Cambridge Assessment International Education

Practical Booklet 12

Separation and analytical techniques

Cambridge International AS & A Level Chemistry 9701

Version 2.0

Cambridge Pathway

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Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

- plan experiments and investigations
- collect, record and present observations, measurements and estimates
- analyse and interpret data to reach conclusions
- evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To determine a partition coefficient for iodine between two immiscible solvents and to investigate separation of suitable mixtures by chromatography and electrophoresis.

Outcomes

Syllabus section 7.3, 22.1(a), 20.3(c) and 21.4(d), and a link with 22.2, 22.3, 22.4 and 22.5 as well as experimental skills 1, 2, 3 and 4

Skills included in the practical

A Level skills	How learners develop the skills
Planning	identify a safe and efficient procedure that when followed would lead to a reliable result describe all the steps necessary to carry out the procedure show an understanding of how and why the procedure suggested will be effective
Analysis	calculation from quantitative data obtained
Evaluation	suggest modifications to a procedure that will improve its accuracy estimate the uncertainty in a quantitative experiment and express the uncertainty as a percentage error
Conclusions	carry out appropriate calculations from data obtained make scientific explanations of data obtained

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How learners develop the skills
MMO collection	make measurements using pipettes, burettes and other common laboratory apparatus
MMO quality	obtain results that are close to those of an experienced chemist
MMO decisions	identify where repeated readings are appropriate
PDO recording	record quantitative results appropriately in a table, with appropriate headings and units
ACE conclusions	draw conclusions from interpretations of observations, data and calculated values

Method A, B and C

- **Learners must wear eye protection for these investigations.**
- The laboratory should be well ventilated**.**
- Method A provides an opportunity for learners to revise the various skills required to carry out a titration accurately. In the experiment they titrate an aqueous and organic layer, both of which contain iodine, in order to determine the partition coefficient of iodine between these two solvents.
- Learners should carry out this experiment individually. The teacher should also carry out the experiment at the same time, so that the learners' accuracy can be assessed by comparing their value for the partition coefficient to the teacher's value.
- In a titration involving iodine and sodium thiosulfate, it is normal to not add the starch indicator at the start of the titration. It is better to add the starch when most of the iodine has reacted, shown by the solution becoming yellow. Then the starch is added. By doing this, the blue-black colour (caused by an iodine-starch complex) decomposes more easily, making the end-point sharper to observe.
- Since iodine is much less soluble in water than in *Volasil 244*, the organic solvent chosen, the sodium thiosulfate solution used to titrate the iodine in the organic layer must be diluted before the aqueous layer of iodine is titrated.
- It is important that the aqueous layer does not become saturated with iodine. If this happens there is no longer an equilibrium of iodine partitioning between the two solvents. This error is avoided by limiting the mass of iodine used.
- The experiments involving chromatography and electrophoresis (Methods B and C) are suitable for learners to work in pairs, in which case it may be helpful to have a 'circus' of experiments. If time is short, learners could work in groups, so that each learner or pair of learners should carry out one of the experiments described. The learners should describe to others in the group how they used the apparatus and what results they obtained from each experiment.
- Learners should become familiar with common techniques used in organic extraction and in some forms of analysis and they should gain confidence in handling hazardous materials safely.

Results

- Learners should record their burette readings correct to 0.05 cm^3.
- They should record the distances travelled by solvent front and spots to the nearest 0.1 cm.
- They should note any differences in the order of spots when using different solvents or different buffer solutions.

Interpretation and evaluation A

- The values of *K* obtained by the learners can be collected and reasons for discrepancies discussed (equilibrium not reached; inaccurate end-point; inaccurate dilution; too much starch added so some I_2 locked).

- The reaction mechanism for the reduction of nitrobenzene can be revised.

- Adding strong base to liberate a weaker base from its salt can be compared with the similar acid displacement reaction.

- The reasons for using several small portions of organic solvent (rather than one large portion) to extract the organic compound can be discussed and this may provide an introduction to partition chromatography.

  [5(i) $x/50 \div (4-x)/50 = 30$; $x = 3.87$ g]

  [5(ii) $y/25 \div (4-y)/50 = 30$; $y = 3.75$ g; mass remaining in aqueous solution = 0.25 g; $z/25 \div (0.25-z)/50 = 30$; $z = 0.23$ g; total extracted = 3.98 g]

Typical results

Titre: I_2(org) with 0.10 mol dm^{-3} $Na_2S_2O_3$ = 14.15 cm^3

Titre: I_2(aq) with 0.010 mol dm^{-3} $Na_2S_2O_3$ = 5.90 cm^3

$[I_2(org)] / [I_2(aq)]$ = titre (org) / (titre (aq) ÷ 10) = 14.15 / 0.59 = 24.0

Interpretation and evaluation B

- The calculation to find R_f values can be introduced or revised.

