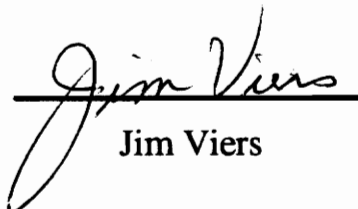
MASTER OF SCIENCE
in
Chemistry

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C. 2

EFFECT OF COLUMN TEMPERATURE ON ELUTION ORDER IN GAS CHROMATOGRAPHY

(Abstract)

This study concerns the effect of column temperature on selectivity in gas chromatography (GC). Reversal of peak elution order at different temperatures is observed when selectivity is sufficiently changed by changing the temperature.

A study of homologous functional groups and the elution order of a test mixture was made at several temperatures, by keeping constant operating chromatographic parameters such as column head pressure, split ratio, sample concentration, injector and detector temperature. The homologous series studied were esters, carboxylic acids, ketones and alkanes. The stationary phases used were Carbowax (Supelcowax 10), and 5% diphenyl and 95% dimethyl silicone (HP Ultra 2). The identification of each compound was confirmed by two methods : comparing retention times, and spiking the sample.

The results of an investigation for accurately determining the dead time is also presented. The experimental results of using 10% methane in helium and the results of a theoretical model based on Poiseuille's equation were studied and compared. It is concluded that dead time calculations are a major source of error for the calculations in this work.

In addition, a derived thermodynamic equation based on the Van't Hoff equation was obtained and used to calculate the molar enthalpy and entropy of compounds over a range of temperatures. The experimental thermodynamic values were compared with the theoretical values obtained from the Clausius- Clapeyron equation.

From the data obtained, a graph of ln k' (retention factor) versus 1/ temperature for a homologous series, known as a Van't Hoff plot, predicts a reversal of elution order where an intersection of two lines occur. Selectivity of the stationary phase has changed solely due to temperature effects. This can only occur when different solute/solvent interactions are present.

This work is an attempt to predict when peak inversion should occur based on the Van Hoff's plot. Experimental result are presented that confirm the prediction.

Abstract

Dedication

To celebrate the birth of my niece Florence Aline Mangeret

Born August 15, 1993 at 6:45

3.75 Kg and 50 Cm.

ACKNOWLEDGMENTS:

She would like to honor all her family members, especially her parents for their financial investment in her education and for providing her an unselfish place in their heart. I would to thank my sister, Alice and her spouse, Philippe who provided a home during my undergraduate studies in Irving, TX.

Finally, to the three roommates, Plato, Athos and Rita who have shared the author's short temper and joy during her graduate school years. Thank you for your patience, cooperation and understanding.

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INTRODUCTION:

Usually, in the introductory GC theory, selectivity is viewed as a property inherent to the stationary phase and independent of temperature. This statement is true in most cases, but exceptions can be found in the literature. For instance, Jennings [1] showed that methyl hexanoate eluted before 2-methyl 1-butanol at 100°C on a homemade glass capillary column coated with Carbowax 20 M. When the column temperature was increased to 120°C, the two compounds reversed their elution order, 2-methyl 1-butanol eluting before methyl hexanoate. Recently, extensive evidence of selectivity dependence on temperature has been observed. Mehran and co-workers [2] showed that on several stationary phases reversal of elution order as temperature changes is quite common. The effect of temperature on the selectivity of the bonded stationary phase is an important factor in the chromatographic analysis.

In the work described here, a study was undertaken to predict the behavior of the selectivity of the stationary phase at different temperature. It is focused on the effect of temperature on the selectivity of the bonded stationary phase.

HISTORICAL REVIEW:

First, a partial list of historic events in chromatography is presented to recognize the earlier achievements. In 1906, Tswett introduced a novel separation technique to the scientific community by separating the chloroplast pigment of plant leaves on a calcium carbonate ($CaCO_3$) column using petroleum ether as a mobile phase. Because the colored bands were visible along the glass column, he called the technique, "chromatography," meaning "colored writing [3]".

It took forty-six years before the second leap came. James and Martin [4] published a gas-liquid chromatography application in 1952; they described the separation of a mixture of fatty acids by partitioning them between nitrogen gas and a stationary phase of silicone oil containing 10% w/w stearic acid supported on kieselguhr. A few years later, Golay [5] introduced the modified Van Deemter equation for capillary columns at the 1958 Amsterdam Symposium. This equation described the influence of various parameters on column performance. At the same meeting Golay showed two chromatograms that were obtained on a 45 m x 0.25 mm i.d. tygon capillary coated with a dodecyl phthalate liquid phase. The reduced column diameter gave an efficiency exceeding 50,000 plates with an analysis time of only a few minutes, compared to a maximum of 6000 plates for a standard 6 feet packed column. Due to the smaller diameter of the column, the typical capillary volumetric flow rate is 1 mL/min. compared to 30 mL/min. for an 1/8 inch O.D. packed column. The thermal conductivity detector (TCD) used at that

time had too large an internal volume and thus lacked the sensitivity needed to optimize capillary column results. Fortunately, the flame ionization detector (FID) also made its debut about the same time and it immediately became the detector of choice for capillary columns because of its very small dead volume, high sensitivity, and fast response [6]. Due in large part to these early achievements, gas chromatography (GC) rapidly became the most widely employed analytical technique, and remains so today [7].

Chromatography has constantly evolved and expanded since its beginnings. It is primarily a separation technique that involves a series of equilibria between two phases. By combining any two phases (gas, liquid, and solid), various chromatographic techniques can be obtained, as shown in Table (1) [8]. For example, a combination of a mobile liquid phase and stationary solid phase is called liquid solid chromatography (LSC). It encompasses thin layer, ion exchange, and paper chromatographies. Since the work in this thesis is only concerned with gas liquid chromatography (GLC), other types of chromatography will not be discussed further.

The GC process is applicable only for the separation of compounds which are both volatile and thermally stable at the analysis temperature. In GLC analysis, a column contains the liquid phase; a flowing gas stream is the mobile phase. When the sample is vaporized in the injector port, it partitions between the liquid (stationary) and gas (mobile) phases. The liquid phase, which coats the capillary wall, retains the sample components depending on

Table **1**- Chromatographic Techniques

Chromatographies Types	Techniques
Adsorption	Liquid-Solid Chromatograph (LSC) Gas-Solid Chromatography (GSC)
Partition	Liquid-Liquid Chromatography (LLC) Gas Liquid chromatography (GLC)
Ion exchange	Liquid Solid Chromatography (LSC) Ion Chromatography (IC)
Size exclusion	Gel Filtration Chromatography (GFC) Gel Permeation Chromatography (GPC)

Reference: Grob R. *Modern Practice of Gas Chromatography* (John Wiley & Sons. Inc.) 1985.

the degree of the interactions between each component molecules and the liquid phase. Dispersion, induced dipole, dipole-dipole forces and hydrogen bonding are the different types of intermolecular attraction forces found between molecules; they are all temperature dependent but to different degrees. The intermolecular interactions are cited in order of their increasing 'bonding' strengths. A continuous repetition of the partitioning process between the two phases causes the separation of the complex sample into its individual components. The mobile phase constantly flowing through the column carries the components at their migration speeds. Depending on the stationary phase used, any of the intermolecular forces may or may not be present and contribute to the column selectivity. The selectivity of a given stationary phase towards a pair of compounds is a relative measure of the interactions and is roughly the ratio of the solubility of the two sample components in the liquid phase at a given temperature. The success of a GC analysis depends on the column where the separation process takes place. Either by increasing the column efficiency and/or its selectivity, the separation can be improved. Since this work is focused on the temperature effect on column selectivity, a discussion of some typical stationary phases used in GC analysis is helpful.

TYPICAL GC COLUMNS:

"The column is the heart of the chromatograph," is a well-known quotation often cited in the chromatography literature. The two main types of GC columns are packed and capillary. Both GSC and GLC analysis make use of packed columns. A packed column consists of a tube (usually of stainless steel or glass) packed with coated or uncoated solid particles. The packing material for GSC is a solid adsorbent without any liquid phase; it consists of materials such as silica, alumina, and charcoal. In the case of GLC, an inert solid support is covered with a high boiling liquid phase. The GSC separation mechanism relies adsorption whereas the mechanism of GLC relies on partitioning between the bulk liquid and gaseous phase. A typical packed column is 3- 6 feet long, has a 1/8 or 1/4 inches O.D., and packing particle diameters of 0.1- 0.3 mm. This type of column has a typical sample capacity of a few milligrams, but it has a large H.E.T.P (0.5 cm). H.E.T.P is an abbreviation which stands for height equivalent to one theoretical plate. It measures the column length required for one partitioning process to occur. A high H.E.T.P value means that the column is less efficient. The applications [9] of the classical packed columns are simple separations, trace analysis, preparative GC, and fixed gas analyses. Classical packed columns were used in GC before the development of Golay's wall coated open tubular columns (WCOT). The WCOT columns overcame the shortcomings of the packed columns and rapidly became the most popular column type.

Various tubing materials have been used for the preparation of open tubular columns. Stainless steel, glass, and fused silica have all been used. Stainless steel columns were popular in the early days of capillary GC. They have good thermal conductivity and are rugged, however their inner wall is often rough, dirty, and active toward polar compounds. After Desty [10] introduced the glass drawing machine, the glass column gradually replaced the stainless steel column. Glass has the advantages of being cheaper and more inert than stainless steel columns. Also, by chemical treatment, the glass column can achieve a coherent and stable film for polar stationary phases. But, it has a short column life and it is extremely fragile and its leak free connection to others parts of the GC are not easy [9].

In 1979 Dandeneau [11] introduced the fused silica column. This column is inert toward polar compounds; it has a high wettability factor for non polar phases which improves the surface coating. It is flexible due to its high tensile strength and almost unbreakable due to its outer polyimide protective coating. On the negative side, it is more difficult to modify the surface than for a glass column. The common dimensions of a WCOT column are: 10- 50 m long, internal diameter of 0.25- 0.53 mm with a 0.2- 5.0 μm coating (film thickness). The WCOT columns had been selected because their chromatographic properties listed above are critical in this work.

CAPILLARY'S SURFACE:

A description of the inner column surfaces and the formation of cross linked stationary phases in the fused silica column is reviewed because they play an active role in column selectivity. The fused silica used to manufacture capillary columns is obtained from two main sources: naturally occurring Brazilian quartz rock crystals and synthetically produced SiO_2. Both sources produce an inert pure silica surface without needing further treatment and possessing a high degree of uniformity. The presence of surface SiOH groups can be either beneficial or induce harmful effects depending on the sample. There are three different types of silanol groups, the lone silanols (normal), the vicinal silanols (active), and geminal silanols or silanediols (active). These structures are shown in figure (1). Free silanols are acidic and can react with an electron donating species and, hence, depending on the column temperature and film thickness, they may promote adsorption. Free silanols and inert siloxane bridges all may be found on the surface of the fused silica column. To deactivate the inner column wall, a chemical reaction (usually silanization) of the silanol surface is carried out. Recently [6] it has been feasible to chemically bond the stationary phase directly to the surface of the column. The chemical reactions that occur on the wall surface is important for an understanding of the chemistry of the stationary phase. To obtain a cross-linked phase, a free radical initiator (peroxide) or diazo compound is added to the methyl silicone coating solution before coating. Afterwards, a thermal treatment is applied to the freshly coated capillary. At higher temperature the

Introduction

methyl groups easily form free radicals which initiate a cross linking reaction with another layer of the methyl silicone. The bonding and the cross-linking has been possible for nonplar and semi-polar silicone-type phases; it has not been as successful for the polar phases. During the coating process, uneven film thickness and droplets may be formed because of the polar bonded phase's high surface.

tension. The manufacturing of GC capillaries with the bonded polar phases still remains under development.

