UTA-740
Forensic Investigations with Chromatography

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Objective: To identify an accelerant used on wood through the use of gas chromatography mass spectrometry and analyze ink samples with paper chromatography methods.

NOTE: You must bring a pencil (as well as your pen) for this experiment.

INTRODUCTION

Forensic science (forensics) encompasses a broad spectrum of sciences and technologies used in the investigation of criminal and civil law. How does chemistry apply to forensic science? We use chemistry to analyze the physical evidence left at the scene of a crime. Chemists analyze substances such as blood, DNA, gunpowder, arson remnants and more to determine when and by whom a crime was committed.

In this experiment, you will be using the method of chromatography to analyze common samples often found at a crime scene: arson remnants and ink samples. First, you will use gas chromatography-mass spectrometry (GC/MS) to analyze a charred wood sample which was soaked in accelerant prior to burning. Your goal is to identify the accelerant used. The second portion of the experiment will be analyzing ink samples with paper chromatography.

I. Arson Accelerant Analysis

Gas Chromatography- Mass Spectrometry

Accelerants are used to rapidly spread a fire and initiate combustion of other materials such as paper, wood, rags or plastic. Household chemicals are commonly used by arsonists as accelerants due to availability. A sample of the accelerant is poured and ignited to start the fire. The liquid itself does not burn; rather a thin hydrocarbon vapor layer above the surface of the liquid is ignited. On porous or absorbent surfaces, such as wood and carpet, accelerants are absorbed into the material. Most accelerants soak into bare wood two to three millimeters. If the wood is lightly charred, accelerant traces can be detected. Accelerants are composed of mixtures of organic compounds. In this laboratory, you will be separating and identifying these organic compounds through gas chromatography-mass spectrometry to determine the identity of your accelerant.

Gas chromatography-mass spectrometry (GC/MS) incorporates the separation properties of gas chromatography (GC) with the identification ability of a mass spectrometer (MS). First, the mixture travels through the GC column, which separates the compounds by molecule-column interactions. Then, the molecules enter the MS, which measures masses and abundance of ions in the gas phase. The combination of GC/MS is used to determine the identity of each separated component.

In general, chromatography is an experimental analytical technique in which a mixture of compounds is separated into their individual compounds due to molecular interactions of the sample with a stationary phase and a mobile phase. The stationary phase is a high temperature liquid coated onto a tubular column. Analytes adsorb onto the stationary phase, separating the different compounds in a sample mixture. The mobile phase is a carrier gas, specifically helium. Helium is an inert gas that does not interact with the sample or the stationary phase. Manipulation of these phases causes separation of the individual compounds.

The GC uses a capillary column. The stationary phase is adsorbed on the interior walls of a thin fused-silica capillary tubular column with a small diameter (Figure 1). The column dimensions are 30m x 0.25nm x 0.25µm.

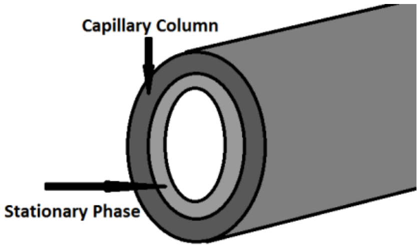

Figure 1: Capillary GC column.

The column is polysiloxane coated, comprised of 5% diphenyl groups (Figure 2A) and 95% dimethyl groups (Figure 2B) making this a nonpolar column. This polysiloxane coating is a liquid but has a high boiling point which prevents it from bleeding off the column during the experiment.

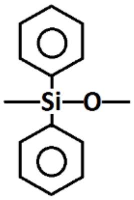

Figure 2A: diphenyl siloxane (5%)

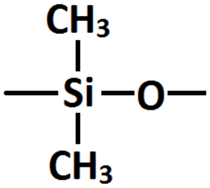

Figure 2B: dimethyl siloxane (95%)

In GC, the **retention time**, t_R, of an analyte is the time the analyte spends in the chromatographic system. Separation of individual components is dependent on their time travelling down the column in the mobile phase versus the time they are adsorbed onto the stationary phase. In general, GC is used for separation of volatile liquid and gas compounds.