- The increase in mass of solute extracted by successive small portions of solvent can be linked to paper chromatography as a process happening very many times as fresh mobile phase solvent passes over the stationary phase.

- The differences in polarity of mobile phase solvents and relative solubilities of solutes in them can be discussed. The suitability of the learners' suggestions for further experiments can be discussed. (Keep everything the same except for the solvent. Use solvents such as ethanol (polar) and petroleum spirit / cyclohexane / *Volasil 244* (non-polar) and compare results.)

- Discussion about greater separation may include smaller pore size (use chromatography grade paper), different polarity of solvent / mixture of solvents and length of strips.

- Differences in speed of solvent front, separation of components and 'tailing' of spots may be discussed for the two types of chromatography (paper and TLC).

- Learners could discuss which technique(s) are suitable to analyse the food colour and what apparatus would be needed. The use of reference spots of pure tartrazine yellow and curcumin and comparison of R_f values with the spot from the orange squash can be discussed. The possibility of cutting out each spot formed from the orange squash, extracting it from the paper / adsorbent (TLC) with a suitable solvent and then analysing it can be mentioned.

- The techniques of analysis and the information each will give may be introduced or revised (mass spec: M_r and m/z for fragments; IR: groups; nmr: number and type of environments.)

Interpretation and evaluation C

- The structures of the α-amino acids used can be discussed and their systematic names can be suggested. (–R groups for gly, lys, asp and glu respectively: –H, $–(CH_2)_4NH_2$, $–CH_2COOH$ and $–(CH_2)_2COOH$)

- The formation of zwitterions can be introduced or revised and the reasons for the different isoelectric points / charges in different pH buffers can be discussed. The direction of travel in an electric field can be discussed.

- Learners might suggest the effect of the size of the ion on the speed of migration (aspartic acid is smaller).

- Other factors affecting the speed of migration can be brainstormed (buffer pH leading to nature of charge on ion, size of charge (lysine +2 in pH 4), shape of ion (–R group with benzene ring or chain), pore size of medium (e.g. amount of cross linking of polyacrylamide)).

- The hydrolysis of proteins can be introduced. The use of gel electrophoresis (often polyacrylamide for peptides, agarose for nucleic acids) in identifying peptides and nucleic acids can be discussed (e.g. genetic fingerprinting).

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Information for technicians

Method A

Each learner will require:

(a) Eye protection

(b) 1 x 100 cm^3 beaker (or larger)

(c) 1 x separating funnel (tap funnel)

(d) 2 x 50 cm^3 measuring cylinder

(e) 1 x burette

(f) 1 x stand and burette clamp

(g) 1 x filter funnel (for filling burette)

(h) 3 x 25 cm^3 pipette

(i) 1 x pipette filler

(j) 1 x 250 cm^3 volumetric (graduated) flask

(k) 2 x 150 cm^3 or 250 cm^3 conical flask

(l) 1 x white tile

(m) 1 x spatula

(n) 1 x weighing boat (or similar)

(o) 1 x stoppered bottle with tightly fitting stopper or bung (≥ 100 cm^3 capacity)

[MH] [N] (p) iodine

[MH] (q) 40 cm^3 *Volasil 244* (or other non polar solvent)

(r) 50 cm^3 0.10 mol dm^{-3} sodium thiosulfate

(s) 2 cm^3 starch indicator

(t) 50 cm^3 distilled water

(u) access to balance weighing to at least 1 dp

(v) paper towel

Technician's notes, *continued*

Method B

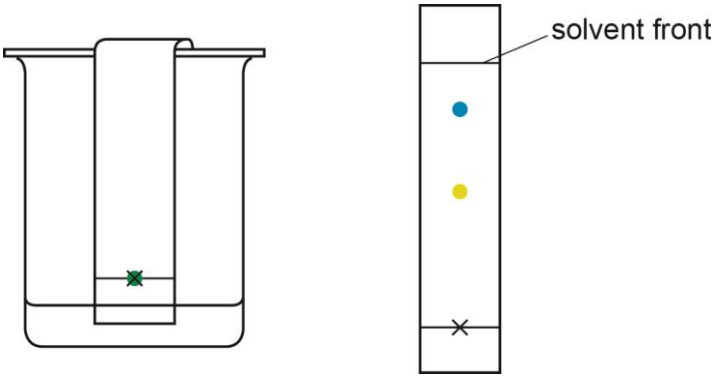
Each learner will require:

(a) Eye protection
(b) 2 x 100 cm^3 beaker
(c) 2 x chromatography paper or filter paper
(d) 2 x thin layer chromatography plate
(e) 2 x glass rod
(f) 2 x watch glass or plastic wrap (cling film) (to cover beaker)
(g) 1 x melting point tube (glass capillary tube)
(h) access to scissors, pencil and ruler
(i) 1 cm^3 screened methyl orange indicator (or mix of other pH indicators)
[F] [MH] (j) 25 cm^3 propanone
(k) 25 cm^3 distilled water
(l) mineral wool (approx 1 cm cube)
(m) paper towel

Additional Instructions

Optional experiment: glass tube approx. 2 cm diameter, 20 cm length fitted with rubber tubing and gate clip at one end; stand and clamp; aluminium oxide (fine powder); ethanol **[F] [H]**; mineral wool.

Equipment set-up



Technician's notes, *continued*

Method C

Each learner will require:

(a) Eye protection
(b) 3 x chromatography paper or (fine pore) filter paper
(c) 3 x microscope slide
(d) 3 x test-tube
(e) 3 x melting point tube (glass capillary tube)
(f) 2 x crocodile clips
(g) 1 x 100 V d.c. supply
(h) 2 x connecting wires
(i) 1 x large beaker or tub to protect the slide and crocodile clips
(j) access to scissors, pencil and ruler
(k) glycine – small amount only
(l) lysine – small amount only
(m) aspartic acid – small amount only
(n) glutamic acid – small amount only
(o) 5 cm^3 pH 6 buffer solution
(p) 5 cm^3 pH 4 buffer solution
(q) 5 cm^3 pH 9 buffer solution
[MH] (r) ninhydrin spray
(s) paper towel

Additional Instructions

If gel electrophoresis is available then this would be preferable to paper electrophoresis.
Good ventilation of the laboratory or use of a fume cupboard is needed.

Technician's notes, *continued*

Hazard symbols

 GHS02 (*flammable* **F**)	 GHS03 (*oxidising* **O**)	 GHS05 (*corrosive* **C**)
 GHS06 (*acutely toxic* **T**)	 **GHS07 (*moderate hazard* MH)**	 GHS08 (*health hazard* **HH**)
	 GHS09 (*hazardous to the aquatic environment* **N**)	

Worksheet

Aim

To determine a partition coefficient for iodine between two immiscible solvents and to investigate separation of suitable mixtures by chromatography and electrophoresis.

Method

Safety:

- Wear eye protection.
- iodine **[H]**
- *Volasil 244* **[H]**
- propanone **[F] [H]**
- ethanol **[F] [H]**
- ninhydrin spray **[H]**

Hazard symbols

H = harmful or irritating substance **F** = highly flammable substance

Experiment A

The laboratory must be well ventilated.

1. Use a measuring cylinder to transfer 35 cm^3 distilled water into a bottle which has a correctly fitting stopper or bung.

2. Use a second measuring cylinder to transfer 35 cm^3 *Volasil 244* (or other suitable organic solvent) into the same bottle.

3. Weigh out approximately 1 g of iodine and carefully add the solid to the mixture in the bottle.

4. Stopper the bottle and shake it for several minutes to dissolve the iodine.

5. Leave the bottle until the colour intensity in each layer does not change.

6. Transfer both liquid layers from the bottle into a separating funnel. Stopper the funnel and clamp it vertically until you are ready to run off one of the layers.

Titrating the organic layer

You will only be able to carry out **one** titration for each solution so care is needed when approaching the end-points.

7. Wash the burette with a little 0.10 mol dm^{-3} sodium thiosulfate and discard the washings. Then fill the burette. Make sure that the region under the tap is full.

8. Take a reading at eye level of the position of the bottom of the meniscus on the scale. Record the initial burette reading to the nearest 0.05 cm^3.

Worksheet, *continued*

9. Remove the stopper from the separating funnel. Run off the lower (aqueous) layer into a 100 cm^3 beaker. Close the tap as the interface enters it. The solution left in the separating funnel should now have no aqueous layer present. You will use the aqueous solution of iodine in step 18.