Free silanol

OH

Si — O — Si — O — Si —

Silica surface

Lone Silanols (Normal)

Free silanols

OH   OH

Si — O — Si — O — Si — O — Si —

Silica surface

Vicinal Silanols (Active)

Free silanols

HO   OH

Si — O — Si — O — Si — O — Si —
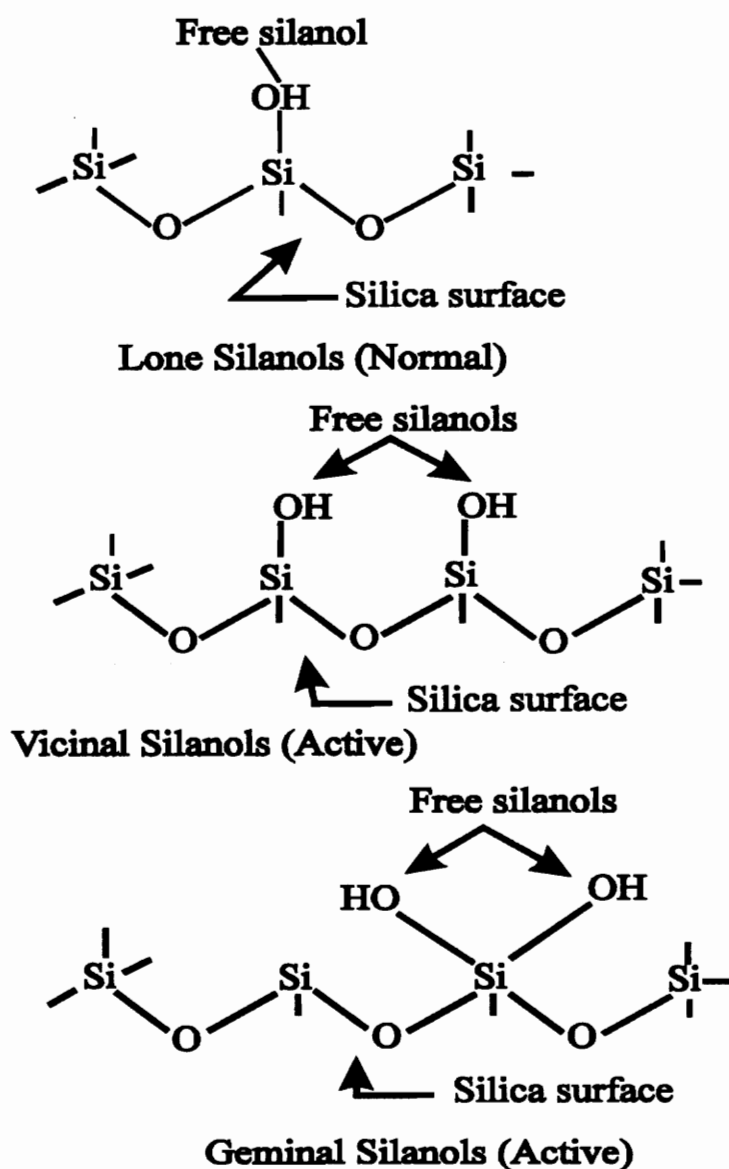
Silica surface

Geminal Silanols (Active)

Figure **1**-Different types of silanol groups

Reference Grob R. *Modern Pratice of Gas Chromatography* (John Wiley & Sons. Inc.) 1985.

CLASSIFICATION OF SELECTIVITY:

Selectivity as defined by Rohrschneider[12] constants determines the degree to which a series of compounds are retarded by any liquid phase relative to their retention on a nonpolar phase. Rohrschneider classification does not account for temperature effects on selectivity, but it does give a good picture of the stationary phase polarity. In addition, the selectivity of the interactions depends not solely on the stationary phase, but also on the analyte (thus a single number will not suffice). Each Rohrschneider constant (x) is the difference of the Kovats Retention Indices (R.I.) [13] between the selected and a reference (nonpolar) phase divided by 100 as shown in equation (1).

$$x = [\text{R.I.}_{\text{selected}} - \text{R.I.}_{\text{nonpolar}}]/100 \, (\text{eq.} 1)$$

Retention indices were introduced to overcome the difficulties of exchanging data between different laboratories, and setting experimental conditions. The R.I. expresses the relative retention of any substance in a defined n-paraffins scale. The retention indices for n-paraffins are defined as 100 times the number of carbon atoms. For example the R.I. of heptane and octane are always 700 and 800 respectively on any liquid phase. However, it is extremely important that the stationary phase and column temperature be specified. It is experimentally found that the retention index of benzene is 649 on a 20% squalane column with Chromosorb W AW at 100 °C. This means that benzene eluted between hexane and heptane on this column,

notwithstanding that it elutes between heptane and octane on 20 % dinonyl phthalate column at $100°$ C (retention index is 733). This example stresses the importance of the selected stationary phase.

To characterize the column, Rohrschneider chose five standard compounds (test probes): benzene (x), ethanol (y), methyl ethyl ketone (z), nitromethane (u), and pyridine (s). The selected reference column is always squalane, a non polar column (only dispersion forces). He defined the ΔI term as the sum of $ax + by + cz + du + es$. The letters a, b, c, d, e represent constants for the test probes in the order cited above. The constant a for benzene is equal to 100, and b, c, d, e are zero by definition. Similarly, the constant b for ethanol is equal to 100, and a, c, d, e are equal to zero [12]. The ΔI term is a measurement of polarity. This classification is a method to relate retention data of different liquid phases based on their interactive forces. Each Rohrschneider constant displays a type of solute-solvent molecular interaction as summarized in table (2). For example, ethanol (y) represents the electron attractors category (alcohols, nitriles, and acids). The y value is equated to the degree of retardation of the electron attractors on a stationary phase. The higher the y value the longer it takes for alcohols, nitriles, and acids to elute when compared to the nonpolar phase. Table (3) shows the McReynolds constants for the stationary phases used in this work. McReynolds system is similar to Rohrschneider's principle except that McReynolds' constants were calculated at a different temperature, $120°C$

Table 2- Molecular Interactions Represented By Rohrschneider Constants.

Compound Symbol	Benzene x	Ethanol y	Methyl Ethyl Ketone z	Nitromethane u	Pyridine s
Molecular Interaction Types	Induced Dipole	Electron Attractors	Electron Repellers	Complex	Complex
Type of Compounds Affected by the Molecular Interactions	Aromatics Olefinics	Alcohols Nitriles Acids $CHCl_2$ CCl_3 CH_2Cl NO_2	Ketones Ethers Aldehydes Esters Epoxides	Nitro-Methane	Pyridine Dioxane

Reference: Supina W. *The Packed Column in Gas Chromatography* (Supelco, Inc. Pennsylvania) 2nd printing 1979.

Table **3** - Tables of McReynolds Constants For The Selected Stationary Phase Used.

Mc Reynolds Constants					
Column Type	Benzene	n-Butanol	2-Pentanone	Nitropropane	Pyridine
HP Ultra 2	32	72	65	98	267
Supelcowax	305	551	360	562	484

Reference: HP *Chromatography Users Catalog* 1994

Supelco *Chromatography Products* 1994

instead of 100°C. The McReynolds constant are the difference of the R.I. of the selected and reference phases. All characteristics mentioned for Rohrschneider constants apply to McReynolds constant.

SELECTIVITY:

The selectivity (α) of a stationary phase is defined in GC as the ratio of the adjusted retention times of two selected compounds at a given isothermal temperature as shown in equation (2).

$$\alpha = \frac{t_{R2}'}{t_{R1}'} \qquad \text{(eq.2)}$$

where t_R' is the adjusted retention time of a compound. Selectivity can also be defined as the ratio of the retention factor (k') of two selected compounds.

$$\alpha = \frac{k_2'}{k_1'} \qquad \text{(eq.3)}$$

The retention factor is the adjusted retention time divided by the gas hold up time (t_M).

$$k' = \frac{t_R'}{t_M} \qquad \text{(eq.4)}$$

By convention α is always greater than 1. Selectivity is related to the interactions of the solutes with the liquid phase. Selectivity is also a relative expression; it always refers to two specific compounds. It will be shown in the theory section that the retention factor is the derived parameter needed to investigate temperature effects on selectivity from the Van't Hoff's equation. Since the retention factor involves the gas hold up time, the latter has to be

determined accurately. The next section covers its experimental measurement and theoretical calculation. An investigation of its behavior on both a non polar and a polar stationary phase was undertaken.

GAS HOLD UP TIME:

Since the inception of GC, there have been numerous proposed theories and experiments to measure the gas hold up time, also called the dead time. The dead time is defined as the time required for a non retained compound to pass through the column. For a non-retained compound, molecular interactions, partitioning, and adsorption are assumed to be absent. One experimental method, introduced by Peterson and Hirsch [14], is based on the linear relationship between the logarithm of the adjusted retention time and the carbon number of the members of a homologous series. The gas hold up time is estimated by extrapolation of the curve at C_0 (hypothetical alkane, with a zero carbon number).

This method is tedious and inaccurate at low temperatures[15] . The dead time is experimentally determined by injecting a non- retained compound such as methane, or argon or air. It has to be pointed out that methane has a slight interaction with the methyl silicone stationary phases. This interaction can be neglected as discussed in the results and discussion section. The theoretical method to obtain the dead time (within the limits of the instrumentation's error) is to derive an equation that fits the GC pneumatic

system. The Poiseuille's equation, mentioned in the theory section, takes into account any fluctuations during the analysis time such as gas viscosity, temperature, and pressure drop across the column [16]. This equation was selected to be compared with the methane injection technique.

VAN'T HOFF PLOT:

The behavior of column selectivity at different temperatures can be measured. Its graphical presentation is summarized based in the Van't Hoff plot. The Van't Hoff equation describes an equilibrium state; it was modified in the theory section of this work to account for the GC system. The plot (ln k' vs 1/temperature) for any compounds displays a straight line behavior and accounts for an overall view of k' effect with temperature. The sum of the molar enthalpy and entropy of a specified compound can be calculated from the slope and the y-intercept of the line.

The Van't Hoff plot of a homologous series shows a trend of parallel lines within the range of experimental temperature used. A plot of two or more compounds may be used to predict the reversal of elution order. When there is a crossing between the lines as shown on figure (2), the elution order had changed.

An extension of the theory of the reversal of elution order predicted with the combination of the Van't Hoff plot and the calculated thermodynamic values is reviewed in the results and discussion section.
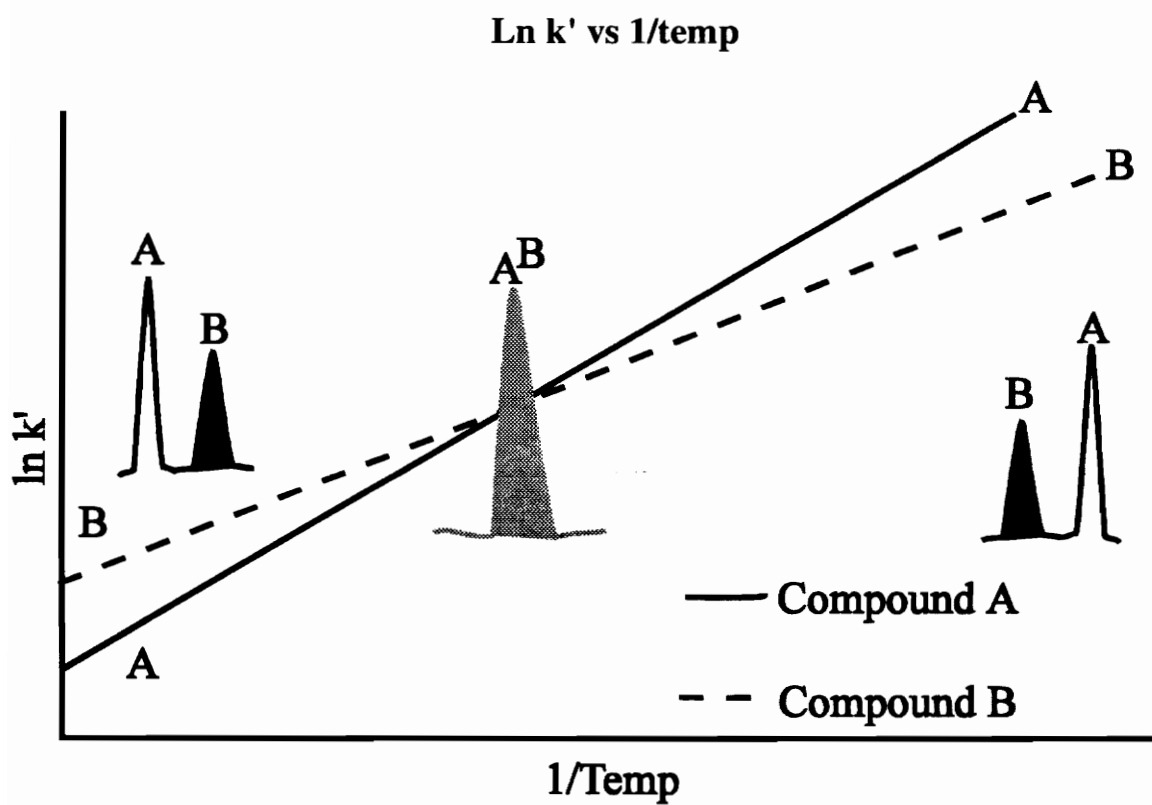
Figure **2** - Reversal of elution order from the Van't Hoff plot.

