Experimental Studies in Temperature Programmed Gas Chromatography

by

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(Abstract)

Temperature programmed gas chromatography (TPGC) is commonly used for the analysis of complex samples with a wide range of boiling points. It is estimated that 80% of GC users implement TPGC on a regular basis.[1] In 1962, John Calvin Giddings was the first to publish a simple model for TPGC.[2] His theories concerning TPGC are still accepted as the benchmark for explaining the underlying theory.

The purpose of this research was to investigate, as speculated by Giddings, if temperature programming rate (β) is the dominant contribution in determining fundamental chromatographic values, such as retention time, retention temperature and resolution. Comparison of these effects was made by studying column length and linear velocity in conjunction with temperature programming rates. Experimental determinations using a combination of three different column lengths, five linear velocities and three ramping rates on a three-component sample were investigated. A late eluting peak, C_{14}, was evaluated by statistical analysis to determine the dominant contribution on retention time, retention temperature and resolution.

Results from statistically analysis show that temperature programming rate (β), column length and linear velocity all have contributions on retention time, retention temperature and resolution, however β dominates at high programming rates.

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Chapter 1 Introduction

Gas Chromatography (GC) is defined as a physical technique for the separation of volatile and semi-volatile compounds by passing a mobile carrier gas over a stationary phase.[3] Separation occurs as analytes partition in and out of the stationary phase while being carried through the column by the moving mobile phase. The major factor in separation is due to analytes having different affinities for the stationary phase.

Types of Chromatography

There are numerous different chromatographic methods used for analytical separations, as shown in Figure 1. Two methods of gas chromatography include the use of packed columns or open tubular columns. Packed columns have either a solid particle or liquid stationary phase coated onto a particle support. Gas-Solid Chromatography (GSC) refers to a solid stationary phase whereas Gas-Liquid Chromatography (GLC) refers to a liquid stationary phase. Solid packings are usually comprised of silica gel, molecular sieve or charcoal. If the stationary phase is a liquid there is a thin film absorbed onto the surface of an inert solid support.[3]

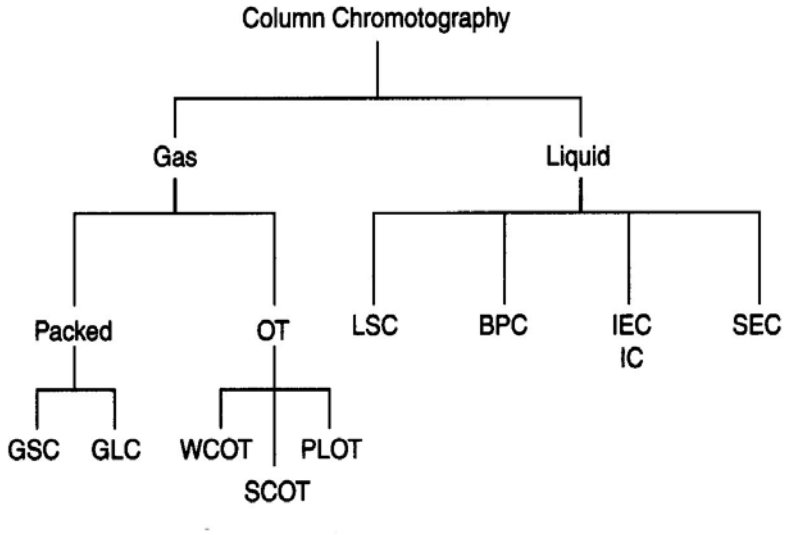

Figure 1: Classification of chromatographic methods[4]

Today, however, the most prominent form of gas chromatography employs open tubular (OT) columns made of fused silica.[4] In 1957, Marcel Golay revolutionized gas chromatography when he introduced open tubular, or capillary columns.[5] Golay proposed that packed columns be replaced with open tubular columns where the walls are coated with a thin layer of stationary phase termed wall coated open tubular or WCOT. Use of open tubular columns lend to a vast improvement in resolution due to the decreased flow resistance of the mobile phase, and the ability to use longer, more efficient columns.[6] The other two types of open tubular columns are porous-layer open tubular (PLOT) and support-coated open tubular (SCOT). PLOT columns have a porous solid absorbent like silica gel or molecular sieve or alumina deposited on the inner surface of a fused silica column. SCOT column have a thin film of Celite coated with a liquid phase attached to the inner surface of the column and they are only

available in stainless steel tubing. SCOT columns have solid support particles that have a liquid phase coated on to a solid support.[7] Therefore SCOT columns have more surface area covered with liquid phase and have a high sample capacity.[4] Figure 2 illustrates the differences between packed and capillary columns.

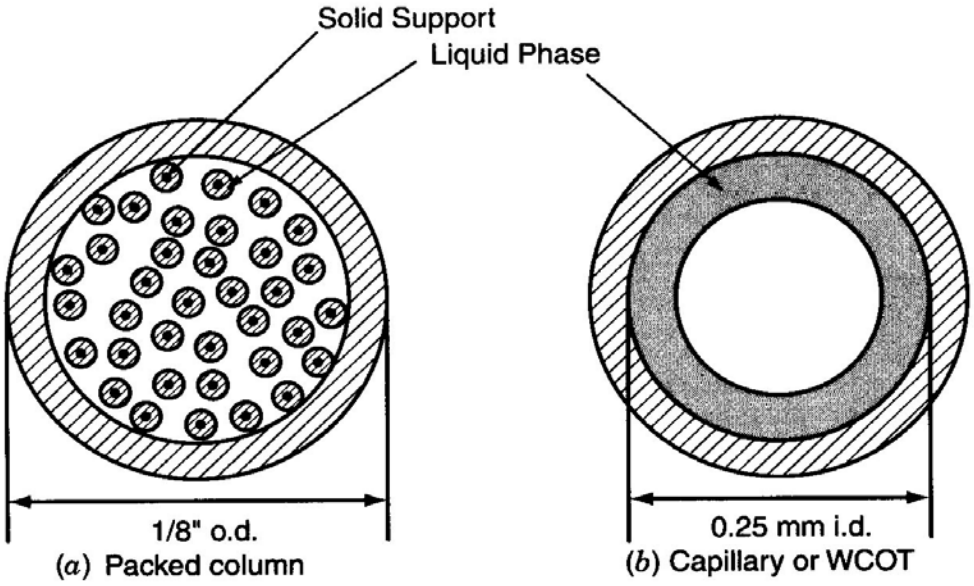

Figure 2: Cross-section schematic of (a) packed and (b) capillary columns[4]

Instrumentation

Eight basic components of a gas chromatograph are shown in Figure 3. Each component plays an important roll in the separation process. The gas cylinder is a source of high purity carrier gas used as the mobile phase which carries the sample through the chromatographic system. The two stage regular serves two purposes. The first stage indicates the pressure remaining in the tank whereas

the second indicates pressure of the gas being applied to the chromatograph. The flow control valve controls the carrier gas flow. The injection port serves as a sample introduction port whereby the analytes are placed into the vapor phase for analysis. The oven regulates the temperature of the column during a chromatographic separation. The column is the heart of the chromatography; this is where the separation process occurs.[4] The detector senses analytes in the effluent exiting the column and sends a signal to the data system which collects the signal and generates a chromatogram. There are numerous types of data systems to collect data including chart recorders, integrators and computer systems. However, computers are the most frequently used type of data system used to generate a chromatogram.[1] A chromatogram is a plot of time versus the output signal generated from the detector as individual sample components elute from the column. The output signal increases and decreases as a function of analyte concentration or mass detected.

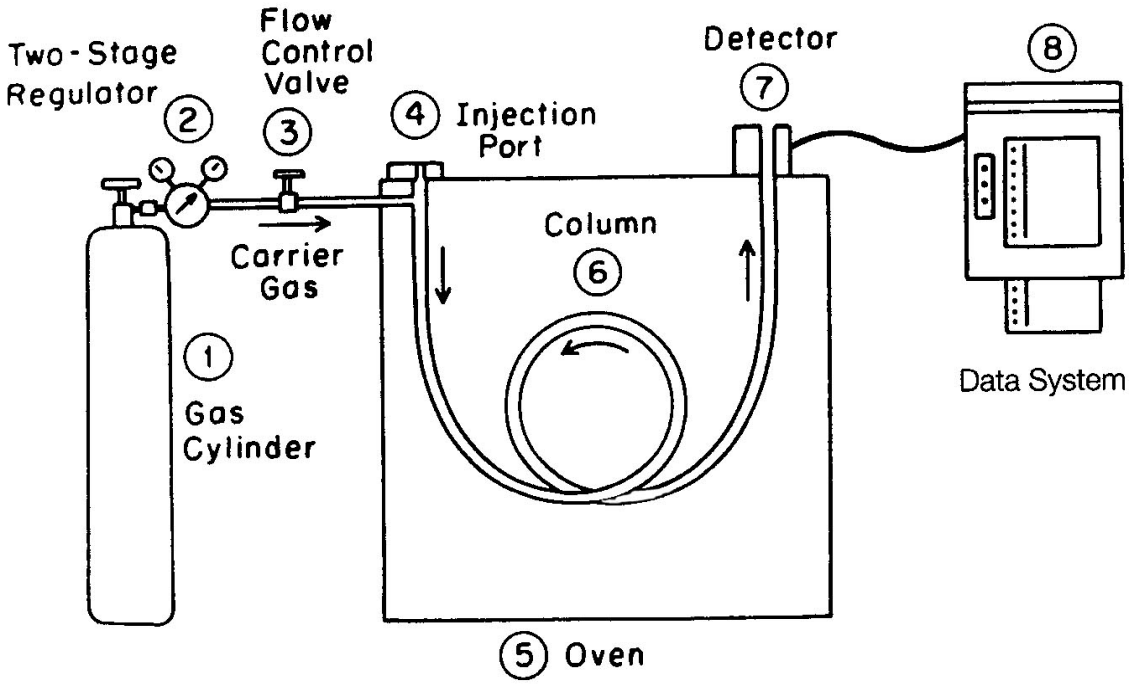

Figure 3: Basic components of a gas chromatograph[4]

The sample is introduced into a hot injection port via a syringe where the analytes and solvent are vaporized. A silicone rubber septum, at the top of the injection port, keeps the system leak free. The vapors are carried by the carrier gas through the column containing a stationary phase where analytes are sorbed onto the thin layer of stationary phase on the surface of the column. Separation occurs as sample components repeatedly partition between the stationary phase and the flowing mobile phase. The sample components are selectively retained by the stationary phase depending on their affinity for the stationary phase.[3] This affinity is measured by a distribution coefficient, K, as shown in Equation 1, which relates the ratio of solute in the stationary and mobile phase. The result is analyte

separation into discrete bands within the column. The carrier gas continues to move analytes along the column until they reach the detector. Here, the analytes are detected and the signal is recorded by the data station, generating the chromatogram.

$$K = \frac{[A]_S}{[A]_M} \qquad \textbf{Equation 1}$$

K = distribution coefficient
A = concentration of solute
S = stationary phase
M = mobile phase

As the value for K increases the analyte with greater affinity for the stationary phase will remain longer in the stationary phase. The result is a longer retention in the column and an increase in retention time. If something is not retained in the stationary phase the distribution coefficient will be equal to zero.

Fundamental Relationships

Concepts and theory in GC are described by numerous fundamental relationships. In this section, a brief overview of relevant relationships and equations is presented.

The number of theoretical plates (N) is a measure of column efficiency. A theoretical plate is defined as the average distance traveled in one distribution step, or partition of the analyte from the mobile phase into the stationary phase and vice versa.[3] The plate number can be directly calculated from the retention

time and standard deviation of a normal Gaussian peak as shown in Equation 2. Figure 4 illustrates the standard deviation of a normal Gaussian peak through the band broadening process occurring during separation. A smaller standard deviation, or the narrower the peak, results in an increase in the number of theoretical plates and thus to a more efficient separation.

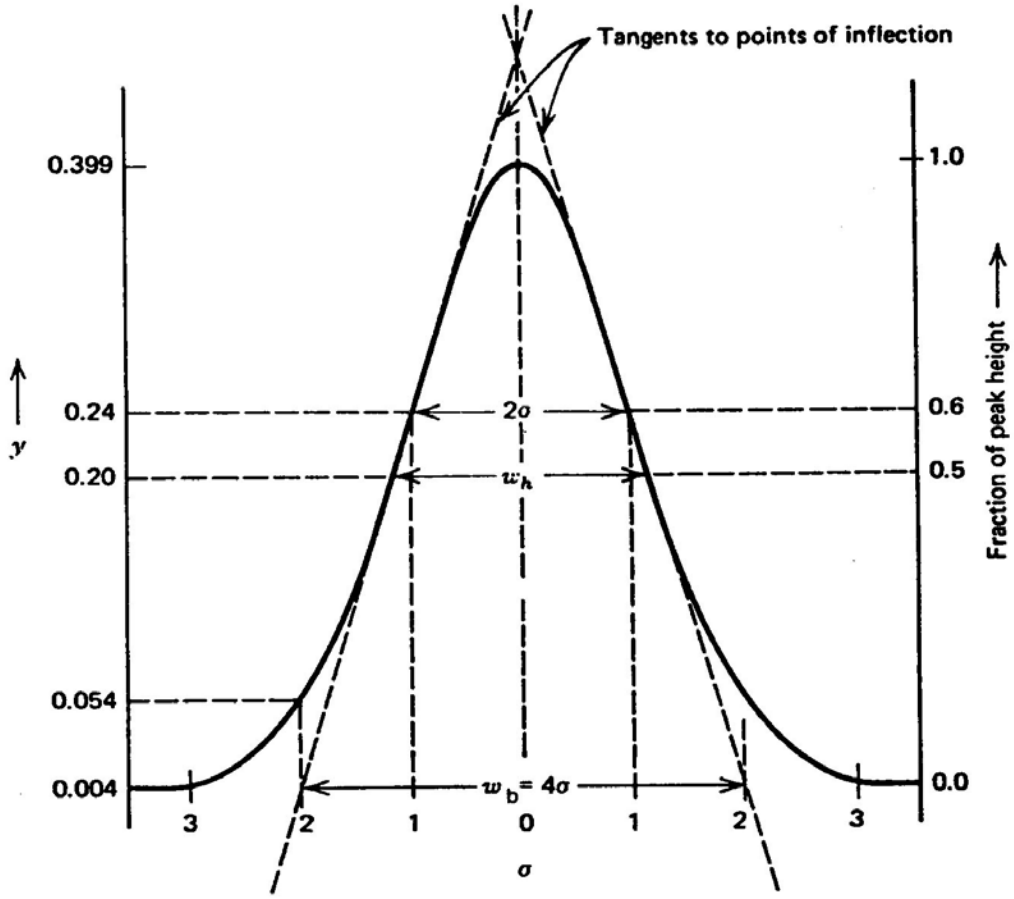

Figure 4: Normal Gaussian peak[4]

