31. Separation techniques

Student Sheet

In this exercise, you will have the opportunity to learn about four different techniques for separating substances in a mixture. All of these techniques are widely used in the outside world.

Intended lesson outcomes

By the end of this exercise you should be able to:

- use solvent extraction techniques and purify by recrystallising
- perform a titration and use a separating funnel
- determine a partition coefficient
- use and understand paper chromatography
- use and understand **two-way** chromatography
- use and understand electrophoresis
- understand amino acid structure and zwitterions

1 Solvent extraction of caffeine from tea or coffee

Background information

Caffeine, a heterocyclic base ($C_8H_{10}N_4O_2$, m.p. 235–237 °C), is found in tea and coffee, and is also present in cola drinks. It is readily soluble in trichloromethane and so this solvent can be used to extract caffeine from an aqueous solution of caffeine. Both tea and coffee provide a ready source of caffeine but the extraction from tea goes more smoothly, since trichloromethane emulsions form less readily and there are fewer coloured impurities. The extraction of caffeine from the tea or coffee into water is 'solvent extraction'; the extraction of caffeine from the water layer into the trichloromethane layer is an example of 'partitioning'.

Safety

- Eye protection should be worn throughout the exercise
- Take care when handling the hot extract
- Points 11, 12 and 13 below **must** be performed in a **fume cupboard**

	**You must wear eye protection throughout this experiment**
	Lead ethanoate (lead acetate) is **toxic**
	Sulphuric acid and ammonia are **corrosive**
	Trichloromethane (chloroform) is **harmful**
	Lead ethanoate and ammonia are **dangerous for the environment**

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Procedure

1. Weigh out about 50 g of tea or roast ground coffee, or 20 g of 'instant' coffee, and warm it in a beaker with 200 cm^3 of water, boiling gently for 15 minutes.
2. Remove the solids by filtering through muslin using, if possible, vacuum filtration; wash the solids with a little hot water and combine the washings with the original filtrate.
3. Heat the filtrate to boiling and add 100 cm^3 of aqueous lead ethanoate to precipitate any albumin and acids present.
4. Filter a small sample through a cotton-wool plug.
5. Use this small sample of your filtrate to check that the precipitation is complete by adding to it a little aqueous lead ethanoate. If any further precipitate forms, add more aqueous lead ethanoate to the original filtrate and repeat steps 4 and 5 until you are convinced that all albumin and acids have been removed.
6. Filter using, if possible, vacuum filtration, placing muslin over the filter paper to prevent clogging.
7. Add dilute sulphuric acid to the filtrate to precipitate all lead ions and remove the lead sulphate precipitate by filtration.
8. Add dilute aqueous ammonia to the filtrate until it is neutral to litmus.
9. Evaporate this solution to 100 cm^3; allow to cool a little.
10. Add 5 g of decolourising charcoal and bring to the boil cautiously. Filter using, if possible, vacuum filtration to remove the charcoal.

The steps which follow involve the use of trichloromethane and must be performed in a fume cupboard

11. Using a separating funnel, extract the filtrate by adding a 40 cm^3 portion of trichloromethane and, rather than shaking vigorously (which may cause emulsification), invert the funnel frequently over a period of 30 seconds. Allow the layers to separate, and remove the lower trichloromethane layer. Repeat the process with a second 40 cm^3 portion of trichloromethane and combine it with the first trichloromethane extract.
12. Dry the trichloromethane solution by adding **anhydrous sodium sulphate;** leave the mixture to stand for 15 minutes, or longer if possible, and then filter it.
13. Distil off most of the trichloromethane, and then evaporate the remaining solution to dryness in a beaker on a water-bath ***in a fume cupboard***. About 1 g of caffeine should remain.

Purification

14. Recrystallise from the **minimum volume** of hot water.
15. Assess the purity of your sample of caffeine by determining its melting point. Compare your value with the data book value of 235–237 °C.

Question

Suggest why it is an advantage that emulsions form less readily when using tea as the source of caffeine.

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2 Partition coefficients – the distribution of iodine between hexane and water

Background information

If a solute is added to a mixture of two immiscible solvents then the solute will dissolve in both solvents. The degree to which the solute dissolves in each solvent will depend on the solubility of the solute in each solvent. It is unlikely, therefore, that the concentration of the solute will be the same in both solvents. The ratio between these two concentrations is known as the **partition coefficient**.