CHAPTER 2 THEORY:

As described in the introduction section, in GLC the analyte distributes itself between the liquid and gaseous phases. The distribution constant or partition coefficient (K) expresses the ratio of the concentration of the analyte in the stationary phase to its concentration in the mobile phase.

$$K = \frac{[analyte]_{stationary}}{[analyte]_{mobile}} \qquad (eq.5)$$

The partition coefficient of an analyte on a given stationary phase depends on both the temperature and the analyte but is independent of its concentration. A derived expression of the partition coefficient in term of retention factor (k') and phase ratio (β) is given by :

$$K = k'\beta \qquad (eq.6)$$

The phase ratio is defined as the volume ratio between the gas and the stationary phases :

$$\beta = \frac{V_{mobile}}{V_{stationary}} \qquad (eq.7)$$

The volume of the mobile phase is expressed as :

$$V_{mobile} = (r_c - d_f)^2 \pi L \qquad (eq.8)$$

where r_c, d_f and L are the radius, the film thickness and the length of the column respectively. The volume of the stationary phase is :

$$V_{stationary} = (2r_c \pi)Ld_f \qquad (eq.9)$$

By substituting equations (8) and (9) into equation (7) and simplifying, we obtain

$$\beta = \frac{r_c - 2d_f}{2d_f} + \frac{d_f}{2r_c} \qquad (eq.10)$$

Since $r_c \gg d_f$ (~ 500 to 1000 times larger), equation (10) can be simplied to

$$\beta \approx \frac{r_c - 2d_f}{2d_f} \qquad \text{(eq.10a)}$$

For thin films, equation (10a) can be further simplified to

$$\beta \approx \frac{r_c}{2d_f} \qquad \text{(eq.10c)}$$

While the partitioning coefficient measures the concentration ratio of the analyte between the phases, the retention factor (k') expresses the ratio of time spent in the two phases. As stated in the introduction section, the retention factor is defined by :

$$k' = \frac{t_R'}{t_M} \qquad \frac{\left[\text{time in liquid phase}\right]}{\left[\text{time in gas phase}\right]} \qquad \text{(eq.4)}$$

k' depends on temperature, analyte, and the stationary phase. Equation 10a shows that β is independent of temperature. Since k' can be directly measured from the chromatogram, it can be substituted for K in the calculations involving the partition coefficient.

If an FID detector is connected to the end of the column, then the output result is a chromatogram. The chromatogram is a valuable source of data to the analyst. A typical chromatogram is shown in figure (3). The first peak corresponds to the unretained compound (methane) used to measure the gas hold up time (t_M). The t_M is that time which all analytes are in their vapor state inside the column. For any analytes, its residence time in the mobile phase is fixed at a specified temperature and chromatographic conditions. An understanding of the temperature dependance of t_M is critical in this work.
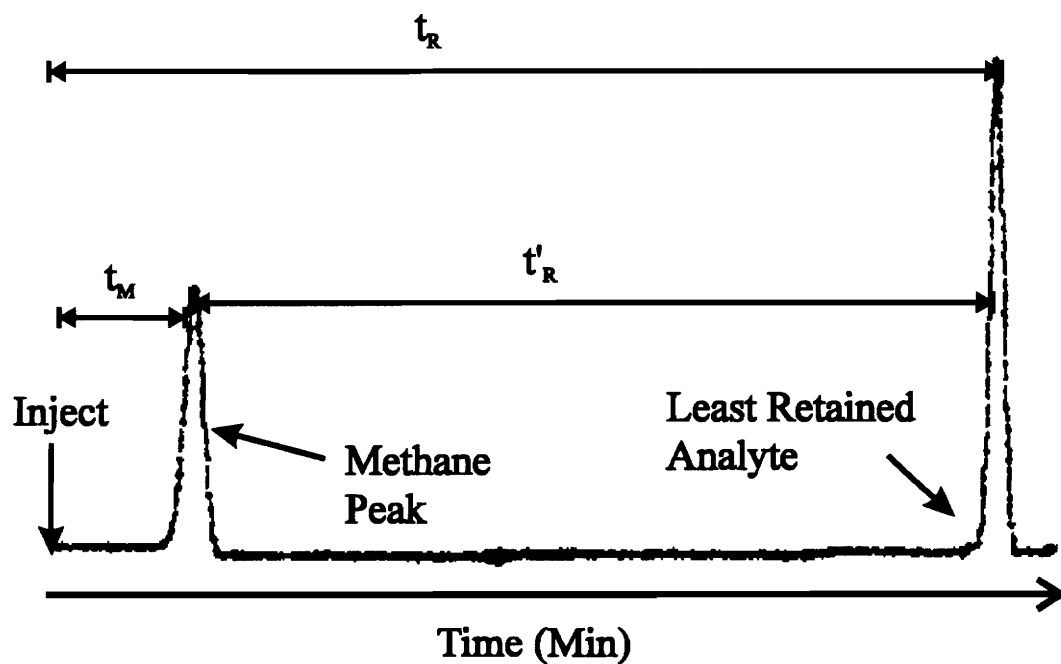
Figure **3** - A typical chromatogram

t_M : the gas hold up time, t'_R : the adjusted retention time, t_R :the retention time.

Harris and Habgood [16] derived t_M from Poiseuille's law with respect to the capillary column.

$$t_M = \frac{32\eta L^2}{3r_c^2} \frac{p_o}{p_i^2 - p_o^2} \frac{P^3 - 1}{P^2 - 1} \qquad (eq.11)$$

η is the viscosity of the carrier gas (helium); p_o and p_i are the column outlet and inlet pressures. The inlet pressure reading must be adjusted for the ambient atmosphere. P is the ratio of the inlet to the outlet pressure. This equation accounts for the change of the gas viscosity with temperature, and the pressure drop along the column.

Viscosity is defined as a resistance to flow; it is a temperature dependent parameter. It is well known that temperature has a direct relation to the gas viscosity. A longer gas hold up time is observed as the column temperature increases. It is difficult to express the exact relationship of viscosity with temperature [17]. An empirical method relates the ratio of gas viscosity at two temperatures to the temperature ratio raised to a power (x)

$$\frac{\eta_i}{\eta_o} = (\frac{T_i}{T_o})^x \qquad (eq.12)$$

As given by Ettre and Hinshaw [18], the value of x for helium is 0.646. This equation had been used in this work for the calculation of η in equation (11) and thus for the calculation of t_M.

The second peak shown on the chromatogram (figure 3) is the least retained analyte for the chosen stationary phase and temperature. The elution time of the peak (t_R) is the total time the solute spends in the column. It combines : the gas hold time (t_M) and the adjusted retention time (t'_R). The

latter part corresponds to the time the analyte actually spends in the stationary phase where all molecular interactions occurr. t_R' is expressed by:

$$t_R' = t_R - t_M \qquad \text{(eq.13)}$$

VAN'T HOFF'S EQUATION:

The basic equilibrium equation for the reaction between the gas and the liquid phase (analyte $_{gas}$ <==> analyte $_{liquid}$) is expressed in terms of Gibbs free energy:

$$\ln K = -\frac{\Delta G}{RT} \qquad \text{(eq.14)}$$

K is the equilibrium constant; R is the gas constant (8.314 J/mol K); and T is temperature in degrees Kelvin. ΔG is Gibbs' free energy of the transition from liquid to gas. ΔG is the difference of Gibbs' free energy (G) between products and its reactant states. ΔG measures the driving force of a reaction; it predicts whether or not a reaction is spontaneous. The Gibbs- Helmholtz equation relates the temperature dependence of ΔG to ΔH and ΔS expressed as:

$$\Delta G = \Delta H - T\Delta S \qquad \text{(eq.15)}$$

ΔH and ΔS are the change in enthalpy and entropy respectively. ΔH represents the heat flow in a chemical reaction carried out at constant pressure. Enthalpy (H) is a thermodynamic property which reflects a system's capacity to exchange heat (q_p) at constant pressure with its surrounding. The first law of thermodynamics states that:

$$\Delta E = H + P\Delta V \qquad \text{(eq.16)}$$

ΔE is the change of internal energy of the system. P is the pressure and ΔV is the change of the volume of the analyte from liquid to gaseous state. Enthalpy is equal to the change of internal energy of the system minus the term PΔV. The term PΔV is a measure of work done on the environment. Enthalphy is a part of the internal energy possessed by the molecules in the system, such as molecular interactions, bonding, translation, rotation, vibration, kinetic, and potential energies. ΔH is the difference between the energy in the sum of the product to the reactant states. ΔH can be interpreted as the sum of the molecular interactions.

ΔS is the difference of entropy between the sum of the product to the reactant states. Entropy (S) is a measure of the randomness or disorder in a system.

The temperature dependency of the partition coefficient can be expressed by :

$$\log K = \frac{A}{T} + B \qquad\qquad (eq.17)$$

where A and B are constants. In the case of GLC, Dose [19] suggested that the equilibrium constant may be substituted by the partition coefficient. By substituting equation (6) for the equilibrium constant and equation (15) for Gibbs' free energy, in equation (14). We obtain

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \beta \qquad\qquad (eq.18)$$

Equation (18) is a derived form of the Van't Hoff equation. It is the foundation of the present work. Equation (18) is written in the linear form. Then by plotting ln k' versus 1/T for any solute, straight lines should be

observed, the term $-\Delta H/R$ being the slope of the line and the y-intercept being equal to $\Delta S/R + \ln \beta$, (figure 4).

Both ΔH and ΔS are constant since they are the slope and y-intercept of the linear equation.

Claussius-Clapeyron Equation:

Any phase change involves the addition or removal of energy as heat from the substance. For the vaporization process to occur, enough energy is required to break most of the intermolecular attractions from its liquid state and escape into the vapor. The change of the enthalpy for the vaporization of a liquid is called heat of vaporization (ΔH_{vap}). The ΔH_{vap} of a liquid can be obtained from the vapor-pressure data listed in the *handbook of chemistry and physics*[20], for example.

As the temperature increases, the vapor pressure of a liquid increases exponentially. To obtain a linear relationship between the vapor pressure and temperature, the two variables are shifted and modified. A plot of the logarithm of the vapor pressure ($\log_{10} P$) versus the repicrocal of the absolute temperature (1/T) is found to be linear. The equation of the straight line is

$$\log_{10} P = A - \frac{\Delta H_{vap}}{2.30RT} \qquad (eq.19)$$

where P is the vapor pressure of the analyte. A is a constant that is specific for a particular analyte. ΔH_{vap} is the heat of vaporization of the analyte. R and T are the gas constant and the temperature.