10. Using a pipette filler wash a 25 cm^3 pipette with a **small** portion of your solution of iodine in the organic solvent (which is left in the separating funnel) and discard the washings. Pipette 25.0 cm^3 of this solution into a conical flask. Touch the bottom of the pipette against the wall of the flask or onto the surface of the solution to deliver the correct volume. (Put the stopper back in the separating funnel to trap the vapour from any remaining organic solvent.)

11. Place the conical flask on the white tile under the burette. Add about 10 cm^3 of distilled water and swirl the flask to allow some iodine to enter the aqueous layer.

12. Run sodium thiosulfate from the burette in small portions and swirl the flask between additions. As soon as the iodine colour fades to yellow add a few drops of starch indicator so the mixture turns blue-black.

13. Add sodium thiosulfate, a few drops at a time, from the burette until the solution **just** becomes colourless.

14. Measure and record the new volume of the sodium thiosulfate in the burette. Calculate and record the volume of the 0.10 mol dm^{-3} sodium thiosulfate needed to react with the iodine in the organic solvent.

15. Pour the contents of the conical flask into an organic residues bottle together with any remaining organic solvent from the separating funnel. Wash the conical flask with water and discard the washings.

Titrating the aqueous layer

16. Firstly you will need to dilute the solution of sodium thiosulfate. Using a pipette filler wash a second 25 cm^3 pipette with a little of the 0.10 mol dm^{-3} sodium thiosulfate and discard the washings. Pipette 25.0 cm^3 of this solution into a 250 cm^3 volumetric flask and add distilled water up to the mark. Mix the diluted solution thoroughly before use.

17. Rinse the burette with water and then rinse with your diluted sodium thiosulfate. Then fill the burette with this solution and record the initial burette reading to the nearest 0.05 cm^3.

18. Wash a third 25 cm^3 pipette with a **small** portion of your solution of aqueous iodine (in the beaker) and discard it. Transfer 25.0 cm^3 of this solution into a second conical flask and place the flask on the white tile.

19. Repeat steps 12 and 13.

20. Record your final burette reading and discard the contents of the conical flask. Record the volume of your diluted sodium thiosulfate needed to react with the iodine in the aqueous layer.

Worksheet, *continued*

Results – experiment A

Record **all** your observations.

Record your burette readings correct to 0.05 cm^3.

Interpretation and evaluation – experiment A

1. Use your titre and the original concentration of the sodium thiosulfate solution to calculate the number of moles of iodine dissolved in 25.0 cm^3 of the organic solution.

2. Use your titre, the original concentration of the sodium thiosulfate and the dilution factor to calculate the number of moles of iodine dissolved in 25.0 cm^3 of the aqueous solution.

3. The iodine is in the same molecular state in the two solvents.
   The partition coefficient, K, is given by

$$K_{ow} = \frac{[I2(org)]}{[I2(aq)]}$$

   Calculate the partition coefficient for iodine between your two solvents at the temperature of your laboratory.

4. What are the errors in single readings for each piece of apparatus that you have used?
   Estimate the maximum percentage error in the value of the partition coefficient you have calculated.

5. The partition of solute between two immiscible solvents is used in extracting organic compounds from an aqueous solution which contains inorganic compounds. For example, one of the stages in preparing phenylamine from nitrobenzene is an ether extraction.

   Nitrobenzene is refluxed with tin and concentrated hydrochloric acid. The salt $C_6H_5NH_3^+Cl^-$ (aq) is formed. What is added to the mixture to displace the phenylamine from its salt?

   To extract the phenylamine from the ionic products of the reaction, the mixture is shaken with ether (ethoxyethane), an organic solvent which is immiscible with water.
   This process is called ether extraction.

   Assume that the partition coefficient for phenylamine between ethoxyethane and water, K_{ew} = 30 at the temperature of the laboratory. Assume there is 4 g of phenylamine present in 50 cm^3 of aqueous solution.

   (i) What mass of phenylamine will be found in the organic layer if an equal volume of ethoxyethane is added to the aqueous solution and the distribution of phenylamine between the two layers is allowed to reach equilibrium?

   (ii) What mass of phenylamine will be extracted if the same volume of ethoxyethane is used but in two successive extractions? That is, 25 cm^3 of ethoxyethane is used initially and then a further 25 cm^3 is shaken with the aqueous layer to remove more of the phenylamine.