Iodine is soluble in both water and hexane. Water and hexane are immiscible (they do not mix to any appreciable degree) so, when they are both added to a flask, two layers are formed; water forms the bottom layer as it is more dense than hexane. If solid iodine is added to this beaker, it dissolves in both solvents, forming an orange/yellow colour in the water and a darker red/purple colour in the hexane.

As the solid iodine falls to the bottom of the beaker, it dissolves first in the water. Slowly, the colour in the hexane layer develops as iodine moves from the water layer into the hexane layer. Shaking the mixture can accelerate this process. Eventually, the colours stabilise and equilibrium is reached.

$$I_2(aq) \rightleftharpoons I_2(hexane)$$

At the interface between the two solvents (the solvent boundary) iodine is continually moving backwards and forwards between the two solvents. The rate at which iodine move from hexane to water will eventually become equal to the rate of iodine moving in the opposite direction. Thus, an equilibrium is established.

The equilibrium constant for this process is shown below. The subscripts after the equilibrium constant symbol, *K*, define the solvents used, i.e. ‘h’ = hexane and ‘w’ = water, and ‘hw’ showing that the order of the concentration ratio.

$$K_{hw} = \frac{[I_2(hexane)]}{[I_2(aq)]}$$

Safety

Materials data safety sheets should be consulted so that the correct action can be taken in event of a spillage and/or accident

- Take care when handling the hot extract.

	**You must wear eye protection throughout this experiment**
  	Hexane is **highly flammable**, **harmful** and **dangerous to the environment**. Your teacher will tell you how to dispose of it safely

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Procedure

1. Measure 30 cm^3 of hexane and 30 cm^3 of water into a 100 cm^3 conical flask.
2. Add about 1 g of iodine crystals to the flask, insert the bung and shake the mixture until the colour in each layer is stable.
3. Decant the mixture into a separating funnel and run off the two layers into separate 100 cm^3 beakers.
4. Using a pipette, transfer a 25.0 cm^3 portion of the aqueous solution of iodine to a 250 cm^3 conical flask.
5. Fill the burette with the solution of sodium thiosulphate provided.
6. Titrate the aqueous solution against the sodium thiosulphate solution. Add starch when the solution is a pale straw colour.
7. Repeat the titration using the hexane solution. Before you start this titration, add about 10 cm^3 of distilled/deionised water. You will need to shake the flask after each addition and allow time for the iodine to transfer from the hexane into the aqueous sodium thiosulphate layer. At the end of this titration the hexane layer will be colourless.
8. Record your results in an appropriate table.

Note: great care should be taken with the titrations, as you will not be able to repeat them.

Calculation

As equal volumes of the two solutions were titrated, the ratio of the titres will be equal to the ratio of the concentrations of iodine in the two solvents. Therefore:

$$K_{hw} = \frac{[I_2(\text{hexane})]}{[I_2(\text{aq})]} = \frac{\text{titre value for hexane solution}}{\text{titre value for aqueous solution}}$$

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3 Chromatography – the chromatographic separation of amino acids

Background information

The experiment is designed to give you experience and understanding of an important analytical chemical tool. To completely separate and identify some materials, for example amino acids, by paper chromatography might take several days, so this brief exercise can only suggest the potentialities of the method.

You are to separate a mixture of dyes or other organic molecules. If the components of the mixture you are separating are colourless, you will need to treat your chromatogram after the separation in order to detect the spots.

If chromatographic separation is to work effectively, it is essential that:

- the spots are small and not overloaded with material
- no spot is placed less than 1.5 cm away from the side or bottom edges
- spots are dry before adding more solution (if the solution used is very dilute), or before standing the paper cylinder in the solvent
- the edges of the cylinder must not touch
- the solvent level must be below the bottom of the spots
- to maintain a saturated atmosphere, the container must be covered
- the paper is only handled by its edges, preferably by gloved hands, and is only placed down on clean blotting paper, NOT on the bench top. Any contamination on the paper, either liquid or solid, will ruin the chromatogram
- when developed, the solvent front must be marked; the paper must be **thoroughly** dried (an oven produces better results than air drying) and then, if required, **lightly** sprayed
- each spot on the chromatogram is outlined in pencil, as the spots may fade with time

Clearly then, to obtain satisfying results you will have to work with care and keep the experimental materials scrupulously clean. Touch the chromatography papers only on their top corners and never lay them down except on a clean sheet of blotting paper.

