Skills

During the Chemistry 1 Laboratory Course you will learn many of the basic laboratory techniques required by a practising chemist. Rather than repeat these sets of instructions each time they are used in an experiment, they are all presented here. When a particular skill is required for an experiment, you will be told to refer to it in this section. By the end of the course you should be proficient in the following skills.

1 Risk assessment and safety

2 Keeping a record of your work

3 Weighing

- 3.1 Use of the Mettler top-loading balance
- 3.2 Use of the Mettler analytical balance

4 Volumetric analysis

- 4.1 Equipment and washing
- 4.2 Using the pipette filler
- 4.3 Using the pipette
- 4.4 Using the volumetric flask and making a standard solution
- 4.5 Dilution
- 4.6 The burette
- 4.7 Indicators
- 4.8 Performing a titration
- 4.9 Use of the micropipette

5 Use of the separating funnel

6 Filtration

- 6.1 Gravity filtration
- 6.2 Vacuum filtration

7 Attaching rubber tubing

8 Distillation

9 Recrystallisation

Skill 1: Risk assessment and safety

No experiment in the School of Chemistry may be undertaken without first completing a HIRAC (Hazard Identification, Risk Assessment and Control). This encompasses all aspects of the experiment including the nature of all reagents and products, inherent dangers in procedures and use of equipment, and the disposal of wastes. This has already been performed for you for all experiments in this course, so you will not be required to do it. However, for safety reasons you must prepare for each experiment:

- by completing the on-line pre-work associated with each experiment; and
- by reading over the experiment before your lab session.

Special notes are given in each experiment to guide you in the disposal of wastes. These are to be followed meticulously. In general:

- broken glass should be placed in the sharps container at the front of the laboratory.
- filter paper and other solid material should be disposed of in the rubbish box beneath your sink.
- organic solvents must be placed in the appropriate waste solvent container in the fume hoods.
- strongly acidic or alkaline solutions should be washed down the sink with large volumes of water.
- silver, mercury, chromium and other toxic metal wastes must be placed in the appropriate waste containers in the fume hoods.

Skill 2: Keeping a record of your work

Your logbook is your record of all of your results, observations, calculations, discussions, etc. for the Chemistry 1 Laboratory Course. It is an official document and another person should be able to pick up your logbook and be able to read it, understand it and to repeat any procedures described. Therefore, your writing should be neat and tidy, have the correct spelling and be in sentences and paragraphs.

Guidelines for Your Logbook

Each page of your logbook needs to be numbered and NO pages may be removed.

The date on which the experimental work was carried out must be given.

You need to write in PEN, but pencil may be used for diagrams and graphs.

Unless otherwise stated you should NOT be copying information from these laboratory notes into your logbook.

Informative titles and sub-titles for each experiment, data set and accompanying discussion should be given.

An example of part of an exercise written up in a logbook can be found on the following pages.

Page number → ①

Exercise 43 ← Exercise number

Date → 9/7/08

Purification by Evaporation and Freezing ← Exercise name

Purification of 2-propanol by distillation ← Sub-title

Results ← Sub-title

Table number and title

Table 1: Results of time and temperature for 2-propanol

Time (min)	Temp (°C)
0	25
1	35
2	50
3	60
4	70
5	80
6	82
7	82
8	82
9	82
10	82
11	82
12	82
13	85
14	88
15	

Column titles, including units if appropriate

The data collected from the experiment are presented in a Table. This makes it easier for you and other people to follow your results from the experiment.

A description of what you saw happening in the laboratory.

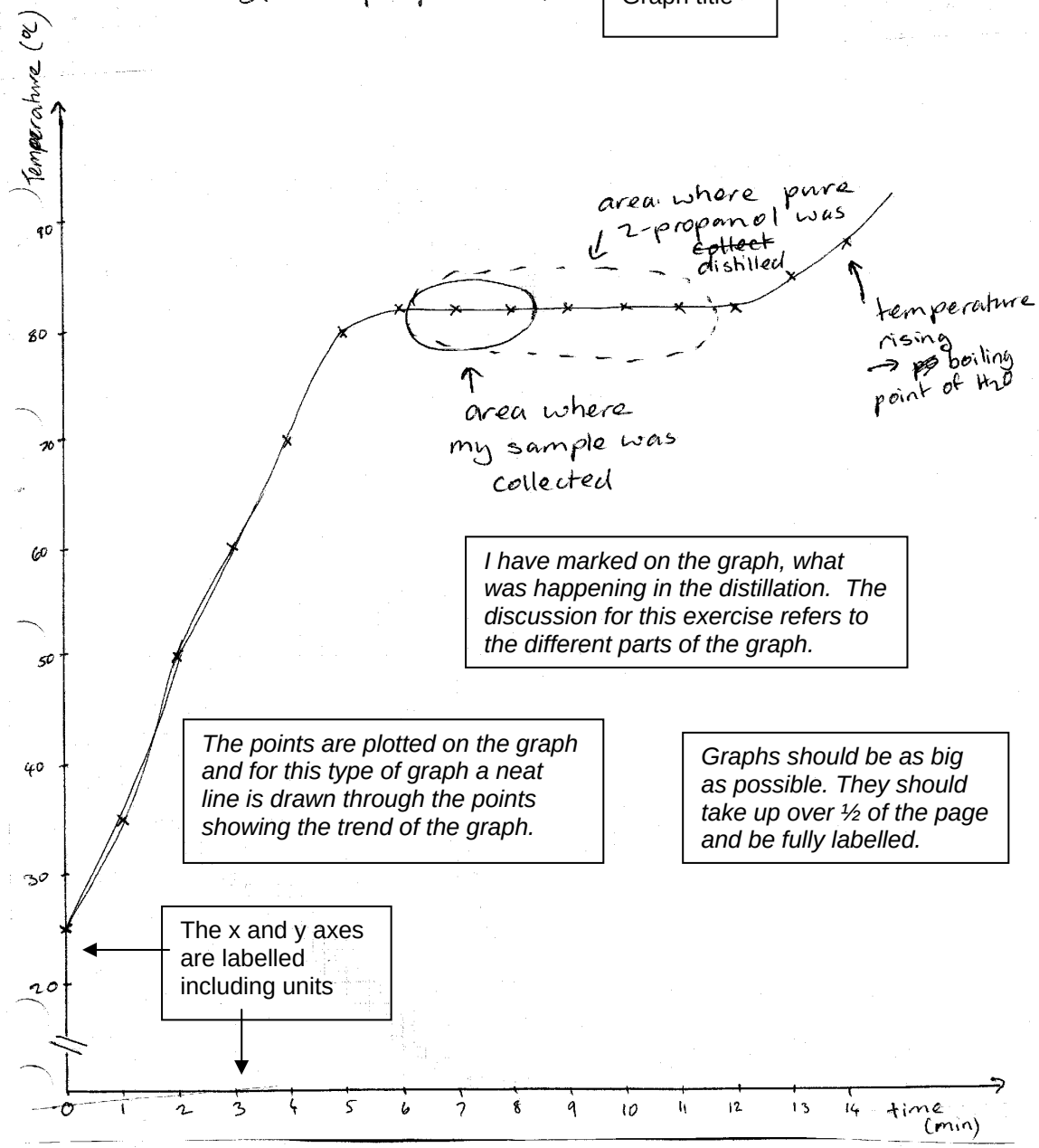
The vapour in the distillation apparatus could be seen moving up the flask and the distil head. As this was happening the temperature was also increasing. When the ~~sol~~ distillation reached 82°C the vapour reached the condenser and was condensed back into a liquid. After the first couple of drops, I collected ~ 30 mL's of 2-propanol. The distillation ~~co~~ continued to run until the temperate started to rise. The temperate stayed constantly at ~~88~~ 82°C for awhile.