Theory

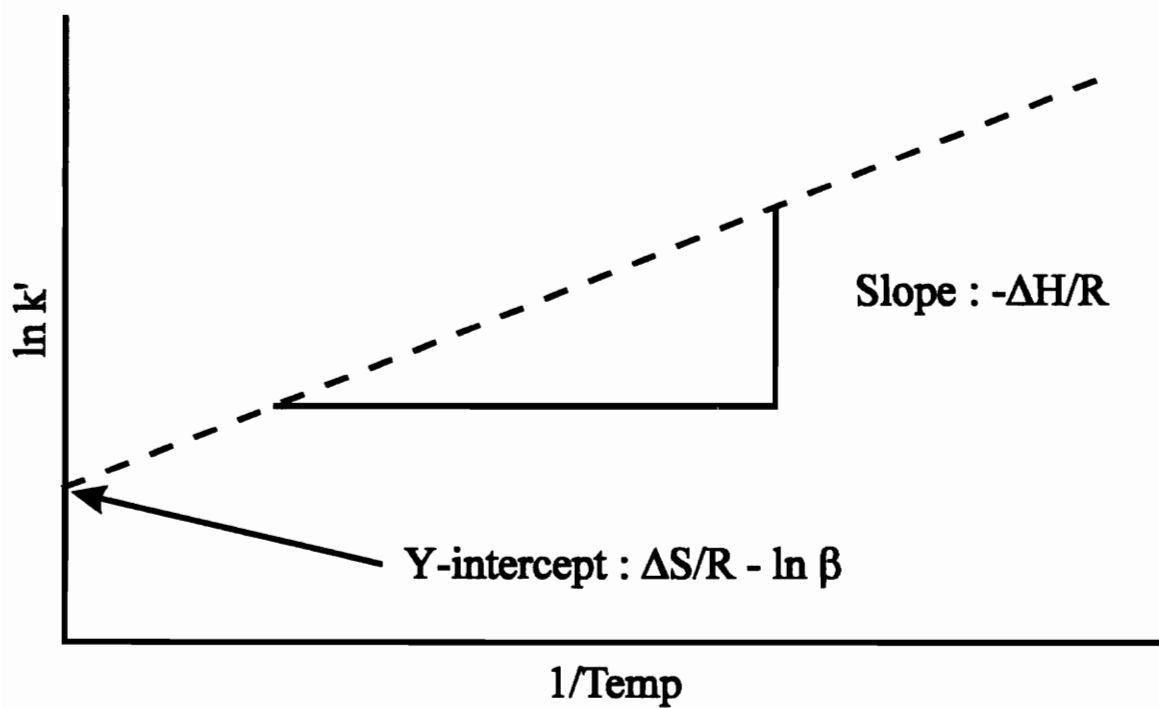$$\ln k' = -\Delta H/RT + \Delta S/R - \ln \beta$$

Figure **4** -Van't Hoff Plot.

To eliminate the specific constant (A) in the equation above, the Clausius-Claypeyron equation is often used to determine ΔH_{vap} using two points equation relating the vapor pressure (P_2) at one temperature (T_2) to (P_1) at another temperature (T_1). After substracting, and rearranging, we obtain

$$\log_{10} = \frac{P_2}{P_1} = \frac{\Delta H_{vap}}{2.303R}\left(\frac{T_2 - T_1}{T_2 T_1}\right) \qquad \text{(eq.20)}$$

where P_2 and P_1 are the vapor pressures of the analyte at two temperatures. T_2 and T_1 (in degrees Kelvin). This form of the Claussius-Clapeyron equation assumes that the gas behaves perfectly and the molar volume of the gas is so much greater than the molar volume of the liquid. The ΔH_{vap} does not change within the temperature range selected.

PROPAGATION OF RANDOM ERRORS:

In experimental work, the result of the gas hold up time, and retention factor are determined from a combination of measurements that have errors due to the precision of the instruments and materials available. Random error has a natural tendency to fluctuate on both sides (plus or minus) of the average values. The precision of the final result is estimated by determining the standard deviation of the result (ε_R). The result of the standard deviation from a combination of measured quantities A and B is determined by the propagation error equation in the general term:

$$\varepsilon_R = \sqrt{\left(\frac{\partial R}{\partial A}\right)_B^2 \varepsilon A^2 + \left(\frac{\partial R}{\partial B}\right)_A^2 \varepsilon B^2} \qquad \text{(eq.21)}$$

where ε_R, R, are standard deviation of the result, and result. $A\pm\varepsilon_A$ and $B\pm\varepsilon_B$ are experimental measurements with their respective standard deviations. The standard deviation of the result for addition and substraction operations is given by :

$$\varepsilon_R = \sqrt{\varepsilon_A^2 + \varepsilon_B^2} \qquad \text{(eq.21a)}$$

Also the standard deviation of the result of a multiplication or division operations is expressed:

$$\left(\frac{\varepsilon_R}{R}\right)^2 = \left(\frac{\varepsilon_A}{A}\right)^2 + \left(\frac{\varepsilon_B}{B}\right)^2 \qquad \text{(eq.21b)}$$

Equation (21a) and (21b) are general equations. Notice that variance is used to calculated the standard deviation of the result because of its additive property. Variance is defined as the square of the standard deviation. The overall result of the standard deviation of the independently measured quantities is equal to the sum of the variance or the relative sum of the variance.

To calculate the standard deviation of the theoretical measurement of t_M, equation (21) has been applied. Its overall standard deviation is found by solving :

$$\varepsilon_{t_M} = \sqrt{\left(\frac{\partial t_M}{\partial L}\right)_{r_c,\eta,P_i}^2 \varepsilon L + \left(\frac{\partial t_M}{\partial r_c}\right)_{L,\eta,P_i}^2 \varepsilon r_c + \left(\frac{\partial t_M}{\partial \eta}\right)_{r_c,L,P_i}^2 \varepsilon\eta + \left(\frac{\partial t_M}{\partial P_i}\right)_{r_c,L,\eta}^2 \varepsilon P_i} \qquad \text{(eq.21c)}$$
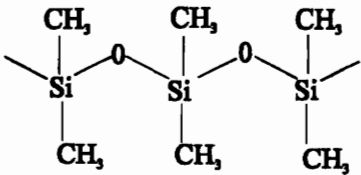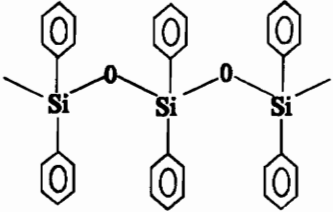
The calculated t_M value is $t_M \pm \varepsilon_{t_M}$.

Theory

The experimental error data are treated according to equation (21). A summary of the experimental results with the standard deviation is discussed in detail in the results and discussion section in this work.

Table **4**. Column Parameters

Specifications:	Supelcowax 10	HP Ultra 2
column length	3160.8 cm	2509.2 cm
I.D.	0.32 mm	0.32 mm
Film thickness	1.0 μm	0.52 μm
Stationary phase	bonded carbowax HO-(CH$_2$-CH$_2$-O)$_n$-H	95% dimethyl siloxane 5% diphenyl siloxane

MATERIALS:

Homologous series of esters, carboxylic acids, ketones and alkanes were purchased from Aldrich Chemical Co. (Milwaukee, WI) (Table 5).

Esters: methyl-, ethyl- , propyl-, and butyl propioniates.

Carboxylic acids: propionic, butyric, valeric and hexanoic acids.

Ketones: 2 propanone, 3 pentanone, 3 hexanone, and 3 heptanone.

Alkanes : decane, undecane, dodecane, tridecane, and tetradecane. Hexane solvent was obtained from Fisher Scientific (Pittsburg,PA). The standard mixture for the FID detector test was provided by Perkin Elmer Company (Norwalk,CT). It contains nonane, 2-octanone, decane, 1-octanol, undecane, 2,6-dimethylphenol, 2,4-dimethylaniline, naphthalene, and dodecane in isopropanol. The chemical structures are showed on figure (5). The Grob test mixure was bought from Fluka Company (Buchs, Switzerland). The 10% methane in helium was obtained by Scott Specialty Gases (Plumsteadville,PA).

SOLUTION PREPARATIONS:

All solutions were prepared in a 250 mL volumetric flask and stored in a glass container. The calculated concentration is about 250 ppm. All except the standard test mixture were diluted approximately 1:20 (v/v). The accuracy

Table **5**: Structures of the Homologous Series.

	General Form	First Hom. Member	Next Homologous. Member
Alkanes	$C_{nc}H_{(2nc+1)}$	$CH_3(CH_2)_8CH_3$	$CH_3(CH_2)_{n+1}CH_3$
Ketones	$RCO(CH_2)_nR$	$CH_3CH_2COCH_3$	$CH_3CH_2CO(CH_2)_{n+1}CH_3$
Esters	$RCOO(CH_2)_nR$	$CH_3CH_2COOCH_3$	$CH_3CH_2COO(CH_2)_{n+1}CH_3$
Carboxylic acids	$R(CH_2)_nCOOH$	CH_3CH_2COOH	$CH_3(CH_2)_{n+1}COOH$

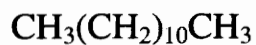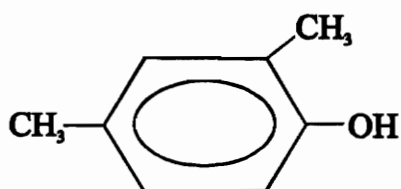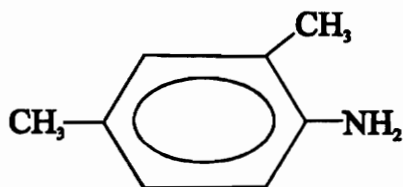nc is the number of carbon; n is the number of methyl group.

Experimental

$CH_3(CH_2)_7CH_3$

Nonane

$CH_3(CH_2)_8CH_3$
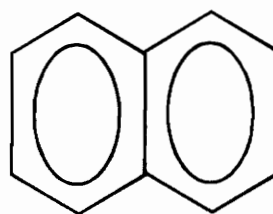
Decane

$OHCH_2(CH_2)_6CH_3$

1-Octanol

$CH_3(CH_2)_{10}CH_3$

Dodecane

2,4 Dimethylaniline

2,4 Dimethylphenol

Naphthalene

Figure 5 : Structure of the standard mixture from Perkin Elmer.

Experimental

of the sample concentration was not critical in this work, as this work is concerned only with compound retention time. The solution concentrations were kept in the range of the capacity of the column and tested for the linear range of the detector. The volume injected, column flow rate, oven and detector temperatures, affecting retention time were carefully controlled.

EXPERIMENTAL METHOD:

All columns used in this work were first tested with the Grob test mixture I using the published procedure [21] for the performance quality of the column. The chromatographic methods were not optimized, but isothermal temperatures were selected to produce analysis time of 60 minutes or less. All the peaks exhibited baseline resolution unless it is specified otherwise. The separations were performed under isothermal conditions at temperatures of 90-180° C for the alkanes, 65-110°C for the ketones, 150-210°C for the carboxyclic acids, and 65-120°C for the esters on the Supelcowax. Table (6) shows the GC experimental conditions which were kept constant throughout this work. The oven temperature was stabilized for at least one hour between runs at different temperatures. The Van't Hoff equation is valid for isothermal temperature runs only. The retention times reported represent the mean values of five repetitive determinations for each homologous series.

The column length was determined by counting the number of loops on the column cage. The length of one loop was measured carefully.