Both the retention time and standard deviation have units of time thus making plate number dimensionless.

$$N = \left(\frac{t_R}{\sigma}\right)^2$$ **Equation 2**

N ≡ efficiency of a column
t_R = retention time of the peak
σ = standard deviation of the peak

Both retention time and plate number are directly proportional to the length of the column. The longer the column, the longer the retention time and the higher the plate count. Since the total number of plates for a particular column is based on its length, it is useful to express column efficiency in terms of height equivalent to a theoretical plate (HETP or H). This height, expressed in micrometers, is the height of one theoretical plate. A small HETP allows for more plates per column and greater efficiency. HETP can be calculated by dividing the length of the column by the number of theoretical plates as illustrated in Equation

$$H = \frac{L}{N}$$ **Equation 3**

H = height equivalent to a theoretical plate
L = length of the column in micrometers
N = efficiency of a column

Resolution is another measure of column efficiency. It is a quantitative measure of the degree of separation between two adjacent peaks.[4,7] Ideally, the resolution must be equal to or greater then 1.5 to be considered baseline resolved.[1,4] There are many ways to calculate resolution (R), but the calculation for resolution demonstrated by Equation 4 is the most widely accepted way.[1]

$$R = \left(\frac{2\Delta t}{W_1 + W_2} \right)$$ **Equation 4**

R = degree of separation between two adjacent peaks
Δt = the time interval between peaks
W_1 = width of peak one at the base
W_2 = width of peak two at the base

If one considers an isothermal separation, both the number of theoretical plates and the retention time of the analytes are directly proportional to the length of the column. Thus, if column length is doubled, then both retention time and plate number will double. However, resolution is only proportional to the square root of length. The square root of two, for doubling the length of the column, equals 1.41 and only leads to a 41% increase in resolution. It should be noted that these relationships only hold true for isothermal separations.

van Deemter plots are another way to evaluate column performance. The simplified van Deemter equation, shown by Equation 5, has three different terms that each refer to band broading phenomena that occur in the column during separation.

$$H = A + \frac{B}{\mu} + C\mu$$ **Equation 5**

H = height equivalent to a theoretical plate
A = eddy diffusion coefficient
B = molecular diffusion
C = mass transfer term
μ = average linear velocity

$$\mu = \left(\frac{L}{t_o}\right) \qquad \textbf{Equation 6}$$

μ = average linear velocity
L = length of the column in centimeters
t_0 = dead time in minutes

The A term represents Edie diffusion, a type of band broadening which occurs in packed columns due to the multiple paths traveled by the analytes. This form of band broadening is dependent on the particle size and the packing efficiency. The B term represents molecular diffusion of the solute in the carrier gas. Diffusion by nature wants to spread the analytes away from one another and is dependant on analytes diffusion coefficient in the mobile phase, column temperature and viscosity of the mobile phase. The C term represents mass transfer, which is the movement of the analyte from one phase to another within the column. When Golay invented the capillary column he derived a modified van Deemter equation to describe band broadening since the van Deemter equation applied only to packed columns called the Golay equation.

$$H = \frac{B}{\mu} + (C_S + C_m)\,\mu \qquad \textbf{Equation 7}$$

H = height equivalent to a theoretical plate
B = molecular diffusion term
C_s = mass transfer in the stationary phase
C_m = mass transfer in the mobile phase
μ = average linear velocity

The differences in these two equations can best be illustrated by expanding both the van Deemter and Golay equations shown by equation 8 and 9 respectively.

Van Deemter Equation

$$H = 2\lambda d_p + \frac{2\gamma D_G}{\mu} + \frac{8kd_f^2\mu}{\pi^2(1+k)^2 D_S} + \frac{\omega d_f^2 \mu}{D_G} \qquad \textbf{Equation 8}$$

A term B term C terms

H = height equivalent to a theoretical plate
λ = packing factor
d_p = particle diameter
γ = tortuosity factor
D_G = diffusion coefficient for the solute in the mobile phase
μ = linear velocity
k = retention factor
d_f^2 = film thickness of the stationary phase
D_S = diffusion coefficient for the solute in the stationary phase
ω = obstruction factor for packed bed

Golay Equation

$$H = \frac{2D_G}{\mu} + \frac{2kd_f^2\mu}{3(1+k)^2 D_S} + \frac{(1+6k+11k)^2 r_C^2 \mu}{24(1+k)^2 D_G} \qquad \textbf{Equation 9}$$

B term C_S term C_M term

r_C^2 = radius of the column
All other terms are as previously defined following equation 8.

The van Deemter and the Golay equations for band broadening differ in three ways: 1) the Golay equation does not have an A term; 2) the van Deemter has a single C term; and 3) the Golay equation does not account for packing factors in its B term. The Golay equation was derived for capillary columns without packing and thus only one flow path and no Edie diffusion. Given that capillary columns lack packing, a tortuosity factor is also unnecessary. In addition, the Golay equation takes into account mass transfer in both the stationary and mobile phases. The mass transfer in packed columns is dominated by transfer in the stationary phase therefore making the transfer in the mobile phase negligible.[1]

Isothermal vs. Temperature Programmed Separations

The research presented here will focus on the differences between isothermal and temperature programmed separations and how they affect separation results (T_R, t_R, and R). Isothermal refers to maintaining constant temperature of the oven during the chromatographic run as shown in Figure 4. Temperature programming refers to the temperature of the oven increasing during the chromatographic analysis. This is usually done in linear fashion (constant programming rate).

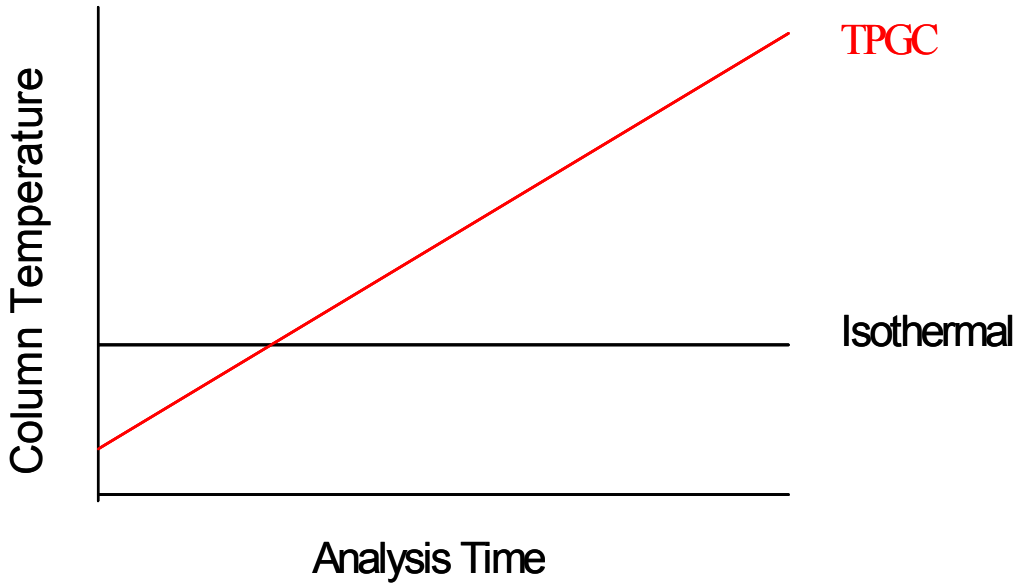

Figure 5: Isothermal vs. temperature programmed

Figure 6 shows a chromatogram of a conventional isothermal separation of the hydrocarbons C_6 through C_{21}. The sample has a wide range of boiling points, ranging from 69^0 to 360^0C. This wide range of vapor pressures makes it difficult to select a single temperature suitable to separate all components. In this isothermal separation, the lighter components coelute because the temperature was too high. The higher boiling components eventually elute but the peaks are so broad that they are indistinguishable from the baseline.

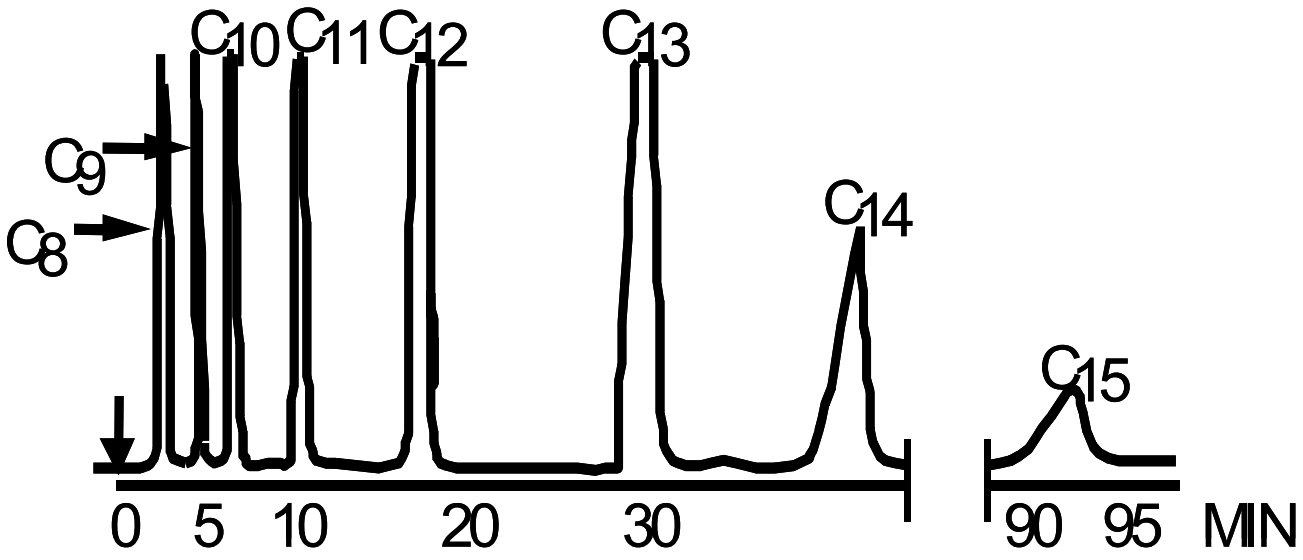

Figure 6: Isothermal separation of hydrocarbons at 130ºC[8]

The chromatogram above was significantly improved by implementing a temperature program from 50 - 250^{0}C at 8^{0}C per minute as shown in Figure 7. Now the peaks are well resolved, equally spaced and all the peaks elute in one third of the original analysis time.

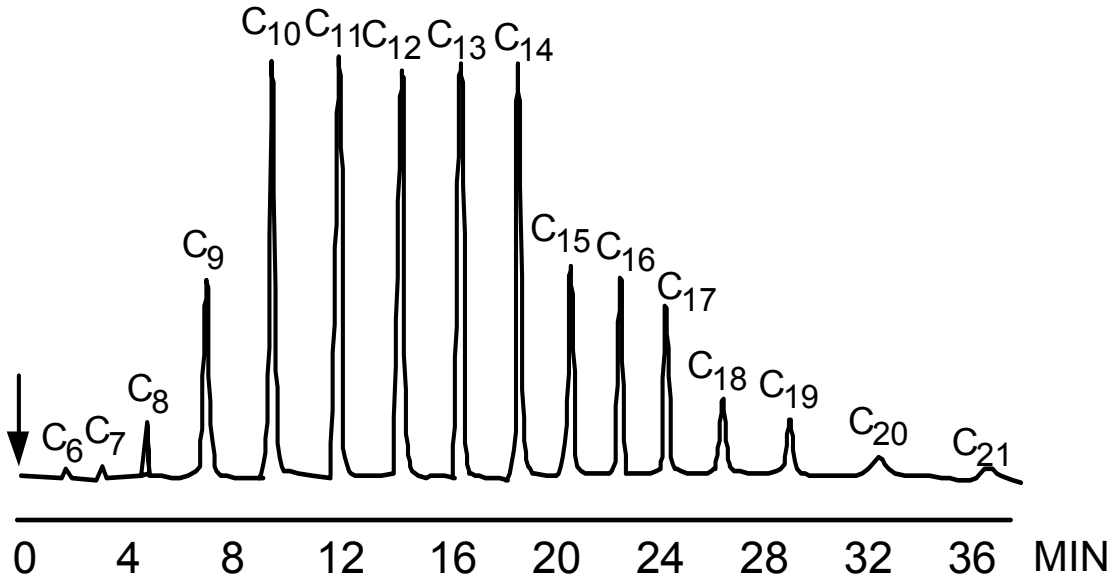

Figure 7: Temperature programmed separation of hydrocarbons[8]

Temperature programming has many advantages as well as disadvantages. Advantages include added versatility in complex sample analysis, faster method development and improved detection limits and precision for late eluting peaks. The late eluting peaks actually reach temperatures high enough to allow migration from the head of the column and thus decrease the amount of band broadening previously seen in the isothermal separation. For this reason late eluting peaks are shaper, leading to better detection limits and precision, as illustrated in Figure 7.

There are a few disadvantages of using temperature programming. There must be three separate well controlled heated zones, the injector, column oven and detector. The temperature of the injector and detector compartments must

remain constant independent of a programmed column oven temperature. This increases the expense for instrumentation. The liquid stationary phase of the column must have a low vapor pressure over the entire range of the temperature program. If the vapor pressure of the stationary phase is too high for the oven temperature used, then the stationary phase can slowly elute from the column, a phenomena called column bleed. Column bleed results in high background noise from the detector.[4] Also, the liquid phase must have a reasonable viscosity at low temperatures to generate high plate counts needed for column efficiency. This leads to a limited number of liquid phases available. Lastly, extra time is needed to re-equilibrate the instrument to the initial temperature after the final temperature is reached.

