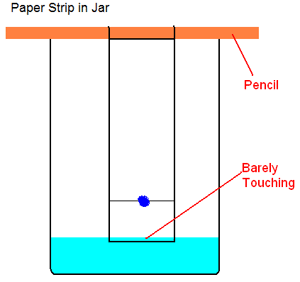
Chromatography

See how many different colours there are in pen ink

The outline for the main method of chromatography is below, explain why each step is followed

* Put a thin pencil line 1cm up from the bottom of the paper

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* Put a pencil dot in the middle of the line and using a capillary tube put at least 3 spots of your sample on the dot, waiting for it to dry in between

………………………………………………………………………………………………………

* Place into jar with solvent in making sure the solvent is just below the line you have drawn

……………………………………………………………………………………………………………………………………..

* Put a lid on top of the jar

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* Wait for the solvent to get to the top, when it does remove the paper and draw a pencil line to show where the solvent has got to and wait for it to dry.

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|  |  |  |  |
| --- | --- | --- | --- |
| **Colour** | **Distance travelled by spot** | **Distance travelled by solvent** | **Rf Value**  **(distance of spot ÷ distance of solvent)** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

## Rf values

The higher the Rf value, the higher the spot has moved. We can use Rf values to compare with other samples to see if they contain the same components. What can we use chromatography for?

The Rf value also tells us about how well the sample has dissolved in the solvent. The more it dissolves in the solvent, the further it will move up. The less it dissolves the less it will move. We used water for the pens which is a very polar solvent. The more the inks moved the more polar they were.

## Paper chromatography of amino acids

Compare how far the acid moves up the paper with known amino acids, use ninhydrin to help you see the amino acids

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Distance travelled by spot** | **Distance travelled by solvent** | **Rf Value**  **(distance of spot ÷ distance of solvent)** | **Actual Rf value (published)** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

For published values see: <http://www.biotopics.co.uk/as/amino_acid_chromatography.html>

## Extracting plant pigments using paper chromatography and TLC (thin layer chromatography)

Most leaves are green due to chlorophyll. This substance is important in photosynthesis (the process by which plants make their food). In this experiment, the different pigments present in a leaf are separated using paper chromatography.

### Method

1. Finely cut up some leaves and fill a mortar to about 2 cm depth.

2. Add a pinch of sand and six drops of propanone from the teat pipette.

3. Grind the mixture for at least three minutes.

4. On a strip of chromatography paper, draw a pencil line 3 cm from the bottom.

5. Use a fine glass tube to put liquid from the leaf extract onto the centre of the line. Keep the spot as small as possible.

6. Allow the spot to dry, then add another spot on top. Add five more drops of solution, letting each one dry before putting on the next. The idea is to build up a very concentrated small spot on the paper.

7. Put a small amount of propanone in a beaker and hang the paper so it dips in the propanone. Ensure the propanone level is below the spot.

8. Leave until the propanone has soaked near to the top.

9. Mark how high the propanone gets on the paper with a pencil and let the chromatogram dry.

## Results for paper chromatography

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample colour** | **Distance travelled by spot** | **Distance travelled by solvent** | **Rf Value**  **(distance of spot ÷ distance of solvent)** | **Plant pigment (look at the table on the next page)** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

### Questions

1. How many substances are on the chromatogram?

………………………………………………………………………………………………………………………………………………………

2.what is the stationary phase and mobile phase in this chromatography?

………………………………………………………………………………………………………………………………………………….

## Results for TLC

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample colour** | **Distance travelled by spot** | **Distance travelled by solvent** | **Rf Value**  **(distance of spot ÷ distance of solvent)** | **Plant pigment (look at the table on the next page)** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

### Questions

1. How many substances are on the chromatogram?

………………………………………………………………………………………………………………………………………………………

2. What is the stationary phase and mobile phase in this chromatography?

…………………………………………………………………………………………………………………………………………………..

3. Did the spots move as far as with the paper chromatography? And why do they move differently?

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Summary of Rf values and visible colors for chlorophyll plant pigments

|  |  |  |
| --- | --- | --- |
| **Pigment name** | **Visible color** | **Rf** |
| Carotene | Yellow | 0.98 |
| Xanthophyll | Yellow | 0.86 |
| Xanthophyll | Red | 0.80 |
| Phaeophytin a | Dark grey | 0.67 |
| Phaeophytin b | Light grey | 0.60 |
| Xanthophyll | Yellow | 0.50 |
| Chlorophyll a1 | Light blue-green | 0.48 |
| Chlorophyll a | Dark blue-green | 0.46 |
| Chlorophyll b1 | Light yellow-green | 0.30 |
| Chlorophyll b | Dark yellow-green | 0.25 |
| Xanthophyll | Yellow | 0.15 |

Summary of Rf values for carotenoid plant pigments

|  |  |  |
| --- | --- | --- |
| **Pigment name** | **Visible color** | **Rf** |
| -carotene | Yellow-orange | 0.97 |
| -carotene | Yellow orange | 0.94 |
| ;Lycopene | Red-orange | 0.81 |
| Leutein | Yellow-brown | 0.75 |
| Violaxathin | Yellow-brown | 0.66 |
| Neoxathin | Yellow-brown | 0.28 |

## Different types of Chromatography

On the following table, fill in the mobile and stationary phases for the different types of chromatography. Draw a sketch of the results which you might obtain and explain when it might be used:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type | Mobile phase | Stationary phase | Diagram of results | What it is used for |
| Paper chromatography |  |  |  |  |
| Thin layer chromatography |  |  |  |  |
| Gas chromatography |  |  |  |  |
| Ion exchange chromatography |  |  |  |  |
| High performance liquid chromatography |  |  |  |  |

## Interpreting chromatograms

What does looking at a chromatogram tell us, try to think of a couple of things:

* ……………………………………………………………………………………
* …………………………………………………………………………………..

Why do you think some substances move further than others ?

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Chromatography is very handy to make comparisons between mixtures of substances, but it also gives us some idea about the types of intermolecular forces in the substances.

If the mobile phase is very polar, what type of substances will mix with the mobile phase and move further up the paper?

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If the stationary phase is non-polar what type of substances will stay with the stationary phase and not move very much?

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## Evaluation of chromatography

What are the difficulties you found when carrying out chromatography (think about both paper chromatography and TLC):

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