Page → ②

Date → 9/7/08

Mistake neatly crossed out

~~Graph~~ Temperature Vs Time for the distillation of 2-propanol ← Graph title



I have marked on the graph, what was happening in the distillation. The discussion for this exercise refers to the different parts of the graph.

The points are plotted on the graph and for this type of graph a neat line is drawn through the points showing the trend of the graph.

Graphs should be as big as possible. They should take up over ½ of the page and be fully labelled.

The x and y axes are labelled including units

Graph 1. ← Graph numbered

Page number → 3

Date → 9/7/08

Testing the purity of 2-propanol. ← Sub-title

Results

Volume of distilled 2-propanol — 25.00 mL

Mass of distilled 2-propanol — 20.0 g ← Results listed, better if tabulated

From Graph 1 in the Laboratory Notes

20.0 g of ~~2-prop~~ distilled 2-propanol = ~0.075 % water in the sample.

The ~~distilla~~ orginal solution that I distilled contained 20% water and 80% 2-propanol. My purified sample of 2-propanol contained ~ 0.075% water. Therefore, I have removed the majority of the water from the ~~iso~~ 2-propanol sample, purifing it.

*Statement about **what I think** about the purity of **my** 2-propanol sample.*

You would continue with new subtitles, tables, graphs, etc. *to the end of the exercise, recording your results and observations as shown above.*

*Make another sub-title for the Discussion and **in your logbook write down your thoughts and opinions about the discussion questions. Your thoughts and opinions should be based on your results and observations from the laboratory exercise.***

Skill 3: Weighing

Skill 3.1 Use of the Mettler top-loading balance

This balance reads masses up to 1000 g with an accuracy of 0.01 g. This type of balance also has provision for taring; that is, a variable zero adjustment that can compensate for the mass of an empty container. The result is that the mass of material added to an empty beaker is indicated directly. There are a number of Mettler top-loading balances on the side benches in each of the laboratories.

Part A Direct weighing of a clean, dry and chemically-inert object.

- Make sure that the balance-pan is clean and dry.
- Ensure that the power supply to the balance is switched on.
- Adjust to zero position (0.00 g).
- Place the sample centrally on the balance-pan.
- Read the direct-reading scale to obtain the mass.
- Record the mass of the object in your logbook.
- Switch off the balance.

Part B Weighing out of a substance

- Make sure that the balance-pan is clean and dry.
- Ensure that the power supply to the balance is switched on.
- Place a clean dry beaker or other appropriate container centrally on the balance-pan.
- Tare the container so that the balance reads 0.00 g.
- Use a nickel spoon to add the substance carefully to the container until the required mass has been added, as indicated by the direct-reading scale. If there is any risk of the substance spilling on to the balance-pan, all additions and removals of the substance must be done with the container removed from the balance-pan.
- Record the mass of compound weighed in the appropriate place in your logbook.
- Switch off the balance.
- Clean up any mess you have made.

Skill 3.2 Use of the Mettler analytical balance

High precision weighing (to 0.0001 g) is required when conducting volumetric analysis work (titrations). This is obtained by use of the Mettler single-pan analytical balance, Type AE260. These delicate and valuable instruments require a shock- and draught-free environment and a dry, non-corrosive atmosphere. These conditions, provided by the balance-rooms at the back of each laboratory, can only be maintained by constant care and effort.

The difference-method must be used when precision is required. The difference-method leads to the highest possible precision and the lowest risk of contamination of the analytical balance.

Part A Weighing by difference

- Take the following into the balance-room:
  - the stoppered weighing-bottle containing the substance to be weighed out;
  - a clean, dry 100 mL beaker containing a clean nickel spoon and covered by a watch-glass;
  - your logbook and pen.
- Tare the stoppered weighing-bottle (press the re-zero bar) so that the balance reads 0.0000 g.
- With the bottle removed from the balance-case, use the spoon to transfer without loss an estimated quantity of the substance to the beaker. Leave the spoon in the beaker and replace the watch-glass cover. Restopper the weighing bottle.
- Place the weighing-bottle containing the residual substance on the balance pan and read the direct-reading scale. This will give a negative number representing the mass of the substance removed from the weighing-bottle and now present in the beaker.
- If the quantity transferred lies outside the acceptable range, a further transfer without loss in either direction can be made, and the stoppered bottle re-weighed.
- Record the mass of compound transferred in your log book.
- Switch off the balance.

