

## THE QUANTITATIVE ANALYSIS OF BUTANOL BY GAS CHROMATOGRAPHY

**DISCUSSION:** With the introduction of gas chromatography (GC) in the late 1950's, the separation of small quantities of volatile liquids with narrowly separated boiling points has become a routine procedure. The principal advantages of gas chromatography are: 1) small sample size, and 2) high degree of separation. In addition, gas chromatography can be used quantitatively. The Shimadzu GC-8A gas chromatographic instrument used in this experiment is shown in Figure 1.

Figure 1 Shimadzu GC-8A Gas Chromatograph

Both liquid and gaseous mixtures can be analyzed in gas chromatography. The process is termed *gas chromatography* because a flow of gas is used to carry the sample through the separating column. This carrier gas is termed the **mobile phase**. The separating column is packed with an immobile solid material which is called the **stationary phase**. In this experiment helium gas is used as the mobile phase and the stationary phase is a commercial packing material sold under the name 80/10 CarboPac C, 0.1% SP-1000.

As the mixture of components to be separated is carried through the column, separation is affected because of the varying attraction each component of the mixture has for the stationary phase. Substances that are more strongly attracted to the stationary phase will be carried down the column at a slower rate than those with a lesser attraction. Care must be taken to choose an appropriate packing material to achieve the best separation. The temperature of the column and, to a lesser degree, the flow rate of the mobile phase also affect the degree of separation. The time required for a particular component of a mixture to pass from the injection port, through the column, to the detector is its **retention time**. Under identical conditions, the same substance will have the same retention time in repeated analyses. For this reason, retention time can be used qualitatively to identify a substance in a mixture.

If a quantitative determination is desired, the method of **internal standards** is normally employed. An internal standard is needed when conditions such as sample size or gas flow cannot be replicated exactly between trials. A compound is chosen to serve as an internal standard that has physical properties similar to those of the substance you wish to measure. A calibration curve is constructed in which the ratio of the peak area of the compound of interest (the analyte) to that of the internal standard is plotted on the y-axis versus the concentration of the analyte on the x-axis. The peak area ratios will be proportional to the analyte concentration, whereas the peak area of the analyte alone will not be. Peak areas are calculated by the Chromatopac<sup>®</sup> electronic integrator. A typical Chromatopac<sup>®</sup> print-out is shown in Figure 2.

#### START

<b>CHROMATOPAC GR6A</b>				<b>FILE</b>	<b>0</b>	
<b>SAMPLE NO 0</b>				<b>METHOD</b>	<b>41</b>	
<b>REPORT NO 3605</b>						
<b>PKNO</b>	<b>TIME</b>	<b>AREA</b>	<b>MK</b>	<b>INDO</b>	<b>CONC</b>	<b>NAME</b>
<b>1</b>	<b>0.438</b>	<b>1346409</b>				<b>58.312</b>
<b>2</b>	<b>1.093</b>	<b>708625</b>		<b>V</b>		<b>30.69</b>
<b>3</b>	<b>2.6</b>	<b>253940</b>		<b>V</b>		<b>10.998</b>
		-----				-----
	<b>TOTAL</b>	<b>2308974</b>				<b>100</b>

Figure 2 Typical Peak area output from the Chromatopac<sup>®</sup> Electronic Integrator

**EXPERIMENTAL  
PROCEDURE:**

There are two parts to this experiment. Part A concerns the quantitative analysis of butanol in a butanol:methanol mixture. Part B concerns the optimization of the carrier gas flow rate and the construction of a van Deemter curve.

**Part A: The Quantitation of Butanol**

Prepare a series of standards ranging from 20% to 80% butanol as indicated below using a **10 mL buret** to deliver each liquid:

standard	volume of butanol	volume of methanol
1	2.0 mL	8.0 mL
2	4.0 mL	6.0 mL
3	6.0 mL	4.0 mL
4	8.0 mL	2.0 mL

After the four standards have been prepared, add 5.0 mL of propanol to each mixture via pipet. Propanol will serve as the **internal standard** in this analysis.