Thesis Statement

This research is designed to investigate the influence of temperature programming rate (β), column length and linear velocity, on fundamental chromatographic results, such as retention time, retention temperature and resolution.

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Chapter 2 GC Historical Background and TPGC Theory

Historical Background

The origin of chromatography can be traced to 1905 when W. Ramsey separated a mixture of gases on adsorbent charcoal.[9] Soon after, Michael Tswett, a Russian botanist, published his first work on the separation of chloroplast pigments by adsorption liquid chromatography.[10] The intensely colored plant pigments led Tswett to call the technique ”color writing” or chromatography. Tswett later became known as the “father of chromatography”.

Some work was done with liquid solid chromatography in the following years, but a major step was made in 1941 when two biochemists, A. J. P. Martin and R. L. M. Synge developed partition chromatography for the separation of amino acids.[11] Martin and Synge won a Nobel Prize in 1952 for their pioneering work in partition chromatography. Martin and Synge continued to contribute to the field of chromatography by deriving the relationships between the separation factor and distribution coefficient. Their investigation of column efficiency showed that height equivalent to a theoretical plate (HETP) was dependent on flow rate and diffusivity of the sample in the mobile and stationary phases.[11]

In 1952, Martin collaborated with A. T. James to introduce gas liquid chromatography (GLC), which they used to separate volatile carboxylic acids and amines.[12] The separation was performed with a gaseous mobile phase and a stationary liquid that had been coated onto an inert support. The treated support was then packed into a metal tube that was heated.

Another milestone of chromatography occurred when M. J. E. Golay introduced wall coated open tubular (WCOT) capillary gas chromatography columns in 1958 at the International GC symposium held in Amsterdam.[13] The introduction of capillary columns revolutionized chromatography by vastly improving separation efficiency. These columns were open tubes and had a small pressure drop allowing for higher flow rates and longer columns which led to more efficient separations. The increased efficiency allowed for faster analysis and the separation of more complex samples then that done previously on packed columns. The capillary column, however had a limited capacity and was manufactured initially from copper or stainless steel which were to active for many polar compounds.

Fused silica columns were introduced 1979 by R. D. Daneneau.[14] Fused silica capillary columns were composed of ultra pure fused silica with a liquid stationary phase thinly coated on the wall of the capillary column. The fused silica column dimensions allowed the columns to be more flexible leading to easier use and installation.

The introduction of temperature programmed gas chromatography allowed for faster and more complex sample analysis. The first published temperature program chromatogram was obtained by J. H. Griffiths et al. and is shown in Figure 8.[15]

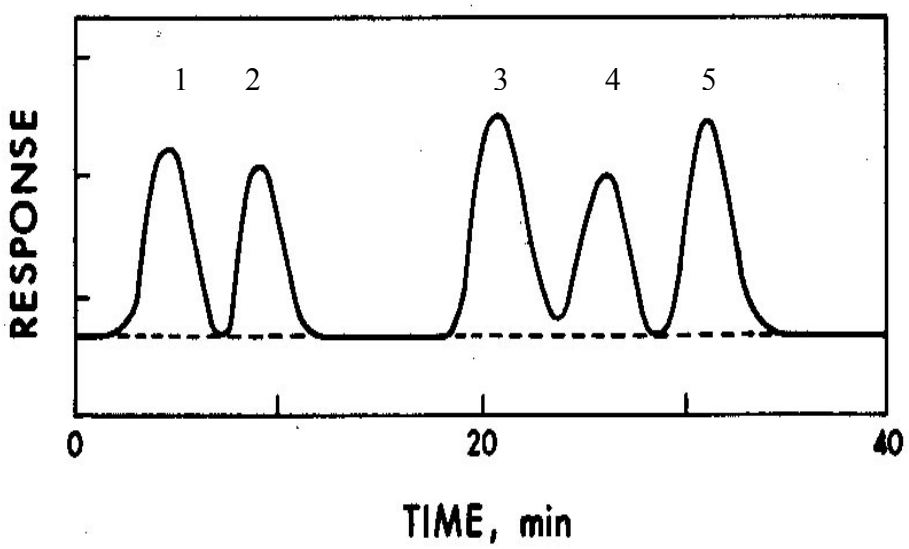

Figure 8: First temperature programmed chromatogram[16] Peaks are listed above; (1) Isopropyl chloride; (2) n-propyl chloride; (3) n-butyl chloride; (4) chloroform; (5) ethylene dichloride.

The 5- component sample was separated on a tricresyl phosphate column using a temperature program from 0° C to 50° C at a ramping rate of 1.25° C/min. Isopropyl chloride, n-propyl chloride, n-buytl chloride, chloroforms and ethylene dichloride were separated in approximately 35 minutes. The peak broadening seen in Figure 8 is due to the limitations of packed columns GC. A slow ramping rate of 1.25° C/min was necessary due to the difficulties of temperature control with large ovens and metal columns. Current instrumentation employs small mass capillary columns and ovens and provides much better temperature control and faster programming rates.

Temperature Effects in GC

Before the theory of TPGC is discussed, the effects of temperature in isothermal gas chromatography should be addressed. The two most important parameters in GC are the column temperature and the stationary phase. The column is the heart of the chromatograph: this is where the separation process occurs.[4] However, the temperature has a greater affect on the chromatographic process than any other single variable.[16] The temperature effects the gaseous and liquid diffusion, flow rate and pressure drop, retention volume, partition coefficient, retention time and retention temperature.

Pressure Drop and Flow Rate

During isothermal chromatography the inlet pressure must be higher than outlet pressure in order to force the eluent from the inlet to the outlet of the column. If the pressure drop is constant then the flow rate must change or vice versa. The pressure drop between in the inlet and outlet can be calculated using equation 10.

$$\Delta p = p_i - p_o \qquad \textbf{Equation 10}$$

Δp = pressure drop across the column
p_i = inlet pressure
p_o = outlet pressure

The pressure drop across the column becomes important due to the carrier gas compressibility which leads to linear velocity variations with in the column. In temperature programming an increase in temperature causes a decrease in flow rate at constant pressure due to the increased viscosity of carrier gas as shown in Figure 9.

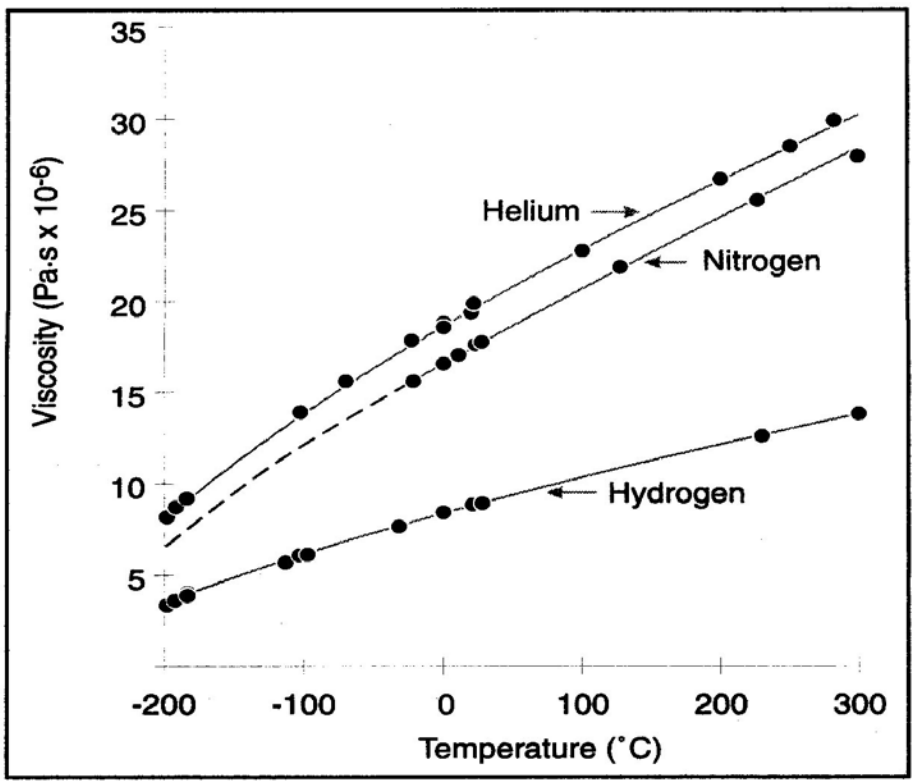

Figure 9: Viscosity as a function of temperature for three common GC gases[17]

During a temperature program if the inlet pressure is constant the linear velocity decreases while the carrier gas viscosity increases due to the direct relationship. So if the initial linear velocity is at its optimum value, according to the van Deemter curve, then the final linear velocity will fall below this optimum level. One option is to set the linear velocity higher then the optimum range to insure

an adequate rate at the higher temperature. Another option would be to implement hydrogen as the carrier gas because it has the lowest viscosity change during temperature increase and has the lowest pressure drop. Darcy's Law can be used to show the direct relationship between linear velocity and the pressure gradient in the chromatographic bed and the inverse relationship to viscosity. This is illustrated in equation 11.

$$\frac{F_2^T}{A} = \frac{-B}{\eta}\frac{d_p}{d_z} \qquad \textbf{Equation 11}$$

F_2^T = volumetric flow rate
A = cross sectional area of empty tube
B = permeability of the bed
η = viscosity
$\frac{dp}{dz}$ = pressure gradient in bed over a distance z

Outlet flow rate also changes during temperature programming according to equation 12.

$$F_O = F_{oTo}\left(\frac{T_o}{T}\right)^{1.7} \qquad \textbf{Equation 12}$$

F_o = outlet flow rate
$F_{o\,To}$ = flow rate at intial temperature
T = temperature

The outlet flow rate varies with a 1.7 power of temperature at constant inlet and outlet pressure as illustrated in Figure 10. The curve is theoretical and corresponds to the 1.7 power relationship to temperature.

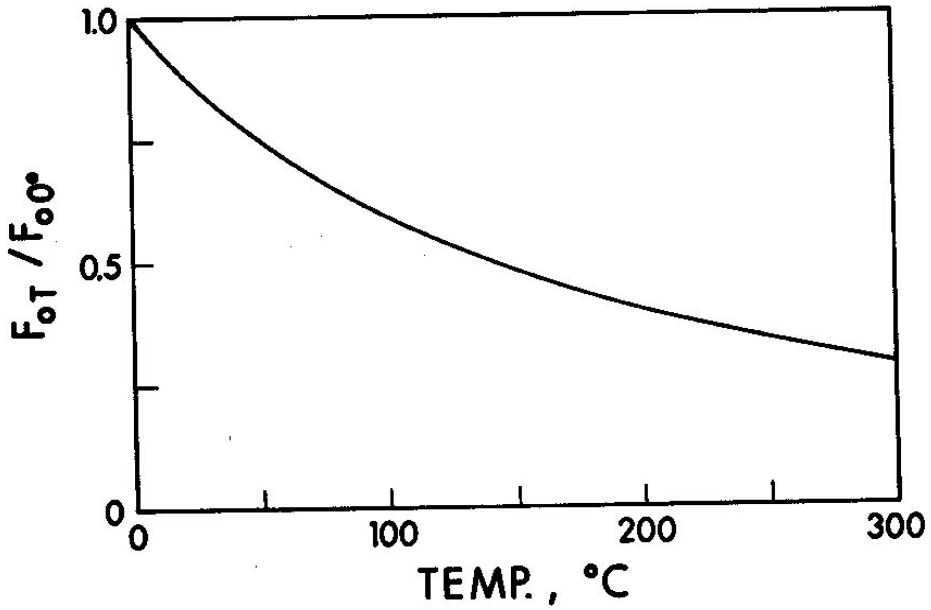

Figure 10: Flow rate as a function of temperature[16]

Retention Volume and Partition Coefficient

Retention volume is defined as the volume of mobile phase required to elute the peak maximum of a compound and is calculated by multiplying the retention time by the flow rate as shown in equation 13. [8]

$$V_R = t_R \times F_c \qquad \textbf{Equation 13}$$

V_R = retention volume
t_R = retention time
F_c = column flow rate

The adjusted retention volume, V'_R, is calculated by subtracting the column dead volume as shown equation 14.

$$V_R^{'} = V_R - t_O$$ **Equation 14**

V'_R = adjusted retention volume
V_R = retention volume
t_o = dead volume

The dead volume of the column is the volume required to transport a nonretained peak (like air) through the column. It is usually measured by injecting either methane or butane, two compounds that are generally not retained by the stationary phase.

Temperature also effects the distribution of the sample between the mobile and stationary phase and hence on the retention volume of a solute peak.[16] The increase in temperature decreases the dead space volume[16] used to calculate the adjusted retention volume.

The partition coefficient of an analyte is defined as the concentration of analyte in the stationary phase divided by the concentration of the analyte in the mobile phase as shown in equation 1 on pg. 5. The partition coefficient of an analyte is affected by temperature using the relationship in equation 15.

$$\log K = \frac{A}{T_c} + B$$ **Equation 15**

K = partition coefficient of an analyte
A and B = constants
T_c = absolute column temperature

As the column temperature increases the partition coefficient of an analyte decreases and K values decrease. This causes other chromatographic results to change. The retention time and the capacity factor k will decrease due to less time spent in the stationary phase. Other chromatographic results have easily defined relationships in isothermal separation but are more complicated in temperature programmed Gas Chromatography.

Theory of TPGC

In 1962, John Calvin Giddings derived a theoretical explanation for TPGC.[2] His concepts concerning TPGC are still accepted as the benchmark for explaining the underlying theory. Giddings described temperature programming by saying

> "In the programmed temperature analysis of a wide boiling mixture one obtains, in succession, a satisfactory temperature range for the separation of each narrow boiling point fraction. If care is taken in selecting the heating rate, each group of substances automatically selects its own ideal temperature at which to migrate and separate within the column. Prior to reaching the workable temperature range, each substance is essentially dormant, or frozen at the head of the column." [18]

This section will describe Giddings explanation of TPGC. Giddings noted that temperature programming differs from an isothermal separation in that the temperature, T, is increased as a function of time usually in a linear fashion. This is shown in equation 16

$$T - T_o = \beta t$$ **Equation 16**

T = temperature of the column
T_o =initial temperature
β = temperature ramping rate in ^oC
t = retention time

Using this equation, the temperature of the column when the peak elutes can be directly calculated from the ramping rate, retention time, and the starting temperature. When developing a TPGC method one must choose the initial and final column temperatures and the ramping rate at which the analysis is best suited. The boiling point range of the sample components is an indicator of the initial and final temperatures. The initial temperature should be 60^{o}C to 90^{o}C lower than the lowest boiling point of the sample components. The final temperature should be slightly above the retention temperature of the latest eluting peak but not higher than the column temperature maximum. Ramping rate (β) is a critical parameter to determine during method development. As ramp rate increases, analysis time as well as resolution decreases. It is this compromise between analysis time and resolution that determines the ramping rate. Giddings theory indicates that β is the dominate factor in determining chromatographic results.

