# Lab 2D: Separation of a Mixture by Paper Chromatography

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Student Self Evaluation</th>
<th>Teacher Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective: Clearly states the aim of the experiment, written in your own words and briefly outlines the related theory.</td>
<td>/3</td>
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<tr>
<td>Procedure: correctly references textbook or handout making notes of any changes.</td>
<td>/9</td>
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<tr>
<td>Flow Chart: a visual representation of the procedure, to be completed before the lab.</td>
<td>/18</td>
<td>/18</td>
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<tr>
<td>Pre-Lab Questions: displays a critical understanding of the background theory.</td>
<td>/7</td>
<td>/7</td>
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<tr>
<td>Data, Results &amp; Calculations: (handwritten neatly) Provides results/observations (and diagrams where appropriate) that are presented in correctly annotated tables and/or graphs. Scientific tables &amp; graphs are numbered (eg Table 1... or Graph 1...) and include descriptive titles.</td>
<td>/2</td>
<td>/2</td>
</tr>
<tr>
<td>Follow-up Questions: Correctly identifies and explains the theory relating to the experiment and supports this with accurate observations, data and/or calculations.</td>
<td>/3</td>
<td>/3</td>
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<tr>
<td>Conclusion: Identifies and defines important concepts and principles relevant to the experiment by relating back to the objective and hypothesis. Be sure to address the points listed in the lab handout when answering the conclusion.</td>
<td>/5</td>
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</tr>
<tr>
<td>Presentation: Practical report is presented in the correct format, is written fluently and provides appropriate section headings and accurate referencing. Tables &amp; graphs have numbered headings. Data &amp; calculations may be hand written, however the remainder of the report is to be word-processed.</td>
<td>/5</td>
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</tr>
<tr>
<td>Safety: (teacher assessed during practical lab work) Arrives to class prepared with pre-lab complete. Demonstrates an organized and safe approach to experimental work &amp; meticulously executed methodology to a high degree of accuracy.</td>
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<tr>
<td>Punctuality: Report is submitted in full on the due date. (-1 per day until drop date)</td>
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**Results Summary:**

We will be doing the lab on **Thursday**. In order to be ready to go, you need to complete the following sections of your lab report:

- **Objective**
- **Flow Chart**
- **Pre-Lab Questions**
- **Data & Observations:** Draw & set up Table 1 into your lab notebook (leave lots of space to write and perform calculations).
Background Information:

Food dyes have been used extensively for more than 100 years. Would you eat maraschino cherries if they were their natural color of beige instead of red? In this laboratory experiment you will explore the properties of artificial food dyes with this chromatography activity.

The use of color additives increased dramatically in the United States in the second half of the nineteenth century. As the economy became more industrial, fewer people lived on farms, city populations grew, and people became more dependent on mass produced foods.

Food dyes were initially used to make food more visually appealing to the consumer and, in some cases, to mask poor-quality, inferior, or imitation foods. For example, meat was colored to appear fresh long after it would have naturally turned brown. Jams and jellies were colored to give the impression of higher fruit content than they actually contained. Some food was colored to look like something else—imitation crab meat, for example. Many food colorings and additives were later discovered to be harmful or toxic.

Food colorants were initially added to food with little or no health testing. In 1907, the USDA reduced the number of synthetic food dyes approved for use from 695 to just seven. Only two of the original dyes from 1907 are still accepted for use today. Five others have been added between 1907 and 1971. Only seven dyes are approved for use in the United States today. All of the FD&C approved food dyes are charged, water-soluble organic compounds that bind to natural ionic and polar sites in large food molecules, including proteins and carbohydrates.

Food dyes can be separated and identified by paper chromatography. Paper chromatography is an example of a more general type of chromatography called adsorption chromatography. The paper acts as an adsorbent, a solid which is capable of attracting and binding the components in a mixture (see Figure 1). The mixture to be separated is "spotted" onto the surface of the paper and a solvent is allowed to seep or flow through the paper by capillary action. If one of the components in the mixture is more strongly adsorbed onto the paper than another, it will move up the paper more slowly than the solvent. Components that are not strongly adsorbed onto the paper will move up the paper at a faster rate. This "partitioning" of the components of a mixture between the paper and the solvent separates the components and gives rise to different bands or spots. If the components of the mixture are colored, like food dyes or pigments in an ink, the colored bands are easily distinguished.