Safety

Depending on which mixture you are working with different risks will apply. There are potentially hazardous substances involved in this exercise and so you must follow all health and safety instructions given to you by your teacher. Operations requiring the use of a fume cupboard MUST be done in a fume cupboard. Materials data safety sheets should be consulted so that the correct action can be taken in event of a spillage and/or accident.

	**You must wear eye protection throughout this experiment**
	Ethanol and propanone are **highly flammable**
 	Methanol is **flammable** and **toxic**
	Hydrochloric acid, nitric acid and glacial ethanoic (acetic) acid are **corrosive**

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Appendix 2

	Butan-1-ol, ninhydrin and copper(II) nitrate are **harmful**
 	Ammonia is **corrosive** and **harmful**
 	Petroleum spirit is **harmful** and **highly flammable**
	Copper(II) nitrate and ammonia are **dangerous for the environment**. Your teacher will tell you how to dispose of them safely.

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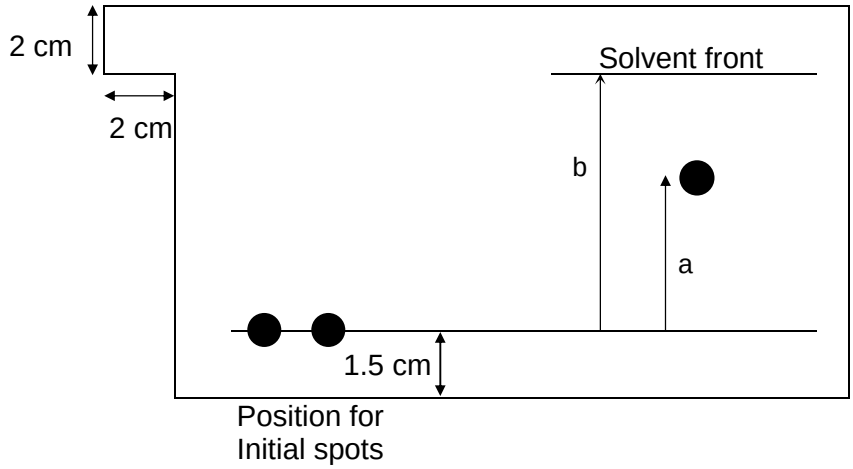
Procedure

1. Put spots of your solutions 1.5 cm from the bottom edge of the chromatography paper (cut to the dimensions shown in the diagram) and well spaced out. To do this, dip a ***clean*** capillary tube in the stock solution and apply a small drop to the chromatography paper, using a quick delicate touch. Practise on a piece of ordinary filter paper until you can produce spots **not more than 0.5 cm** in diameter. You should not place a spot less than 1.5 cm from the edge of the paper.



2. Make identification marks in ***pencil*** at the top of the paper above each spot.
3. Allow the spots to **dry** thoroughly. If you need to add more material to a spot, you must let the spot **dry before** applying a second small drop of the solution. Otherwise, the size of the spot will grow too large.
4. Place the solvent mixture you are to use in a 1 dm^3 beaker; covering the beaker to produce a saturated atmosphere. The depth of the solvent in the beaker **must be below** the bottom of each spot on the paper when the paper is in place.
5. Roll the chromatography paper into a cylinder and secure it with a paper clip. The 'tab' shown in the diagram allows you to clip the two ends of the paper together **without** the two sides below the tab **touching**. If they do touch, the solvent will not 'run' properly at the edges of the paper.
6. Stand the cylinder in the covered solvent beaker and leave it for the solvent to ascend to nearly the top of the paper. If time is limited, you may not be able to allow the solvent to rise the full distance.
7. Remove the chromatography paper from the beaker and **mark** the solvent level.
8. Dry the paper (without unfastening it), in an oven if possible, but ***not*** over a Bunsen flame, as many of the solvents used are pungent, flammable or both.
9. It may be necessary to 'detect' the substances in your mixture. If this is the case, follow the instructions given in the table below.
10. Determine the R_f (relative front) values for the components in your sample. R_f values should be constant, providing standard conditions are used, and are obtained by using the expression:

$$R_f = \frac{\text{distance moved by sample}}{\text{distance moved by solvent front}} = \frac{a}{b}$$

Note: When a mixture of amino acids is separated using **one-way** chromatography, as described above, the separation is incomplete. The full separation of all the amino acids

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requires the use of **2-way** chromatography, in which the chromatogram is developed in one direction in one solvent system, followed by a second development at right angles to the first in a different solvent system.