Intermolecular forces cause certain liquid compounds to volatilize more than others. Liquid molecules are held together by attractive forces. As the temperature of a liquid is increased, the forces decrease. When the boiling point is reached, the molecule easily escapes the liquid phase and moves into the gas phase. Accelerants have a mixture of various components in the liquid phase. The molecules with the weakest attractive forces are easier to separate from one another so they will enter the vapor phase more quickly than molecules with strong attractive forces. Upon entering the GC, the compounds are in the vapor phase. The interaction between these vaporized compounds and the GC column is dependent upon polarity, “like dissolves like”. In addition to polarity interactions, small molecules tend to move more quickly through a GC column than larger molecules.

Once the sample is injected into the instrument, the individual components of the sample can adsorb onto the stationary phase and be retained. Separation is due to intermolecular interactions with the stationary phase in the GC column. As the temperature in the column increases, the samples desorb from the stationary phase, return to the mobile phase and are carried further down the column. The components that interact more strongly with the stationary phase will elute at a later point. Different compounds within the same sample will interact at different rates with the stationary phase, leading to separation of the individual components within the mixture. In GC, the flowing mobile phase carries the sample through the column towards the detector.

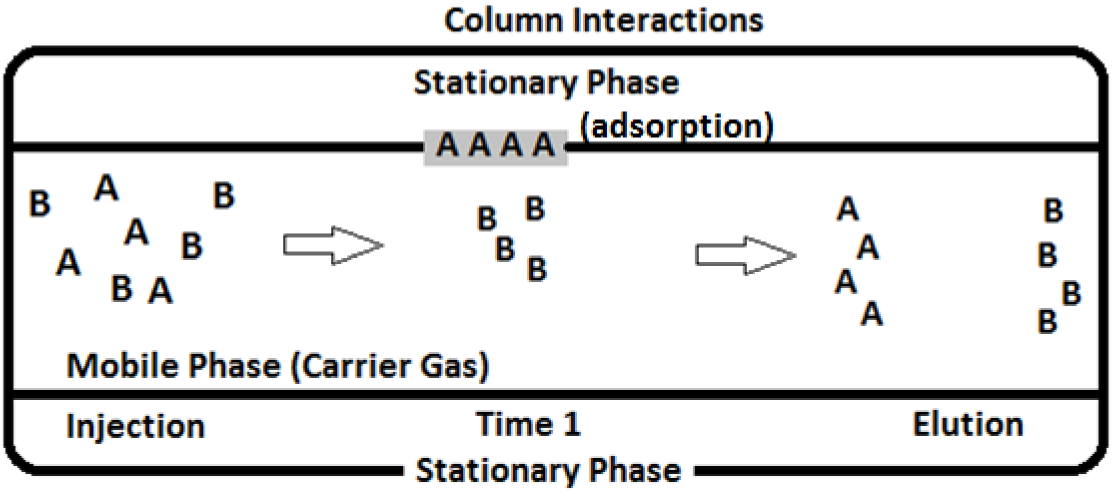

Figure 3: Column interactions of individual solute compounds **A** and **B**.

The rate at which the compounds move through the column is dependent on the interaction between the compound and the stationary phase. In figure 3, although both compounds (**A** and **B**) of the mixture are injected at the same time, the first sample to elute from the column is **B**, meaning this compound has the least interaction with the stationary phase. The second compound to elute from the column is **A**, meaning this compound has a stronger interaction with the stationary phase. At the injection, compounds **A** and **B** are mixed in the sample. At Time 1, compound **A** is adsorbed onto the stationary phase and compound **B** is in the mobile phase (not interacting with the stationary phase). Therefore, compound **B** elutes first followed by compound **A**. The mixture (**A** and **B**) has been separated into the individual components. Column temperature and carrier gas flow rate affect this rate of elution.