$\text{CH}_3\text{OH}$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$
methanol	propanol	butanol

Swirl each mixture thoroughly to ensure complete mixing, then cover each solution to avoid evaporation.

Obtain 10.0 mL of an unknown butanol:methanol mixture from your instructor. Using a 5.0 mL pipet, add exactly 5.0 mL propanol to the unknown. Swirl the mixture thoroughly and cover to retard evaporation. Note: The volume of each unknown will be exactly 10.0 mL when issued.

**SETTING UP THE SHIMADZU GC! 8A AND CHROMATOPAC®**

You will use the Shimadzu GC-8A gas chromatograph, shown in Figure 1, in conjunction with the Chromatopac® integrator and recorder, shown in Figure 3. Steps 1 ! 6 listed below should already be completed for you by the instructor. If this is so, proceed to step 7, otherwise you will need to start with step 1.

First, open the main valve on the helium tank. This is the valve on the top of the tank itself. The reducing valve with its gauges will be correctly set and should not require adjustment. If so, you would do this in step 5.

The following adjustments are made on the GC. Refer to Figure 1 for the location of the controls:

1. Turn the power switch on.
2. Set port (INJ) temperature to 150°C.
3. Set column (INITIAL COL) temperature to 130°C.
4. Adjust PRIMARY pressure to 6 kg/cm<sup>2</sup>.
5. Adjust CARRIER GAS pressure 1 and 2 to 1 kg/cm<sup>2</sup>.
6. Set the detector current to 100 mA

The following adjustments are made on the Chromatopac<sup>®</sup> recording integrator:

Figure 3 The keyboard of the Chromatopac<sup>®</sup> Integrating Recorder

Turn on the Chromatopac<sup>®</sup> unit. The switch is located on the upper back side.

8. Press **METHOD** on the Chromatopac<sup>®</sup> unit.

9. Press "4" then "1"; then **ENTER**.
10. Press **ATTEN**, press "7", then press **ENTER**.
11. Press **PRINT**.
12. Press control (**CTRL**) and **LEVEL** simultaneously, then press **ENTER**.
13. The Chromatopac<sup>®</sup> unit will print a number indicating the background level. This number should be between ! 1000 and + 5000; if it is not, adjust the **COARSE ZERO** knob on the GC until the value is between ! 1000 and + 5000.
14. Repeat steps 11 and 12 until the background level is between ! 100 and + 5000.
15. Depress the key labeled **ZERO** (not the number "0" or the letter "o"); press **ENTER**.

You are now ready to proceed to the GC analysis.

#### THE ANALYSIS:

16. Rinse a 10.0 : L glass syringe well with the sample to be analyzed.
17. Inject 1.0 : L of the sample to be analyzed into the injection port (ask your instructor which port to use) and **simultaneously** press the **START** button on the Chromatopac<sup>®</sup> to initiate the run.
18. Each analysis will require approximately 3 minutes and will produce 3 peaks on the chromatogram. After the third component has been eluted, press **STOP**.
19. Analyze two samples of each standard mixture and use the average values to prepare the calibration curve.
20. Analyze two samples of the unknown mixture.

#### CALCULATIONS:

1. Calculate the ratio of peak area values of butanol to propanol.
2. Using Quattro Pro<sup>®</sup>, prepare a calibration curve by plotting the concentration of analyte on the x-axis versus the average of the ratios of the peak area of butanol to peak area of propanol.
3. Use Quattro Pro<sup>®</sup> to perform a regression analysis on the calibration curve .

4. Calculate the concentration butanol in the unknown sample from the regression analysis basing your calculation on the straight line equation form,  $y = m x + b$ .
5. Turn in the graph of the calibration curve, the regression analysis, the calculation of the unknown and the Report Sheet.