The distance a sample moves along the chromatography paper is compared to the overall distance the solvent travels—this ratio is called the Rf or rate of flow. In general, food dye molecules that are more highly charged, that is, have more ionic binding sites and are more polar, will be attracted to the paper more strongly and will have lower Rf values. (Flinn Scientific, 2016)

The Wilton Food Company uses FD&C approved food dyes in highly concentrated forms for their icings. Table 1 shows a list of icing colours and which FD&C dyes they contain.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon Yellow</td>
<td>Yellow #5</td>
<td>Pink</td>
<td>Red #3</td>
<td>Royal Blue</td>
<td>Blue #1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yellow #5</td>
<td></td>
<td>Red #3</td>
</tr>
<tr>
<td>Golden Yellow</td>
<td>Yellow #5</td>
<td>Red (no-taste)</td>
<td>Red #40</td>
<td>Kelly Green</td>
<td>Yellow #5</td>
</tr>
<tr>
<td></td>
<td>Yellow #6</td>
<td></td>
<td></td>
<td></td>
<td>Blue #1</td>
</tr>
</tbody>
</table>

Figure 1. Adsorption of solute particles onto the surface of a solid.

\[ R_f = \text{retention factor} \]
Objective: (to be typed and added to formal report)

Flow Chart: Summarize the steps that you will follow in the lab. You will find this information on the attached pages, which give the "procedure" for the lab. These steps should be VERY simple, and easy to follow. You will not be permitted to carry books, and binders to your lab bench. So imagine the lab is not beside you. You will require THIS FLOW CHART to see what steps will follow.

An example flow chart is shown below.

Note: your flow chart may include diagrams/pictures; should include measurements & amounts required.

Pre-lab Questions: (to be answered in full sentences)
Carefully read the pre-lab discussion, and the procedure BASED ON Heath Chemistry page 24-29. These pages are attached for reference & include supplementary information you may find helpful.

1. What is chromatography used for?
2. What differences allow substances to be separated using chromatography?
3. What two features are shared by all forms of chromatography? In paper chromatography, what comprises these features?
4. Why do some components travel farther than others during a chromatogram?
5. What does R stand for? How is it calculated?
6. What range is possible for R values? What unit does it have?
7. Two chemists perform a chromatography procedure on the same substance and get quite different R values. How is this possible?
8. Would paper chromatography be suitable for separating large amounts of mixtures? Explain.

Materials:

Apparatus
- 600 mL beaker
- Large filter paper (chromatography paper)
- 25 mL measuring cylinder
- 50 mL beaker
- Disposable pipette
- Funnel
- Pencil
- Ruler
- scissors

Reagents
- Set of Winston FoodLabels
- Ethanol solution
**Procedure:** This lab is an excerpt from *Nestle Chemistry*, a textbook of laboratory experiments. Information regarding the lab and detailed procedure are provided on the following pages.

1. Obtain a circular piece of filter paper. Fold and cut to make an approx 20cm by 12cm piece of chromatography paper. 1cm from the bottom edge draw a pencil line along the long axis of the paper. Starting 1cm from the left side, make pencil marks every 2cm. Label the first seven spots as shown. Label the last spots as shown. Label the last spots with the unknowns you are going to test in the lab.