Skill 4: Volumetric analysis

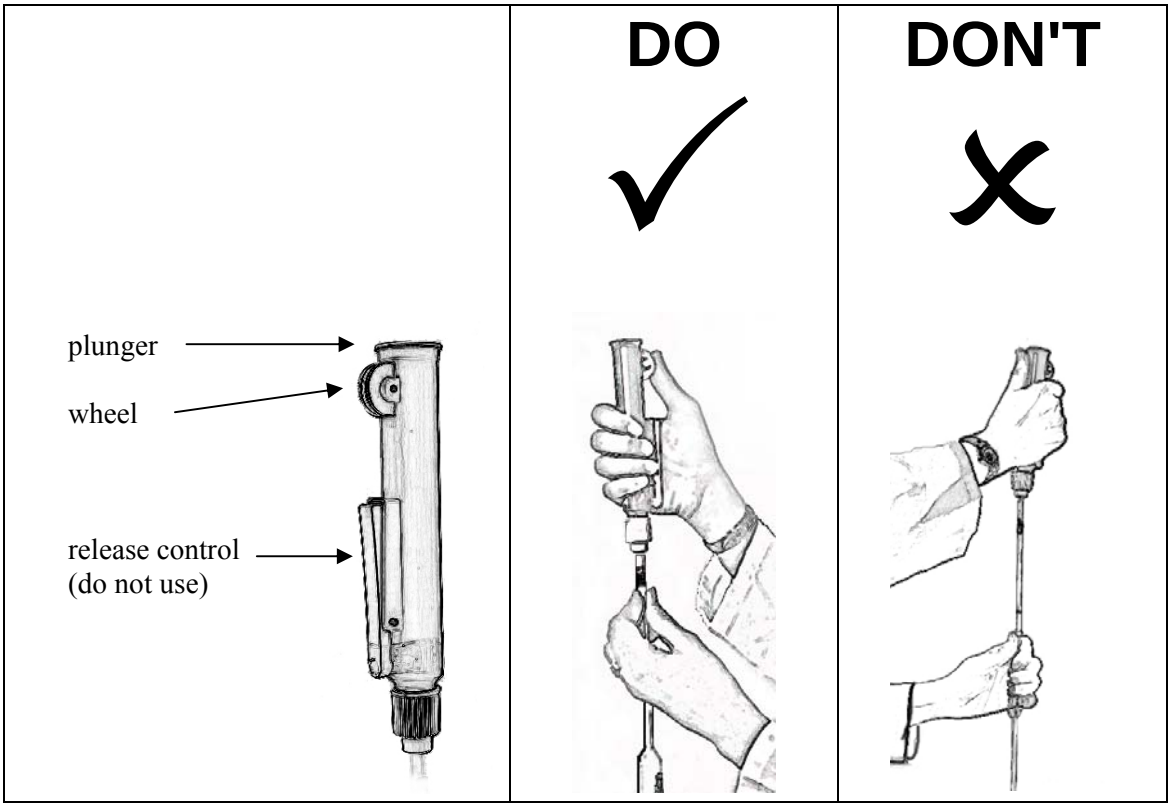
Volumetric analysis involves the making-up, storage and delivery of solutions of known concentrations and/or volumes. It is intrinsic to this process that all equipment and vessels that are used are thoroughly clean and have been rinsed with the appropriate liquid, which, as outlined below, depends on the purpose of the equipment.

Skill 4.1 Equipment and washing

Pipettes, **burettes** and **storage bottles** are designed to store, transport or deliver solutions of a *specific concentration*. Before use, they must be washed, rinsed with deionised water and then thoroughly rinsed 3 times **with the solution they are to contain**.

Volumetric flasks and **reaction vessels** (conical flasks) are designed to receive *known amounts (mass and/or volume)* of reagents. Before use, they must be rinsed 3 times **with deionised water**.

Skill 4.2 Using the pipette filler



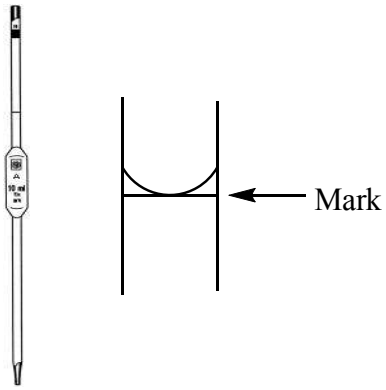
4(a) 4(b) 4(c)

Figure 4-1 Shows a diagram of (a) pipette filler; (b) the correct way to hold and insert a pipette into a pipette filler; (c) an incorrect way to hold and insert a pipette into a pipette filler

Ensure the plunger is fully down. Hold the pipette about 2 cm from the top and gently push it into the end of the filler until it stops. A complete seal is all that is required - it should not be pushed any further once an increase in resistance is felt and a complete seal obtained.

Do not hold the pipette by the bulb or below the bulb. This will put undue stresses through the bulb, which is the weakest part of the pipette. If the pipette breaks whilst being inserted into the filler, sharp pieces of glass usually get embedded in one or both hands, resulting in a trip to Student Health or hospital.

Skill 4.3 Using the pipette



The pipette is a device for delivering a fixed volume of solution. Rinse it with a small amount of water, drain, dry the outside of the tip, and then use the pipette-filler to suck-up a few mL of solution. Use this solution to rinse the entire inside surface of the pipette up to the graduation mark - hold the pipette horizontally and rotate it through at least 360°. Drain. Repeat this rinsing process twice more.

Once the pipette has been rinsed as explained above, it is then carefully filled to a level well above the graduation line. Ensure the tip of the pipette is well submerged during filling and that there is enough solution to fill the pipette completely. Do not suck liquid into the pipette filler. Remove the pipette from the filler, preventing outflow of the liquid by placing your dry forefinger (not thumb) firmly over the top. This takes practice - you need to be able to do this fast enough so that the level of liquid stays above the graduation line. (With one hand, hold the pipette at the very top with your forefinger level with the top. Quickly pull the filler from the pipette with your other hand and immediately place your forefinger over the opening.)