### Part B: The van Deemter Plot ! the Importance of Flow Rate

The various column processes that contribute to band broadening can be described in simple terms by the van Deemter equation.

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

Where:

- H = plate height (or HETP)
- A = eddy diffusion constant
- B = longitudinal or axial diffusion constant
- C = non-equilibrium mass transfer constant
- $\mu$  = flow rate of mobile phase

A plot of H versus  $\mu$  is shown below illustrating the component parts of the van Deemter equation as well as the overall or composite result (in bold).

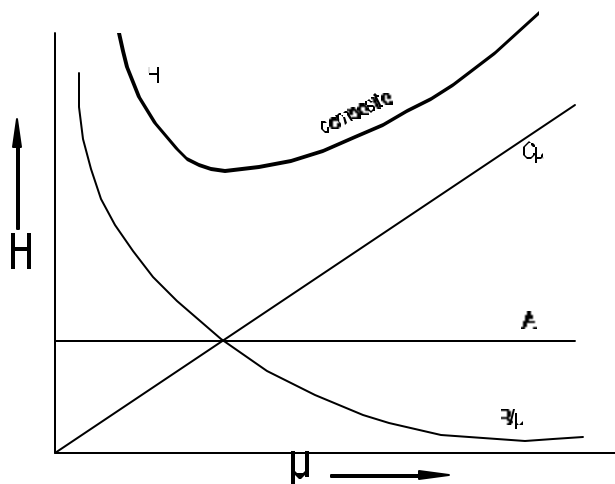


Figure 4 The contributions of eddy diffusion (A), axial diffusion (B) and non-equilibrium mass transfer (C) on plate height (H)

The minimum value of H extracted from the composite curve gives rise to the optimum flow rate to minimize band broadening.

In this portion of the experiment you will construct a van Deemter curve to determine the optimum flow rate to provide the best separation of a homologous series of alcohols.

#### CALIBRATION OF FLOW RATE

1. Adjust the carrier gas pressure valve to read  $0.25 \text{ kg/cm}^2$  on both gauges 1 and 2.
2. The bubble flow meter shown in Figure 5 should already be assembled and located near the GC. Attach the free end of the tygon tube to vent #2 on the top of the GC. Squeeze the bulb until soap bubbles come up into the buret, then slowly release the bulb and let the gas carry the soap film up the buret. When a soap bubble reaches the 0.00 mL mark start timing; stop when it reaches the 5.00 mL mark. Repeat the measurement and record the average flow rate in units of mL/min.
3. Repeat the flow rate measurement using carrier gas pressures of 0.50, 0.75, 1.00, and  $1.25 \text{ kg/cm}^2$ . Perform each measurement two times, recording the average flow rates.
4. Complete the table of **carrier gas pressure** versus **average flow rate** on the Report Sheet.

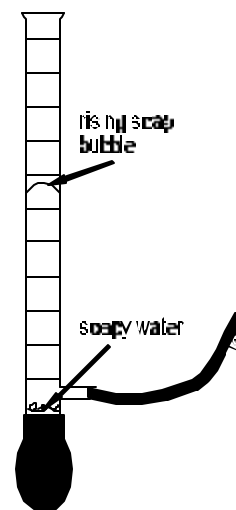


Figure 5 Carrier gas flow meter

#### CONSTRUCTING THE VAN DEEMTER PLOT

1. Obtain a bottle of propanol and the appropriate syringe from your instructor. Rinse syringe with propanol before starting injections.
2. Adjust the carrier gas pressure to  $0.25 \text{ kg/cm}^2$  on gauges 1 and 2.
3. Set **ATTENUATION** on the Chromatopac<sup>®</sup> to 7.