The first assumption stated is that the migration rate of the analyte through the column is proportional to its vapor pressure. Giddings used the integrated Clausius-Claperyon equation, shown in equation 17, to relate retention temperature with the programming rate (beta).

$$\ln\left(\frac{P_2}{P_1}\right) = \frac{\Delta H}{R} * \frac{T_2 - T_1}{T_1 * T_2}$$ **Equation 17**

P_2 = pressure at temperature two
P_1 = pressure at temperature one
ΔH = heat of vaporization
R = gas constant
T_1 = temperature one
T_2 = temperature two

Using this equation Giddings calculated that temperature increase needed to double the vapor pressure of the analyte which in turn doubles the migration rate. Doubling the vapor pressure, the natural log of 2 is 0.693 as shown in equation 18

$$\ln 2 = 0.693 = \frac{\Delta H}{R} * \frac{\Delta T}{T_1 * T_2}$$ **Equation 18**

In order to solve the equation, Giddings made a second assumption; the use of Trouton's rule. The rule states that the latent heat of vaporization is approximately constant for a number of liquids.[16] Trouton's rule states that $\Delta H/T$, (molar entropy of vaporization), is equal to 23 (kcal / mol K) for hydrocarbons. The molar entropy of vaporization is derived from the second law of thermodynamics.

$$\Delta G = \Delta H - T\Delta S$$ **Equation 19**

ΔG = free energy
ΔH = heat of vaporization
ΔS = entropy
T = temperature in Kelvin

Using this equation the entropy of vaporization is equal to the heat of vaporization divided by temperature as shown in equations 20.

$$\Delta_{vap}S = \frac{\Delta H}{T}$$ **Equation 20**

The molar entropy of vaporization along with the assumption that the temperature is 500^{o}K and the gas constant is 1.99 results in a 30^{o}C change in temperature, ΔT, required to double the migration rate of the analytes. While trying to describe the theory behind TPGC, Giddings stated "the exact theory of TPGC, from the calculation of plate height to the prediction of retention time is riddled through with difficult integrals whose solutions reside in numerical methods rather than in simple and direct equations." [18] Giddings simplified the theory by showing that the relative rate of migration as a function of temperature can be described by a step function approximation. Figure 9 illustrates the step function approximation.

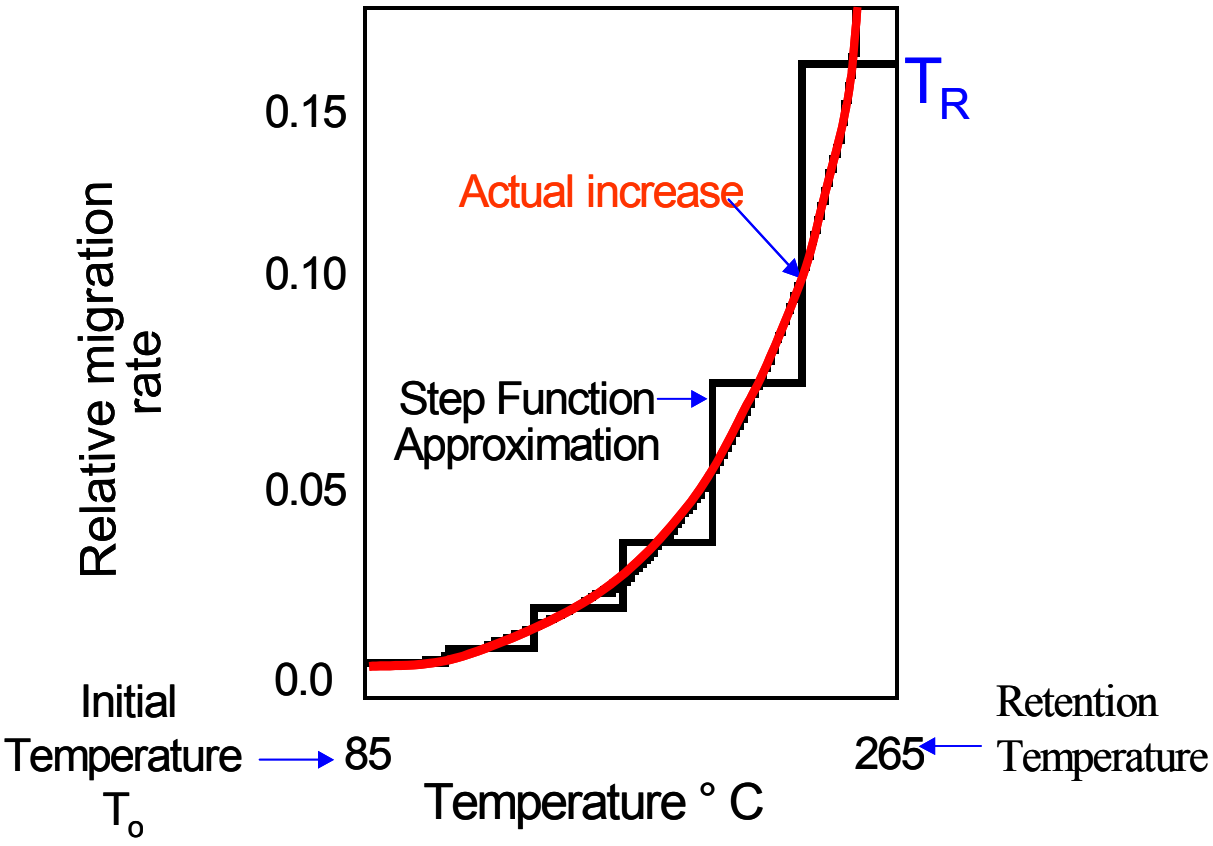

Figure 11: Giddings step function approximation[18]

The red line that increases logarithmically with the increase of temperature highlights the actual increase of migration rate. The exact numerical methods to solve for this smooth curve in temperature programming were later described by Rowan[19], Habgood and Harris[20], and Dal Nogare and Langlois[21].

Giddings assumed that there would be an isothermal hold at the initial temperature, T_o, for a fixed period of time for a migration rate designated by an "X". In actuality there is a continual increase in the migration rate of the analyte through the column. However, Giddings simplified the theory by breaking it into individual steps. The next 30°C change in temperature will result in the migration rate doubling (2x). Note that the change in temperature to double the vapor

pressure of the analyte or migration rate is a 30^{o}C change in temperature as doubled from the previous rate. As the temperature continues to increase the migration rate will also double until the peak elutes at a point which Giddings described as retention temperature, $T_{R.}$ Retention temperature is that temperature at which the peak maximum elutes from the column. This step function approximation illustrates that in five steps the final rate of migration is sixty four times that of the initial migration rate.

Giddings also introduced a peak migration model to illustrate migration rate of the analyte thought the column. In TPGC, the analytes are placed on a column at low temperatures. The analytes are essentially frozen at the head of the column. As the column temperature increases analytes reach a temperature where its vapor pressure is sufficient to allow for migration down the column to begin. The peak migration model is illustrated in Figure 10.

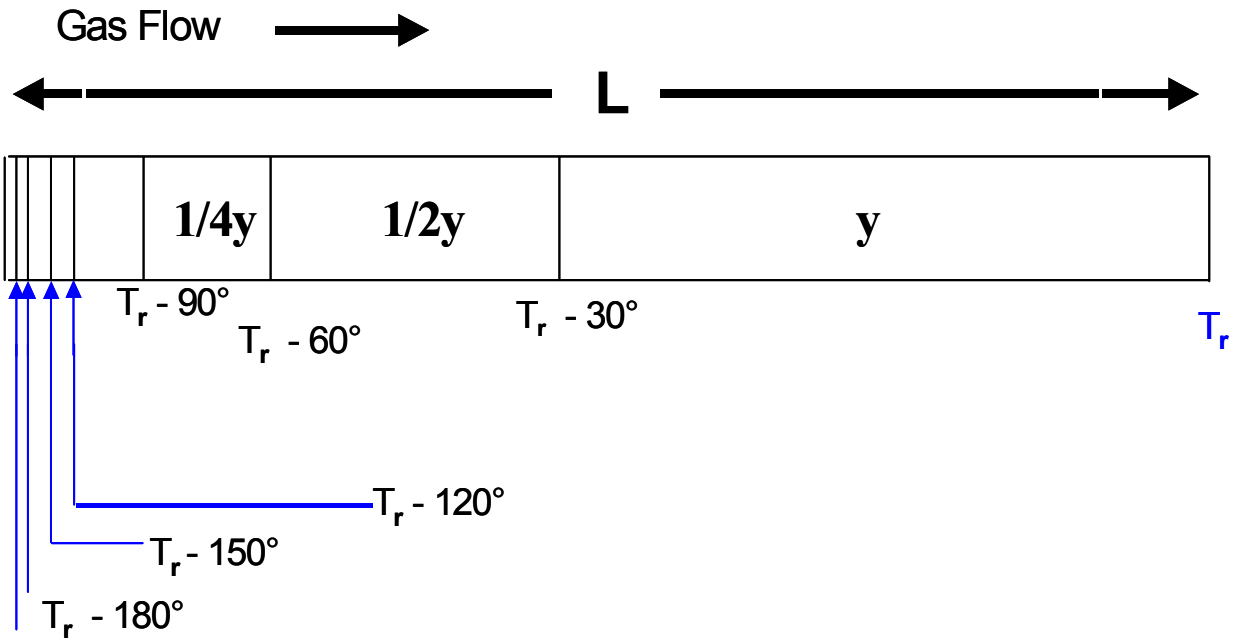

Figure 12: Giddings peak migration model[18]

In the peak migration model "L" represents the column length where analyte migration occurs from left to right. The analytes are placed on a cold column and are "frozen" at the head of the column. Each pair of adjacent vertical lines represents the distance traveled in the 30°C temperature change interval. The diagram shows that most of the migration occurs in the last three intervals; 90°C before the elution temperature. If the letter "y" designates the distance traveled in the last 30oC interval the previous 30°C intervals will be 1/2y, 1/4y, 1/8y, 1/16y. The sum of these intervals approaches two as a limit.[18] The total distance traveled is two times the distance migrated in the last 30°C interval. The analyte has migrated through 50% of the column in the last 30°C interval; 75% in the last

60°C interval; and 88% in the last 90°C before elution. This demonstrates that nearly all migration occurs near T_R and leads to a third assumption made by Giddings that the length of the column is a secondary factor in determining chromatographic results. If the column length were doubled the analytes would essentially be blown through the new column, with little to no retention since they had already reached a temperature high enough for rapid migration. If the initial temperature, T_o, is too high analytes are not initially frozen at the head of the column.

While analytes may not be frozen at the head at the column, if the R value approaches a value of one the initial temperature is independent of the retention temperature for late eluting peaks. R is define by equation 21.

$$R = \alpha e^{-\Delta H / RT} \qquad \textbf{Equation 21}$$

R = the fraction of solute that is in the vapor state
α = defined in equation 14
ΔH = change in enthalpy in the solute going from the liquid to gas phase
R = gas constant
T= temperature

$$\alpha = \frac{A_M}{A_S} e^{\Delta S / R} \qquad \textbf{Equation 22}$$

A_M = area of the mobile phase
A_S = area of the stationary phase
ΔS = entropy change for solute going from the liquid to gas phase

R the fraction of solute that is in the vapor state quantifies the amount of solute soluble in the stationary phase. This is illustrated by data generated by H. McNair in 1986 as shown in Table I.

Table I: Retention temperature as a function of initial temperature

	Retention Temperature		
T_0 (^oC)	**Carbon # 16**	**17**	**18**
140	209	254	276
160	229	253	276
180	231	254	276
200	234	255	276

Table I shows that the retention temperature for carbon number eighteen is independent of the initial temperatures provided the T_o "freezes" the analyte. Carbon number eighteen eluted at 276^{o}C regardless of the four different initial temperatures. The boiling point for octadecane, C_{18}, is 306^{o}C, so it is essentially "frozen" at all T_o values in Table I.

In summary Giddings made several assumptions to describe the simplified mechanism behind temperature programmed gas chromatography. The first assumption stated that the migration rate of the analyte through the column is proportional to its vapor pressure. This assumption was used to solve for the

change in temperature required to double the migration rate of an analyte or doubling its vapor pressure. Giddings made another assumption; the use of Trouton's rule to solve for the change in temperature needed to double the migration rate which resulted in a 30 ^{o}C increase. Giddings simplified the mechanism by showing that the relative rate of migration as a function of temperature can be described by the step function approximation. This step function approximation illustrates that in five steps the final rate of migration is sixty four times that of the initial migration rate when the peak elutes at its retention temperature. Giddings also introduced a peak migration model to illustrate migration rate of the analyte thought the column. The diagram shows that most of the migration occurs in the last three intervals; 90^{o}C before the elution temperature. Along with Giddings assumptions to explain the theory behind TPGC Giddings also stated that the ramping rate,β, is the dominant factor in TPGC whereas flow rate and length are secondary factors in obtaining chromatographic results.

The purpose of this research was to investigate, as speculated by Giddings, if temperature programming rate (β) is the dominant contribution in determining fundamental chromatographic values, such as retention time, retention temperature and resolution. Comparison of these effects was made by studying column length and flow rate in conjunction with varying temperature programming rates.