To use **two-way** chromatography:

1. Using square chromatography paper, spot your sample in the **bottom-right-hand** corner of the paper, about 3 cm from the corner.
2. Allow it to dry, roll the chromatography paper into a cylinder and place it in the first solvent system.
3. When the solvent has reached almost to the top of the paper, allow the paper to dry thoroughly.
4. Re-roll and clip the paper so that the original spot is at the **bottom-left-hand** corner.
5. Develop the paper using the second solvent system.
6. When complete, dry the paper thoroughly and detect the amino acids by spraying with ninhydrin, as for the **one-way** process.

If **two-way** chromatography is to be successful, cleanliness is crucial. Cover your workbench with clean blotting paper, wear gloves, and only handle the paper by its edges.

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Experiments

Use the materials given in the table below, and the instruction given above, to complete the task you have been set.

mixture	preparation	solvent system	detection
ink	• ballpoint pen, use ink straight • coloured inks, dilute 1:3 with water	**Solvent system A** water (15 cm^3); saturated ammonium sulphate (2 cm^3); ethanol (3 cm^3) **or** **Solvent system B** methanol (15 cm^3); concentrated hydrochloric acid (2 cm^3); water (2 cm^3)	No further treatment is required. Compare the effectiveness of the two solvent systems, **A** and **B**, in separating your inks.
Amino acids	Prepare solutions (0.01 mol dm^{-3}) of the amino acids to be tested. For **one-way** chromatography, just use solvent system **C**. For **two-way** chromatography use solvent system **C** and then solvent system **D**. In both cases, detect and then preserve the spots produced.	**Solvent system C** butan-1-ol (12 cm^3); glacial ethanoic acid (3 cm^3); water (6 cm^3) **Solvent system D** ethanol (36 cm^3); '0.880' ammonia (2 cm^3); water (2 cm^3)	• Spray sparingly with aqueous ninhydrin (0.02 mol dm^{-3}) ***in a fume cupboard***. • Heat in an oven at 110 °C for 10 mins. • The amino acid spots will be purple. • Preserve the spots by spraying with the following mixture: copper(II) nitrate (1.0 mol dm^{-3}) (1 cm^3); methanol (19 cm^3); nitric acid (2 mol dm^3) (1 drop). • Expose to the fumes from '0.880' aqueous ammonia ***in a fume cupboard***. • The background will be blue and the spots orange.
chlorophylls etc.	• Grind about 1 g of fresh nettle leaves (or similar) with sand. • Soak in 5 cm^3 propanone for 5 minutes and filter into a separating funnel, using a cotton-wool plug. • Add 5 cm^3 of petroleum spirit, shake, remove top layer and dry it using anhydrous sodium sulphate.	petroleum spirit (40–60 °C) (17 cm^3); propanone (3 cm^3)	No further treatment is necessary although exposure to UV light may assist visualisation. Colours, from top: • carotene • phaeophytins • xanthophylls • chlorophyll A • chlorophyll B

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Smarties, M&Ms or similar	• Using a moist artist's paintbrush, remove colour from the surface. • Spot onto chromatography paper.	water	none
organic acids	• Prepare solutions (0.05 mol dm^{-3}) of the sodium salts of the organic acids to be tested.	Shake together butan-1-ol (30 cm^3) and 1.5 mol dm^3 aqueous ammonia (30 cm^3). Use the upper, organic layer (20 cm^3).	Spray ***lightly*** with bromothymol blue [0.1 g in 0.01 mol dm^3 NaOH (1.6 cm^3) diluted to 100 cm^3]. • background – green • acids – yellow