4. Inject 1  $\mu$ -liter of propanol into the GC while **simultaneously** pressing **START** on the Chromatopac<sup>®</sup> unit.
5. Observe the chromatogram, and once the propanol sample has eluted completely, push STOP on the Chromatopac<sup>®</sup>.
6. Repeat using four other carrier gas pressures; 0.50, 0.75, 1.00 and 1.25 kg/cm<sup>2</sup>.
7. Using the data obtained, construct a van Deemter plot of flow rate on the abscissa versus HETP (**H**eight **E**quivalent **T**heoretical **P**late) on the ordinate. Use the flow rates measured previously for each carrier gas pressure.

The following equations will be useful:

$$\text{HETP} = \frac{L}{N} \quad \text{and} \quad N = 16 \left( \frac{T_r}{W} \right)^2$$

Where **L** is the length of the column ( 2 meters );  
**T<sub>r</sub>** is the retention time (given by Chromatopac<sup>®</sup> printout);  
 and **W** is the width of the peak at the base line, measured from the printout. Make certain you convert **W** into units of time. This can be obtained by knowing the chart speed (which is usually set at 1.0 cm/min).

8. Find the lowest HETP value in your plot, and use the corresponding flow rate pressure as your **optimum flow rate** for the remainder of the experiment.

#### CHROMATOGRAM OF A HOMOLOGOUS SERIES OF C<sub>1</sub> TO C<sub>5</sub> ALCOHOLS

1. Adjust the carrier gas pressure to obtain the optimum flow rate obtained from the van Deemter plot.
2. Rinse the GC syringe and inject 1 microliter of the mixture obtained from the instructor, while **simultaneously** pressing **START** on the Chromatopac<sup>®</sup> unit.
3. After all five alcohols have eluted, press STOP.
4. Use the plotting feature of Quattro-Pro<sup>®</sup> (see Appendix A! 4) to construct a plot of "Log of Retention Time" on the ordinate versus the "Number of Carbon Atoms" in the alcohols on the abscissa.

**REPORT SHEET:****THE QUANTITATIVE ANALYSIS  
OF BUTANOL BY  
GAS CHROMATOGRAPHY**Name \_\_\_\_\_ Date: \_\_\_\_\_ Sample No.: \_\_\_\_\_  
Please print; last name firstName \_\_\_\_\_  
Please print; last name first**Part A: The Quantitation of Butanol**

%(v/v) Butanol	Butanol peak area	Propanol peak area	Butanol/Propanol Ratio	Average Ratio
20.00%	1) _____ 2) _____	1) _____ 2) _____	1) _____ 2) _____	
40.00 %	1) _____ 2) _____	1) _____ 2) _____	1) _____ 2) _____	
60.00 %	1) _____ 2) _____	1) _____ 2) _____	1) _____ 2) _____	
80.00 %	1) _____ 2) _____	1) _____ 2) _____	1) _____ 2) _____	
unknown	1) _____ 2) _____	1) _____ 2) _____	1) _____ 2) _____	

Y intercept = \_\_\_\_\_ R-squared = \_\_\_\_\_ Slope = \_\_\_\_\_

%(v/v)-Butanol in the unknown: \_\_\_\_\_ (State to three significant figures.)

Show all calculations clearly on the back side of this page.

**Part B: The van Deemter Plot ! the Importance of Flow Rate**

CALIBRATION OF FLOW RATE	
Carrier Gas Pressure	Average Flow Rate
0.50 kg/cm <sup>2</sup>	
0.75 kg/cm <sup>2</sup>	
1.00 kg/cm <sup>2</sup>	
1.25 kg/cm <sup>2</sup>	

CONSTRUCTING THE VAN DEEMTER PLOT
<p>Attach the van Deemter plot of "flow rate" versus HETP to this Reportsheet.</p> <p>Optimum flow rate obtained from the van Deemter plot: _____</p>

CHROMATOGRAM OF THE C <sub>1</sub> TO C <sub>5</sub> ALCOHOLS
<p>Attach the plot of "Log of Retention Time" versus "Number of Carbon Atoms" to this Report Sheet.</p> <p>Write your interpretation of the plot on the back of this page.</p>