Experimental

Table **6**. Experimental GC conditions

Oven temperature:	Isothermal
Injector temperature:	250 °C
F.I.D. detector temperature:	250 °C
Volume injected:	1.00 µL (autosampler)
Split ratio:	50:1
Sample concentration:	250 ppm

CHAPTER 4 RESULTS AND DISCUSSION:

This chapter initially discusses the results of the dead time study including a statistical study of error in treating this data. A discussion of the limitations and the assumptions taken in the thermodynamics study are also reviewed. Next, a discussion of the ketone homologous series and its Van't Hoff's plot is covered in detail. A summary of the alkanes and carboxylic acids is given to contrast the changes of enthalpy (ΔH) and entropy (ΔS) with temperature. A comparison of the molar enthalpy obtained from the Van't Hoff plot and the extrapolated values from the Claussius-Clayperon equation is discussed. It concludes with a prediction of reversal elution order in the test mixture from Perkin Elmer on the HP Ultra 2 capillary column based on the Van't Hoff plot. A thermodynamic explanation is proposed for the observed case.

GAS HOLD UP TIME:

It was shown in the theory section that the Poiseuille's equation allows to predict the gas hold up time from a theoretical approach. The equation is reprinted from the theory section for discussion.

$$t_M = \frac{32\eta L^2}{3r_c^2} * \frac{p_o}{p_i^2 - p_o^2} * \frac{P^3 - 1}{P^2 - 1} \qquad (eq.11)$$

The accuracy of t_M is hindered by the error found in the column dimensions, the gas viscosity calculation, and the inlet pressure reading.

The column dimensions are important factors in the Poiseuille's equation, since both L and r_c appear as their second power. The standard deviation of the column's diameter is ±12µm for the Supelcowax column and ±3µm for the HP column [22]. Applying equation (20c), the standard deviation of t_M can be determined. Each measured parameter that contributes to the t_M's standard deviation is listed below. The deviation due to the column radius from Supelcowax on t_M is 8%. Figure (7) shows calculated t_M based on the column radius with standard deviation and the methane t_M. The methane t_M lies within the range of the calculated t_M.

The column length is another possible source of error. The estimated column length is obtained by the method described in the experimental section. The column has a length between ±20 cm. The calculated standard deviation is less than 1%; the error associated with the column length has a minimal effect to the overall errors.

The gas viscosity value is calculated according to equation (12). The contribution of error due to the viscosity is found by comparing the viscosity values given by Ettre and The *Handbook of Chemistry and Physics* [20]. The deviation is also less than 1% between the t_M values.

Finally, the inlet pressure gave a 3.5% standard deviation from its precision reading.
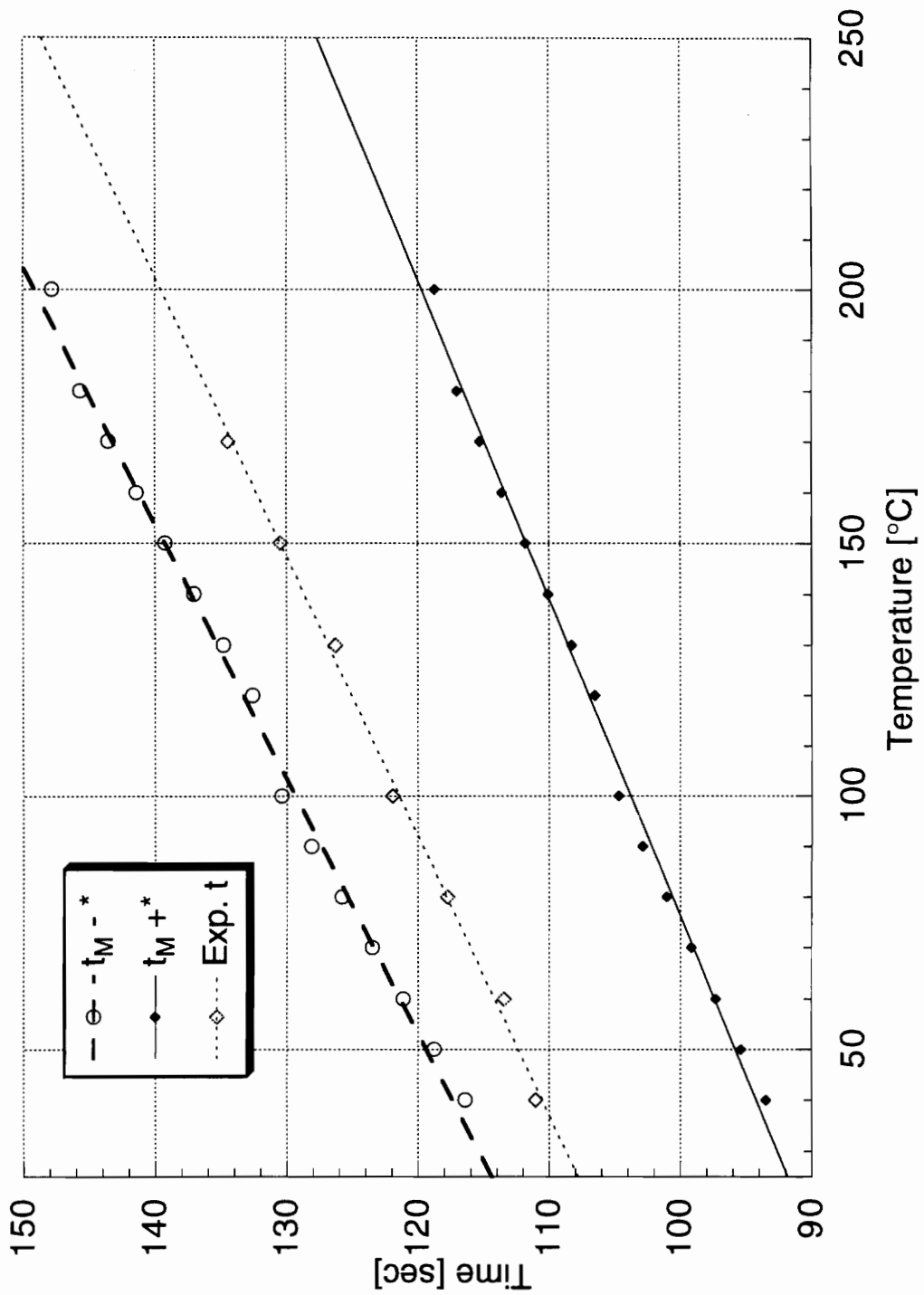
Results and Discussion

Figure **7** - A comparison of the gas holp up time between column radii limits (tM-*/tM+*) and experimental measurements (Exp. t)on Supelcowax column.

Each measured parameter contributes to t_M's standard deviation. The standard deviation in the t_M theoretical calculation is the sum of all measured parameters. Table (7) shows the summary of the percent standard deviation for each parameter and the t_M standard deviation for the Supelcowax and HP Ultra 2 column. The overall standard deviation of the calculated t_M is 13% for the Supelcowax column and 6% for the HP Ultra 2 column.

In the methane t_M data, the error due to the integrator is the major source of error. Having only one possible measured data (integrator's time), the standard deviation of methane t_M is smaller than the calculated value.

In this work, it is assumed that the measured methane t_M accounted for all deviations mentioned in the calculated t_M value. It yields a time closer to the true t_M. The determination of methane t_M is used to calculate the retention factor (k'). Furthermore, it is simpler and faster to obtain and does not require tedious calculations.

ASSUMPTIONS:

In this work, several assumptions are applied to reduce tedious thermodynamic calculations and to simplify the defined GC system. First, the carrier gas (helium) behaves like an ideal gas. Therefore, the mobile phase does not take part in any molecular interactions. The calculated ΔH and

Table **7** - Gas Holp Up Time With Its Standard Deviations.

Column Errors	Supelcowax Column	HP Ultra 2 Column
Radius	8%	2.4%
Length	less than 1%	less than 1%
Viscosity	less than 1%	less than 1%
Inlet pressure reading	3.5%	2%

ΔS are constant in the range of temperature used. The pressure drop across the capillary column does not affect the ΔH calculation.

Since the data are treated from a standpoint of thermodynamics, the intermolecular interactions between the analytes and the liquid phase cannot be broken down into their specific interactions (hydrogen, dipole-dipole and induced-dipole bonding). The molar enthalpy is considered as the sum of the intermolecular interactions occurring in the GC system as explained in the theory section.

It is also assumed that at any moment in a GC column, the equilibrium is established between the gas and stationary phases. There is a negligible amount of adsorption at the interface between liquid and gas phases. No adsorption occured during the separation process.

HOMOLOGOUS SERIES :

For any compound, the plot of ln k' versus 1/temperature (Van't Hoff plot), according to equation (18) appears to be a straight line.

