OCR (A) Biology A-level

Module 1: Development of practical skills in Biology

PAG 6: Chromatography OR Electrophoresis

Please note: You only need to do one from each PAG, and you don't need to do the PAGs listed here, as long as you show the same skills that these are testing (see 5f of the specification for more information). However, you need to at least be able to design your own method for most of these experiments in the exam.

Chromatography – theory:

Chromatography is an **analytical method** used to **separate a mixture into the different biological molecules**. There are two components: the **stationary phase** and the **mobile phase**.

The **stationary phase** is either a **TLC plate or chromatography paper**. Chromatography paper is made out of cellulose, a TLC plate is usually a plastic sheet coated in a layer of aluminium hydroxide or silica gel.

The **mobile phase** is the **solvent for the biological molecules**. The mobile phase **travels up the stationary phase and carries the biological molecules with it.**

Each type of biological molecule will be **adsorbed onto the surface of the TLC by different strengths**. Molecules which are **adsorbed more** will **travel up the plate slower**, meaning that **different molecules will travel at different speeds up the plate**, being **separated** as they do.

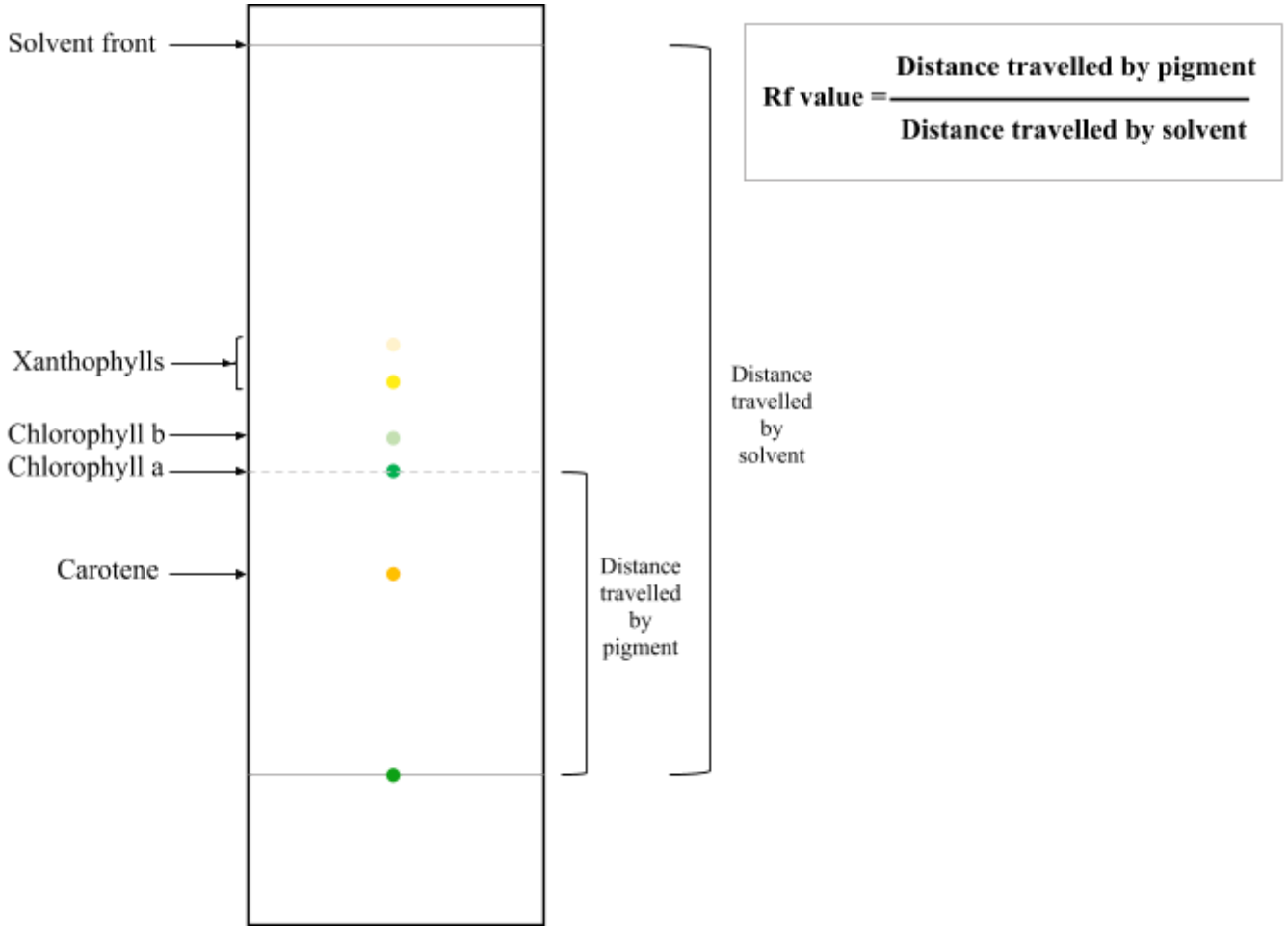
Adsorption = where molecules bond **to the surface of a substance** – do not confuse this with absorption!

