Your teacher may watch to see if you can:

- follow instructions carefully
- draw conclusions from your results.

Introduction

Many inks contain a mixture of dyes. This method can be used to identify inks – for example inks from crime scenes or from documents that may have been forged.

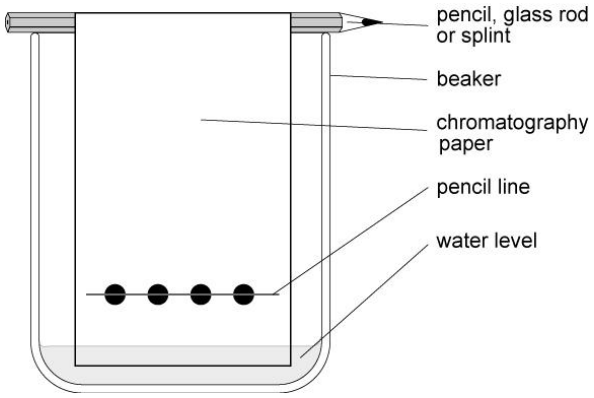
Aim

You are going to test some inks to see how many dyes they contain. You will then plan your own investigation.

Method

Apparatus

- beaker
- **chromatography** paper attached to rod
- 4 black marker pens or felt-tip pens



A Check that your chromatography paper hangs close to the bottom of the empty beaker without touching it (as shown in the diagram).

B Take the paper out of the beaker and draw a pencil line on the paper, about 2 cm from the bottom.

C Put a small spot of ink from each pen on your pencil line.

D Label underneath each spot with a pencil.

E Pour some water into the beaker to a depth of about 1 cm.

F Lower the chromatography paper into the beaker so that the bottom of the paper is in the water, but the water level is below the spots.

G Leave the paper in the beaker until the water reaches near the top of the paper.

H Take the paper out and immediately use a pencil to mark the location of the solvent front (the level the water has reached) before it evaporates. Leave it to dry.

Recording your results

1 Describe the coloured dyes that mixed to produce the black ink in each pen.

2 Measure the distance the solvent has risen from the pencil line.

3 Measure the distance that each dye spot has risen from the pencil line. Write your results in a table.

Considering your results

4 Calculate the **R_f value** for each separate colour in the four inks.

5 Were any of the black inks a pure colour? Explain your conclusion.

6 Did the same coloured dyes appear in more than one ink? If so, do you think they were the same chemical compound? Explain your answer.

Evaluation

7 Why was the starting line drawn in pencil?

8 Why did you have to label the spots?

9 Why is the chromatography paper hung with the bottom just in the water?

10 Why must the water level in the beaker be below the spots?

11 How easy was it to identify the level to which each coloured dye had travelled? How would this affect the accuracy of the R_f values you calculated?

Chromatography investigations

Now you have seen how to investigate the dyes used to make some black inks, you can use the same techniques for other investigations.

1 Choose one of the following questions:

- Do any inks used in felt-tipped pens or marker pens consist of just one dye?
- Are some coloured inks usually a mixture of more dyes than other colours of ink?
- Are similar mixtures of dyes used in the black ink in pens with permanent inks? ('Permanent' inks are those that do not dissolve in water, so you will also need to investigate the best solvent to use.)

2 Write a plan for your investigation, explaining which inks you will test and how you will analyse your **chromatogram**.

⚠ Safety

Many solvents are flammable.

3 Show your plan to your teacher before you start.

Poison pen letter

The police have been asked to find out who has been writing nasty letters to people. It is not easy to obtain ink samples from a letter, so only one chromatogram can be made using this ink. You will be allowed to look at this chromatogram.

The police have several suspects, and have taken pens from their houses. Your task is to test the ink in the pens to see if any of them match the ink used on the letter.

4 Write a plan to explain how you will try to find out if any of the pens could have been used to write the letter. In your plan you should explain:

- why you will need to calculate R_f values for the different dyes in the inks you will test
- why you need to use the same solvent and same kind of paper in your investigation as the solvent and paper used for the chromatogram made using ink from the letter
- whether or not the results of your investigation will *prove* that one of the suspects wrote the letter.

5 When you have carried out your investigation, write a short report for the police. Your evidence may be used in court, so your report should include:

- a brief description of what you did to test the inks, and how you decided if any of them matched the ink from the letter
- an evaluation of your results to show how confident you are about your conclusion.