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \beta \qquad (eq.18)$$

For example, in the ketone homologous series, the elution order is 2-butanone, 3-pentanone, 3-hexanone, and 3-heptanone. Similar trends are obtained for the carboxyclic acid, ester, and alkanes homologous series. Graphs (7) to (10) summarize the experimental data trends. Within a homologous series, the intersection between the lines does not occur within
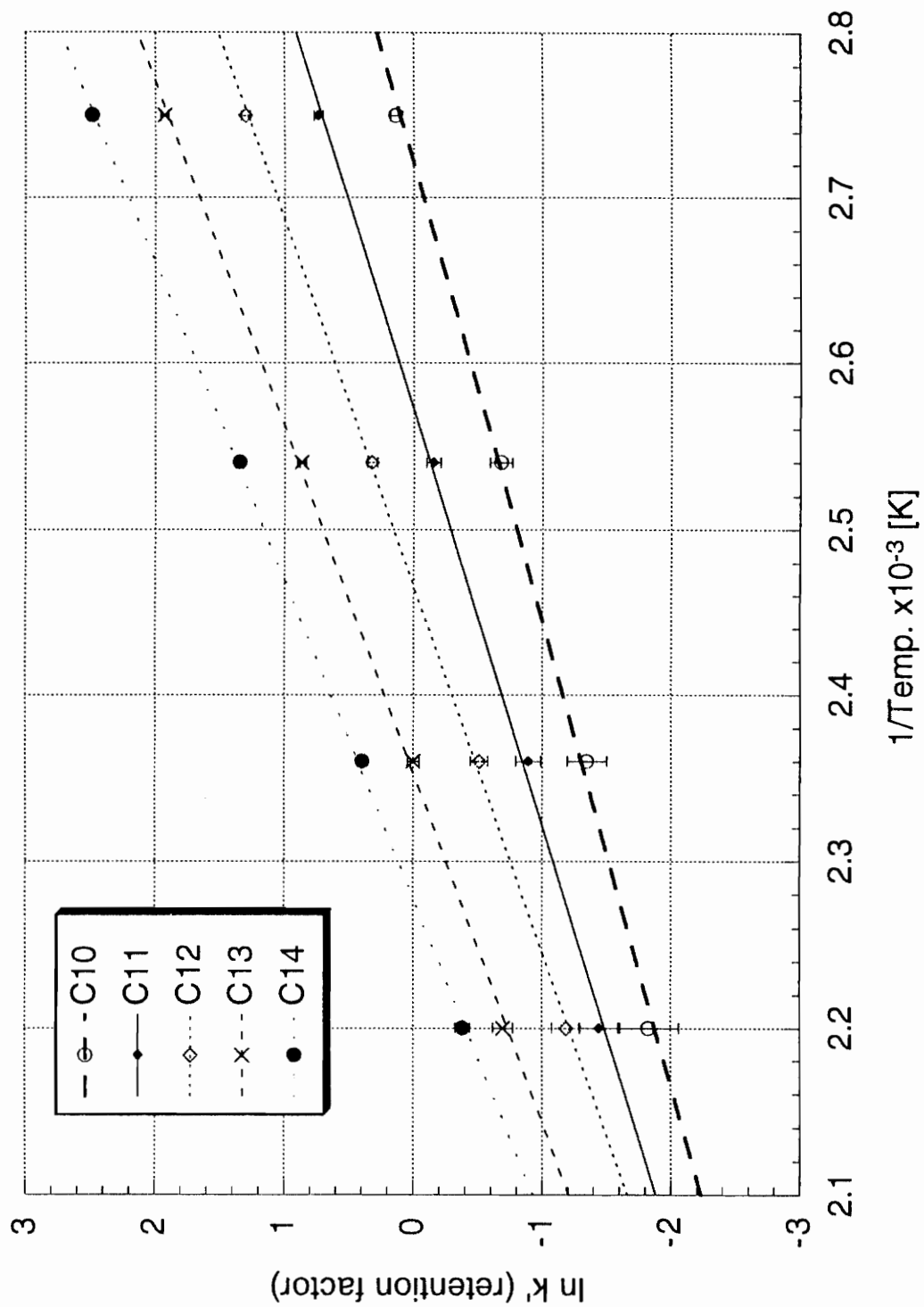
Figure **7** -Van't Hoff graph alkanes.
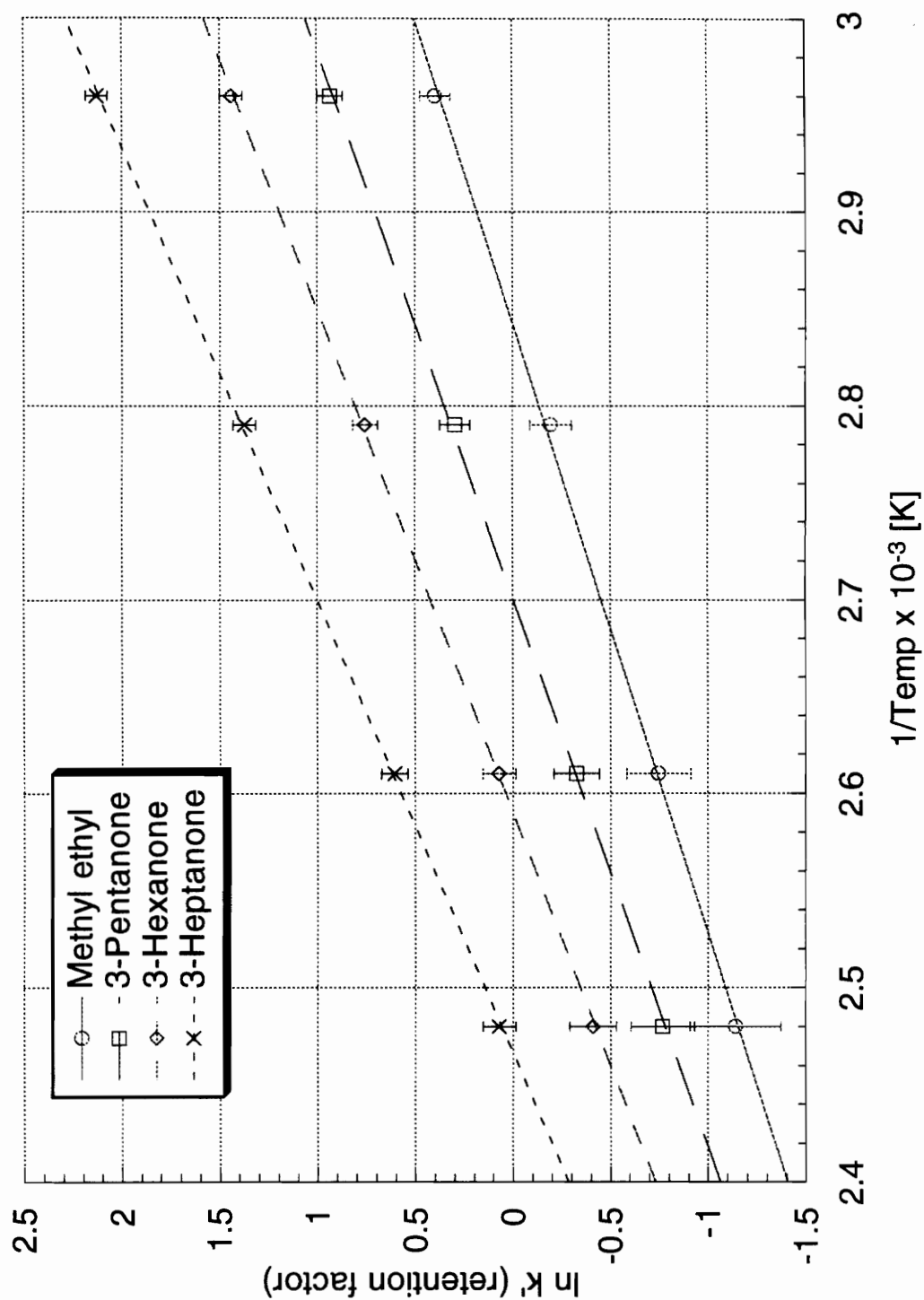
Figure **8** -Van't Hoff graph ketones
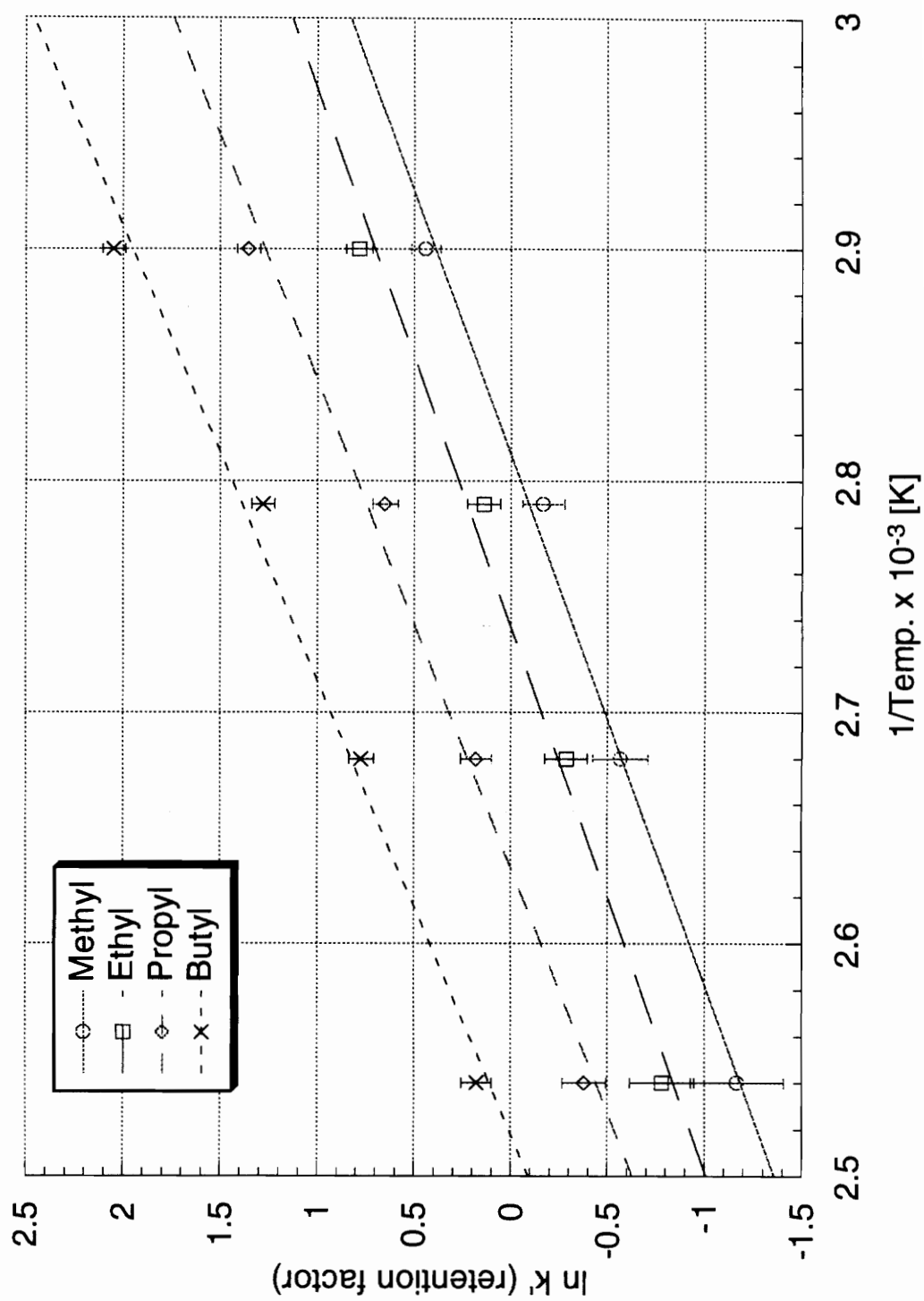
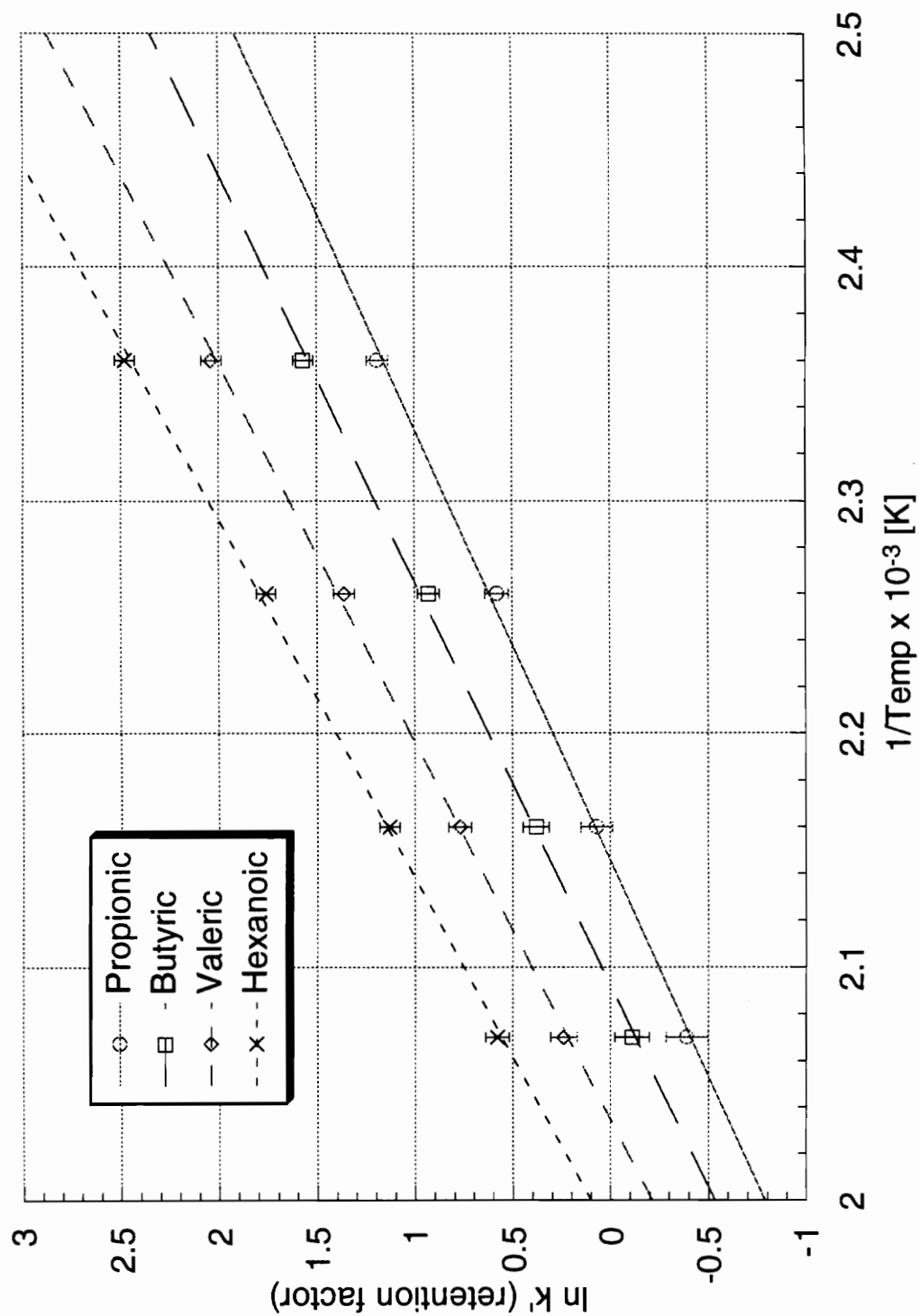Figure **9** - Van't Hoff graph esters

Figure **10** -Van't Hoff graph carboxylic acids

the column temperature studied. When temperature alters the selectivity of the stationary phase, the homologous compounds are equally influenced. The ΔH (slope of the compound) difference between two consecutive members of a homologous series is shifted by a constant value, independent of the compound functional group. This trend had been observed previously in the Kovat's retention index. Table (8) showed the molar enthalpy (ΔH, and the slope) and molar entropy, ΔS (y-intercept) of the selected homologous series. The carboxylic acids have the highest ΔH values while alkanes have the lowest values on the Supelcowax column. Acids have a lower vapor pressure; their molecular interactions are strongest with the stationary phase. These effects are reflected in the ΔH experimental data. The ΔH is the sum of all kinds of molecular interactions possessed by molecules.