Limitations of chromatography include the fact that similar molecules may have similar Rf values, meaning that it may be difficult to distinguish between them.

Chromatography – chlorophyll pigment method:

1. **Grind up the leaves using a pestle and mortar**, with some anhydrous sodium sulphate and then add some propanone as a solvent.
2. Transfer the liquid to a test tube and add some petroleum ether and shake. Two layers will form – the top layer is the pigments mixed with petroleum ether.
3. Transfer some liquid from the top layer with some anhydrous sodium sulphate
4. **Draw a line on the TLC plate with a pencil** about 2cm from the bottom.
5. **Use a capillary tube to take up some of the pigment solution, and then use this to place a dot of pigment in the middle of the pencil line**.
6. **Allow the dot to dry** (you have to allow it to dry to prevent the pigment from spreading out into a larger dot), **then add another spot over the first dot. Repeat this until you have a concentrated spot of pigment.**
7. **Add a small amount of solvent in a beaker** (less than 2cm depth – **the pencil line should be above the solvent line**). The solvent should be a mixture of propanone, cyclohexane and petroleum ether.
8. **Place the TLC plate in the beaker** with the pencil line towards the bottom.
9. **Place a watch glass over the beaker to stop the solvent evaporating**. This should be done in a fume cupboard.
10. The solvent will move up the plate, and the pigments will separate out.
11. When the solvent almost reaches the top, take the paper out and mark the **solvent front** with a pencil. Also **mark at the side of the plate the locations of the pigments, as these may also disappear as the solvent dries.**
12. Leave the paper to dry.

13. Measure with a ruler the **distance travelled by each pigment up the plate**, as well as the **distance the solvent moved**.
14. Calculate the **Rf value** for each spot by **dividing the distance travelled by the pigment by the distance travelled by the solvent** (note: you should always get a value less than 1!! – e.g. 0.75)
15. If you are identifying pigments, **compare the obtained Rf values with reference values.**



Chromatography – amino acid method:

1. **Draw a pencil line near the bottom of a piece of chromatography paper**.
2. Put a concentrated spot of the mixture of amino acids on the paper.
3. **Add a small amount of solvent** (butan-1-ol, glacial ethanoic acid and water) to the beaker. **Put the chromatography paper in** and **ensure the pencil line is above the solvent line**.
4. Place a **watch glass over the beaker to stop the solvent evaporating**. This should be done in a fume cupboard.
5. As the solvent moves up the paper, the amino acids separate out.

6. When the solvent almost reaches the top, take the paper out and mark the **solvent front** with a pencil.
7. **Leave the paper to dry.**
8. Amino acids aren't coloured so **spray the amino acids with ninhydrin solution, which makes them go purple**. This should be done in a fume cupboard.
9. Measure the **distance the solvent moved** and the **distance each spot moved up the plate**.
10. Calculate the **Rf value** for each spot by dividing the **distance travelled by the spot by the distance travelled by the solvent** (note: you should always get a value less than 1!! – e.g. 0.75).
11. **Compare experimental values to known values to identify the unknown amino acids**.

Electrophoresis:

1. The DNA first has to be cut into fragments by **restriction enzymes**. This could take up to an hour.
2. Pour **agarose gel** into a gel tray a leave it to solidify.
3. A row of wells is created at one end of the tray.
4. Put the gel tray into a gel box/tank.
5. Make sure the end of the gel tray with the wells is closest to the **negative electrode** on the gel box.
6. Add **buffer solution** to the reservoirs at the sides of the gel box so that the surface of the gel becomes covered in the buffer solution.
7. Using a **micropipette**, add the same volume of **loading dye (10 ul)** and DNA sample and put in the bottom of a well. **The loading dye helps the DNA fragment sink to the bottom of the well and make the fragments more visible.**
8. Do not stick the micropipette too far in the well because it will pierce the bottom.
9. Repeat this process for each DNA sample, using a clean micropipette for each different DNA fragment.
10. Record which DNA sample goes into which well.
11. Put the lid on the gel box and connect the leads from the gel box to the power source.
12. Turn on the **power source** and set it to 100V.
13. **DNA fragments are negatively charged so will move towards the positive electrode.**
14. Smaller DNA fragments will move through the gel **faster than larger fragments**, so the it will travel further in the same time. This will separate and order the fragments from smallest to longest.
15. Let the gel run for 30 minutes or until the dye is about 2 cm from the end of the gel.
16. Remove the gel box and tip off any excess buffer solution.
17. Wearing gloves **stain the DNA fragments** by covering the surface of the gel with a staining solution then **rinsing the gel with water.**
18. The bands of different DNA fragments will now be visible.

19. This method can be used with **RNA fragments and proteins** too. Proteins are positively and negatively charged so a chemical is added to make them all negatively charged.
20. **The bands of DNA** in each sample can be **compared** for similarities and differences.