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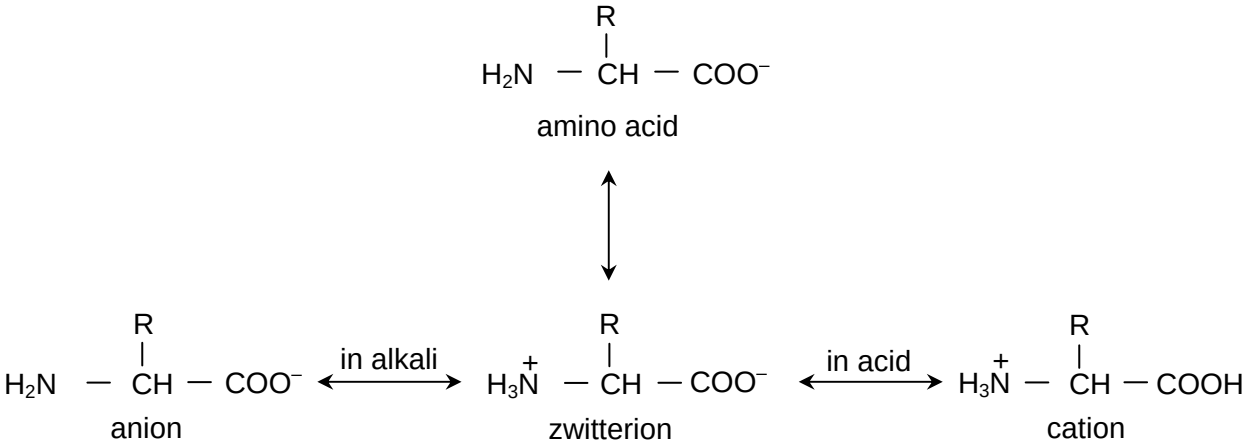
4 Electrophoresis – the separation of amino acids

Background information

In an electrolytic cell, positive ions are attracted to the cathode, while negative ions move towards the anode. The current is carried through the electrolyte by the ions. At the electrodes, electrons are transferred to or from the ions. The overall reaction is a redox reaction.

Electrophoresis works in essentially the same way. A piece of filter paper is soaked in an electrolyte and connected into a d.c. circuit using crocodile clips. A mixture of ions is spotted into the centre of the paper and the circuit is switched on. The positive ions migrate towards the cathode and the negative ions towards the anode. The rate at which different ions will migrate will vary according to the mass, charge and shape of the ions; thus different ions are separated as they migrate. The distance which each amino acid moves, under controlled conditions of pH and electric field, can be measured and compared with standard values.

This technique is particularly useful in the separation of amino acid and protein mixtures. The structure of an amino acid is shown below. However, amino acids predominantly take the form of a **zwitterion**, that is a double ion which is neutral overall. In alkaline solution the $-NH_3^+$ ion is deprotonated, leaving the molecule negatively charged overall and in acid solution the $-COO^-$ group is protonated, leaving the amino acid positively charged overall.



In an electrophoresis experiment a molecule that is uncharged will not move towards either pole. The R group of the simplest amino acid, glycine, is simply H, so the only groups affecting its movement are the NH_3^+/NH_2 and $COO^-/COOH$ groups. But many other amino acids have charged side chains, for example arginine has an amino group, and these groups will affect the overall charge of the molecule. These differences in charge can be used to separate amino acids in the electrophoresis experiment. At neutral pH, glycine will not be charged and will not move in an electric current, but arginine will be positively charged owing to its side chain, and will move towards the cathode.

For each amino acid there is a pH at which its overall charge will be neutral and it will not move in an electric current. This is termed its “isoelectric point” or “pI” value. You would expect the pI value of glycine to be 7 (neutral). In actual fact it is closer 6, although it will only move very slowly at pH 7.

In this experiment you will investigate the effect of pH on the movement of different amino acids in an electrophoresis experiment.

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Safety

Point 9 requires the operation to be performed in a fume cupboard.

	**You must wear eye protection throughout this experiment**
	Ninhydrin and copper(II) nitrate are **harmful**
 	Methanol is **highly flammable** and **toxic**
	Nitric acid is **corrosive**
 	Ammonia is **corrosive** and **harmful**
	Copper(II) nitrate and ammonia are **dangerous to the environment**. Your teacher will tell you how to dispose of them safely.