The order of decreasing molecular interactions, according to the ΔH values is acids > esters > ketones >alkanes. The acids can interact through their hydroxyl and ethers groups of the carbowax stationary phase. Ketones and esters are not subject to hydrogen bondings (no active proton). Alkanes showed the least interactions with the stationary phase mainly with the alkanes methyl groups and ether groups of the stationary phase (induced dipole interaction).

The trend of ΔS within a homologous series is that the longest member of the series has the least disorder (lowest ΔS value). The larger molecules have less motion due to their size.

Table **8**. Results of ΔH and ΔS for the homologous series

Alkanes	Decane	Undecane	Dodecane	Tridecane	Tetradecane
ΔH (kJ/mol) Van't Hoff	30.1±1.0	33.4±0.9	37.9±0.4	40.0±0.8	43.7±0.6
ΔH (kJ/mol) Claus. Clay.					
ΔS (J/mol.K) Van't Hoff	-58.8±2.6	-62.9±2.3	-70.2±1.1	-71.2±0.9	-76.7±1.5

Ketones	2-Butanone	3-Pentanone	3-Hexanone	3-Heptanone
ΔH (kJ/mol) Van't Hoff	26.6±0.8	29.6±.6	32.2±0.6	35.8±0.6
ΔH (kJ/mol) Claus. Clay.	34.1±0.4	46.9 ± 0.4		
ΔS (J/mol K) Van't Hoff	-52.5±2.1	-56.8±1.7	-60.4±1.6	-65.4±1.6

Results and Discussion

Table **8**. Results of ΔH and ΔS for the homologous series (continue)

Esters	methyl proprionate	ethyl proprionate	propyl proprionate	butyl proprionate
ΔH (kJ/mol) Van't Hoff	31.9±1.2	31.5±0.4	34.9±.2	37.6±0.4
ΔH (kJ/mol) Claus. Clay.	36.8 ± 0.3	39.5 ± 0.5	41.3 ± 0.5	
ΔS (J/mol K) Van't Hoff	-67.7±3.2	-63.6±1.0	-68.9±0.6	-71.2±1.2

Acids	proprionic	butyric	valeric	hexanoic
ΔH (kJ/mol) Van't Hoff	44.8±0.7	47.5±0.7	50.8±0.7	53.9±0.7
ΔH (kJ/mol) Claus. Clay.	46.3 ± 0.2	52. ± 0.3	55.8 ± 0.1	
ΔS (J/mol K) Van't Hoff	-73.0±0.5	-76.3±1.5	-80.1±1.5	-83.9±1.5

Results and Discussion

A COMPARAISON OF CLAUSSIUS-CLAYPERON BETWEEN VAN'T HOFF VALUES:

From the vapor pressure data in the *Handbook of Chemistry and Physics* [20], the ΔH of the compounds were calculated from the Clausius-Claypeyron equations, as described in the theory section. The graph of log vapor pressure versus 1/temperature are straight lines for all the compounds. Their correlation factors are between 0.90 to 0.95. The slope of the line gives the calculated ΔH of the compound. The results are shown in table (8). The experimental data obtained from the Van't Hoff plot based on the Supelcowax column are found to vary within 10% of the Clausius-Claypeyron's equation. The experimental ΔH values vary due to the column dimensions and stationary phases used. A comparaison of these two ΔH values can not be drawn, due to the variations of both experimental settings. The Van't Hoff plot can be used to estimate ΔH values for different compounds on any columns (for a specific environement).

PREDICTION OF REVERSAL OF ELUTION ORDER:

The homologous series allowed a study of functional group behavior and also served to predict the reversal of elution order. The different series

are overlapped to find a pair of compounds with reversal of elution order as showed on figure (11). Some commercial and home made isothermal test mixture are studied on the different stationary phases available. After various trial and errors, a pair of compound on the FID Perkin Elmer test mixture was found to reverse their elution order on HP Ultra 2 (5% diphenyl, 95% dimethyl silicone) column.

The FID Perkin Elmer test mixture consists of a range of compounds having different functional groups showed on figure (5). The mixture was analyzed on a HP Ultra 2 column. Experimentally, it was found that dodecane eluted before naphthalene at 90°C, but this elution order was reversed at 130°C. The actual chromatograms (90°C and 130°C) are shown in figure (12). To confirm the reversal peak order, dodecane was spiked; figure (13) shows that the dodecane peak had shifted its elution position.

A Van't Hoff plot of the compound pair, dodecane and naphthalene is plotted, figure (14). The ΔH and ΔS of the dodecane and naphthalene are found to be 47.1 kJ/mol, -107.4 J/mol K and 42.2 kJ/mol , -95.1 J/mol K respectively. They have different ΔH and ΔS values, otherwise the two lines will be overlapping each other throughout the temperature range. Dodecane has a greater molecular interaction than naphthalene. However naphthalene has lesser change in entropy than dodecane. Naphthalene molecules can interact with the low percentage of diphenyl group on the stationary phase.
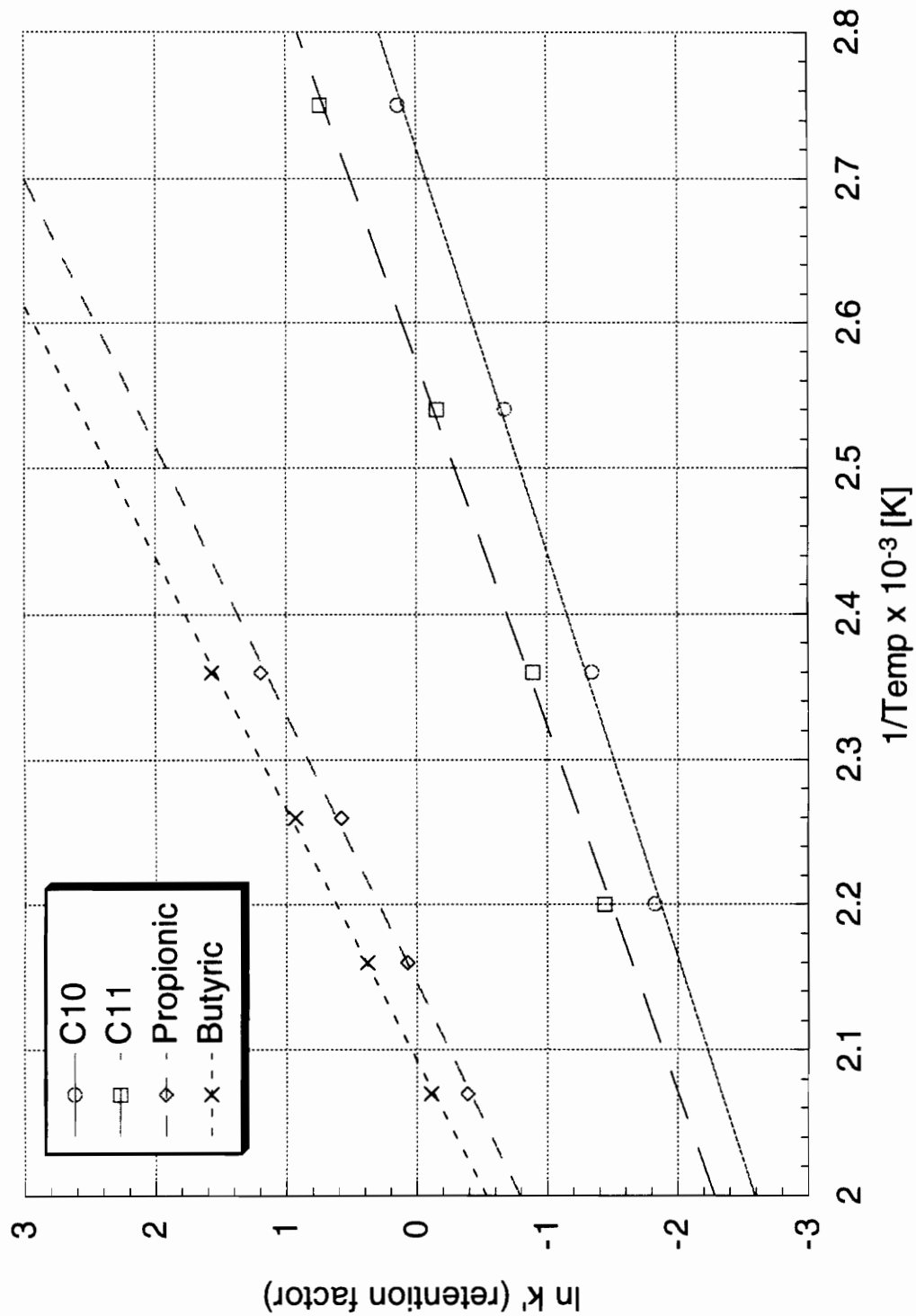
Results and Discussion
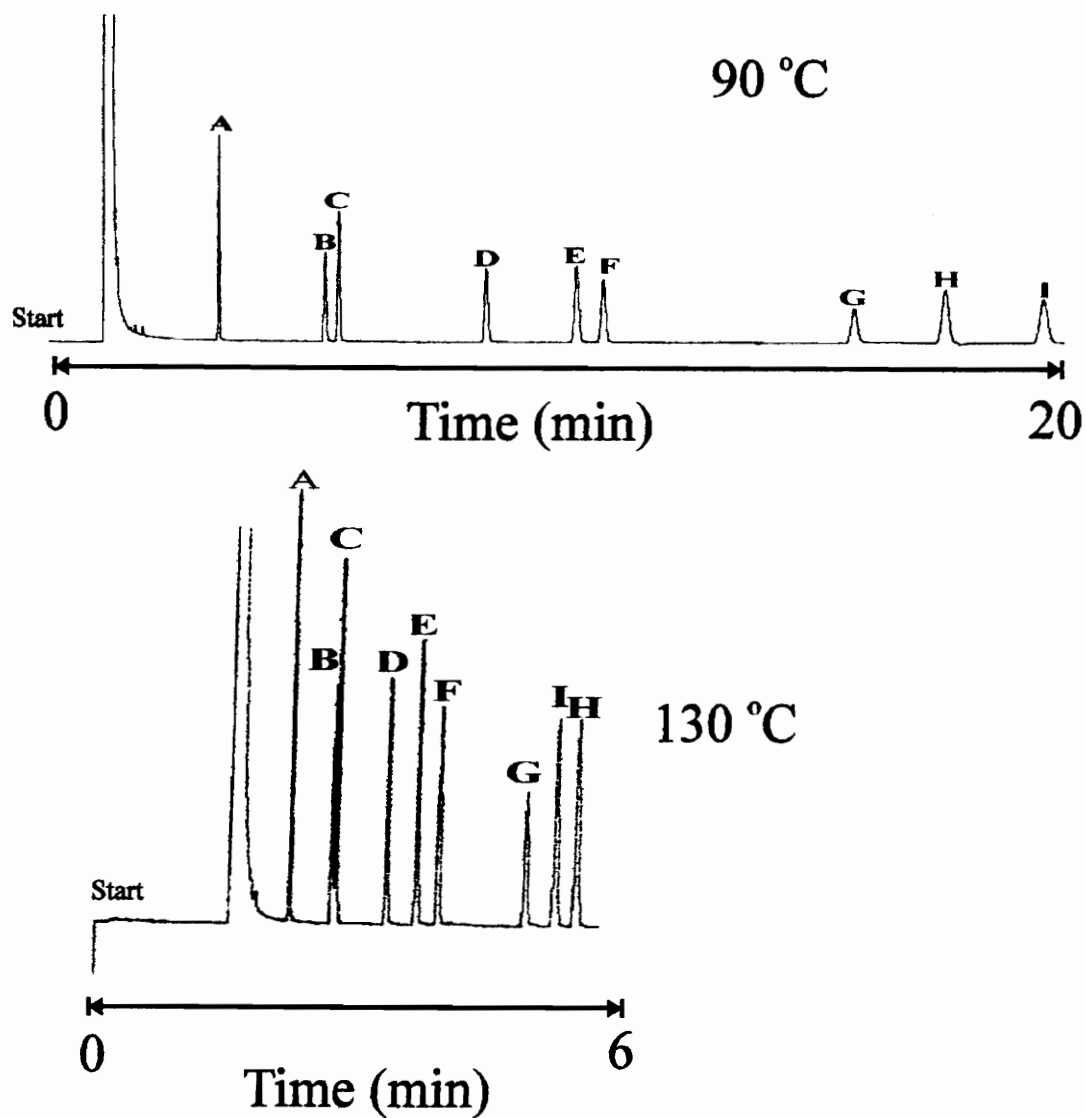
Figure **11** Homologous series overlapping.