Chapter 3 Experimental

Instrumentation

All experiments were performed using a Shimadzu, (Columbia, MD, USA) GC-2010 gas chromatograph with advanced flow control. The system contained an AOC 20i auto injector, AOC 20 sample tray, and a flame ionization detector. The peak integration was performed by a Shimadzu Lab Solution data handling software package. The instrumentation used in this study is shown in Figure 13.[22]

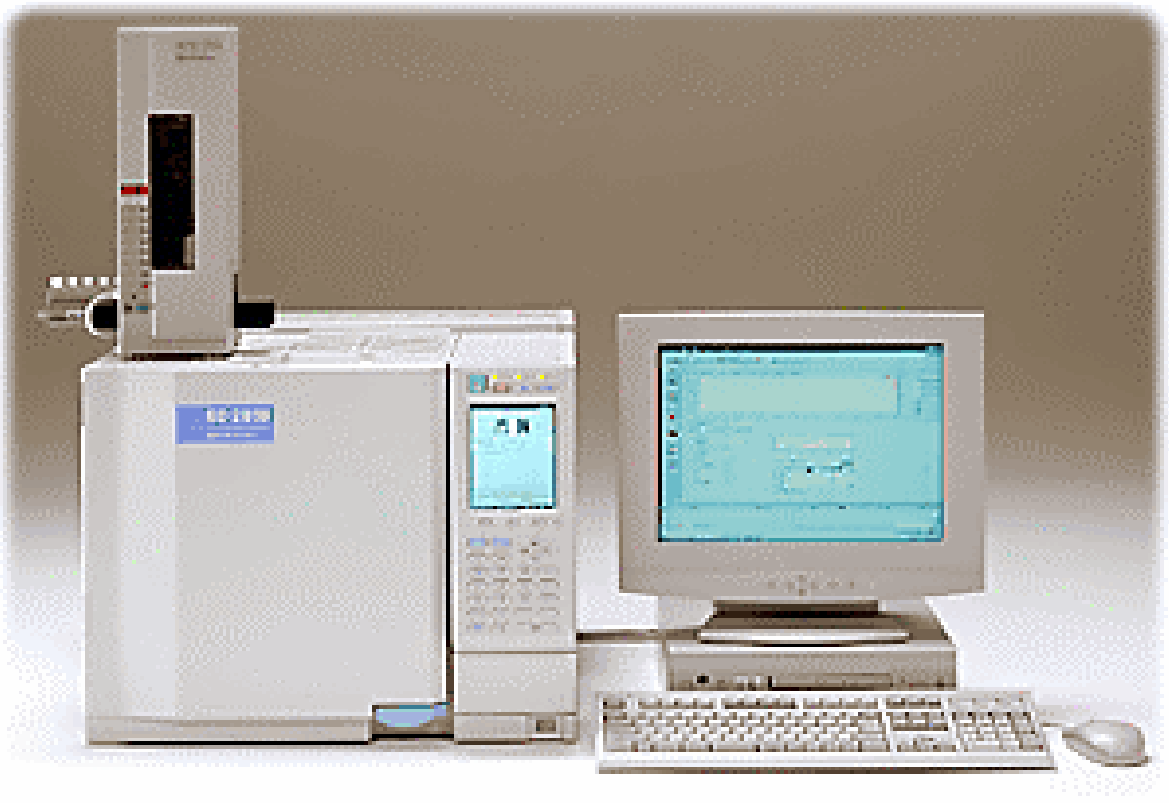

Figure 13: GC instrumentation used for all experiments (AOC 20 sample tray is not shown)

Table II contains instrumental parameters used for all experiments performed in this study. Split ratio and injection volume were optimized by previous experimentations.

Table II: Instrumental Parameters

Instrumental Parameters	
Injector Temperature	250^{o}C
Column-(Phenomenex)	ZB-5; 0.25mm I.D. x 0.25μm df
Detector Temperature	300^{o}C
Carrier Gas	Hydrogen
Split Ratio	100:1
Injection Volume (auto sampler)	0.5μl

Chemicals

A three-component sample was prepared from individual standards of dodecane, tridecane and tetradecane obtained from Sigma- Aldrich (Milwaukee, WI, USA). Hexane obtained from Burdick & Jackson (Muskegon, MI, USA) was used to dilute the sample to 100ppm of each individual standard.

GC columns

Two commercially available thirty-meter capillary columns were obtained from Phenomenex (Torrance, CA, USA). Both columns were Zebron ZB-5 capillary

columns have a stationary phase of 5% phenyl polysiloxane and 95% dimethyl siloxane. The first column was used as is, with the original 30m length. The second column was rolled out, measured and cut into a 15m and 7.5m lengths.

Experimental Design

The purpose of this research was to investigate, as speculated by Giddings, if the temperature programming rate (β) is the dominant contribution in determining fundamental chromatographic values, such as retention time, retention temperature and resolution. Comparison of these effects was made by studying column length and linear velocity in conjunction with the temperature programming rate. Experimental determinations using a combination of three different column lengths, five linear velocities and three ramping rates on a three-component sample were investigated as summarized in Table III.

Table III: Experimental Design

Experimental Design	
Column Lengths	30, 15, 7.5 meters
Linear Velocities	25, 30, 45, 50, 60 cm/sec
Start Temperatures	35, 40, 45^{o}C
Ramping Rates (β)	5, 10, 20 ^{o}C/min
Sample	C_{12}, C_{13}, C_{14} (100ppm) in hexane

*All experiments run in triplicates

The latest eluting peak, C_{14}, was evaluated by statistical analysis to determine the dominant contribution on retention time, retention temperature and resolution. Statistical analysis software was implemented to interpret the data. Results from statistically analysis are discussed in Chapter 4 and complete statistical outputs are located in Appendix I.

Quantitative Analysis

Quantitative analysis was performed on the sample listed in Table III. The sample was run isothermally at 80^{o}C in triplicate. Each individual components area for each of the triplicate runs was averaged in order to generate a relative standard deviation. The sample was run with a 20 ^{o}C/min temperature program in triplicate. The areas for each component were averaged and the relative standard deviation was compared to results in the isothermal separation.

Chapter 4 Results

Temperature Programming Rate

Increasing the temperature is known to decrease the retention time for analytes.[4] Tables IV, V and VI show the effects of ramping rate on retention time, retention temperature and the resolution between sample components tridecane and tetradecane, respectively. The data presented here use a 35°C initial temperature with a carrier gas linear velocity of 25cm/sec for all three column lengths.

Shimadzu Instrumentation as described in Chapter 3 using integration by the Lab Solutions data handling software generated all the results presented here. Microsoft excel was used to compile all the data as well as to calculate retention temperatures, averages, standard deviations and percent relative standard deviation (%RSD).

Effect on Retention Time

Table IV lists the retention times for various temperature programming rates along with the %RSD. The retention times listed are the average retention times for tetradecane for all 135 observations.

Table IV: Average retention time as a function ramping rate

	Retention Times		
Length (m)	5°C/min **(%RSD)**	10°C/min **(%RSD)**	20°C/min **(%RSD)**
30	23.36 **(0.0043)**	13.99 **(0.0109)**	8.59 **(0.0067)**
15	19.93 **(0.0058)**	11.77 **(0.0049)**	6.99 **(0.0082)**
7.5	16.76 **(0.0479)**	9.89 **(0.0254)**	5.83 **(0.0099)**

Table IV clearly shows that retention time decreases with increasing temperature programming rates. Also, it shows that the reproducibility of retention times between runs is excellent with all data points having %RSD less than 0.11% for triplicate runs.

It should be noted that there were 135 different runs due to the use of three different start temperatures, three different column lengths, five linear velocities and three ramping rates on a three-component sample as shown in Table III. The standard deviation of retention time for all runs was 0.0098 with a 0.0878 %RSD.

Effect on Retention Temperature

Table V illustrates the effects of ramping rate (β) on retention temperature (T_R) along with the %RSD. Retention temperature is that temperature at which the peak maximum elutes from the column, a concept introduced by Giddings in his 1962 paper. Retention temperature is calculated from equation 16.

Table V: Retention temperature as a function of ramping rate

	Retention Temperatures		
Length (m)	5°C/min **(%RSD)**	10°C/min **(%RSD)**	20°C/min **(%RSD)**
30	151.8 **(0.003)**	174.9 **(0.009)**	206.8 **(0.006)**
15	134.6 **(0.004)**	152. 7 **(0.004)**	174.9 **(0.007)**
7.5	118.8 **(0.034)**	133.9 **(0.019)**	151.5 **(0.008)**

It can be seen from Table V that increasing the ramping rate causes the retention temperature to increase. No good explanation for this effect is known. As the length of the column decreases the retention temperature also decreases as expected. The standard deviation of retention temperature for all 135 combinations was 0.0681 with a 0.0554 %RSD. The reproducibility of retention temperature is even better than retention times with %RSD of all data points having less than 0.06% deviation.

Effect on Resolution

Table VI is a summary of resolution between tridecane and tetradecane at three different ramping rates. Resolution is another measure of column efficiency. It is a quantitative measure of the degree of separation between two adjacent peaks.[4,7] Ideally, the resolution must be equal to or greater than 1.5 to be considered baseline resolved.[1,4] Resolution can be calculated by using equation 4.

Table VI: Resolution as a function of ramping rate

	Resolution		
Length (m)	5°C/min **(%RSD)**	10°C/min **(%RSD)**	20°C/min **(%RSD)**
30	33.0 **(0.326)**	22.5 **(0.007)**	13.6 **(0.074)**
15	28.6 **(1.590)**	20.7 **(0.206)**	13.4 **(0.110)**
7.5	22.9 **(0.281)**	17.8 **(0.575)**	12.4 **(0.730)**

Table VI shows that resolution decreases with increasing ramping rate. As the length of the column decreases, the resolution also decreases. Although the resolution (R) does decrease the R values are still well above the 1.5 value for ideal baseline resolution. The compromise between analysis time and resolution is a major factor in determining the ramping rate. The standard deviation of resolution for all 135 combinations was 0.1685 with a 0.7160 %RSD. This is still good reproducibility with %RSD less than 1%.

Length Effects

In isothermal operation both retention time and plate number are directly proportional to the length of the column as mentioned previously. The longer the column, the longer the retention time and the higher the plate count. Thus, if column length is doubled, then both retention time and plate number will double. However, resolution is only proportional to the square root of length. The square root of two, for doubling the length of the column, equals 1.41 and only leads to a

41% increase in resolution. It should be noted that these relationships only hold true for isothermal separations.

Effect on Retention Time

Figure 14 shows the retention time as a function of length for three different ramping rates for three columns of different length. The red line indicates the linear relationship between retention time and column length for an isothermal run. The data plotted represents the average retention times of all 135 combination runs (See Table III) for the three ramping rates and column lengths.

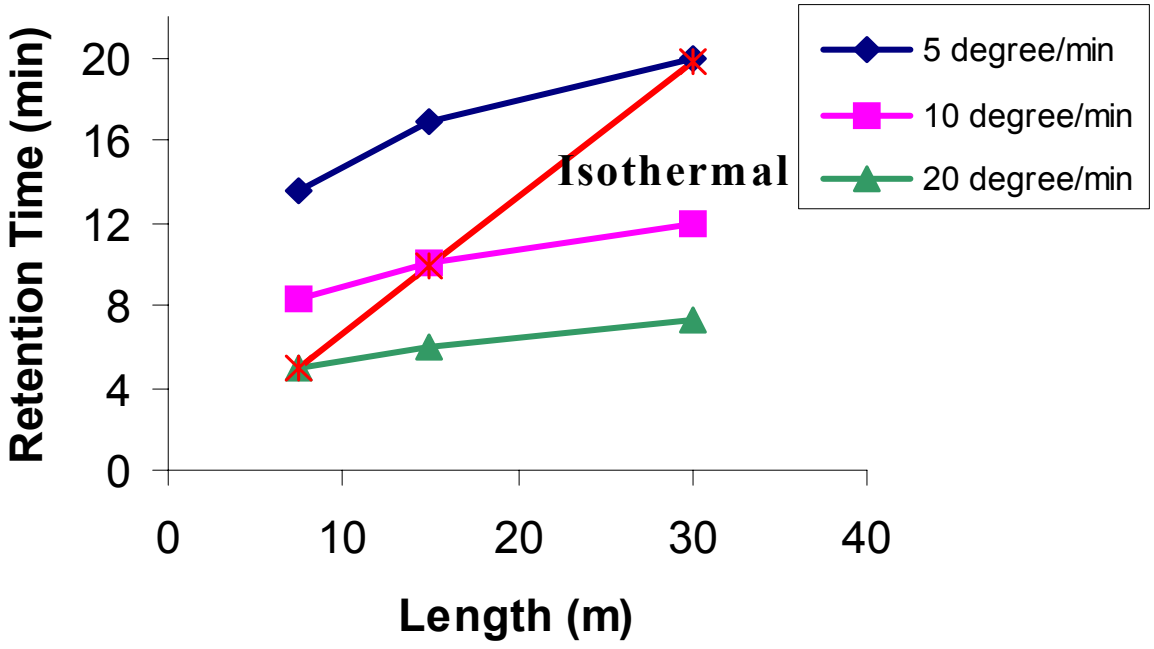

Figure 14: Retention time as a function of column length

The length of the column seems to show little effect on the retention times at higher ramping rates as predicted by Giddings. As the temperature begins to

reach isothermal conditions (i.e. 5°C/min) the slope of the line increases and comes closer to the linear isothermal relationship. Thus the dependence of T_R on length depends on the rate of temperature programming.

Effect on Retention Temperature

As seen previously, Giddings peak migration model demonstrates that 88% of migration occurs in the last 90°C before T_R and leads to a third assumption made by Giddings that the length of the column is a secondary factor in determining chromatographic results. If the column length were doubled the analytes would essentially be blown through the new length at a high temeprature, with little to no retention. Figure 15 illustrates the effects of length on the retention temperature. The data represented are the average retention temperature of tetradecane at each column length.

