Preparative Gas Chromatography

Theory of Chromatography
Chromatography

Chromatography got its name from the fact that it was originally used to separate colored compounds. There are several types of chromatography and all of them can be used for separation and analysis of mixtures of compounds that have no color. We do have to have some method for detecting the presence of the separated compounds if they are not colored.

Chromatography - Definition

Chromatography is defined as the separation of two or more components (molecules or ions) of a mixture by distribution between two phases, one of which is immobile and the other is moving. Just as in extraction, separation comes from differences in distribution of the components in the two phases.

The immobile or stationary phase may be a solid or a liquid. The mobile phase may be a liquid or a gas.
Consider butanol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$, and decanol, $\text{CH}_3(\text{CH}_2)_{9}\text{OH}$, being equilibrated between hexane and water.

The hexane layer would contain most of the decanol (more hydrocarbon-like) while the water would contain most of the butanol (more water soluble).

If we move the hexane layer to a second beaker containing water, most of the decanol and a little of the butanol would be in the second beaker.

After equilibration in the second beaker, the hexane that gets moved to the third beaker would contain only decanol (although some of the decanol is still in the water layers). We have effected separation of pure decanol.
In this model, the hexane is the mobile phase and water is the stationary phase. In chromatography, we don’t have separate beakers, the equilibration between the two phases is continuous as one phase moves with respect to the other.

The types of chromatography that you have encountered or will encounter in this course are:
- Thin-layer Chromatography (liquid-solid chromatography in a thin layer)
- Column Chromatography (liquid-solid chromatography in a column)
- Gas Chromatography (gas-liquid chromatography)

The mobile phase in gas chromatography is a gas. Separation depends upon differences in distribution between the gas and the liquid used as the stationary phase.
Gas Chromatography

7 In gas chromatography, we coat small particles of an inert solid with a high-boiling, viscous liquid and pack a small-diameter column (metal or glass) with the coated particles. The adsorbed liquid is the immobile stationary phase.

7 We then pass an inert gas (helium or nitrogen) through the column at a slow rate to be the mobile phase.

7 We also have to have a mechanism for introducing our mixture into the column (an injection port) and a mechanism for detecting whether a compound is in the gas coming out of the end of the column (a detector).

7 We know that most organic compounds are liquids or solids at room temperature. In order for a significant amount of material to be in the vapor phase, we are going to have to raise the temperature. Therefore, our gas chromatograph has the injection port, column, and detector in an oven that can be heated to a selected temperature.

7 Note that we would not want to heat the column for separation of a mixture of gases (e.g. CH₄ and CO₂).
In a gas chromatographic separation of butanol and decanol, two factors come into play. The higher boiling point of decanol would mean that it would spend more time in the liquid phase than the lower-boiling butanol. Thus, butanol will move through the column faster and emerge (elute) from the column first.

The second factor is the extent of interaction between the compound being separated and the compound used as the liquid phase. Two types of liquids are used: a) non-polar liquids that interact only by van der Waals interactions and b) polar liquids that can interact by hydrogen bonding.

Therefore, non-polar GC columns separate primarily by volatility (related to boiling points), but polar columns separate both by volatility and extent of hydrogen-bonding or polar interactions. We could use a polar GC column to separate a mixture of decanol and a hydrocarbon with identical boiling point because the decanol would interact more strongly with the liquid of the stationary phase than would the hydrocarbon.

We will be using a polar stationary phase in today’s experiment.
Effect of Stationary Phase

The flow of bees past a row of flowers is slowed by interactions with the flowers while the flow of wasps is not slowed!
Several types of detectors have been developed for gas chromatography. The first and simplest one is on the GCs in our lab. They are called thermal conductivity detectors.

A thermal conductivity detector measures the resistance to electrical conductivity of a small heated wire in the path of the gas emerging from the GC column. The low molecular weight carrier gas (helium in our case) cools the wire a significant amount. When an organic compound comes off the column mixed with the carrier gas, the wire is cooled less effectively. This changes the conductivity of the wire.
Detector Response

The change in conductivity results in a ‘peak’ as the compound elutes past the detector. We now record the detector response by computer, and the result can be printed out as the gas chromatogram for our sample.

Preparative GC

A GC analysis can be used to determine the amount of different components in a mixture as you did in the distillation experiment. Importantly, the components eluting from the column are unchanged.
Preparative GC

Therefore, we can isolate pure components by condensing the organic compound(s) at the outlet from the detector. We have to collect only during the period that the desired compound is exiting from the column.

Review the lab manual for the technique we will use today to collect the components of the mixture.

Preparative GC

Preparative GC is limited to relatively small sample sizes even though we are using larger diameter columns than were used in the distillation experiment. More material could be isolated by repeating the injections and collections multiple times.
Preparative GC

7 The picture below shows a collection tube correctly attached to the GC outlet.

Safety

7 The exit port from the GC detector is hot. Be careful not to burn your fingers sliding the Teflon tube onto or off the exit port.
7 DO not attach the collection vial to the tube until after you have collected your sample.
Procedures

For today’s experiment, you will work in pairs to conduct a preparative GC separation of two compounds. Each of you will collect material from one of the two major peaks in the sample.

You will be called into the instrument room in pairs by a student worker to conduct your prep GC separation.

The temperatures used for various mixtures differ because of the difference in volatility of the compounds.

You will then use IR spectroscopy to identify the compound that each of you isolated.

Notes on Prep GC Injections

DO NOT RINSE SYRINGES WITH ACETONE BETWEEN SAMPLES

Rinse your syringe by filling with material from your sample vial and expelling the liquid into a waste vial.

Review the example gas chromatogram shown in the book on top of the GC to help you determine when to start and stop collecting each sample.
Collection of Sample and IR

7 After the GC signal for your component has returned to near baseline (see example chromatogram displayed above the GC), remove the tube from the exit port.

7 Connect the tube to the sample vial using the screw cap to secure it. Do not let the tube extend to the bottom of the vial.

7 You now want to transfer the liquid from the collection tube using a simple centrifuge.

Centrifuge Use

7 Make sure you have two vials and tubes in the centrifuge (opposite compartments) before starting centrifuge. Use an empty vial and tube if you don’t have a partner.
Infrared Spectrum

1. Cap your vial after removal from the centrifuge until you are ready to obtain the IR spectrum.
2. Transfer some of your sample to the ATR plate of the IR using one of pipets provided.
3. After you have obtained your spectrum, clean the ATR crystal using a cotton ball moistened with acetone (don’t drip acetone!).