Objective
Use paper chromatography and acid-base reactions to separate and identify the components in an unknown mixture of pH indicator dyes.

Introduction
The unique abilities of different substances to bind to the surfaces of various solids such as starches or cellulose can be used to separate mixtures of these substances into pure components. In 1906 Russian scientist Michael Tswett successfully demonstrated this concept by separating chloroplasts from plant material. He called this new technique chromatography, which literally means “the writing of colors.” Since Tswett’s original experiments, chromatography has become increasingly more sophisticated and is now one of the most powerful techniques available for separating mixtures into their component parts.

There are numerous different types of chromatography that are currently in use. All chromatographic techniques use similar principles to separate components. A typical chromatographic system consists of a stationary phase and a mobile phase. The stationary phase consists of a porous, adsorbing solid that is fixed in position. The mobile phase is a solvent that is either a liquid (liquid chromatography) or a gas (gas chromatography) that is in constant, direct contact with the stationary phase. As the solvent moves through the stationary phase it competes with the stationary phase for interaction with the solute components. Different solutes have different affinities for the two phases and will separate into individual components based on their attractions to the stationary phase and their solubilities in the mobile phase. With the proper choice of stationary and mobile phases it is possible to separate a complex mixture of chemically similar compounds.

In a chromatographic experiment the sample, a chemical mixture dissolved in a solvent, is deposited on the stationary phase. The components of the mixture are adsorbed onto the stationary phase in varying degrees depending on the nature of the component, the nature of the solid phase, and the temperature. The mobile phase, a solvent, is then allowed to flow through the solid either by capillary action or under pressure (applied or gravitational). As the solvent passes through the deposited sample it carries the components of the mixture with it into and through the stationary phase. The rate of movement of a solute through the solid phase depends on its partition ratio between the mobile phase and the stationary phase. The partition ratio depends on the ratio of attraction (adsorption) of the solute to the stationary phase and its solubility in the mobile phase. A component that is strongly attracted to the stationary phase will move slowly whereas a component that is weakly attracted to the stationary phase will move more quickly. As a result of the chromatographic process, the sample is separated into individual components seen as isolated spots or fractions. The physical result of a chromatographic process is called a chromatogram (see Figure 1).
Figure 1: A diagram of a paper chromatogram before and after the experiment is run. The paper is the stationary phase. The mobile phase (solvent) is drawn up the paper by capillary action and draws the components of the mixture with it at different rates depending on their attraction for the cellulose in the paper and their solubility in the solvent.

The rate of chromatographic migration of a compound is characteristic of that component and remains constant as long as the same experimental conditions are used (stationary phase, mobile phase, column pressure, and temperature). A measure of the rate at which the compound moves through the stationary phase is called its retention factor ($R_f$). The $R_f$ value can be used to identify the components of the mixture. For paper chromatography, the $R_f$ value is calculated as

$$R_f = \frac{\text{distance the solute (component) moved}}{\text{distance the solvent front moved}} = \frac{D}{L} \quad \text{Equation 1}$$

The distance, $D$, should be measured from the start line to the middle of the solute spot although either the top or the bottom of the spot can also be used as long as you are consistent throughout the experiment (see Figure 2).

Figure 2: A chromatogram shown before and after developing indicating the measurements for $D$ and $L$ used in calculating the retention factor, $R_f$.

In this experiment you will use paper chromatography to separate the components of a mixture of pH indicator dyes into individual components. The following indicator dyes will be used, methyl orange (MO), methyl red (MR), phenol red (PR), and
phenolphthalein (PH). You will use pure individual indicators as reference standards. After the separation, you will identify the components of each mixture by measuring their retention factors and comparing them to those of the standards.

The stationary phase used in this experiment is the cellulose of the chromatography paper. The mobile phase is a base, 6M ammonium hydroxide (NH₄OH). The NH₄OH is drawn through the paper by capillary action. At the end of the experiment, concentrated hydrochloric acid (HCl) will be used to create an acidic environment that can also be helpful in identifying the components.

**Safety measures**

NH₄OH and HCL are both caustic and corrosive. Both also have a low vapor pressure so they easily move into the vapor phase and both have a suffocating odor. It is important to use these solvents in a fume hood.

**Procedure**

1. Obtain an 11 X 17 cm piece of chromatography paper. Draw a light pencil line along the wider edge of the paper 1.0 cm from the edge. Starting about 1.5 cm from the side of the paper, mark the line at 2.0 cm intervals and label the segments with a pencil as shown in Figure 3. Do not mark the paper with ink.