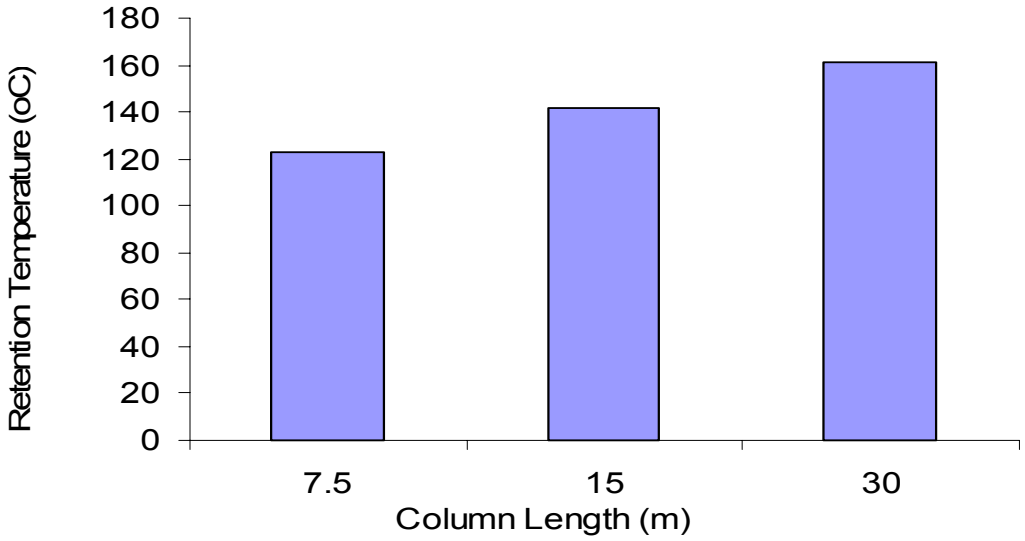

Figure 15: Retention temperature as a function of column length

The retention temperature increases as the length of the column increases but with minimal effects.

Effect on Resolution

Figure 16 illustrates resolution as a function of three different column lengths at three different ramping rates.

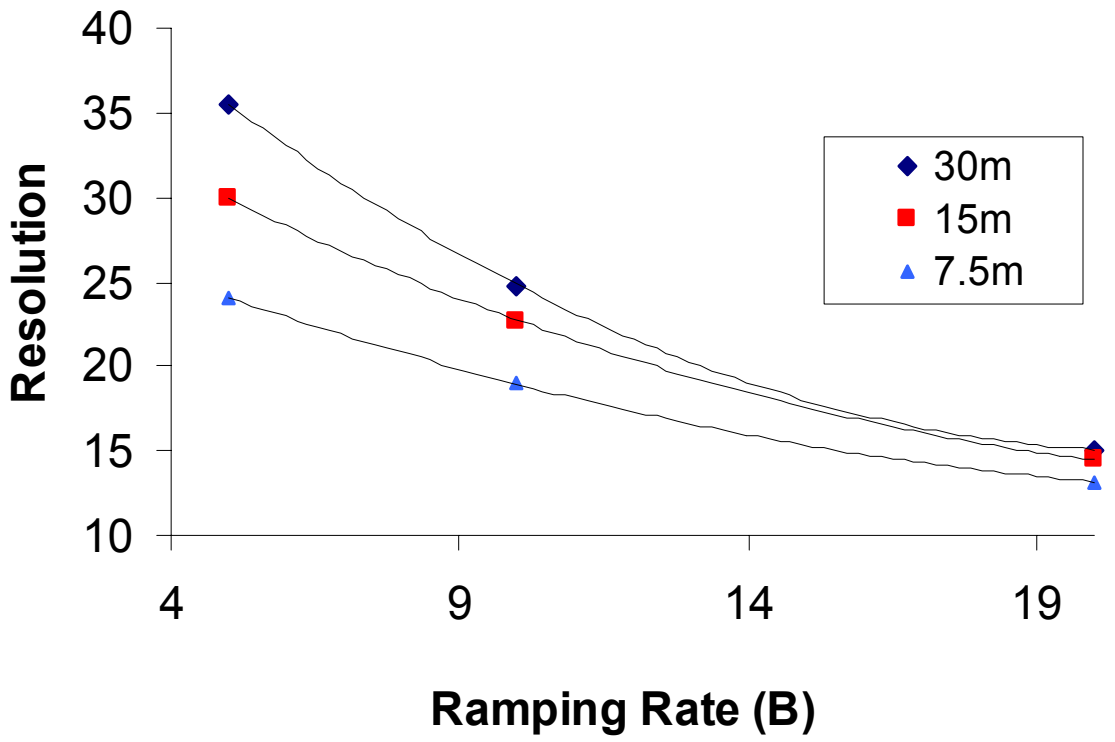

Figure 16: Resolution as a function of length at three different ramping rates

It can be seen from Figure 16 that resolution decreases with decreasing column length as well as with increasing temperature programming. This is illustrated by the 5^{o}C/min data. At ramping rates that approach isothermal conditions the length of the column plays a larger role in the resolution between tridecane and

tetradecane. However, as the ramping rate increases it can also be seen that all three columns have nearly the same resolution for the peak pairs. The compromise between analysis time and resolution will be a major factor in determining the ramping rate and the length of the column.

Linear Velocity Effects

Linear velocity is the mobile phase velocity through the column which can be calculated from the length of the column divided by the retention time of a nonretained peak as shown in equation 4. A sample chromatogram illustrates that increase in linear velocity decreases retention time.

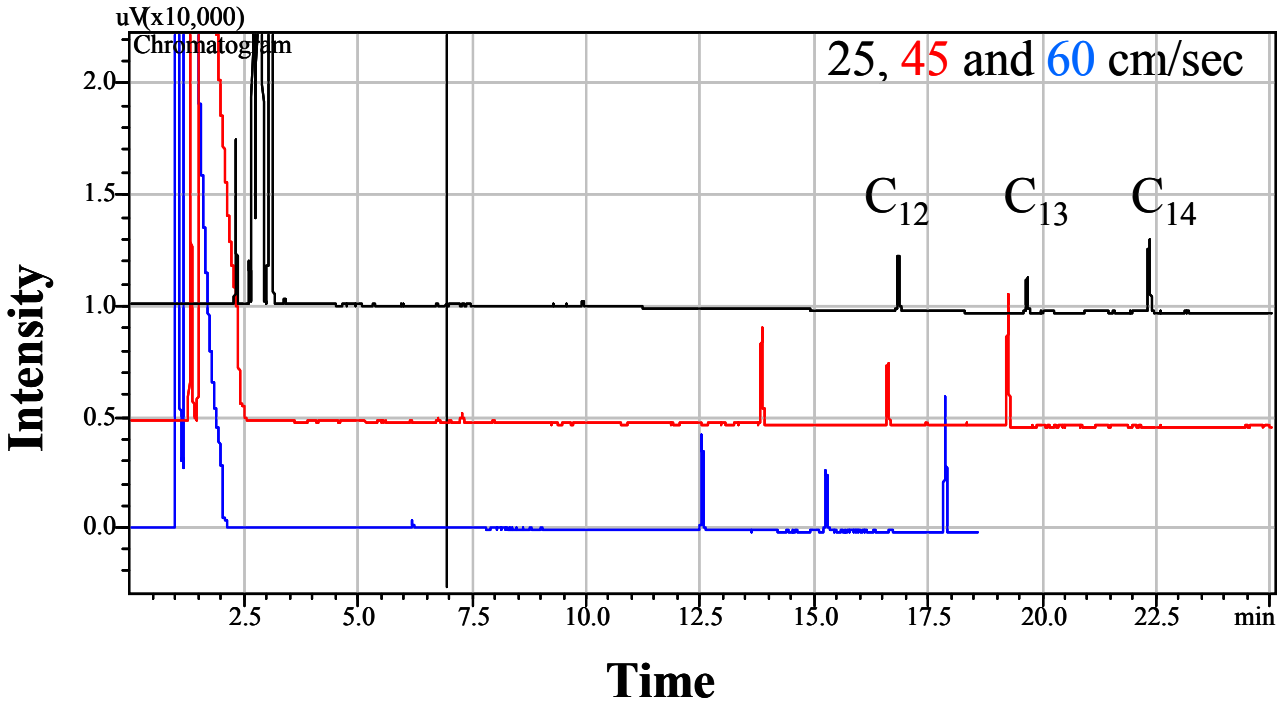

Figure 17: Sample Chromatogram of separation on the 30m column at 5°C/min

Effects on Retention Time

Figure 18 illustrates the change in retention time at five different linear velocities and three different ramping rates on the 30 meter column.

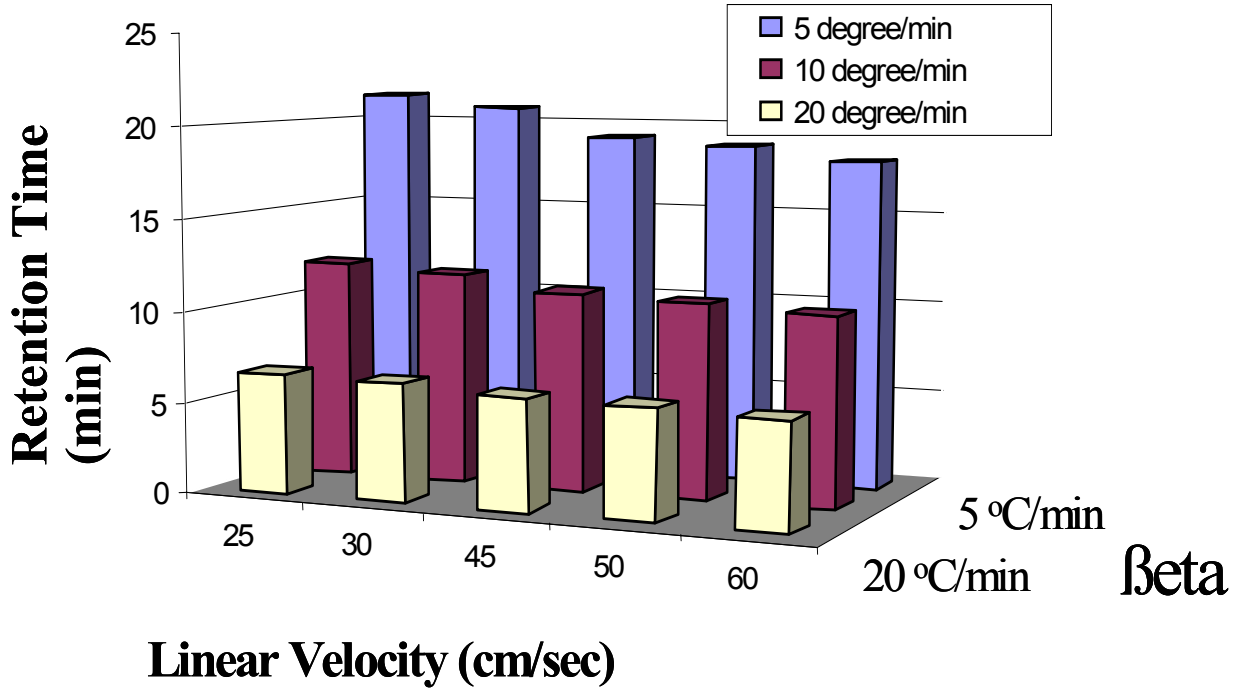

Figure 18: Retention time as function of linear velocity

At 5°C/min or close to isothermal conditions it can be seen in Figure 18 that as the linear velocity of the mobile phase increases that the retention time of tetradecane decreases. As the ramping rate increases the retention times become similar at any of the five linear velocities. At 20°C/min ramping rate there is little to no effect on retention time when the linear velocity is increased, a

phenomenia predicted by Giddings. The same trend is seen for the 15 and 7.5 meter columns as well.

Effects on Resolution

The effects of linear velocity on resolution are illustrated in Figure 20, where the average resolution is plotted at each linear velocity and at each of the three ramping rates.

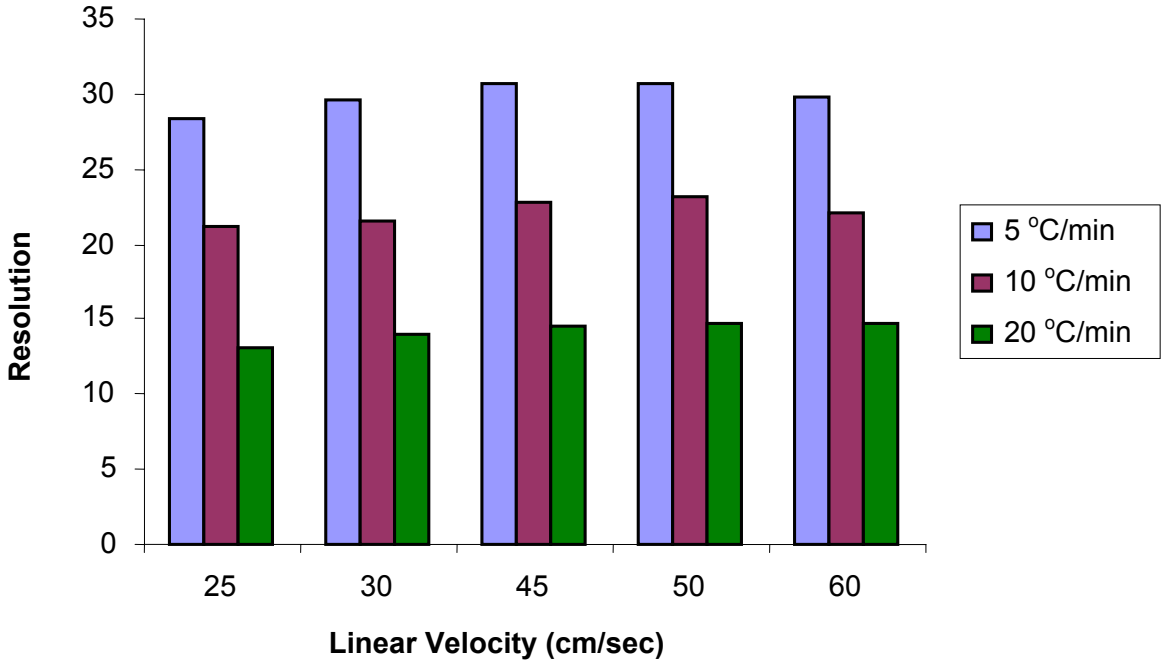

Figure 19: Resolution as a function of linear velocity

Figure 19 shows that at 5°C/min ramping rate the resolution decreases before and after the optimum linear velocity, classical Golay equation behavior. As the linear velocity increases the resolution increases and once past the optimum linear velocity the resolution begins to decrease. However, as the ramping rate

increase the resolution levels off and is practically the same for the entire range of linear velocities tested.