Procedure

1 Draw a faint pencil line across the middle of a strip of filter paper and mark the ends negative and positive. Place the paper on top of a sheet of glass.
2 Soak the paper with the pH 6 buffer solution.
3 Spot the mixture containing lysine, glycine and glutamic acid onto the pencil line.
4 **Carefully** attach crocodile clips to each end of the paper, taking care to attach the negative and positive correctly.
5 Cover the paper with a large beaker for protection.
6 Connect the wires to a 100 V d.c. supply and pass current for 30 minutes.
7 Dry the paper carefully.
8 Detect the amino acid spots by spraying the paper with ninhydrin.
9 Preserve the spots by spraying the paper with a mixture of 1.0 mol dm^{-3} copper(II) nitrate (1 cm^3), methanol (19 cm^3) and 2 mol dm^3 nitric acid (1 drop), followed by exposure to the fumes from '0.880' aqueous ammonia ***in a fume cupboard***.

Questions

1 How would you expect glycine to move? Explain your answer.
2 In which direction would you expect lysine and glutamic acid to move? Explain your answer.
3 Identify the glycine, lysine and glutamic acid spots on your electrophoresis paper.

Procedure (continued)

10 Repeat the experiment using the same mixture but different pH buffers.
11 Repeat the experiment using different mixtures of amino acids.

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Question

4 What difference, in terms of the movement of amino acids, do you observe when different pH buffers are used? Account for these differences.

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31. Separation techniques

Teachers' Notes

This exercise contains a suite of four experiments designed to give practice in a range of separation techniques. It is suggested that the experiments are performed as a **circus**, perhaps over several weeks. The basic techniques involved are relatively straightforward but great emphasis must be placed on safety, as there are hazards associated with several of the solvents used. The use of a fume cupboard is necessary in some instances. If fume cupboard facilities are limited, it may be better to perform the experiments separately, rather than as a circus, so that attention can be focussed on one process at a time. The use of demonstration might be considered; however, a hands-on approach will make the techniques more memorable to the students.

1 Solvent extraction

The 'kitchen sink' nature of this experiment is appealing to students. The basic extraction into water is a simple extension of what happens each time we make a cup of tea or coffee. The extraction into trichloromethane, however, carries some risk and **must** be performed in a fume cupboard.

Extension exercises

If suitable apparatus is available,

- the recrystallised caffeine may be further purified by **column chromatography** using an alumina column. The solvent mixture to use in this process is benzene (3 parts) and trichloromethane (1 part);
- compare the purified sample with a commercial sample of caffeine by **chromatography** on a silica gel slide. Develop the slide using a solvent mixture comprising of trichloromethane (9 parts) and ethanol (1 part). Detect the spots by exposing to iodine vapour.

This extension provides a useful overlap between solvent extraction and chromatography, and provides experience in column chromatography and in TLC (Thin Layer Chromatography).

Answer to question

Emulsions take time, often a long time, to settle and separate into layers. If no emulsion is formed, the two layers form rapidly.

Technical Information

Requirements per student/group

Apparatus

- two 500 cm^3 glass beakers.
- tripod, gauze, Bunsen burner
- vacuum filtration kit – if possible, otherwise use gravity filtration
- glass filter funnel
- a piece of muslin cloth
- cotton-wool
- filter papers
- two 100 cm^3 measuring cylinders
- one large evaporating dish
- one 250 cm^3 separating funnel
- apparatus suitable for carrying out a distillation
- water bath

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- melting point apparatus

Materials

- Access to 0.30 mol dm^{-3} aqueous lead ethanoate
- Access to 2 mol dm^{-3} sulphuric acid
- Access to 2 mol dm^{-3} aqueous ammonia
- Litmus paper
- Activated charcoal
- Access to trichloromethane
- Access to anhydrous sodium sulphate

2. Partition coefficients

This experiment is quite easy to perform and is satisfyingly 'visual'. Students will **see** that the colour in the hexane layer is darker than that in the aqueous layer, and so deduce a difference in solubility. The quantitative analysis which follows later will confirm that iodine is much more soluble in hexane than in water.

You may have to explain why, as this is a titration exercise, it is not necessary to calculate the iodine concentrations. More able student should deduce this intuitively. However, if you wish to practice mole calculations, the concentrations in each solvent could be calculated and their ratio obtained. The fact that the concentration ratio is the same as the ratio of the titres could then be used as a basis for the discussion of the basic theory involved. This should help those students whose understanding of this area is less secure.