The outside of the pipette is then dried by wiping with absorptive tissue or filter-paper. Place the tip in contact with the side of an empty glass beaker and gradually relax your forefinger to control the outflow, until the bottom of the meniscus of the liquid exactly corresponds with the mark. The contents are then allowed to run down the inside of the receiving flask. The pipette should be held vertically, with its tip touching the glass throughout delivery. The flask needs to be held at an angle. When running has ceased, hold the pipette in contact with the side of the flask for 15 seconds to allow for internal drainage. The drop retained inside by surface tension is not expelled - it has been allowed for in calibration.

Skill 4.4 Using the volumetric flask

The volumetric flask is used to make a fixed volume of solution. It can be used to carry out an accurate dilution or for the preparation of a primary standard.

Part A Filling a volumetric flask

- Bring the level of solution in the flask ***nearly*** to the reference-line by addition of deionised water.
- Stopper the flask and shake by inversion and swirling.
- Add deionised water by drops until the bottom of the meniscus corresponds exactly to the line.
- Stopper the flask and shake by inversion and swirling for at least 1 minute to give a homogeneous solution.
- Label the flask or transfer the solution to a labelled storage vessel.

Part B Making a standard solution

- Weigh out precisely the required quantity of primary standard into a clean, dry, covered beaker. (Skill 3.2) ***All*** of this weighed-out quantity must now be transferred to a clean, but not necessarily dry, volumetric flask.
- Add about 50 mL of deionised water to the beaker and stir to hasten dissolution. It is essential that all of the solid is dissolved - the solution may be heated if necessary.
- Pour the solution down a stirring-rod into a small funnel in the neck of the volumetric flask. Rinse the beaker several times with water to ensure all traces of solute are transferred to the volumetric flask.
- Fill the volumetric flask as described in Part A above to give a precise volume of solution of known concentration.

Skill 4.5 Dilution

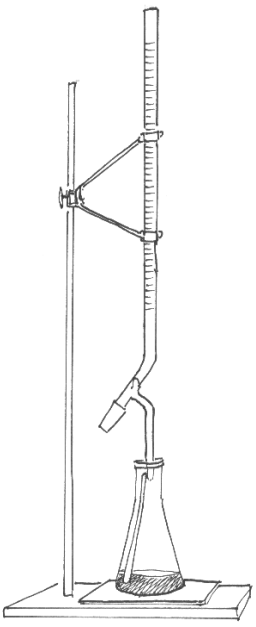
At times we need to dilute a solution to get the desired concentration. To do this we first make up a **stock solution**, which is at a higher concentration than any of the desired concentrations. To make a stock solution you accurately weigh out the required mass of solid and prepare the solution using a volumetric flask as described for standard solutions (Skill 4.4). Lower concentrations may then be prepared by dilution. The required dilutions are done by transferring an accurate volume of the stock solution by pipette (Skill 4.3) into a volumetric flask, and making up to the correct volume for the flask by adding deionised water. If less accuracy is sufficient, use measuring cylinders to measure the desired volumes.

To calculate the volume V_{stock} of stock solution (of concentration C_{stock}) that should be added to make a solution of volume V_{solution} with a concentration C_{solution}, we use the following formula:

$$V_{\text{stock}} = \frac{C_{\text{solution}}}{C_{\text{stock}}} V_{\text{solution}}$$

In this formula the quantity $C_{\text{solution}}/C_{\text{stock}}$ is termed the dilution ratio, *i.e.* the factor by which the stock solution is diluted in order to prepare the required concentration of solution. The above equation can also be used to dilute an already diluted solution further. This is termed **serial dilution**. For example, a 1 M solution can be diluted to a 0.1 M solution and then the 0.1 M solution can be diluted to a 0.01 M solution. Serial dilution is a more accurate way of preparing solutions of low concentration than weighing and dissolving a very small mass of solute.

Skill 4.6 The burette



The burette is a device for measuring the volume of solution delivered. It must drain cleanly and the tap must be in perfect order. Like the pipette, before use it must be thoroughly rinsed with the solution it is to deliver. Remove the burette from its stand, close the tap and pour in about 5 mL of solution. Hold the burette horizontally and carefully rotate it to rinse the entire inside surface thoroughly. Drain by opening the tap. Repeat this rinsing process twice more. Fill by pouring the reactant solution through a small funnel. **DO NOT fill the burette above your eyes**. Remove the funnel. Clamp the burette in its stand with the graduations facing you and with the tip 13 cm above a white tile. Carefully run liquid to waste, so as to remove any air bubbles below the tap and record the initial volume to two decimal places in your log book. The level does not need to be adjusted to 0.00 mL.

Skill 4.7 Indicators

Indicators are used to determine when a reaction is complete. Some coloured titrants (*e.g.* DCPIP and $KMnO_4$) act as their own indicators. However, in the majority of acid/base titrations, both reactants are colourless and an organic compound, whose colour depends on the pH of the solution, is usually added. Two of the most commonly used indicators are methyl-orange (yellow in base, pink in acid) and phenolphthalein (red in base, colourless in acid).

Skill 4.8 Performing a titration

Equipment must be clean and appropriately rinsed. The burette is filled with one reactant solution (not necessarily the standard solution).

A pipette is used to transfer a known volume of the other solution to a conical flask (rinsed with water), which is placed on a white tile under the burette. If required, the appropriate indicator is added.

Solution from the burette is run into the flask, which is swirled throughout the titration so that all parts of the mixture reach the end-point simultaneously. The left hand opens the burette tap and the right hand holds the flask by the neck, imparting a swirling motion. [Left-handed people will find it easier to rotate the burette through 180°, use the right hand to control the burette tap and the left hand for swirling the conical flask. At the completion of the titration, rotate the burette through 180° again in order to read the scale.] Addition is rapid at first, but, as the indicator takes longer to revert to its original colour (with approach of the end-point), progressively decreasing volumes are added. Single drops, each about 0.05 mL, or even half-drops, are added in the last stages. As the end-point is approached, a wash-bottle must be used to wash down half-drops from the burette tip and solution sticking to the inside of the flask. End-point is reached when you see a permanent colour change for at least 30 seconds. The end-point volume is now read as closely as possible to 0.05 mL, and recorded in your logbook. A white paper slide with a broad line may be attached to the burette so that the line is vertical; by using the line, and taking care to avoid parallax error, precise readings of volume can be made. Take care to read the scale correctly. Usually 2 or 3 accurate titrations are performed, and should agree to within 0.10 mL.