Statistical Analysis

Statistical analysis was performed using SAS statistical analysis software. Three replicates of the experiment were run at each combination of five levels of linear velocities, three initial temperatures, three column lengths and three ramping rates. This led to 405 observations that were used in an analysis of variance (ANOVA) model. Each of these four factors and select interactions between them were considered.

Retention Times

An ANOVA model for retention time was run on the four factors (linear velocity (μ), start temperature, column length (L) and ramping rate (β)) and three interactions (μ *β; L * β;μ*L). ANOVA is used to test the null hypotheses (H_o) that the mean retention times at each level of the factor or interaction being considered are equal to one another while the alternative hypothesis (H_A) states they are not equal. Each of these hypotheses is tested assuming that all other factors are held constant. The significance level α was 0.01. This is the probability of falsely rejecting H_0.[23]

The p values for each factor and interaction were less than 0.0001 showing that all factors and their interactions are statistically significant for retention time and should be retained in a model for retention time.

Effects of Linear Velocity

As the ANOVA tests indicated that the mean retention time was not the same for each level of the four factors considered, we used Bonferroni (Dunn) intervals to determine which levels of a factor were different in their mean retention times. All tests were run with an alpha value of 0.01. Bonferroni (Dunn) intervals are used for multiple comparison tests of the null and alternative hypothesis. Levels of a factor with the same Bonferroni grouping (indicated by letters in the SAS output) have mean retention times that are not significantly different at this alpha level.

The Bonferroni intervals for mean retention time (t_R) at each linear velocity (μ) show that there is a difference in mean retention time for each of the linear velocity and that higher retention times occur at lower linear velocities. The minimum significant difference (MSD) was 0.3031, which is the smallest difference in mean retention time that results in different Bon groupings.

Effects of Column Length and Ramping Rate

Separate comparisons of t_R over column lengths and ramping rates each resulted in minimum significant differences of 0.2091. Each column length considered was significantly different from the others in mean retention time. A similar result is true for ramping rate. Bonferroni tests showed that as column length increased the mean retention time increased. The tests also showed that as ramping rate increased the mean retention times decreased.

Retention Temperatures

An ANOVA model was performed with retention temperature as the response and linear velocity (μ), column length (L) and their interaction (μ*L) as factors. The null hypothesis for each factor, $H_{o,}$ in this case states that mean retention temperature over each factor level are equal to one another while the alternative hypothesis (H_A) states they are not equal. For the interaction term, H_o states that there is no interaction between μ and L and the alternative states there is an interaction. If this interaction is present (the null is rejected), the effect of changing column lengths on mean retention temperature is different when different linear velocities are used. The significance level,α, was 0.01.

The p values for the 2 factors considered were each less than 0.0001 showing linear velocity and column length should be retained in the model. The interaction had a p value of 0.9127 suggesting that the interaction between μ and L is not significant and can be removed from further analysis. This means that the effect of changing column lengths on mean retention temperature is the same at every linear velocity considered.

Effects of Linear Velocity and Column Length

Bonferroni (Dunn) intervals separately comparing the effects of linear velocity and column lengths on mean retention temperature were run with an alpha value of 0.01 resulting in minimum significant differences of 8.3459 and 5.7583 respectively. Linear velocities 25-30 cm/sec are not significantly different in their effect on mean retention temperature and resulted in higher mean retention

temperatures than linear velocities 45-60cm/sec, which were not significantly different from each other. The tests show that each column length results in a statistically different mean retention temperature and that longer columns have higher mean retention temperatures.

Resolution

An ANOVA model with resolution as the response and linear velocity (μ), start temperature, column length (L) and ramping rate (β) and three interactions (μ *β; L*β;μ*L) as factors, was run. The significance level,α, was 0.01. The p values were each less than 0.0001 with the exception of start temperature (p value = 0.7626) and a borderline p value of 0.0104 for the interaction of linear velocity and ramping rate. Therefore all factors except start temperature as well as the three interactions are statistically significant for resolution.

Effects of Linear Velocity

Bonferroni (Dunn) intervals for mean resolution over the considered levels of linear velocity resulted in non-significantly different mean resolution for linear velocities 30-60 cm/sec. However the mean resolution for 25 cm/sec was significantly different (lower than the others).

Effects of Column Length

Bonferroni (Dunn) intervals grouped each column length separately due to the difference in mean resolution (MSD = 0.3271). The tests show that longer columns had higher resolutions between tridecane and tetradecane.

Effects of Ramping Rate

Bonferroni (Dunn) intervals grouped each ramping rate separately due to the difference in mean resolution (MSD = 0.3271). Higher resolution resulted when using lower ramping rates.

Statistical analysis shows that all factors (ramping rate, column length, ramping rate and start temperature) are statistically significant for retention time, retention temperature and resolution including the selected interactions. However, start temperature is not significant in modeling mean resolution values.

Quantitative Analysis

Figure 21 illustrates the difference in area %RSD for hydrocarbons dodecane, tridecane and tetradecane when run isothermally and with a 20^{o}C/min temperature program.

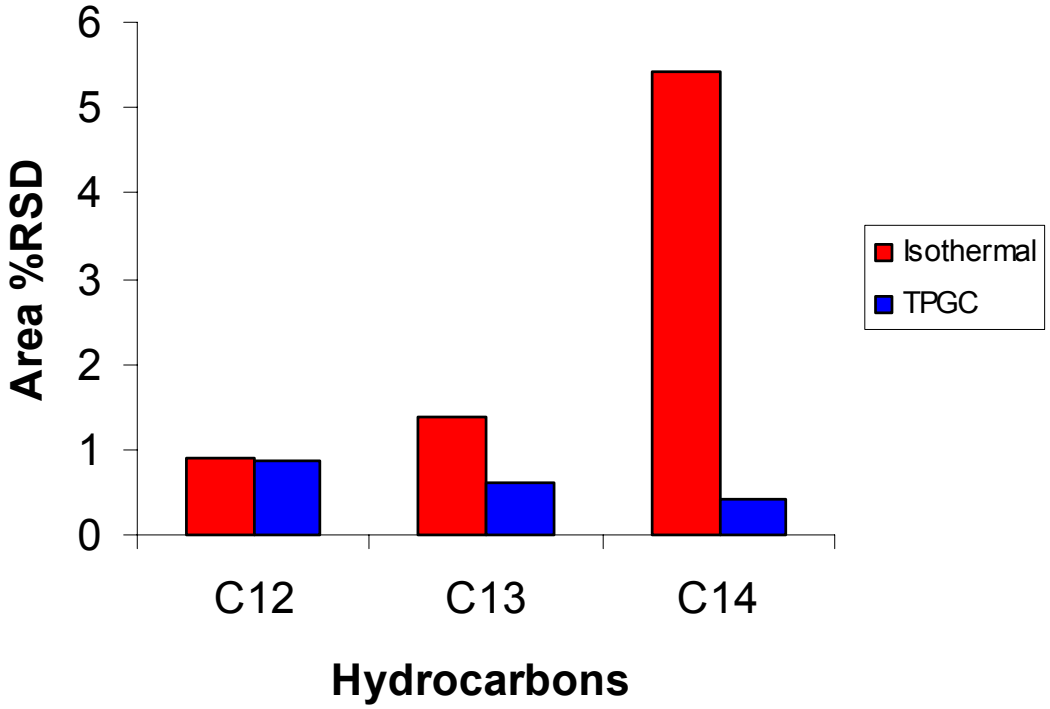

Figure 20: Area %RSD of Isothermal vs. TPGC

Temperature programming leads to improved detection limits and precision for late eluting peaks. In TPGC the late eluting peaks actually reach temperatures high enough to migrate from the head of the column and thus decrease the amount of band broadening previously seen in the isothermal separation. For this reason late eluting peaks are shaper, leading to better detection limits and precision, as illustrated in Figure 21. The area %RSD for the temperature programmed runs were all under 0.9% with the lowest being 0.42% for tetradecane. The %RSD for tetradecane when run isothermal was 5.4%, which is a significant difference. Implementing a temperature program leads to better precision for late eluting peaks.

Chapter 5 Conclusions

Retention Time Effects

Beta Effects on Retention Time

Temperature programming refers to the temperature of the column oven increasing during the chromatographic analysis. As the column temperature increases the analytes vapor pressure increase and thus spend less time in the stationary phase. These results in a reduced retention time than if run at a lower temperature. Using faster ramping rates will cause analytes to reach higher vapor pressures faster and thus elute peaks at even earlier retention times. All data confirmed that retention time decreased with an increasing temperature programming rate.

Length Effects on Retention Time

For an isothermal run, the retention time is directly proportional to the length of the column. However, for temperature programmed runs, the length of the column is a minor contributor to retention time at higher ramping rates as shown in Figure 14. That Figure shows the retention time as a function of length for three different ramping rates for three columns of different length. The length of the column seems to show little effect on the retention times at higher ramping rates. As the temperatures begin to reach isothermal conditions (i.e. 5^{o}C/min) the slope of the line increased and comes closer to the linear isothermal relationship.

Linear Velocity Effects on Retention Time

Average Linear velocity is the mobile phase velocity through the column which can be calculated from the length of the column divided by the retention time of a nonretained peak as shown in equation 4. A sample chromatogram illustrates that increase in linear velocity decreased retention time. Figure 18 illustrates the change in retention time at five different linear velocities and three different ramping rates on the 30 meter column. At 5^{o}C/min or close to isothermal conditions it can be seen that as the linear velocity of the mobile phase increases that the retention time of tetradecane decreases. As the ramping rate increased the retention times became similar at any of the five linear velocities. At 20^{o}C/min ramping rate there is little to no effect on retention time when the linear velocity is increased (see Figure 20) as predicted by Giddings. The same trend is seen for the 15 and 7.5 meter columns as well.

Retention Temperature Effects

Beta Effects on Retention Temperature

Retention temperature is that temperature at which the peak maximum elutes from the column. Retention temperature is calculated from equation 16. This equation illustrated the direct relationship between retention temperature and the ramping rate beta. Increasing the ramping rate resulted in an increase in retention temperature. With faster ramping rates, higher temperatures are reached in shorter periods of time thus causing components to elute at higher retention temperatures when elution occurs. Table V illustrates the effects of

ramping rate (β) on retention temperature (T_R) along with the %RSD. Increasing the ramping rate increased the retention temperature.

Length Effects on Retention Temperature

The retention temperature increased as the length of the column increased but only with minimal effects. As seen previously, Giddings peak migration model demonstrated that 50% of migration occurred in the last 30°C before T_R and lead to a third assumption made by Giddings that the length of the column is a secondary factor in determining chromatographic results. If the column length were doubled in TPGC the analytes would essentially be blown through the new column length, with little additional retention as shown in Figure 10. The diagram shows that most of the migration occurred in the last three intervals; 90°C before the elution temperature. If the distance traveled in the last 30°C interval is designated by the letter "y" the last 30°C interval will be 1/2y, 1/4y, 1/8y, 1/16y. The sum of these intervals approaches two as a limit.[18] The total distance traveled is two times the distance migrated in the last 30°C interval. The analyte has migrated through 50% of the column in the last 30°C interval. Almost 88% of the migration occurred in the 90°C before the retention temperature. This demonstrated that nearly all migration occurs near T_R and leads to a third assumption made by Giddings that the length of the column is a secondary factor in determining chromatographic results.

Linear Velocity Effects on Retention Temperature

As the linear velocity of the mobile phase increased the retention temperature of the analytes decreased. At higher linear velocities, 45-60 cm/sec, the retention temperatures are similar for each column. Figure 19 illustrated that as the linear velocity of the mobile phase increased the retention temperature of the analytes decreased.

Linear velocities 25-30 cm/sec are not significantly different in their effect on mean retention temperature and resulted in higher mean retention temperatures than linear velocities 45-60cm/sec, which were not significantly different from each other.

Resolution Effects

Beta Effects on Resolution

Resolution is another measure of column efficiency. Ideally, the resolution must be equal to or greater then 1.5 to be considered baseline resolved.[1,4] Resolution can be calculated by using equation 4. Data generated showed the resolution decreased with increasing ramping rate has illustrated in Table VI. At faster ramping rates analytes do not spend as much time in the stationary phase due to rapid movement at higher temperatures leading to decreased resolution. It can be seen from Figure 16 that resolution decreased with increasing temperature programming as well as with column length. This is illustrated by the 5^{o}C/min data. The compromise between analysis time and resolution is a major factoring in determining the ramping rate.

Length Effects on Resolution

As the length of the column decreased, the resolution also decreased. The analytes have less stationary phase to partition through leading to less resolution between analytes. Although the resolution does decrease, the R values are still well above the 1.5 value for ideal baseline resolution. It can be seen from Figure 16 that resolution decreased with decreasing column length. The resolution decreased with decreasing column length as well as with increasing temperature programming. At low ramping rates that approach isothermal conditions the length of the column plays a role in the resolution between tridecane and tetradecane. However, as the ramping rate increased data shows that all three columns have nearly the same resolution for the peak pairs. If the column length were doubled the analytes would essentially be blown through the new column, with little to no additional retention. The compromise between analysis time and resolution will be a major factor in determining the ramping rate and the length of the column.

Linear Velocity on Resolution

As the linear velocity increased the resolution increases and once past the optimum linear velocity the resolution begins to decrease as illustrated in Figure 19. However, as the ramping rate increased the resolution levels off and is practically the same for the entire range of linear velocities tested. Figure 19 shows that at 5^{o}C/min ramping rate the resolution decreased before and after the optimum linear velocity. As the linear velocity increased the resolution increased and once past the optimum linear velocity the resolution begins to decrease.

Quantitative Results

Retention Time

The reproducibility of retention times between runs is excellent with all 135 data points resulting in a standard deviation for retention time of 0.0098 minutes with a 0.0878 %RSD. This demonstrated that using different column lengths, start temperatures and linear velocities in a method will still produce reproducible retention times at various temperature programs.

Retention Temperature

The standard deviation of retention temperature for all 135 combinations was 0.0681 ^{o}C with a 0.0554 %RSD. The reproducibility of retention temperature is even better than retention times with %RSD of all data points having less than 0.06% deviation.

Resolution

The standard deviation of resolution for all 135 combinations was 0.1685 with a 0.7160 %RSD. This is still good reproducibility with %RSD less than 1% deviations.

