

Paper Chromatography

Simulation: edtechsims.com/paper-chromatography

Name: _____ Class: _____ Date: _____



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Activities 1 & 2: Setting Up Paper Chromatography

On the sim: In Activity 1, follow the five steps to set up and run paper chromatography. Complete each step in order and read the feedback carefully before moving on.

Before you begin

1. What type of mixture can be separated by paper chromatography?

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2. Fig. 1 shows the apparatus for paper chromatography. Name the parts labelled A to F.

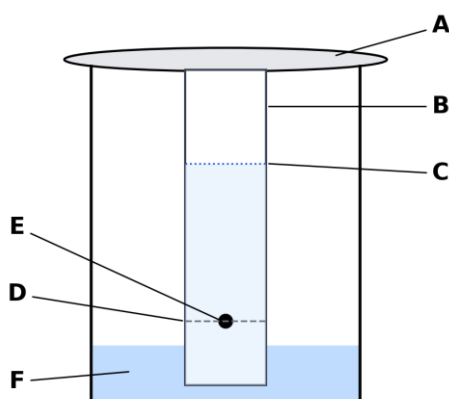


Fig. 1

A:

B:

C:

D:

E:

F:

Step 1 — Draw the Start Line

3. Why is the start line drawn in pencil and not in pen?

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Step 2 — Apply the Sample

4. Why should the sample spot be small and concentrated?

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Step 3 — Insert the Paper into the Solvent

5. Why must the start line be above the level of the solvent? What would happen if it were below?

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Step 4 — Cover the Beaker

6. Why is the beaker covered with a watch glass?

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Step 5 — Observe the Separation

7. For the Black Marker Ink, how many pigments are separated? Name their colours.

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8. Explain why the pigments travel different distances up the paper.

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9. Why should the paper be removed before the solvent reaches the top, and why is the solvent front marked straight away?

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Reflection

10. Number the following steps from 1 to 5 in the correct order.

Step	Order
Cover the beaker with a watch glass	
Apply a small spot of the sample on the start line	
Observe the separation and mark the solvent front	
Draw a start line in pencil near the bottom of the paper	
Place the paper in the beaker with the start line above the solvent	

11. A pure substance and a mixture are both run on the same chromatogram. How would you tell which is pure?

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Activity 3: Investigations — Identifying Unknown Samples

On the sim: In Activity 2, compare the chromatogram of an unknown sample with three reference samples, and identify the match.

Before you begin

1. List **three** features of a chromatogram that you should compare when identifying an unknown sample.

(i).....

(ii).....

(iii).....

Example 1 — Crime Scene Evidence

2. How many spots does the Unknown Ink show? _____

3. Which pen matches the Unknown Ink? _____

4. Explain how you reached this conclusion.

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Example 2 — Food Colouring Analysis

5. Which reference dye is present in the Mystery Mixture? _____

6. Is the Mystery Mixture a pure substance? Explain.

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7. The Yellow Dye reference and the Mystery Mixture both contain a yellow spot. Explain why the Yellow Dye is **not** the same substance as the yellow component of the mixture.

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Example 3 — Marker Authentication

8. Which reference dye is present in the Suspected Counterfeit marker? _____

9. How many components does the Suspected Counterfeit marker contain? Which of its spots do not match any reference?

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Reflection

10. Two different substances may produce spots at the same height in one solvent. Suggest how you could confirm whether they are the same substance.

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Activity 4: Calculating R_f Values

On the sim: In Activity 3, use the scale beside each chromatogram to measure distances from the start line, then calculate the R_f value of each pigment.

Before you begin

1. Write the formula used to calculate the R_f value.

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2. Explain why an R_f value is always between 0 and 1.

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3. From which line must both distances be measured?

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Example 1 — Single Pigment Analysis

4. Record your measurements and calculate the R_f value.

Pigment	Distance moved by spot (cm)	Distance moved by solvent front (cm)	R_f value
Red Dye			

Example 2 — Two Pigment Separation

5. Record your measurements and calculate the R_f values.

Pigment	Distance moved by spot (cm)	Distance moved by solvent front (cm)	R_f value
Blue Component			
Yellow Component			

6. Which component is more soluble in the solvent? Explain using the R_f values.

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Example 3 — Complex Mixture

7. Record your measurements and calculate the R_f values.

Pigment	Distance moved by spot (cm)	Distance moved by solvent front (cm)	R_f value
Pink			
Blue			
Yellow			

Going Further

8. The table shows the R_f values of three dyes in the same solvent. A spot from an unknown dye moves 3.6 cm while the solvent front moves 8.0 cm. Identify the unknown dye. Show your working.