Gas chromatography separates compounds based on molecular properties, i.e. boiling point. The boiling point of a compound is dependent on its intermolecular forces.

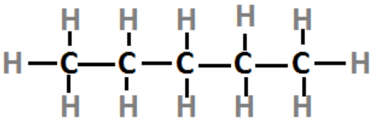	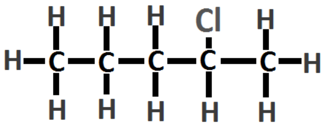	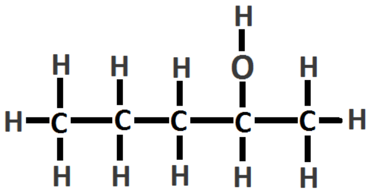
Figure 4A: Pentane, C_5H_{12} Boiling Point: 36.1 °C	Figure 4B: 2-chloro-pentane, $C_5H_{11}Cl$ Boiling Point: 98.3 °C	Figure 4C: 2-pentanol, $C_5H_{12}O$ Boiling Point: 119.3 °C

Although the compounds in Figure 4A-C have similar structures, there is large variation in their boiling points. The variation in boiling point temperature is due to intermolecular forces. Intermolecular forces increase in strength as we increase polarizability of the bonds, therefore London dispersion forces < dipole-dipole < Hydrogen bonding. The pentane molecule (Figure 4A) exhibits London dispersion forces. 2-chloro-pentane (Figure 4B) has London dispersion forces and dipole-dipole interactions due to the polar nature of the molecule. 2-pentanol (Figure 4C) has London dispersion forces, dipole-dipole interactions and hydrogen bonding present, leading to an increase in the overall boiling point for this molecule. Variations in the intermolecular forces have a strong effect on boiling point, and the technique of gas chromatography uses these effects to separate compounds into their individual components.

For arson analysis, GC/MS is used to characterize volatile components in common accelerants through the characterization of the gas/vapor over the sample (headspace). The accelerants used in this experiment are volatile, meaning they easily vaporize at low temperatures from the liquid phase to the gas phase. Since your sample will be placed into a sealed container (the sample vial) we are able to examine the accelerant used by studying the headspace in the sample vial. A burned piece of wood is placed in a vial (Figure 5). The vial is placed into an oven to encourage vaporization of the volatile components of the accelerant. The volatile chemicals in the accelerant (soaked into the wood) escape into the headspace of the vial and are analyzed. A mixture of the gases in the headspace is examined and characterized to identify the accelerant used.

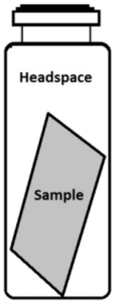

Figure 5: Wood sample in GC/MS sample vial. Volatile chemical components of the accelerant escape into the headspace of the vial.

The sample of the mixture in the headspace is analyzed as follows (Refer to the numbers in Figure 6 on the next page).

1. A sample is injected in the injector port and is carried by a carrier gas (helium gas/mobile phase) to a capillary gas chromatography column in the temperature controlled oven.

2. As the sample travels through the capillary GC column, components of the mixture separate as each component moves at a different speed due to intermolecular forces: attractions, repulsions, and polarity.

3. Each analyte elutes from the column at a different time (retention time, t_R). Retention times of common chemical compounds in accelerants are listed in **Table 1**. As an analyte moves to the mass spectrometer, first it is fragmented into ions in the ion source.