Isothermal vs. TPGC

Quantitative analyses were better when a temperature program were employed versus running isothermally. The area %RSD for the temperature programmed runs were all under 0.9% with the lowest being 0.42% for tetradecane. The %RSD for tetradecane when run isothermal was 5.4%, which is a significant

difference. Implementing a temperature program lead to much better precision for late eluting peaks.

Summary

The purpose of this research was to investigate, as speculated by Giddings, if temperature programming rate (β) is the dominant contribution in determining fundamental chromatographic values, such as retention time, retention temperature and resolution. As Giddings speculated the ramping rate is the major factor in determining chromatographic values, particularly at high ramping rates as was common in 1962. All data confirmed that increasing temperature programming rate decreased retention time and resolution with an increase in retention temperature.

The length of the column seemed to show little effect on the retention times at higher ramping rates. The length of the column was a minor contributor to retention time at higher ramping rates as shown in Figure 14. The length of the column only affected retention times, retention temperatures and resolution when the ramping rates approached isothermal conditions. At high higher ramping rates the length of the column had minimal effects on any of the chromatographic values.

As the linear velocity of the mobile phase increased the retention temperature of the analytes decreased. Data showed that as the ramping rate increased the retention times and retention temperatures become similar at any of the five linear velocities showed very little effect on chromatographic values. As the linear velocity increased the resolution increased and once past the optimum

linear velocity the resolution began to decrease. However, as the ramping rate increased the resolution levels off and is practically the same for the entire range of linear velocities tested.

Statistical analysis showed that all factors (ramping rate, column length, ramping rate and start temperature) were statistically significant for retention time, retention temperature and resolution including the selected interactions. However, start temperature is not significant in modeling mean resolution values.

Appendix 1

Analysis of Retention Time 1
RTime=Retention Time,FR=FlowRate,Beta=Ramping Rate,PipeL=Pipe Length,ST=Start Time
GLM 10:38 Thursday, October 31, 2002

The GLM Procedure

Class Level Information

Class	Levels	Values
FR	5	25 30 45 50 60
ST	3	35 40 45
PipeL	3	7.5 15 30
Beta	3	5 10 20
rep	3	1 2 3

Number of observations 405

NOTE: Due to missing values, only 404 observations can be used in this analysis.

Analysis of Retention Time 2
RTime=Retention Time,FR=FlowRate,Beta=Ramping Rate,PipeL=Pipe Length,ST=Start Time
GLM 10:38 Thursday, October 31, 2002
The GLM Procedure

Dependent Variable: Rtime

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	30	9811.421503	327.047383	969.26	<.0001
Error	373	125.857238	0.337419		
Corrected Total	403	9937.278741			

R-Square	Coeff Var	Root MSE	Rtime Mean
0.987335	5.280781	0.580878	10.99984

Source	DF	Type I SS	Mean Square	F Value	Pr > F
FR	4	391.446183	97.861546	290.03	<.0001
ST	2	99.986366	49.993183	148.16	<.0001
PipeL	2	1118.988303	559.494152	1658.16	<.0001
Beta	2	7938.275042	3969.137521	11763.2	<.0001
PipeL*Beta	4	186.303104	46.575776	138.04	<.0001
FR*PipeL	8	7.993370	0.999171	2.96	0.0032
FR*Beta	8	68.429136	8.553642	25.35	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FR	4	395.325215	98.831304	292.90	<.0001
ST	2	99.544805	49.772403	147.51	<.0001
PipeL	2	1134.252282	567.126141	1680.78	<.0001
Beta	2	7925.154699	3962.577350	11743.8	<.0001
PipeL*Beta	4	186.746580	46.686645	138.36	<.0001
FR*PipeL	8	8.026364	1.003296	2.97	0.0031
FR*Beta	8	68.429136	8.553642	25.35	<.0001

The GLM Procedure

Bonferroni (Dunn) t Tests for Rtime

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	373
Error Mean Square	0.337419
Critical Value of t	3.31680
Minimum Significant Difference	0.3031
Harmonic Mean of Cell Sizes	80.798

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	FR
A	12.42316	81	25
B	11.84648	81	30
C	10.69186	80	45
D	10.19616	81	50
E	9.83775	81	60

The GLM Procedure

Bonferroni (Dunn) t Tests for Rtime

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	373
Error Mean Square	0.337419
Critical Value of t	2.95423
Minimum Significant Difference	0.2091
Harmonic Mean of Cell Sizes	134.665

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	ST
A	11.57999	135	35
B	11.05369	134	40
C	10.36625	135	45

The GLM Procedure

Bonferroni (Dunn) t Tests for Rtime

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	373
Error Mean Square	0.337419
Critical Value of t	2.95423
Minimum Significant Difference	0.2091
Harmonic Mean of Cell Sizes	134.665

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Pipe L
A	13.05164	135	30
B	10.95776	135	15
C	8.97513	134	7.5

The GLM Procedure

Bonferroni (Dunn) t Tests for Rtime

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	373
Error Mean Square	0.337419
Critical Value of t	2.95423
Minimum Significant Difference	0.2091
Harmonic Mean of Cell Sizes	134.665

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Beta
A	16.80274	135	5
B	10.09096	135	10
C	6.06931	134	20

The GLM Procedure

Class Level Information

Class	Levels	Values
FR	5	25 30 45 50 60
PipeL	3	7.5 15 30
rep	3	1 2 3

Number of observations 405

NOTE: Due to missing values, only 404 observations can be used in this analysis.

The GLM Procedure

Dependent Variable: RTemp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	134230.0726	9587.8623	37.46	<.0001
Error	389	99565.4675	255.9524		
Corrected Total	403	233795.5401			

R-Square	Coeff Var	Root MSE	RTemp Mean
0.574134	11.26210	15.99851	142.0562

Source	DF	Type I SS	Mean Square	F Value	Pr > F
FR	4	34068.68286	8517.17071	33.28	<.0001
PipeL	2	99314.43378	49657.21689	194.01	<.0001
FR*PipeL	8	846.95595	105.86949	0.41	0.9127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FR	4	34203.72737	8550.93184	33.41	<.0001
PipeL	2	99285.01331	49642.50665	193.95	<.0001
FR*PipeL	8	846.95595	105.86949	0.41	0.9127

The GLM Procedure

Bonferroni (Dunn) t Tests for RTemp

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	389
Error Mean Square	255.9524
Critical Value of t	3.31571
Minimum Significant Difference	8.3459
Harmonic Mean of Cell Sizes	80.798

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	FR
A	155.479	81	25
A			
A	149.953	81	30
B	138.457	80	45
B			
B	135.111	81	50
B			
B	131.238	81	60

The GLM Procedure

Bonferroni (Dunn) t Tests for RTemp

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	389
Error Mean Square	255.9524
Critical Value of t	2.95344
Minimum Significant Difference	5.7583
Harmonic Mean of Cell Sizes	134.665

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Pipe L
A	161.497	135	30
B	141.420	135	15
C	123.111	134	7.5

The GLM Procedure

Class Level Information

Class	Levels	Values
FR	5	25 30 45 50 60
PipeL	3	7.5 15 30
rep	3	1 2 3
Beta	3	5 10 20
ST	3	35 40 45

Number of observations 405

NOTE: Due to missing values, only 404 observations can be used in this analysis.

The GLM Procedure

Dependent Variable: RTemp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	133383.1166	22230.5194	87.89	<.0001
Error	397	100412.4234	252.9280		
Corrected Total	403	233795.5401			

R-Square	Coeff Var	Root MSE	RTemp Mean
0.570512	11.19536	15.90371	142.0562

Source	DF	Type I SS	Mean Square	F Value	Pr > F
FR	4	34068.68286	8517.17071	33.67	<.0001
PipeL	2	99314.43378	49657.21689	196.33	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FR	4	34208.39538	8552.09885	33.81	<.0001
PipeL	2	99314.43378	49657.21689	196.33	<.0001

The GLM Procedure

Bonferroni (Dunn) t Tests for RTemp

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	397
Error Mean Square	252.928
Critical Value of t	3.31520
Minimum Significant Difference	8.2951
Harmonic Mean of Cell Sizes	80.798

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	FR
A	155.479	81	25
A			
A	149.953	81	30
B	138.457	80	45
B			
B	135.111	81	50
B			
B	131.238	81	60

The GLM Procedure

Bonferroni (Dunn) t Tests for RTemp

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	397
Error Mean Square	252.928
Critical Value of t	2.95307
Minimum Significant Difference	5.7235
Harmonic Mean of Cell Sizes	134.665

NOTE: Cell sizes are not equal.
Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Pipe L
A	161.497	135	30
B	141.420	135	15
C	123.111	134	7.5

The GLM Procedure

Class Level Information

Class	Levels	Values
FR	5	25 30 45 50 60
ST	3	35 40 45
PipeL	3	7.5 15 30
Beta	3	5 10 20
rep	3	1 2 3

Number of observations 405

The GLM Procedure

Dependent Variable: Re

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	20437.85795	928.99354	1082.78	<.0001
Error	382	327.74342	0.85797		
Corrected Total	404	20765.60136			

R-Square	Coeff Var	Root MSE	Re Mean
0.984217	4.197057	0.926265	22.06940

Source	DF	Type I SS	Mean Square	F Value	Pr > F
FR	4	198.19453	49.54863	57.75	<.0001
ST	2	0.45084	0.22542	0.26	0.7691
PipeL	2	2775.44711	1387.72355	1617.46	<.0001
Beta	2	16356.44633	8178.22317	9532.09	<.0001
PipeL*Beta	4	1047.83236	261.95809	305.32	<.0001
FR*PipeL	8	59.48678	7.43585	8.67	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FR	4	198.19453	49.54863	57.75	<.0001
ST	2	0.45084	0.22542	0.26	0.7691
PipeL	2	2775.44711	1387.72355	1617.46	<.0001
Beta	2	16356.44633	8178.22317	9532.09	<.0001
PipeL*Beta	4	1047.83236	261.95809	305.32	<.0001
FR*PipeL	8	59.48678	7.43585	8.67	<.0001

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	382
Error Mean Square	0.857967
Critical Value of t	3.31618
Minimum Significant Difference	0.4827

Means with the same letter are not significantly different.

Bon Grouping		Mean	N	FR
	A	22.8561	81	50
	A			
B	A	22.6584	81	45
B				
B	C	22.1889	81	60
	C			
	C	21.7395	81	30
	D	20.9041	81	25

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	382
Error Mean Square	0.857967
Critical Value of t	2.95378
Minimum Significant Difference	0.333

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	ST
A	22.1147	135	35
A			
A	22.0580	135	40
A			
A	22.0354	135	45

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	382
Error Mean Square	0.857967
Critical Value of t	2.95378
Minimum Significant Difference	0.333

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Pipe L
A	25.1264	135	30
B	22.3493	135	15
C	18.7325	135	7.5

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	382
Error Mean Square	0.857967
Critical Value of t	2.95378
Minimum Significant Difference	0.333

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Beta
A	29.8095	135	5
B	22.1550	135	10
C	14.2437	135	20

The GLM Procedure

Class Level Information

Class	Levels	Values
FR	5	25 30 45 50 60
PipeL	3	7.5 15 30
Beta	3	5 10 20
rep	3	1 2 3
ST	3	35 40 45

Number of observations 405

The GLM Procedure

Dependent Variable: Re

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	20437.40711	1021.87036	1195.63	<.0001
Error	384	328.19426	0.85467		
Corrected Total	404	20765.60136			

R-Square	Coeff Var	Root MSE	Re Mean
0.984195	4.188991	0.924485	22.06940

Source	DF	Type I SS	Mean Square	F Value	Pr > F
FR	4	198.19453	49.54863	57.97	<.0001
PipeL	2	2775.44711	1387.72355	1623.69	<.0001
Beta	2	16356.44633	8178.22317	9568.84	<.0001
PipeL*Beta	4	1047.83236	261.95809	306.50	<.0001
FR*PipeL	8	59.48678	7.43585	8.70	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FR	4	198.19453	49.54863	57.97	<.0001
PipeL	2	2775.44711	1387.72355	1623.69	<.0001
Beta	2	16356.44633	8178.22317	9568.84	<.0001
PipeL*Beta	4	1047.83236	261.95809	306.50	<.0001
FR*PipeL	8	59.48678	7.43585	8.70	<.0001

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	384
Error Mean Square	0.854673
Critical Value of t	3.31604
Minimum Significant Difference	0.4817

Means with the same letter are not significantly different.

Bon Grouping		Mean	N	FR
	A	22.8561	81	50
	A			
B	A	22.6584	81	45
B				
B	C	22.1889	81	60
	C			
	C	21.7395	81	30
	D	20.9041	81	25

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	384
Error Mean Square	0.854673
Critical Value of t	2.95368
Minimum Significant Difference	0.3324

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Pipe L
A	25.1264	135	30
B	22.3493	135	15
C	18.7325	135	7.5

The GLM Procedure

Bonferroni (Dunn) t Tests for Re

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher
Type II error rate than REGWQ.

Alpha	0.01
Error Degrees of Freedom	384
Error Mean Square	0.854673
Critical Value of t	2.95368
Minimum Significant Difference	0.3324

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	Beta
A	29.8095	135	5
B	22.1550	135	10
C	14.2437	135	20

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Vita

Kari R. Urias was born in Martinsburg, WV, on September 22, 1978 to proud parents Roxanne and Elocadio Urias. Kari grew up in Hedgesville, WV, where she attended elementary, middle and high school. After graduation from high school in 1996 she attended Virginia Polytechnic and State University in Blacksburg, Va. In May 2000 she received her bachelors of Science degree in Biochemistry. During her sophomore year while in organic chemistry lab she met her future husband. She decided to further her education by pursuing a Masters degree in Chemistry under the advisement of Dr. Harold McNair. Summers were spent first in Richmond, VA at Whitehall-Robins and the second summer was spent at Shimadzu in Columbia, MD. During her time at Virginia Tech she was involved in teaching undergraduate chemistry and analytical chemistry labs. She was also involved in teaching ACS short course where she gave lectures and instructed lab work. She went back to West Virginia to plan her wedding before her move to NC where she worked in Research Triangle Park while her husband pursued his PhD in Biochemistry. They lived happily ever after.