Figure **12** A test mixture at 90 and 130 C. A: nonane, B: 2-octanone, C: decane, D: 1-octanol, E: undecane, F: 2,6 dimethylphenol, G: 2,4 dimethylaniline, H: naphthalene, I: dodecane.
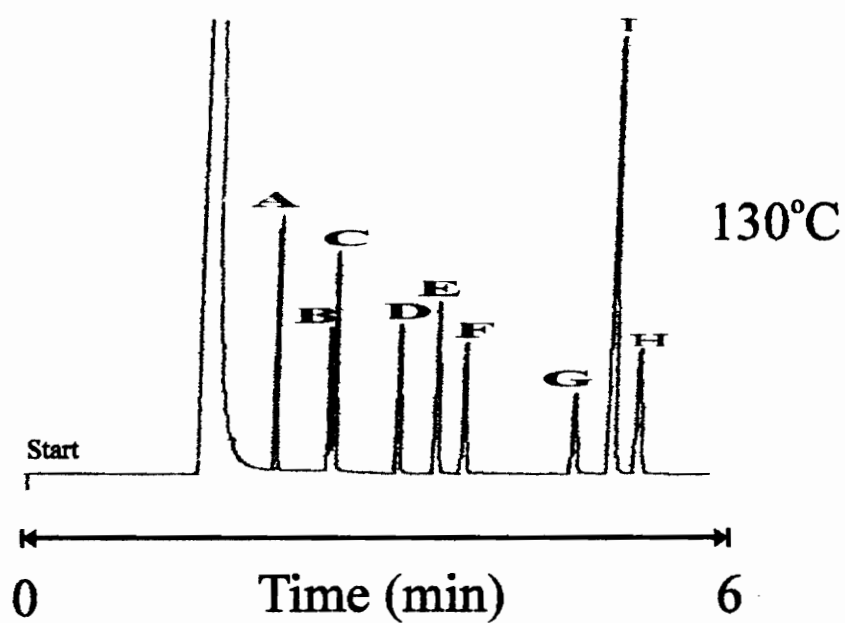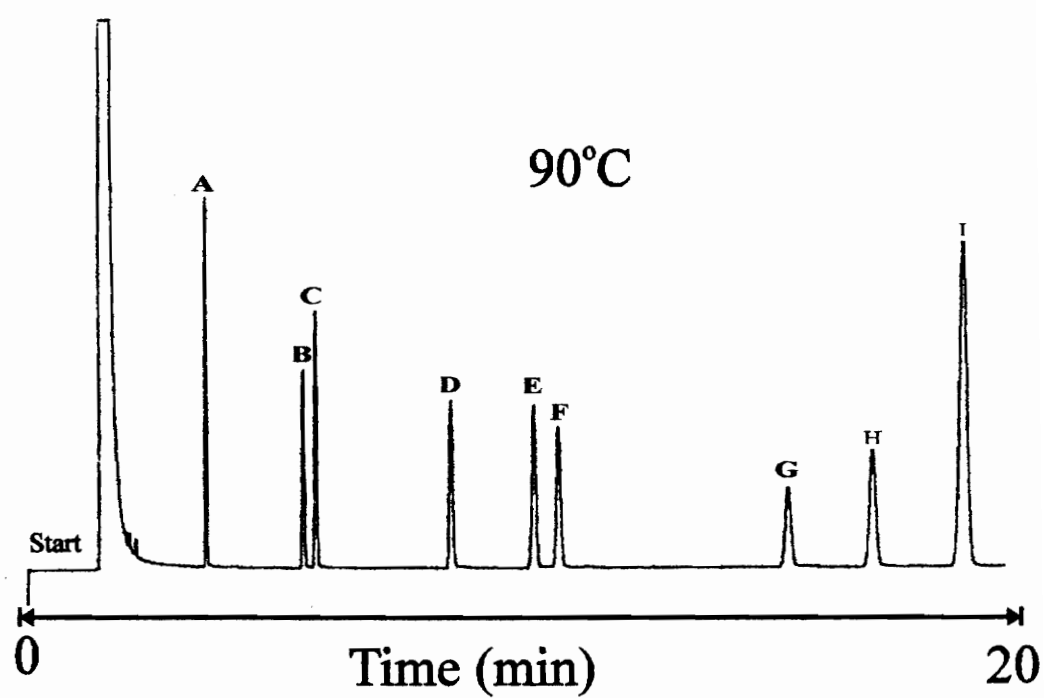
Results and Discussion

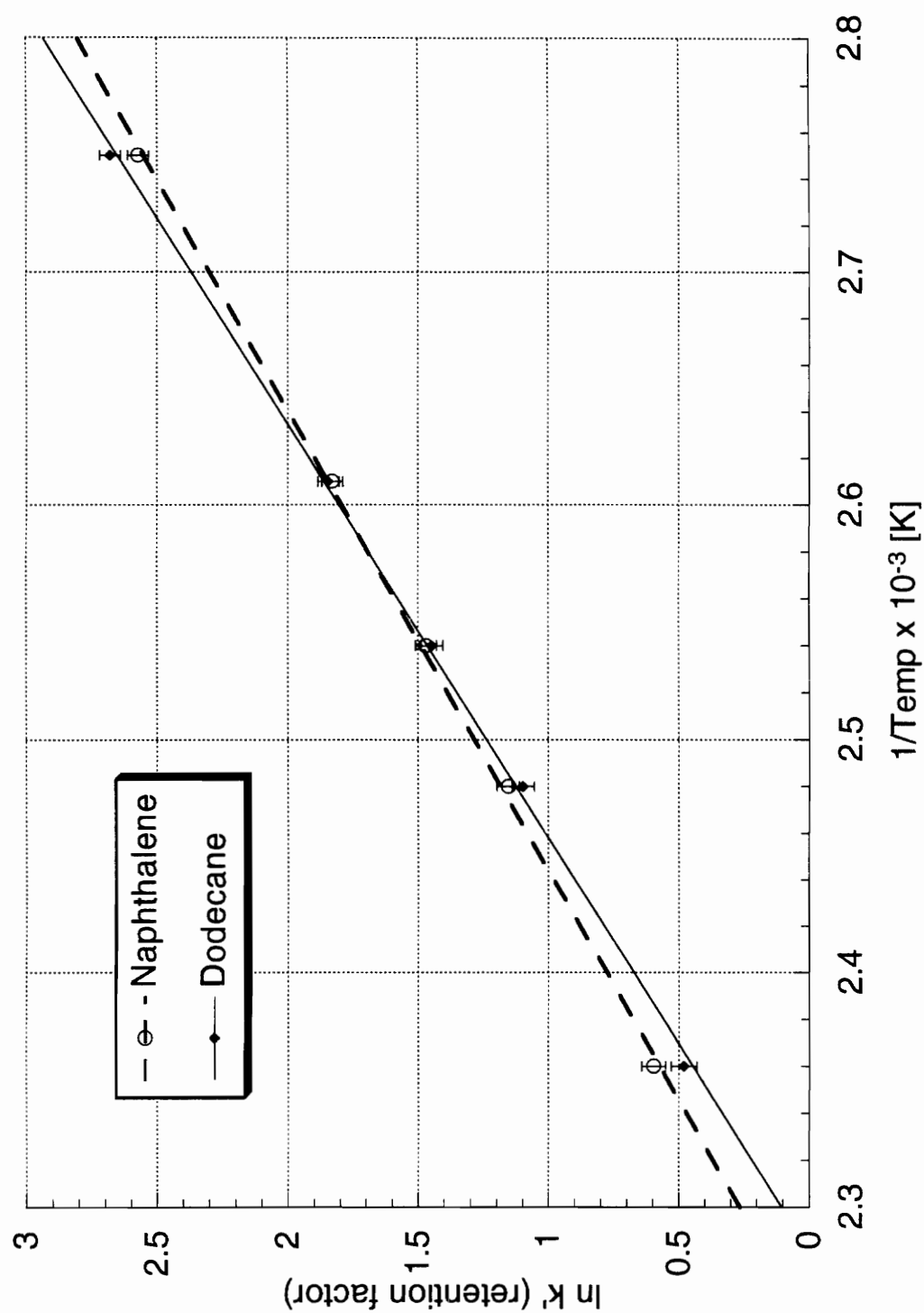Figure **13** A test mixture at 90°C and 130°C spiking with dodecane

Figure **14** Van't Hoff plot of dodecane and naphthalene.

Having a different slope and y-intercept, any two lines crossed at one point, assuming that the two lines are straight. When dodecane and naphthalene lines intersect each other around 115°C, they share a common point. The two compounds are co-eluted, even though their ΔH and ΔS are still different. At the intersection point, their overall sum (ΔH and ΔS) compensate each other and the combination become equal at this temperature, even though they exhibit different temperature effects. After the crossing over, dodecane compound armed with its high molecular interactions overcome its handicap (ΔS value) and it elutes before naphalene. Naphthalene could not held to its advance (ΔS) due to its low molecular interactions, it losses its elution position and elutes after dodecane.

CHAPTER 5 CONCLUSION:

The column temperature may affect selectivity, and hence the elution order, of certain analytes for a given stationary phase. This has been shown for dodecane and naphthalene on an HP Ultra 2 column at 90° and 130°C. This work has also shown that the Van't Hoff graph is a useful tool to predict reversal of elution order. This is accomplished by determining where the intersection of ln k' vs. 1/Temp occurs between any two compounds. The compound pair must show different enthalpy and entropy values which are affected differently by temperature changes.

The reversal of elution order for certain compounds may be explained by the effect of the intermolecular interactions between the analytes and stationary phase. By extrapolating the van't hoff's plots to both temperature extreme (high and low), the lines will intersect each other; the reversal of the elution order had occured at this temperature. However the temperature limits of the capillary colum, thermal stability of the compounds are the limiting factors. The reversal of the elution order is not commonly observed.

From a thermodynamic point of view, the specific molecular interactions cannot be determined independently of each other. In order to determine the fundamental molecular interactions which occur, statistical models must be applied.

This study does however show the importance of the fundamental thermodynamics properties to help explain, and hopefully in some cases even predict unusual chromatographic behavior.

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VITA

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Kien Heng