Note: In some cases, for example when the expected volume is unknown or the end-point unfamiliar, a rapid titration may be performed first to locate the endpoint approximately.

Skill 4.9 Use of the micropipette

Micropipettes are used for dispensing small quantities of liquids. The Lasany micropipette used in the Boden Laboratories is used to dispense variable volumes of 100 µL - 1000 µL, *i.e.* 0.10 - 1.00 mL.

Note 1.000 mL = 1000 µL.

When use correctly, only the disposable plastic tips come into contact with the solutions being handled.

The plunger rests naturally in the 'ready' position and can be depressed relatively easily to the 'first stage' position. Considerably more pressure is required to depress it to the 'second stage' position. Make sure you are confident you know these three positions before you begin to use the micropipette.

- Fix a disposable plastic pipette tip firmly onto the 'tip cone'. The micropipette is now ready for use.
- Set the desired volume, visible in the 'window', by rotating the 'plunger': clockwise to increase or anticlockwise to decrease the volume. **Do not go over 1000 µL nor below 100 µL. The micropipette is not accurate outside this range and more importantly, you risk breaking it.**
- Depress the plunger to the first stage. Dip the tip in the solution and slowly release the plunger. The plunger will return to the ready position and the required volume of solution will be drawn into the plastic pipette tip.
- To dispense the solution, gently depress the plunger to the first stage, wait a few seconds for any remaining solution to run down to the very end of the pipette tip and then depress the plunger to the second stage position. Release the plunger gently so that it returns to the ready position.
- Use a different pipette tip for each different solution you are measuring. The tip can be easily removed just by pulling it firmly (or you can use the 'ejector').

Skill 5: Use of the separating funnel

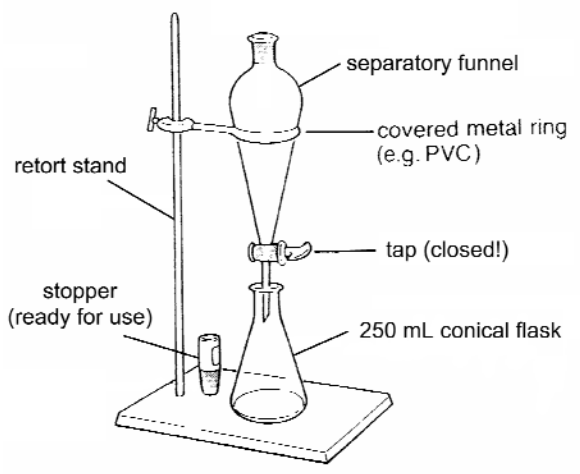


Figure 5-1: Set-up for the separating funnel

Your separating funnel needs to be set-up on your bench as shown in Figure 5-1. With the tap closed, solutions are poured in via the top of the separating funnel.

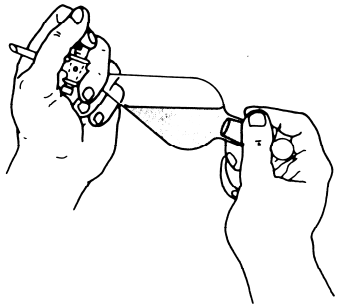


Figure 5-2: How the separating funnel is held.

The separating funnel is carefully removed from the supporting ring and held in both hands as shown in Figure 5-2. The tap is immediately opened to release the pressure of gases inside (venting). **Always** ensure the end of the separating funnel is pointing away from people when venting. Close the tap. The separating funnel is gently shaken, mixing the contents, with frequent venting.

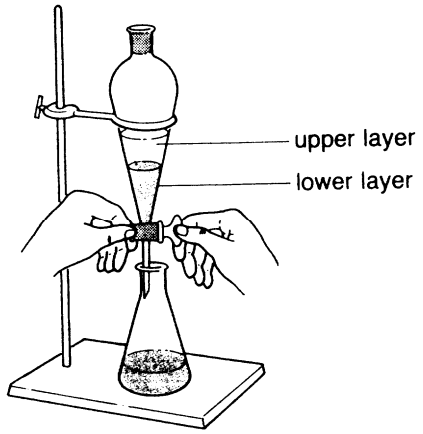


Figure 5-3: The two layers have separated and the lower layer is being removed.

After shaking, the separating funnel is returned to the metal ring and the two layers are allowed to separate as shown in Figure 5-3. Once the two layers have separated, the stopper is removed and the lower layer is run off into a conical flask by opening the tap.

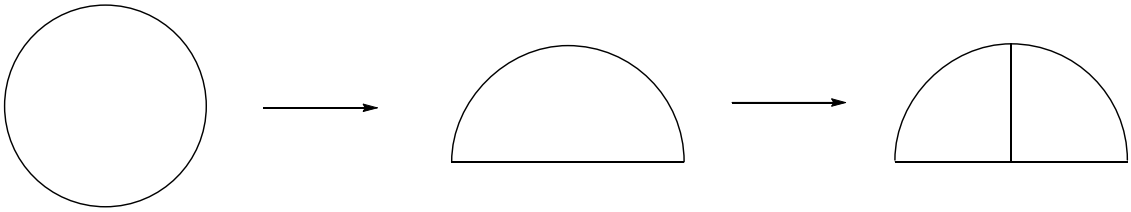
Careful control will ensure the entire upper layer remains in the separating funnel - the lower layer has been run off into the conical flask or been trapped in the passage of the tap. The upper layer is then poured out the top of the separating funnel into a clean and dry conical flask.