![Image of chromatography paper with labels]

2. Lightly curl the paper by running it over the edge of a table so that the paper can stand up by itself.
3. Using a toothpick, place a 1mm spot of the known food dyes on their respective mark. Similarly, spot the remaining 2 marks with your chosen "unknowns" being analyzed.
4. Form the chromatography paper into a loose cylinder so that it doesn't touch itself and staple.
5. Lower the paper into the beaker, taking care not to allow any contact between the paper and the sides of the beaker.
6. Using a 25mL measuring cylinder, measure out 30 mL of ethanol into a 50mL beaker.
7. Pipette about 3mL (approx. 25-30 mL) of the developer mixture (ethanol) into the bottom-centre of the 50mL beaker taking care NOT TO TOUCH THE CHROMATOGRAPHY PAPER.
8. Cover the beaker loosely with aluminium foil to reduce evaporation of the developer.
9. Leave the beaker for approximately 20 minutes as the chromatogram develops.

*NOTE* STEPS #10 + #11 MUST BE DONE SIMULTANEOUSLY AND AS QUICKLY AS POSSIBLE!

10. Remove the chromatogram from the beaker and carefully remove the staples. Lay flat to dry on a sheet of paper (be sure to mark with your group name).
11. Using pencil, IMMEDIATELY mark specific points on the chromatogram!
   - Mark the top edge of each spot
   - The position of the developer front above the spot
   - If a sample has separated into more than one component, mark the top of each component.
12. Make a data table in your notebook using the following headings. For samples that have more than one component, make a data table entry for each component.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Component Colour(s)</th>
<th>Solvent Distance: d₁ (cm)</th>
<th>Solute Distance: d₂ (cm)</th>
<th>( R_f = \frac{\text{distance of solute} (cm)}{\text{distance of solvent} (cm)} )</th>
<th>R_F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Golden Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pink</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Royal Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>Yellow, Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown A</td>
<td>Pink, Red, Orange, Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown B</td>
<td>Pink, Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13. For each sample, measure the distance travelled by the spot (solute) and the distance travelled by the solvent (ethanol solution). Some samples may have separated into more than one component. In such cases, measurements are needed for each component. Enter values in the data table.

14. For each sample, calculate the ratio of fronts (R_f value) and enter in the table.

**Safety:**

⚠️ **NOTE:** all data, observations and calculations are to be completed in numbered data tables with appropriate titles.

👏 **Safety glasses are to be worn at all times for all experiments!**

Reagent Disposal: All waste from this lab is to be rinsed down the sink with lots of water. All glassware used must be washed & rinsed thoroughly in order to be used in following reactions.

Clean Up: clean up all materials, wipe lab bench with disinfectant and wash hands well with soap and water before you leave the lab each day.
Safety glasses must be worn at all times for all experiments!

Reagent Disposal: All waste from this lab shall be collected in the designated waste container. All glassware should be rinsed thoroughly in order to be used in following reactions.

Clean Up: clean up all materials, wipe lab bench with disinfectant and wash hands well with soap and water before you leave the lab each day.

<table>
<thead>
<tr>
<th>Table 1:</th>
<th>&quot;Descriptive title&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Component Colour</td>
</tr>
<tr>
<td>Rf</td>
<td>Tucka</td>
</tr>
</tbody>
</table>

Y:
- yellow Rf: ........
- orange Rf: .......

Hand in only 1 copy per group.

Follow Up Q's:
1. Identify the dyes that appear on the chromatogram in Figure 20.5.3 (Consult Table 4 for Rf values.) The original sample was orange food coloring. (2 marks)
2. A pharmaceutical chemist runs a chromatography test on a substance and identifies two of its components by comparing their Rf values against certain standards. If the two components have Rf values of 1.0 and 0.41, and the solvent front has traveled 12.0 cm from the sample's origin, what is the separation distance on the chromatogram? (2 marks)
3. A chemist performs an R calculation, obtains a value of 1.2, and decides that the answer is unacceptable. Why? (1 mark)
4. In paper chromatography,
   a. If a molecule is very soluble in the liquid phase, and very non-reactive with the solid phase, how would it migrate? (1 mark)
   b. If a molecule is very insoluble in the liquid phase, and very reactive with the solid phase, how would it migrate? (1 mark)

Calculations:

\[ R_a = R_f = \frac{4.21}{6.31} = 0.667 \]

Conclusion:
Your conclusion should summarize your experimental results and answer your objective.

Describe your experimental (not human) error.