Technical Information

Requirements per pupil/group

Apparatus

- one spatula
- two 50 cm^3 measuring cylinders
- two 100 cm^3 beakers
- one 100 cm^3 conical flask + bung
- one 250 cm^3 conical flask
- one separating funnel
- one burette
- one funnel
- one 25.0 cm^3 pipette and pipette filler

Materials

- access to solid iodine
- access to hexane
- a supply of distilled/deionised water
- access to 1% starch solution
- a supply of aqueous sodium thiosulphate

The concentration of this solution will, to some measure, depend on the ambient room temperature, as the solubility of iodine is temperature dependent, and on the time allowed for the iodine to dissolve. When you trial the experiment, start with a concentration of 0.100 mol dm^{-3} but be prepared to change this in the light of experience.

Iodine is much more soluble in hexane than in water, therefore the amount of sodium thiosulphate required to react with the iodine dissolved in the aqueous layer will be **much smaller** than that needed to react with the iodine dissolved in the hexane layer. The sodium

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thiosulphate concentration should be such that the titre with the hexane solution is **not greater than the capacity** of the burette.

When titrating the aqueous layer, two possibilities exist. Either:

(i) use a diluted solution of the original stock aqueous sodium thiosulphate or
(ii) use the original stock aqueous sodium thiosulphate solution

The advantage of (i) is that the titre value can, by appropriate dilution, be arranged to be similar to that in the hexane titration, thus minimising the burette error factor; however, the dilution process will introduce an additional error factor. Also, the students will have to be supplied with two different solutions of sodium thiosulphate, with the danger that they will use the wrong one for a given titration, and will have to change from one to the other during the experiment.

The advantage of (ii) is that the same stock solution is used, so that dilution is not necessary and there is no need for students to change solutions; however, the titre obtained will be **much smaller** than that for the hexane titration and so the burette error factor will be **much more significant**.

If method (i) is used, the dilution factor will have to be determined when you trial the experiment; it is likely to be around a dilution factor of 10. It is crucial that this dilution is performed accurately. A burette or pipette must be used to measure the volume of the stock solution into a volumetric flask, which is then made up to the mark with distilled/deionised water. The volumetric flask must then be inverted several times to ensure thorough mixing. The diluted solution must be made up in **one batch** and the volume prepared must be sufficient for the needs of all the students who need to use it.

If method (ii) is used, the concentration of the stock solution should be such that the titre with the hexane solution is **not greater than the capacity** of the burette nor is the titre with the aqueous solution **too small**. Again, this concentration should be determined when you trial the experiment.

The actual concentration of the stock solution is not critical, as it is the ratio of the titre values which gives the K_{hw} value; appropriately scaled if method (i) is used. What **is critical** is that, in method (ii), the **same stock solution** is used for both titrations or, in method (i), that the dilution factor is accurately known.

Note: Arguably, method (i) will give the more reliable/accurate results, but method (ii) is more straightforward. For this reason, method (ii) has been used when writing the instructions on the Student Sheet. Students could be asked to discuss the errors in each method and decide themselves which to use.

3 Chromatography

This experiment provides a range of chromatographic opportunities, ranging from the fun ‘Smartie’/‘M&M’ experiment, where the colours on the outer sugar shell are separated, to the much more complex **two-way** chromatographic separation of amino acids. The solvent and spray systems used in some separations use hazardous chemicals, and so great care must be taken to ensure that safety is not compromised.

It is worth practising the techniques involved using filter paper before using the more expensive chromatography paper. Some paper, e.g. ‘Whatman CRT/1 paper’, has vertical slits cut into it in order to physically separate the different mixtures being tested; such paper works well.