4. Fragmented ions travel through the mass analyzer.

5. Fragmented ions are detected as they arrive at the detector.

6. Reports are generated by the computer.

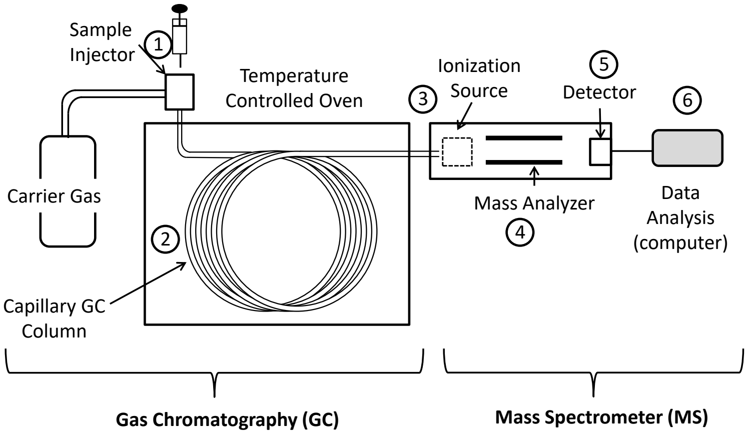

Figure 6: Schematic Diagram of GC/MS. The numbers in the diagram are referred in the main text.

Table 1: Retention times of common household accelerants.

Accelerant	Volatile Compound(s)	Approximate Retention Time
Ethyl Acetate	ethyl acetate	1.5 min
Gasoline component	isooctane (2,2,4 trimethyl pentane)	1.7 min
Lacquer Thinner	acetone, ethyl acetate, toluene	multiple peaks 1.3 - 2.2 min
Nail Polish Remover	acetone	1.3 min
Paint Thinner	petroleum distillates (hydrocarbons)	multiple peaks: 2.8-3.4 min
Turpentine	turpentine molecules	2.9, 3.0, 3.1 min

II. Ink Analysis

Paper Chromatography

We are not always able to classify something as a pure substance or a mixture based on visual inspection. If we are given a material, how can we determine if it is a pure substance or a mixture? **Chromatography** is a technique used to separate and identify the components of a mixture. The mechanism of separation in paper chromatography is similar to that of gas chromatography (GC). In paper chromatography, a very small amount of a sample (a mixture to be separated) is applied near the end of the chromatography paper (see Figure 7a). Chromatography paper, an absorbent paper, is the stationary phase. The point of sample application on the paper is called the origin, near the bottom of the paper. The paper is then placed vertically in a solvent called eluent. In paper chromatography, the eluent is the mobile phase. Eluent composition is dependent upon the analytes you are examining and your paper source. Common eluents include water and alcohols. Different eluents are used as their interactions with the analytes vary, causing variation in their ability to “pull” along the analytes in the mixture being separated. As shown in Figure 7a, the origin should always be above the surface of the solvent. The eluent rises up the paper due to capillary action (Figure 7b). When the eluent reaches the origin, the analytes in the sample begin to interact with the eluent. The analyte components will rise at different rates depending on this interaction between the mobile and stationary phases.

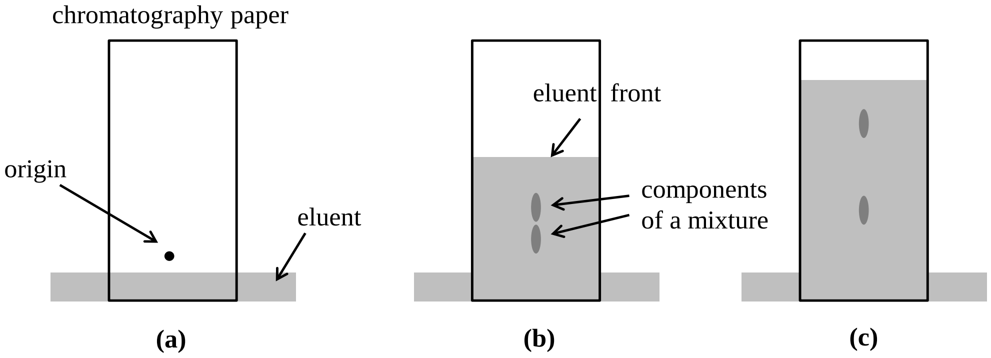

Figure 7: Schematic pictures of separation processes by paper chromatography are shown in chronological order (from left to right). Two spots (dark gray) in (b) and (c) represent different components in the mixture applied at the origin (Black point in (a)).