Skill 6: Filtration

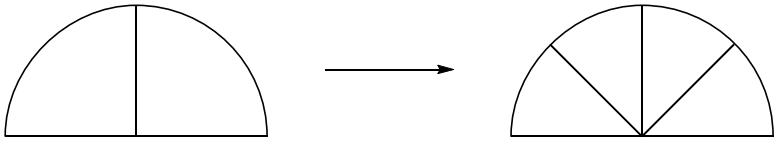
Skill 6.1 Gravity filtration

Gravity filtration is generally used to remove insoluble impurities from a solution. A fluted filter paper greatly increases the rate of filtration by maximising the contact area between the filter paper and the solution and minimising the contact area between the filter paper and the funnel. To flute a filter paper, follow the instructions below.

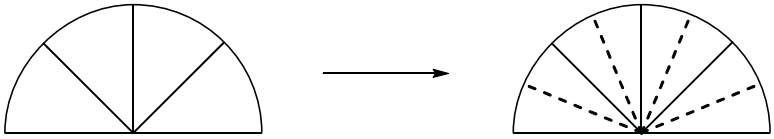
Fold a filter paper into halves and then into halves again.



Open to the half-folded position and fold into quarters so that all the creases are in the same direction.



Now fold each of the four sections into halves with a crease in the opposite direction.



The filter paper should fold like a concertina into 8 segments.

Open it fully, place it in the funnel supported by a retort ring and moisten with a little solvent.

Skill 6.2 Vacuum filtration

Vacuum filtration is usually used to isolate a solid product. Figure 6-1 shows you how to set up the vacuum filtration. Two different types of funnels can be used; the Hirsch funnel or the Büchner funnel, which is used for larger amounts of product.

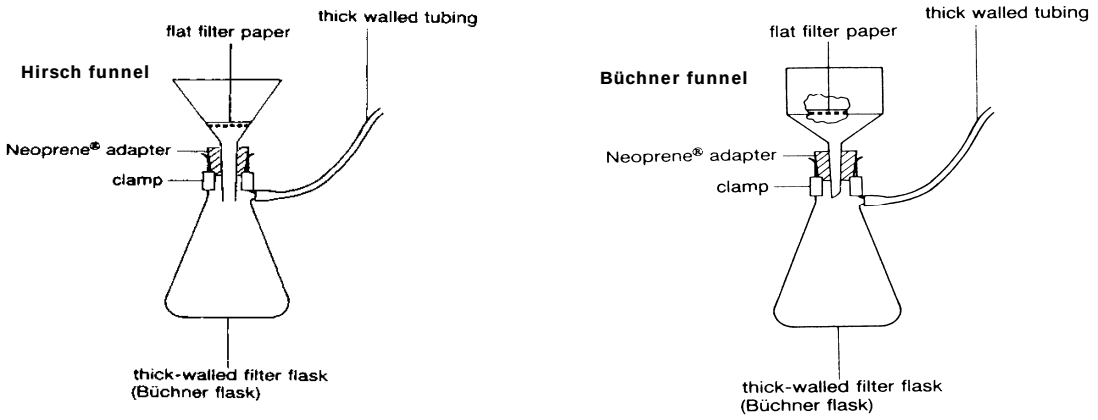


Figure 6-1. Diagram showing how to set up a vacuum filtration.

Make sure the filter flask is clean and then rinse it with the solvent you are using. Dampen the filter paper circle with a little of the solvent as well. Apply the vacuum and check that a good seal is obtained. Then collect your crystals by swirling the flask to suspend them and pouring the suspension into the filter funnel, attempting to leave as little product behind in the flask as you can. Your cold filtrate is a saturated solution of your compound - it may be recycled and used to assist in the transfer of the crystals because the product will not dissolve in it. Do not, however, use fresh solvent for this procedure - your product probably has an appreciable solubility in pure solvent and much of it will dissolve and the yield will decrease accordingly. Disconnect the vacuum before you remove the Büchner or Hirsch funnel and recycle the contents of the filter flask.

After the product has been filtered off, it is normally left to ‘dry at the pump’ for another 5 - 10 minutes by using the vacuum pump to draw air through the compound.

Skill 7: Attaching rubber tubing

Thick rubber tubing is used to connect filter flasks to the vacuum line. Thin rubber tubing is used to connect water condensers to the water supply. In both cases, the tubing needs to be attached to a piece of glassware. **This simple procedure is one of the major sources of injury in undergraduate labs** so it is important that you learn the correct procedure. If too much stress is put on the glass it will break and sharp bits of glass will almost certainly get embedded in your hand.

1. Wet the end of the rubber tubing to be attached.
2. Wet the glass tube around the end where the tubing is going.
3. Hold the glass tube as close to that end as possible.
4. Gently slide the tubing on over the end. **Do not twist**. Keep your hand as close to the end of the glass tube as possible throughout the process.

Skill 8: Distillation

Distillation is used to purify liquids or to remove the solvent from a solution. The required set-up of the apparatus is shown in Figure 8-1. The condenser consists of a hollow tube surrounded by a water jacket. When the solvent is heated to its boiling point, the solvent vapours rise up the still head and enter the condenser. On contact with the water-cooled tube, the vapours condense and the purified liquid solvent can be collected.

Make sure all the equipment is clean and dry before you begin. As most organic solvents are immiscible with water, this is usually done by rinsing the equipment with small amounts of acetone. Acetone dissolves many organic compounds and is also miscible with water, so performs both functions - cleaning and drying. It also has a relatively low boiling point, so the last traces of acetone can be easily removed by either warming the equipment in your hands or by passing a current of air through it.

Set up the distillation apparatus as shown in Figure 8-1. Boiling chips promote even boiling of the solvent and help to prevent it from **superheating**, a potentially dangerous phenomenon. A superheated liquid is hotter than its boiling point, so has the potential to vaporise explosively.