Worksheet, *continued*

Experiment B: chromatography

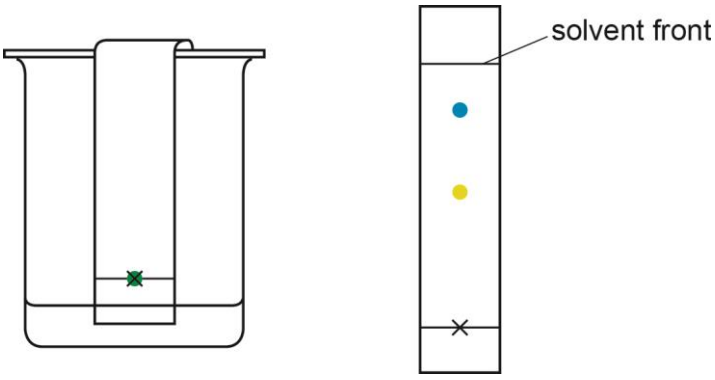
1: paper chromatography

1. Cut two strips of filter paper approximately 2 x 10 cm. Draw a pencil line about 1.5 cm from the bottom of each strip and parallel to the base. Put a pencil cross at the centre of the line.

2. Set up two beakers, one containing about 1 cm depth of water and the other containing about 1 cm depth of propanone. Cover the tops of the beakers with plastic film (or a watch glass) so the vapour in the beaker becomes saturated with solvent.

3. Dip the end of a melting point tube (glass capillary tube) into screened methyl orange indicator (or a mix of pH indicators) and pick up about a 2 mm depth of solution. Touch the tube onto the pencil cross on one strip of filter paper. Repeat the process for the second strip.

4. Fasten the top of each filter paper strip to a glass rod or a wooden spill so that the bottom of the paper enters the solvent in the beaker but the coloured spot will be above the liquid level (see diagram.)



5. Cover the tops of the beakers with plastic film and leave them until the solvent level is close to the fixing point of the strip. (The two solvents are unlikely to travel upwards at the same rate.)

6. Remove the filter paper strips from the beakers and use a pencil to mark the height of each solvent front. Let the paper dry then measure the distance from the base pencil line to (a) the solvent front, (b) the coloured spots.

Worksheet, *continued*

2: thin layer chromatography

1. Set up two beakers to act as chromatography tanks as in experiment 1.

2. Draw a pencil line across the two TLC plates (as experiment 1).

3. Spot the same coloured substance onto a pencil cross on the line.

4. Place the plates in the two beakers and cover both beakers.

5. Remove the plates when the solvent front nears the top of the plate and mark the position of the front in pencil.

6. Measure the distances travelled by the solvent fronts and the coloured spots.

Extension

These experiments may be carried out with the colour from dark green leaves (chlorophyll). Use a pestle to grind up dark green leaves with sand and about 5 cm^3 propanone in a mortar. Extract the colour in a non-polar solvent such as 5 cm^3 petroleum spirit **[F] [H]** using a separating funnel. A suitable mobile phase (solvent) is a 3:17 by volume mixture of propanone and petroleum spirit. If the colour of the spot is not dark enough, dry the paper / plate then add a second portion at the same position. Repeat as necessary. If the resulting colour or position of the different spots is not clear then shine a UV lamp onto the paper / plate.

Other suitable substances include coloured inks or the colouring extracted from the coating of chocolates or sweets, such as Smarties, M&Ms and Skittles. (A wider strip of paper should be used and the different colours spotted onto separate pencil crosses on the pencil line. The order of the colours applied should be recorded. It is then possible to see whether the same dyes are used in more than one ink or sweet.)

3: column chromatography

1. Mix alumina (aluminium oxide) with ethanol to make a slurry (like thin mud).

2. Set up a tube of approximately 2 cm diameter and 20 cm length with rubber tubing and a gate clip at one end. Place a small ball of mineral wool at the bottom end of the tube. (Part of a broken burette is suitable for this experiment, especially if the tap is still intact.)

3. Clamp the tube vertically with the mineral wool at the bottom. Gradually fill the tube with the slurry, tapping it to remove any air bubbles and with the gate clip open for excess ethanol to run off into a beaker to be used again. Leave a 1 – 2 cm gap at the top of the column.

4. Use a dropper to place about 5 drops of screened methyl orange indicator on the top of the alumina column without disturbing the surface and allow them to soak in.

Worksheet, *continued*

5. Add a thin layer of sand to the top of the alumina to protect it. Then add ethanol so there is always at least 0.5 cm depth of liquid above the sand. Keep the tap open.