In this example, the mixture is composed of two substances. When the eluent moves closer to the top of the paper (Figure 7c), two analytes are clearly separated. The eluent should not move all the way to the top of the paper, and the paper should be removed from the eluent beforehand. The top of the eluent (the line made by the boundary between wet and dry part of the paper) is called the **eluent front**. In this system, the stationary phase is the paper the sample has been plotted on and the mobile phase is the solvents used for analysis.

A liquids ability to flow in narrow spaces in opposition to external forces such as gravity is known as capillary action. Paper chromatography uses this method, where liquids are drawn up in a porous material such as paper. The intermolecular forces between the liquid and solid surfaces dictate this ability. The combination of surface tension (a result of cohesive forces within a liquid) and adhesive forces between the liquid and paper allow the liquid to rise up the paper. The solvent (mobile phase) moves vertically up the paper (stationary phase) via capillary action. In the case of a water based solvent, the water molecules, which are polar, are attracted to the polar alcohol bonds in the paper fibers, allowing the solvent to climb the paper. When examining ink, the different colors of the ink travel up the paper, causing separation of the individual components of the ink. The distance the components of ink travel is dependent on their degree of solubility in water (mobile phase) and their attractions to the paper (stationary phase).

Figure 8 shows an example of a finished chromatography paper (called **chromatogram**). In this chromatogram, three samples are applied simultaneously at different origins on the same chromatography paper. For the first sample (left in Figure 8) shows representing separated components of a mixture. For the second (center in Figure 8) and the third (right in Figure 8) samples, a pure substance X and a pure substance Y were used respectively.

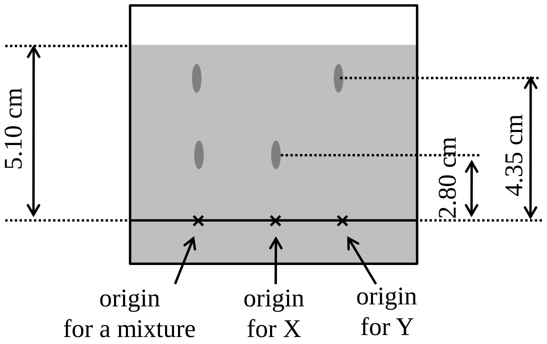

Figure 8: An example of a chromatogram shows the application of three samples. In this example, two known pure substances (X and Y) are applied to the right side. The first two spots on the left side are separated components of a mixture.

According to this chromatogram, you can conclude that X moves slower than Y for this particular eluent used. The position of the analyte is described by the retention factor, R_f. The retention factor is the ratio of the distance travelled by the analyte to the distance moved by the mobile phase, and can be calculated for each spot in the sample:

$$R_f = \frac{\text{distance traveled by } \textbf{the spot} \text{ from the origin}}{\text{distance traveled by } \textbf{the eluent front} \text{ from the origin}}$$

The distance is measured to the center of the spot (figure 8). If the spot has an irregular shape, attempt to locate the center of mass for the measurement.

It is important to specify the natures of eluent used in an experiment since the values of retention factors and the order of elution are dependent on a type of eluent. For example, X might move faster than Y for another type of eluent. Once the R_f value is known, the substance can be identified by comparing its R_f value with those reported in literature dependent on eluent used.

Example: Calculate a retention factor (R_f) for X and Y in Figure 8.

For X: $R_f = \frac{2.80\,\text{cm}}{5.10\,\text{cm}} = 0.549$ For Y: $R_f = \frac{4.35\,\text{cm}}{5.10\,\text{cm}} = 0.853$

A retention factor can be used to determine the identification of substances in a mixture. In Figure 8, the values of retention factors for two spots from the mixture (Left in Figure 8) can be compared to those of X and Y. If their values are close enough such as in Figure 8, you could conclude the identities of substances in the mixture as X and Y.

Hazardous waste disposal: All excess accelerant as well as the paper chromatography solvent are disposed of in the Non-Halogenated Waste container.