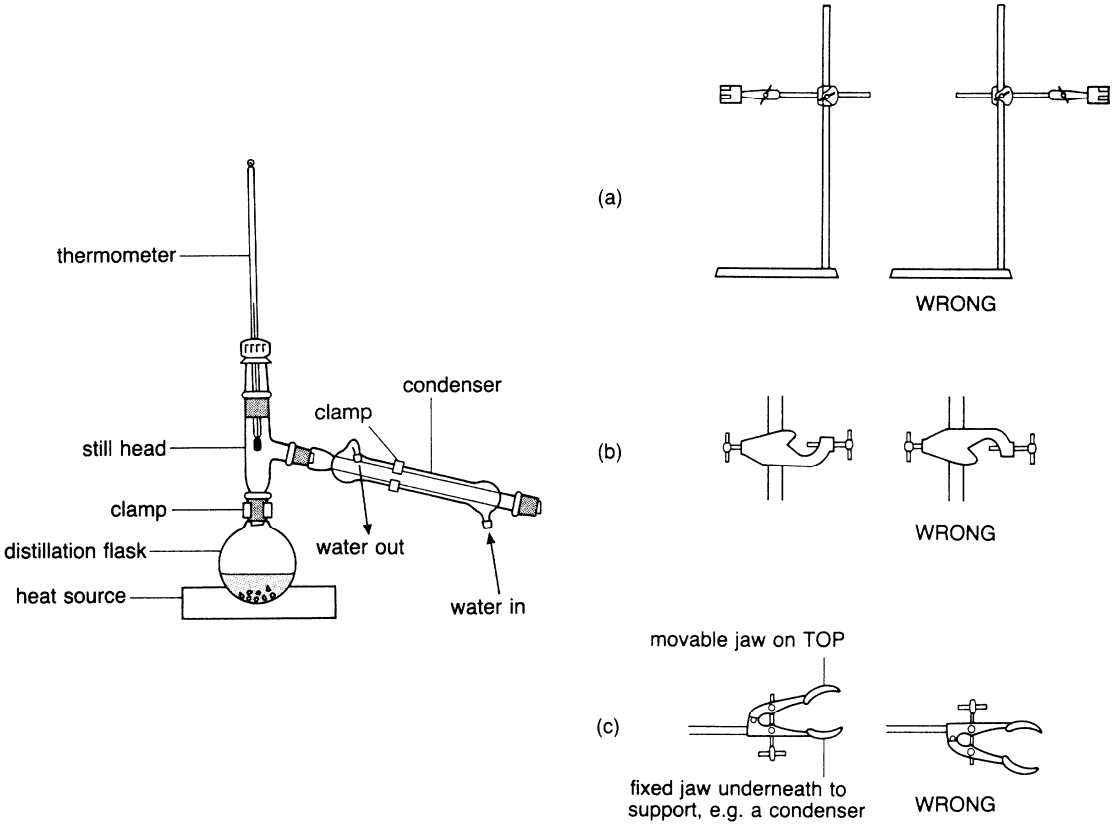


Figure 8-1. Set up of distillation equipment

Make sure that:

- all ground glass joints are connected properly with no gaps;
- the water supply is connected properly - in at the bottom, out at the top;
- the water is flowing slowly and the rubber outlet tube is in the sink, not lying on the bench;
- the thermometer scale is facing you;
- fresh boiling chips have been added to the distillation flask.

Get your demonstrator to check your set-up before you begin. Once you have turned on the heat source, do not leave the apparatus unattended. Once the solvent begins to boil, the heat source should be controlled so that a steady flow of distillate is achieved. If for any reason the solvent stops boiling before the distillation is complete, the boiling chips will deactivate. You need to let it cool slightly, then add more boiling chips (remove the thermometer, drop them in, replace the thermometer) before you reapply the heat source.

Skill 9: Recrystallisation

Purification of solids is most readily achieved by recrystallisation. This process involves identifying a suitable solvent in which the material to be recrystallised has a high solubility at an elevated temperature (typically near the boiling point of the solvent) and a low solubility at low temperature. The solid (or mixture) is dissolved in a minimal volume of hot solvent, filtered to remove any insoluble components in the mixture and the solution is slowly cooled to allow the product to crystallise.

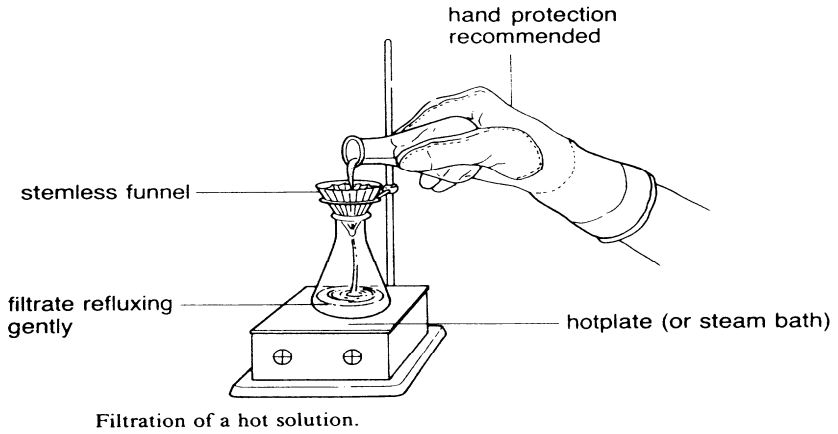


Figure 9-1. Diagram showing the hot filtration of the dissolved compound.

Recrystallisations from water or ethanol may be done on your bench - all others must be done in the fume hoods. Three flasks are generally required for a recrystallisation and 2 boiling chips must be added to each. The receiving flask has a stemless funnel containing a fluted filter paper suspended above it (Figure 9-1). It contains a small amount of boiling solvent so that the vapours heat the entire flask and funnel. A second flask contains a supply of boiling solvent, whilst the third contains the compound to be recrystallised.

Boiling solvent is added in small portions to the compound to be recrystallised until it has just dissolved and then a further ~ 10 % more solvent is added. The hot solution is filtered to remove any insoluble impurities, as shown in Figure 9-1.

When the filtered solution is allowed to cool, the pure compound crystallises whilst the soluble impurities are left in the solution (known as the “mother liquor”). The crystals are then filtered off using vacuum filtration and dried at the pump (Skill 6.2). A second crop of crystals can often be obtained from the mother liquor by further concentration, but this will not be required for any of the experiments in this book.