Dye P	Dye Q	Dye R
$R_f = 0.25$	$R_f = 0.45$	$R_f = 0.70$

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9. Amino acids are colourless. Explain how their spots can be seen on a chromatogram.

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10. A student compares R_f values from two experiments that used different solvents. Explain why this comparison is not valid.

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Answer Key: Paper Chromatography

Activities 1 & 2

- 1 A mixture of soluble substances, such as the coloured dyes in ink or food colouring.
- 2 A: watch glass (lid). B: chromatography (filter) paper. C: solvent front. D: start line (in pencil). E: sample spot. F: solvent.
- 3 Pencil (graphite) does not dissolve in the solvent, so it will not move up the paper and interfere with the results. Pen ink would dissolve and separate.
- 4 A small, concentrated spot gives clear, well-separated spots. A large spot spreads out and the components overlap.
- 5 If the start line is below the solvent level, the sample dissolves into the solvent in the beaker instead of travelling up the paper, so no separation is seen.
- 6 To stop the solvent from evaporating, so the air inside is saturated with solvent vapour and the solvent rises evenly, giving reproducible results.
- 7 Three pigments: pink, blue and yellow (check against the sim).
- 8 Each pigment has a different solubility in the solvent and a different attraction to the paper. Pigments that are more soluble (less attracted to the paper) travel further.
- 9 If the solvent reaches the top, the solvent front cannot be marked and the spots may run off the paper. The solvent front is marked immediately because it is needed to calculate R_f values and becomes invisible once the solvent evaporates.
- 10 Cover the beaker: 4. Apply the sample: 2. Observe and mark the solvent front: 5. Draw the start line: 1. Place the paper in the beaker: 3.
- 11 A pure substance gives only one spot; a mixture gives two or more spots.

Activity 3

- 1 (i) Number of spots; (ii) colours of the spots; (iii) height of each spot / R_f value.
- 2 2 spots (blue and purple).
- 3 Pen A.
- 4 Pen A has the same number of spots (two), the same colours (blue and purple) and the spots are at the same heights (same R_f values) as the Unknown Ink. Pens B and C have different colours and/or heights.
- 5 Red Dye.
- 6 No. It separates into three spots (yellow, red and blue), so it is a mixture.
- 7 The spots are at different heights, so they have different R_f values in the same solvent. The same substance would travel the same distance.
- 8 Yellow Dye.
- 9 Three components (blue, yellow and light green). The blue and light green spots are at different heights from the Blue Dye and Green Dye references, so they do not match any reference.
- 10 Run both substances again using a different solvent (and compare R_f values), or run them side by side on the same paper. If they are the same substance they will give matching spots in every solvent.

Activity 4

- 1 R_f = distance moved by the substance (spot) $\div$ distance moved by the solvent front.
- 2 The substance cannot travel further than the solvent front, so the top of the fraction is always smaller than (or equal to) the bottom.
- 3 The start line (the pencil line where the sample was spotted).
- 4 Red Dye: R_f = 0.75.
- 5 Blue Component: R_f = 0.80. Yellow Component: R_f = 0.47.
- 6 The Blue Component. It has the higher R_f value, so it travels further with the solvent.
- 7 Pink: R_f = 0.79. Blue: R_f = 0.58. Yellow: R_f = 0.35. (Distances from the sim's scale; accept R_f within ± 0.01 .)

Going Further

- 8 R_f = $3.6 \div 8.0$ = 0.45, so the unknown dye is Dye Q.
- 9 Spray the dried chromatogram with a locating agent (e.g. ninhydrin), which reacts with the amino acids to form coloured spots.
- 10 R_f values depend on the solvent used (and conditions such as temperature). The same substance has different R_f values in different solvents, so values can only be compared if the same solvent is used.