Experiment V. Separation of an unknown two-component mixture by distillation and identification of the components by GC analysis.


Topics and Techniques
i) methods, techniques and separation of liquids using the process of distillation
ii) gas chromatography, relative response factors and analysis
iii) monitoring separation of distillate fractions by gas chromatography

The experiment (overview)
In this experiment, your TA will assign students to pairs. Each couple will be given 100 mL of an "unknown two component mixture". One student will perform a fractional distillation of the unknown mixture (50 mL) and the second student will perform a simple distillation of the unknown mixture (50 mL). Each distillation will generate four 10 mL fractions to be analyzed using gas chromatography for mole fraction composition. In order to analyze the mixture each couple will have to obtain relative response factors from a "standard" of four-component known mixture. Employing these relative response factors to the results of the unknown mixture will give the amount of each component.

Approximate boiling points of the low and high boiling components comprising the "two-component unknown mixture" can be obtained from the fractional distillation data and/or gas chromatography analysis. These data will permit the students to identify the two unknowns comprising their "unknown mixture". The names of the students must be written on the first page of their laboratory experiment in each student laboratory notebook. Note: you work, collect and share data as a team, but each student writes his/her own laboratory report. It is each student's responsibility to obtain all laboratory data, so make arrangements (i.e.; immediately at the end of the laboratory) to have all laboratory data including photocopied gas chromatograms.

Liquid unknowns
Your "unknown two-component mixture" will be a mixture of two of the four hydrocarbons:
- n-Hexane
- Cyclohexane
- n-Heptane
- n-Octane

Safety aspects
1. Fume Hood. It is best to set up the distillation in a fume hood in order to minimize your exposure to the vapors. Because there is less room in the fume hoods than needed for the entire class, only about half of the class will be able to perform the distillation in the fume hoods. However, with only half of the operations in the fume hoods the total concentration of organic vapors in the air will be substantially reduced. When setting up in the fume hood, consider the cooling effect that air flowing through the hoods will have on the distillation.
2. Heating Closed Vessels. These distillations are performed with the collection port open to the atmosphere. Warning, be sure never to heat a closed vessel. Remember PV=nRT can have "explosive effects".
Part A: Simple Distillation

Add 50 mL of your mixture (1:1 by volume) and one or two boiling chips to a 100 mL round bottom flask. Position the flask in a cool disconnected heating mantle and clamp the flask securely. Alternatively, a sand bath can be used as a heat source and in this case, make sure that the round bottom flask is positioned deep in the sand. Connect the distillation still head to the flask.

![Diagram of distillation setup](image)

**Figure 5.1 Assembly of Simple and Fractional distillation systems.**

Connect two hoses to a water-cooled condenser, with water entry into the lower tap and water exit out the upper tap of the condenser. Attach the condenser to the distillation head (using a small amount of glycerol) and hold it securely in position with a compression cap. Also, fit a bent adapter to the end of the condenser using a compression cap to keep it in position.

Carefully position a **non-Mercury** thermometer in the thermometer adapter and join the adapter to the top of the distillation head. Make sure that the rubber thermometer adapter fits tightly over the lip of the glass thermometer adapter. Properly position the thermometer so that the bulb is efficiently positioned below the bottom of the side arm of the distillation head. **An accurate reading of the thermometer can only be determined if the bulb of the thermometer is bathed efficiently with a flow of distillation vapor during the distillation.** Use a clamped graduated cylinder as a collection flask. Start a gentle stream of water through the condenser. If a sand bath is used, place a second thermometer securely in the sand bath so that the thermometer bulb is buried below the surface of the sand. If a heating mantle is used, there is no need for the second thermometer. **Do not starts the distillation until the TA approved you setup!**

Raise the temperature of the heat source and bring the solution to a gentle boil. Maintain the rate of distillation at approximately one drop per second from the tip of the bent adapter into the
graduated cylinder. Record the temperature at distillation head vs. the volume of distillate collected every milliliter. When 10 mL of distillate is collected, collect the next 10 mL fraction in another vial. Cap the vials tightly.

As the distillation progresses, the temperature of the heat source should be adjusted upward because the liquid mixture in the distillation flask is enriched with the less volatile component.*

After you have collected a total of 40 mL, the distillation should be stopped, the heating source turned off and the equipment allowed to cool.