2. Obtain a 1 X 12 well strip. Transfer about 2 drops of 0.1% solutions of each of the four indicator dyes into the first four wells. You do not need to label the wells if you fill them in the same order as shown in Figure 3. Take care not to spill the solutions; they may stain clothes and skin. Place two drops of unknown X in the fifth well and two drops of unknown Y in well six. Put several drops of distilled water in each of the other wells.

3. Obtain a capillary tube. Practice spotting technique by dipping the end of the tube into one of the solutions and touching it momentarily to a piece of scratch filter paper (do not use the prepared chromatography paper). The liquid from the capillary tube should form a spot no larger than 3-4 mm in diameter.
You can clean the capillary by first touching the end of the tube to a piece of absorbent paper to remove the residual dye. Then dip the capillary tube into the water in a well of the tray and touch it to the paper to draw out the water. Repeat the procedure. Make sure all the water has been removed from the tube. If some of the dye won’t wash out you may need to repent the blotting procedure with ethanol.

4. Dip the capillary tube into the first indicator solution and spot it on the line on your chromatography paper in the middle of the space labeled for that solution. Allow the spot to dry then spot it again in the same place with the same dye. Clean the tube as described in step three. Repeat the spotting procedure with the remaining solutions. Remember to clean the tube between solutions.

5. Fold the paper into a cylinder, with spots facing you, and staple the paper edges in such a way that the edges almost meet but do not overlap (see Figure 3). The marked pencil line should meet at the edges.

6. In a fume hood place the formed cylinder next to a 600 mL beaker and pour ammonia solution into the beaker. The solvent level in the beaker must be below the bottom of the largest spot on the paper. Gently place the cylinder in the beaker being careful not to wet the paper above the level of the spots. The paper should not touch the walls of the beaker. Cover the beaker with a piece of aluminum foil. Do not touch or move the developing chamber while the chromatogram is developing. Watch the chromatography as it progresses.

7. When the mobile phase has risen to within about one centimeter from the top of the paper, remove the cylinder from the beaker. Immediately mark the solvent front and the position all spots (top and bottom) with a pencil. Remove the staples and unfold the cylinder into a rectangle. Record the color of each spot on your data sheet. Let the chromatogram partially dry in the hood only until the phenolphthalein spot disappears.

8. Pour a small amount (about 3 mL) of concentrated HCl into a 50 ml beaker. Expose the chromatogram to the acid vapors by passing it over the beaker of acid. Allow sufficient exposure time for each of the spots to change color. Note the new (acidic) colors of the spots on your data sheet.

**Calculations**

1. Determine the \( R_f \) value of each spot on your chromatogram.

2. Identify the indicator dyes present in your unknowns by comparing the \( R_f \) values and both basic and acidic colors of the components in the unknowns with those of the standard solutions. Record all data on your data sheet.
Data Table

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color in base</th>
<th>Color in acid</th>
<th>Distance to spot (D)</th>
<th>Distance to solvent front (L)</th>
<th>RF = D/L</th>
<th>Identity of component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl orange</td>
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<tr>
<td>Methyl red</td>
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<tr>
<td>Phenolphthalein</td>
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<tr>
<td>Phenol Red</td>
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<tr>
<td>Unknown X</td>
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<tr>
<td>Unknown Y</td>
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</tbody>
</table>

**Discussion**

Write a discussion of your results including a statement of your results, the identity of the dyes in the unknowns and a discussion of possible errors and how you could minimize them.
1. The retention factor, R_f, is a quantity used in all types of chromatography to determine effectiveness of solute separation. Describe the mathematical basis of the R_f and tell how it is used.

2. A chromatography experiment was carried out using powdered alumina suspended in a pipet and washed with liquid hexane. A crude organic mixture was put on the column and the various components were washed off the column with hexane. Identify the stationary phase and the mobile phase in this chromatography experiment.

3. In a paper chromatography experiment why is it necessary to apply the spot of the mixture to be analyzed above the level of the solvent (see step six of the experiment).

4. The chromatogram below was obtained by a student doing paper chromatography. Use her data to calculate the R_f values for each spot on the chromatogram.
1. Explain the effect of the following errors on the outcome of a paper chromatography experiment. Be as specific in your answers as possible.
   A. The student was in a hurry and only allowed the solvent front to move 2.5 cm up the 10 cm paper.
   B. The student forgot to clean the capillary tube between application of the Methyl Orange standard and the Unknown.
   C. The student removed the chromatogram from the development chamber and allowed it to dry without marking the spots.

2. Based on your experimental results, why is it possible to identify Phenol Red but not Phenolphthalein in an unknown that contains both?