The white cards (A–M) show the steps needed to separate the different colours in dyes using **paper chromatography**. The grey cards (1–9) have explanations for some of the steps.

1 Cut out the white cards and arrange them in order.

2 Cut out the grey cards and match them up to the correct steps in the method.

A Label the samples on the paper using a pencil.	**B** Allow the paper to dry, then measure the distance between your original pencil line and the line the solvent reached.
C Leave the paper in the solvent until the solvent has nearly reached the top of the paper.	**D** Calculate the **R_f value** for each substance in the dyes by dividing the distance each substance has travelled by the distance the solvent has travelled.
E Measure the distance of each spot from the original pencil line.	**F** Compare the R_f values of different substances to try to identify them.
G Put about a 1 cm depth of solvent into the beaker.	**H** Cut the paper so that it doesn't quite reach the bottom of an empty beaker.
I Draw a pencil line about 2 cm from the bottom of the paper.	**J** Put the paper into the beaker so that the end of it just dips into the solvent.
K Drop a sample of each colouring on the pencil line and let it dry.	**L** Remove the paper from the solvent, and use a pencil to mark where the solvent has reached.
M Fasten a piece of paper to a pencil or splint.	
1 This allows the different substances in each dye to separate as much as possible.	**2** To support the paper in a beaker.
3 This is needed to calculate the R_f value for an individual substance.	**4** If two substances have the same R_f value, they are likely to be the same substance, so long as the **chromatography** was carried out in the same way.
5 The level the solvent reached will not be visible when the paper dries.	**6** This information is needed to calculate R_f values for all the substances in the dyes.
7 The solvent in the beaker must not reach the samples on the paper, or they will just dissolve into it instead of moving up the paper.	**8** Pencil is used so that the mark does not dissolve in the solvent.
9 To make sure all the dyes start at the same level.	

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EASIER

1 The police have taken four orange lipsticks from suspects. Complete these sentences, using words from the box, to explain the steps needed to find out if one of the lipsticks could have made a mark at a crime scene. You can use each word once, more than once or not at all.

compared	dissolve	dyes	evaporate	filter	five	four	insoluble
lipstick	paper	pattern	separate	soluble	solute	solvent	

The police need to test the _____ lipsticks and some _____ from the mark at the crime scene. All _____ samples are put onto a piece of **chromatography** _____, so they can be _____ with each other. The bottom of the chromatography paper is dipped into a _____. The _____ will move up the paper, and the _____ from the lipsticks will _____ in it. The dyes that are the most _____ will move up the paper fastest.

If a lipstick contains more than one dye, the sample will _____ into different spots. Different combinations of _____ will produce different patterns. If one of the lipsticks from the suspects produces the same _____ as the sample from the crime scene, the mark could have been left by that lipstick.

S1 The police have taken four orange lipsticks from suspects. Explain the steps needed to find out if one of the lipsticks could have made a mark at a crime scene.

2 Here are some glossary definitions. What words are they describing?

	Definition	**Word**
a	The part of the apparatus that the solvent and dyes move up.	_____
b	A ratio that compares the distance moved by a dye to the distance moved by the solvent.	_____
c	The solvent that moves up the paper in chromatography.	_____
d	The pattern of spots produced when an ink separates into its different dyes.	_____

HARDER

3 A student carries out a chromatography test on a sample of paint, but no spots appear on the **chromatogram**. Which is the best explanation for this? Tick one box.

A The paint does not have any colouring in it. ☐

B The dyes in the paint do not dissolve in the solvent used. ☐

C The paint has only one dye in it. ☐

stationary phase	**mobile phase**	chromatogram	**R_f value**

E1 A laboratory produces a list of **R_f values** for food colourings. Explain why R_f values are used and what other information is needed for these R_f values to be useful.

1 What measurements do you need to make from a **chromatogram** to calculate an R_f value?

2 How is an R_f value calculated?

3 Ali and Bill follow exactly the same method to investigate the mixture of dyes in blue ink, except that Ali leaves his **chromatography** paper in the solvent for 3 minutes and Bill leaves his in for 4 minutes.

a Describe the difference(s) you would expect to see between the two chromatograms.

b Explain how calculating R_f values for the different spots on their chromatograms will help Ali and Bill to check that they have got the same results.

c Chris carries out a similar test on a different day. Explain what he will have to do if he wants to compare his results to Ali's results.