*) If the boiling ceases at any time, the distillation has to be restarted and one or more boiling chips must be added after cooling of the round bottom flask.

Part B: Fractional Distillation.

Fractional Distillation (overview)

With repeated simple distillation and the combination and recombination of different distillate and condensation fractions, one would eventually separate a mixture of two volatile liquids with close boiling points unless they form an azeotrope. In the end, the more volatile component would be more pure. However, multiple simple distillations would be very tedious and require a large volume of the initial liquid mixture.

Fractional distillation is a simple technique for accomplishing a large number of simple distillation/separations, in a single continuous operation. A fractionation column (fractional distillation column) has an extensive surface area for exchange of heat between ascending vapor and descending liquid (condensate). As the condensate accepts heat from the vapor at any point in the column, the condensate is partially vaporized and the vapor is enriched in the more volatile component. At the same time, as the vapor loses heat to the condensate, a part of the vapor condenses and the condensate becomes enriched in the less volatile component. Eventually, the vapor that condenses at the top of the column is pure or enriched in the more volatile more pure. Similarly, the condensate that has returned to the distillation flask is pure or enriched in the less volatile component.

The ability to separate two liquids depends on the differences in the boiling points of the two liquids as well as a number of other factors, some being the rate of the distillation, insulation of the column and on how efficient the column is. In order to achieve good separation, an even heating is required along with low rate of distillation to maintain as much as possible a thermal equilibrium and a high reflux ratio. Furthermore, the type of column, the column packing and the process of packing a column all determine the efficiency or ability that a column has for separating the components of a liquid mixture.

Pack a fractional distillation column with the copper mesh provided. Your TA will demo this procedure for the class. Be careful not to break the fragile glass fingers at the bottom of the distillation column when positioning or inserting the copper mesh. What is the purpose of adding packing material to the distillation column? Wrap the distillation column with glass wool. You may want to use aluminum foil to help keep the glass wool positioned about the distillation column. What is the purpose of insulating the distillation column? Charge a 100 mL round bottom flask with 50 mL of the unknown liquid mixture and add one or two boiling chips. Assemble the apparatus with the distillation column inserted between the 100 mL round bottom flask and the distillation head. Position the flask in the heating mantle and wrap the top of the flask and the column with glass wool (not cotton) in order to obtain efficient heat transfer. Wrap aluminum foil around the glass wool in order to keep in place. Remember to use the fume hood whenever possible.
Perform a fractional distillation with the column in a similar manner as to that without the column (see part A, simple distillation). Maintain the distillation rate at one drop per second. As before, record the temperature of the distillation head vs. the volume of distillate collected every milliliter. When 10 mL of distillate is collected, transfer the distillate to vial. Collect every 10 mL fraction in the vials and cap the vials tightly. After the fourth 10 mL fraction has been collected, the distillation should be stopped, the heating source turned off and the equipment allowed to cool and lastly, when cooled, then glassware disassembled. Analyze each fraction by gas chromatography.

Part C: Gas chromatography and relative response factors.

Your TA will demonstrate the use of and instruct you on the gas chromatography instrument as well as how to work with GC syringes. Make sure to use the same instrument for the all experiment.

Before analyzing the "standard" or "known four-component mixture" by gas chromatography, record the instrumentation parameters used for the analysis: Your TA will discuss with you each of the settings below.

- Helium air flow meter
- Injector temperature
- Column temperature
- Detector temperature
- Detector current

Consult Rebecca Hwa if there is confusion or uncertainty.

A 5 mL capped-vial of a "standard" or "known four-component mixture" and associated syringe is placed by the GC. The relative volume of the "standard" or "known four-component mixture" is 1.00:1.00:1.00:1.00. Use the micro GC syringe for injecting 1.0 µL (that is 1.0 not 10.0 and micro liters not mls) of the "standard mixture" into the injection port of the Gas Chromatograph. Be very careful with the GC syringe for they are very fragile and very expensive. Make sure you rinse the syringe a couple of times with the standard mixture to be analyzed before each injection. Make sure you press the start button of the plot recorder immediately after you inject. Make sure you place the cap back on the "standard" or "known four-component mixture". How would not placing the cap back on the "standard" vial affect the data obtained? How could not rinsing the GC syringe with the standard/sample being analyzed effect the GC plot or the percent areas reported on the chromatogram? What effect will a 1-minute delay between the injection time and time the start button is pressed on the recorder have on the observed GC retention times or the appearance of the chromatogram?