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Technical Information

Requirements per student/group

Apparatus

- chromatography paper
- scissors
- capillary tubes
- paper clips or stapler
- 1000 cm^3 tall-form beaker / suitable jar with a screw top
- watch glass, or similar, to cover beaker
- access to an oven (around 110 °C) or hair dryer etc
- blotting paper to cover working area
- 100 cm^3 beakers – one for each solvent system to be used
- access to fume cupboard facilities

Materials

- Solvent systems – as listed in the table on the Student Sheet. These could be premixed, or experienced students could make their own mixtures. If this is the case, measuring cylinders and beakers will have to be made available.
- access to appropriate solutions for detecting and protecting the spots – as listed in the table – in spray bottles
- 0.880 ammonia (concentrated ammonia) solution

4 Electrophoresis

It is worth emphasising to your students the importance of this process in normal life, e.g. in 'DNA fingerprinting' and in separating fragments in gene analysis. A simple introduction to this process is provided by the electrophoresis of metal ions; two examples of which are given below:

(i) A potassium manganate(VII) crystal is placed at the centre of paper moistened with water.
(ii) Aqueous silver nitrate is placed at the positive end of the paper (moistened with water), with aqueous potassium chromate(VI) at the negative end.

A 20 V smoothed d.c. supply is used. In (i), the movement of the purple MnO_4^- ions can be tracked, and reversed, if the polarity of the paper is reversed. In (ii), the movement of yellow CrO_4^{2-} ions can be followed until they meet Ag^+ ions and form a red precipitate of Ag_2CrO_4. Details may be found in 'The migration of ions' (Experiment number 34, 'Classic Chemistry Experiments', RSC).

As this experiment uses water, rather than buffer solutions, and the progress of the migrating ions can be followed visually, you may wish to consider using this before tackling amino acid mixtures.

Answers to questions

1 Glycine has a neutral side chain so will not move at pH 6 because it is in its zwitterionic form and there are no other charges.

2 Lysine has a positively charged side chain in solution at pH 6 and so will move towards the negative pole. Glutamic acid has a negatively charged side chain in solution at pH 6 and so will move towards the positive pole.

3 Glycine on start line, glutamic acid towards positive pole, lysine towards negative pole.

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4 In an acid buffer, the $–COO^-$ groups of the zwitterions are protonated. The charge on an amino acid with a neutral side chain is dominated by the $–NH_3^+$ groups and hence will move towards the negative pole. Conversely in an alkaline buffer the $–NH_2$ groups are no longer protonated and so an amino acid with a neutral side chain will have an overall negative charge owing to the $–COO^-$ group and will move towards the positive pole. The effect on amino acids with charged side chains will be in the same direction but modified by the charge on the side chain.

Safety

The main points are included on the Student Sheet. However:

- It is essential that a risk assessment be carried out before a decision is taken to go ahead with this exercise.
- It must be **made clear** to students that potentially hazardous materials are in use and that precautions are needed to minimise the risk to themselves and to others.
- Your **MUST** be prepared to **intervene** if a student seems to be unsure of a procedure, or is performing an unsafe operation.
- MSDS sheets should be consulted so that the correct action can be taken in event of a spillage and/or accident. Any materials dangerous to the environment should be disposed of according to local regulations.

A number of ways in which the exercise may be made less hazardous and more likely to succeed include:

(i) Preparing the solvent and spray mixtures yourself and supplying them to the students in suitably labelled containers.
(ii) Providing adequate access to fume cupboard facilities. This may mean scheduling the exercises so that demand for the fume cupboards is staggered.
(iii) Demonstrating some of the techniques, particularly the spraying of a developed chromatogram.
(iv) Telling the students that their safe working practices are being **assessed**; whether this is true or not!

Technical Information

Requirements per student/group

Apparatus

- filter paper / chromatography paper
- glass plates – microscope slide would do but larger plates would be better
- scissors
- capillary tubes
- large beaker to cover the paper/glass
- two crocodile clips and wires
- a smoothed 100 V (d.c.) supply
- access to an oven (around 110 °C) or hair dryer etc
- blotting paper to cover working area
- 100 cm^3 beakers – one for each buffer solution to be used
- access to fume cupboard facilities

Materials

- access to the mixtures to be tested e.g. a mixture of glycine, lysine and glutamic acid
- access to appropriate buffer solutions. It is suggested that a pH 7 buffer, together with an acidic buffer (pH 6 or pH 5) and an alkaline buffer (pH 8 or pH 9) are used.

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- access to ninhydrin in a spray bottle
- access to a solution containing 1.0 mol dm^{-3} copper(II) nitrate (1 cm^3), methanol (19 cm^3) and 2 mol dm^3 nitric acid (1 drop), in a spray bottle
- 0.880 ammonia (concentrated ammonia) solution

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