4 Two laboratories produce lists of R_f values for food colourings, but the values in the two lists are different.

a Explain why this does not necessarily mean that either of the lists is incorrect.

b What information do the laboratories need to publish with their lists of R_f values to make the values useful to other people testing the food colourings?

5 Use your answers to questions 1–4 to help you to write a short paragraph that answers question **E1**. Your paragraph should be less than 100 words long.

6 Describe three different mixtures that can be separated by chromatography.

7 Use diagrams to help you to explain how a chromatogram can be used to:

a distinguish between a pure substance and a mixture

b identify whether or not two mixtures contain the same substances.

8 The table shows the solubilities of some different food colourings. A chromatogram is made from a mixture of all four. Explain what you would see on the chromatogram.

Colouring	Solubility (g/100 g water)
R176	38
R792	47
Y1438	17
X8649	23

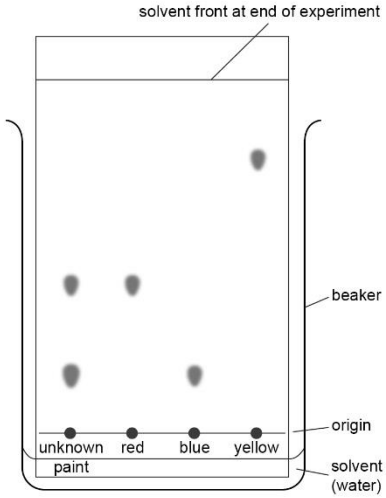
1 Jack did an experiment to look at the pigments in some water-soluble paint. He used **paper chromatography**.

a Why is the level of the solvent lower than the level of the spots?

b Why has he put the red, blue and yellow paints next to the unknown paint?

The diagram on the right shows the **chromatogram** that Jack obtained.

c What colour paints were in the 'unknown' paint that Jack used? Explain your answer.



2 In a **chromatography** experiment to identify the food dyes in a red sweet, the solvent moved 10 cm up the paper. Three spots were seen – spot X had moved 7.6 cm, spot Y had moved 4.6 cm and spot Z had moved 2.6 cm.

a Calculate the **R_f values** for spots X, Y and Z.

Remember that the solvent always moves further than the dye, so the R_f value is always less than 1.

The R_f values for some food dyes are given in the table.

Food dye	R_f value
mauve	0.76
blue	0.55
carmine	0.46
red	0.26
yellow	0.24

b Identify the food dyes in the red sweet.

3 The statements below describe some mistakes made in a chromatography experiment, and what effect the mistakes will have. Match up the mistakes with the correct effects, and write the letters and numbers in your book.

a You leave the paper in the water for too long.

b You use a felt pen instead of a pencil to draw the line across the bottom of the paper.

c You make the spots too close to the bottom of the paper, so that they are below the water level when you put the paper into a beaker.

d You put some of the spots onto the paper above the pencil line.

e You use water to test some permanent inks.

1 The spots will wash out of the paper into the solvent.

2 All of the dyes will reach the top of the paper.

3 Permanent inks are not soluble in water so they will not move up the paper.

4 The colours from the pen will spread out on the paper.

5 The R_f values will not be accurate because the spots will not have moved as far as they appear to have moved.

$$R_f = \frac{\text{distance moved by compound}}{\text{distance moved by solvent}}$$

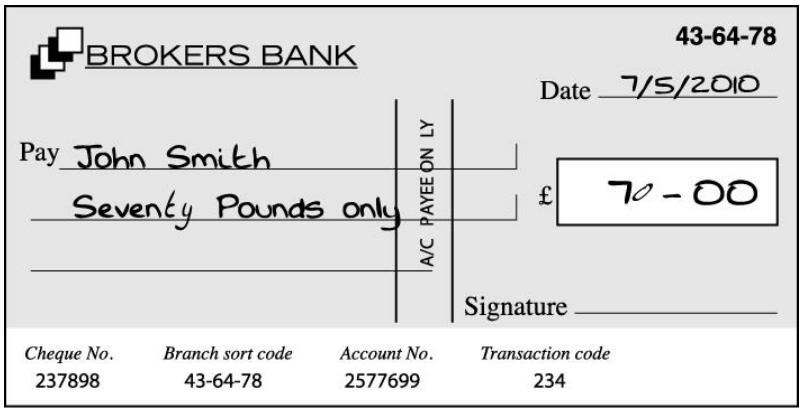
Worked example

If the solvent moves 10 cm and a spot moved 4 cm up the paper:

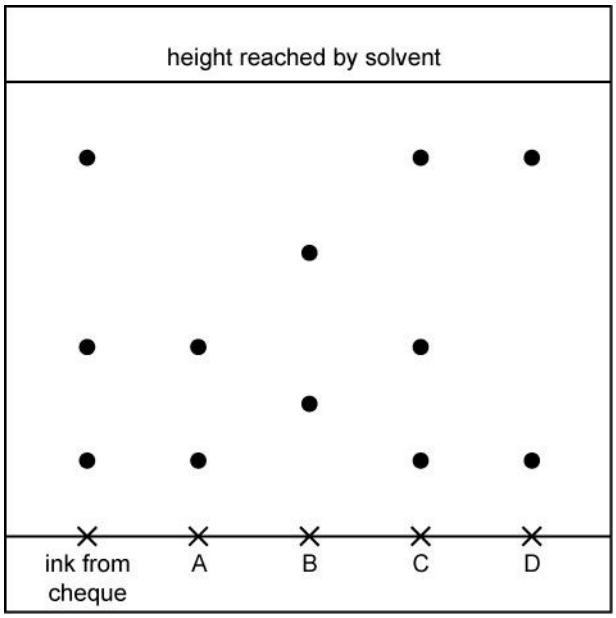
$$R_f = \frac{4}{10} = 0.4$$

A forger altered a cheque by changing the amount from 'seven' to 'seventy'. A forensic scientist was asked to find out who had done it.

The cheque was cut up and the black ink from the 'ty' of the 'seventy' and the '0' of the '70' were dissolved in ethanol. There were four suspects – A, B, C and D.



They were each asked for the black pen they use. A **chromatogram** was produced from the ink on the cheque, and the inks from the pens of the four suspects.



1 Describe the steps that the forensic scientist would have to carry out to make the chromatogram shown.

2 How many dyes were in the ink from the cheque?

3 **a** Which suspect's pen was definitely **not** used to forge the cheque?

b Explain your answer to part **a**.

4 Calculate the **R_f value** for each dye in the ink from the cheque.

5 Explain what will go wrong in each of the following descriptions of **chromatography** experiments.

a You leave the paper in the water for too long.

b You use a felt pen instead of a pencil to draw the start line across the bottom of the paper.

c You make the spots too close to the bottom of the paper, so that they are below the water level when you put the paper into a beaker.

d You put some of the spots on the paper above the pencil line.

e You use water to test some permanent inks.

f You test a sample from a crime scene on one piece of paper, and the dye samples from the manufacturers on a different piece of paper.

Extra challenge

6 In a chromatography experiment to identify the black colouring in some ink, the solvent front reached a height of 10 cm.

Use the R_f values on the right to draw the chromatogram that would be obtained from the mixture of dyes in one type of black ink.

Food dye	R_f value
mauve	0.76
blue	0.55
carmine	0.46
red	0.26
yellow	0.24
brown	0.10

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Progression questions

Answer these questions.

1 How can **chromatography** be used to separate mixtures?

_____

_____

2 What are the differences between mixtures and pure substances on a **chromatogram**?

_____

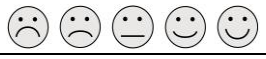
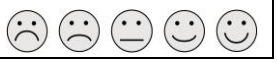
_____

3 How do you calculate an **R_f value**?

_____

_____

Now circle the faces in the 'Start' row in the table showing how confident you are of your answers.

Question	**1**	**2**	**3**
Start			

Assessment

Using a different colour, correct or add to your answers above. You may need to use the back of this sheet or another piece of paper. Then circle the faces in the 'Check' row in the table.

Question	**1**	**2**	**3**
Check			

Feedback

What will you do next? Tick one box.

☐ strengthen my learning ☐ strengthen then extend ☐ extend

Note down any specific areas you need to improve.

_____

Action

You may now be given another activity. After this, note down any remaining areas you need to improve and how you will try to improve in these areas.

_____

_____