Quantitative GC analysis: Relative response factors

We would like to find what the response factors are for each of the components that we have to analyze. Rather than finding absolute numbers, we will use relative response factors, where one of the components is set to have a value of one and the others are relative to that number. The first step is to inject a mixture of standards where the ratio between the components is known to be 1:1 (by volume). In the following example, a two component mixture was injected. The hypothetical chromatogram is presented in the following scheme:

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1 Inject only 0.5 µl from the fractions that you collected.
The area beneath each peak is proportional to the absolute amount of compound that was injected. This area is calculated by an “integrator” and is represented in “arbitrary units” (because its absolute value is meaningless). As you can see, the ratio between the peaks is not 1:1. This is due to the different response of the GC detector to various compounds. In other words, the detector is more sensitive to one compound then to the other. Therefore we need to compensate for this difference by applying a correction factor known as “relative response factor”.

I will try to walk you through these calculations without using any formal formula. The first step is to choose a peak which will be a reference peak. It is convenient to choose the largest area as a reference (peak a in this example). Next we need to divide the area of each peak by the reference area.

For component a: \[ \frac{2500}{2500} = 1 \]
For component b: \[ \frac{2325}{2500} = 0.93 \]

In this example we see that the area of the second peak is only 93% of the first peak’s area. Therefore if we want to “correct” the area to be equivalent to 100%, we need to multiply the area of the second peak by \( \frac{1}{0.93} = 1.075 \). This is our relative response factor.

Relative response factor for component a: \[ \frac{1}{1} = 1 \]
Relative response factor for component b: \[ \frac{1}{0.93} = 1.075 \]

Once we have these numbers we are able to analyze an unknown mixture of the two components. Let’s assume that we have a vial that contains 10 ml of a mixture and we inject 1 µl of this solution into the same GC on which we determined its relative response factors. Our integrator gives the following values:

<table>
<thead>
<tr>
<th>peak</th>
<th>Area (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3300</td>
</tr>
<tr>
<td>b</td>
<td>4700</td>
</tr>
</tbody>
</table>

We need to correct these numbers by using the relative response factors:
For component a: $3300 \times 1 = 3300$

For component b: $4700 \times 1.075 = 5052$

Now we can calculate the percentage of each component in the mixture:

For component a: 
$$\frac{3300}{(3300 + 5052)} \times 100 = 39.5\%$$

For component b: 
$$\frac{5052}{(3300 + 5052)} \times 100 = 60.5\%$$

At this point we need to remember that when we determined the relative response factors we used a mixture of 1:1 based on volume. Therefore, the ratio between the components in our unknown is also based on volume. Multiplying the volume percent by the total volume of the fraction (10 ml) will give us the total volume of each component:

For component a: 
$$0.395 \times 10 ml = 3.95 ml$$

For component b: 
$$0.605 \times 10 ml = 6.05 ml$$

Using the compound’s density (d) and the molecular weight (MW) we can convert the volume to moles:

Number of moles in the sample for component a: 
$$\frac{3.95 \times d}{MW}$$

In the same way we will calculate the number of moles of component b and then we can calculate the mole fraction of each component.

GC data analysis

For each of your fractional distillation samples, convert the ratio of integrated peak areas to a mole fraction. On a single graph, plot the mole fraction of each component in each sample (y-axis) as a function of the midpoint of the boiling range of the sample (x-axis). Draw a smooth curve connecting the data points for each compound. Repeat the procedure for the fractional distillation experiment.

Draw a distillation curve that shows the change of distillation temperature as a function of volume of distillate. (graph the data for simple and fractional distillation on the same plot).

Your report should include your assessment of the efficiency of your fractional distillation in the context of the theory and your understanding of its underlying principles. How might your separation be improved? Turn in your gas chromatograms (or photocopies) with your report.

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2 You also should note that the fact that we injected 1 µl and not 5 µl is not important to our calculation since we are working with ratio and not absolute numbers.