6. Collect and re-use the ethanol that leaves the column without any colour. Collect and keep ethanol containing each of the colours. (There should be a complete separation of the two components of screened methyl orange.)

Results

Record **all** your observations.

Record the distances travelled by the solvent front and spots to the nearest 0.1 cm.

Note any differences in the order of spots when using different solvents or different buffer solutions.

Interpretation and evaluation

Paper chromatography involves partition coefficients.

The stationary phase is the water which is naturally adsorbed onto the paper fibres and which is immiscible with any mobile phase solvent (including water).

In a mixture, the solute with the greater solubility hence concentration in the mobile phase will travel further than one of lower solubility in that phase.

1. Calculate the retardation factor, R_f, values for each spot in the different solvents used in the (ascending) paper chromatography experiment.

2. Suggest why there are differences in R_f values for the same coloured spot in the different solvents. What further experiment could you carry out to test your suggestion?

3. With some mixtures the coloured components are not completely separated. Suggest how better separation might be achieved.

Thin layer, column and gas chromatography are all examples of adsorption chromatography. The components of a mixture are adsorbed onto the solid or liquid stationary phase. The weaker the adsorption the further the liquid mobile phase will move the component.

4. Calculate R_f values for each spot in the different solvents used in the TLC experiment.

5. Describe differences you noticed in the chromatograms formed using the two techniques, paper and thin layer chromatography.

Worksheet, *continued*

Some azo dyes are used to colour foods. Tartrazine yellow is one of these and has been banned as a food additive in some countries as it may be linked to hyperactivity in children. Some companies use β-carotene (in carrots and sweet potatoes) or curcumin (in turmeric) as a food colour instead.

6. A company producing orange squash is accused of using tartrazine yellow in their product to improve its colour, but the company claims that curcumin is being used.
   Design an experiment to determine which food colouring is being used by the company.

7. An advantage of carrying out column chromatography is that the separate components of the mixture may be run out of from the column and analysed. Suggest techniques that can be used in the analysis and what each technique can add to information about the component. (You may select techniques not available in your school laboratory.)

Experiment C: electrophoresis

Gloves may be worn to prevent fingerprints.

1. Use forceps to hold a piece of chromatography paper (or fine pore filter paper) and cut it to the size of a microscope slide. Place it on the slide and draw a pencil line across the middle (shorter length) and mark the ends positive and negative. Clip the (longer length) ends of the paper onto the glass slide with clean non-corroded crocodile clips.

2. Soak the paper with pH 6 buffer solution.

3. Mix together small amounts of the amino acids glycine, lysine, aspartic acid and glutamic acid in a test-tube. Add a small volume of the pH 6 buffer solution to make a concentrated solution. Use a melting point tube to transfer some of the solution to the line on the chromatography paper. Draw the tube along about 1 cm length of the line.

4. Attach wires to the crocodile clips. Cover the slide and crocodile clips with a large beaker or tub to protect the paper. Connect the wires to a 100 V d.c. supply (positive to +), switch on the supply and allow current to pass for at least 30 minutes. Switch off the d.c. supply and disconnect the crocodile clips. Let the paper dry.

5. Make sure that the laboratory is well ventilated before starting the next stage or use a fume cupboard when spraying and drying the paper.
   Spray the paper with ninhydrin and allow it to dry. The positions of the bands (spots) should gradually become visible. Draw around the blue or brown bands in pencil, because the colours will fade. Record the positions of the bands relative to the original pencil line and in which direction they travelled.

Worksheet, *continued*

6. Repeat the experiment twice using buffer solutions of (a) pH 4, and (b) pH 9. Keep everything else the same.

   (A better separation is achieved using a gel such as polyacrylamide or agarose which has been made up in the required pH buffer solution. A hole is made in the gel and the sample placed in the hole.)

Results

Record **all** your observations.

Record the distances travelled by the solvent front and spots to the nearest 0.1 cm.

Note any differences in the order of spots when using different solvents or different buffer solutions.

Interpretation and evaluation

1. Use books or the internet to find the chemical structures of the four amino acids. (If you cannot access this information ask your teacher for help.)

2. Use your results and the structures of the amino acids to identify each spot.

3. Suggest a reason for the different positions of aspartic and glutamic acids with the pH 6 buffer.

4. Suggest two more factors which may influence the movement of the amino acids in an electric field.

5. A practical instruction book suggests using a voltage of 500 V for 1 hour when carrying out gel electrophoresis on a mixture of peptides. Suggest why the instructions are different from the ones given for your experiment.

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