Skill 10: Thin layer chromatography (TLC)

Thin-layer chromatography (TLC) is an important technique in chemistry. TLC can be used to assess the course of a reaction, to assess the purity of a sample and also to identify compounds in an unknown mixture by comparison with standards.

A TLC plate consists of an inert backing material (*e.g.* aluminium) covered with a thin, even layer of adsorbent material (*e.g.* silica) called the **stationary phase**. In TLC, the mixture to be analysed is spotted onto the TLC plate, whereupon the various components in the mixture are adsorbed onto the stationary phase. The TLC plate is then placed into a tank containing the developing solvent, or **mobile phase**. The mobile phase travels up the plate by capillary action and the various compounds in the mixture are carried along with the mobile phase at different rates, depending upon the relative strengths of their attractions to the stationary and the mobile phases.

Ideally, when the solvent front has almost reached the top of the plate, the components should be well-separated and appear in columns of discrete spots. Some compounds are coloured so they are easily seen on the TLC plate with the naked eye. (The word "chromatography" comes from the Greek word for colour, *chroma*.) Many different techniques are currently used to visualise colourless compounds and hence the term chromatography no longer refers exclusively to coloured compounds.

The plates that you will use are prepared commercially and consist of a thin layer of silica on an aluminium carrier plate. The mixture to be analysed is dissolved in an appropriate solvent and this solution is spotted onto the TLC plate using a capillary tube (Figure 10-1). The plate is then placed in a developing tank containing the mobile phase (Figure 10-2).

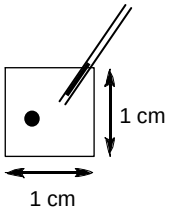


Figure 10-1. Loading a TLC plate.

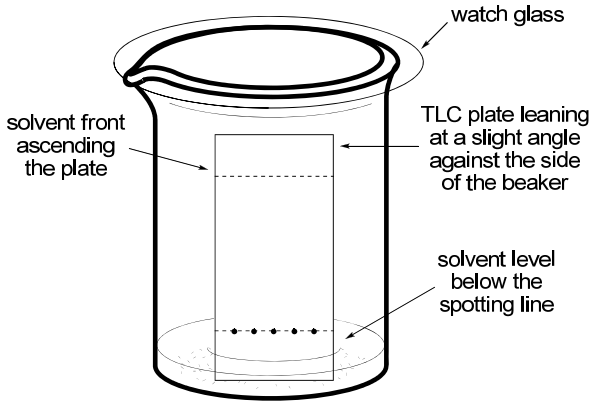


Figure 10-2. Developing a TLC plate.

Ideally, when the solvent front has almost reached the top of the plate, the components should be well-separated and appear in columns of discrete spots (Figure 10-3).

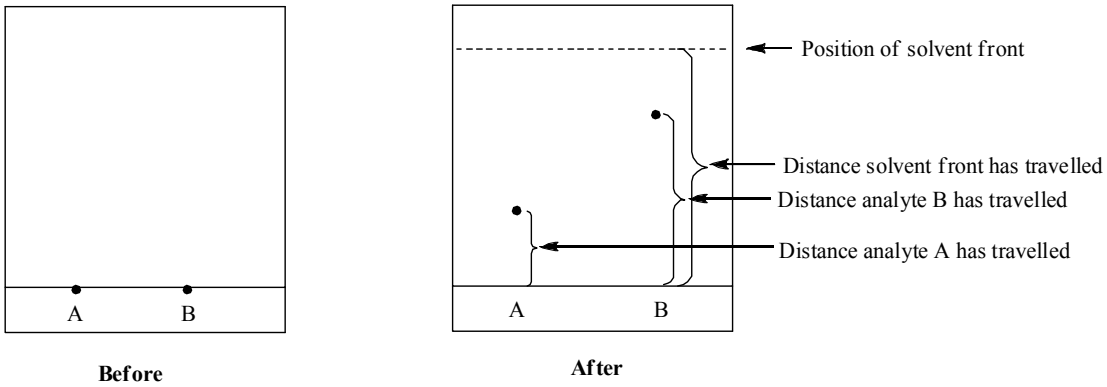


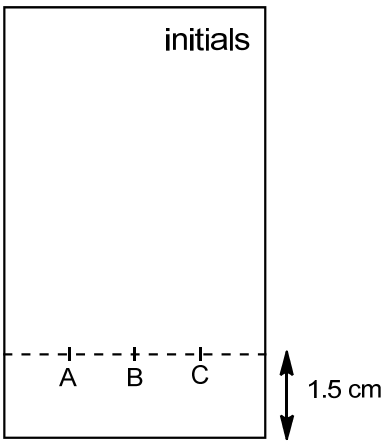
Figure 10-3. TLC plate before and after development.

In Figure 10-3, analyte A is more strongly attracted to the stationary phase (relative to the mobile phase) than analyte B. The position of the analyte spots is defined by their retention factor (R_f):

R_f = distance analyte has travelled / distance solvent front has travelled

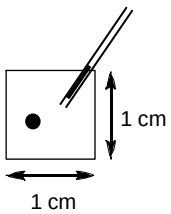
Skill 10.1 Preparation of the TLC plate

1. Handle TLC plates by the edges only, taking care not to touch the white surface with your fingers.
2. Place the plates on a clean dry surface and, using a pencil and a ruler, draw a line 1.5 cm from the edge. Press lightly with the pencil so as not to damage the silica layer.
3. On this line, mark points at intervals as specified in the experiment. Label the points lightly with pencil and include your initials at the top of the plate.



Skill 10.2 Loading the TLC plate

1. A plastic pipette tip is used as a capillary to load the samples onto the plate.
2. Before loading the TLC plate, practise spotting on a practice plate (1 cm × 1 cm) as follows. Dip the capillary into one of your samples. Briefly touch the tip of the capillary onto the plate. The solvent will evaporate in a few seconds. Reapply the tip of the capillary onto the same spot on the plate. Try to keep the spots as small as possible.



3. Run out any residual analyte by touching the capillary tip onto a piece of paper towel.

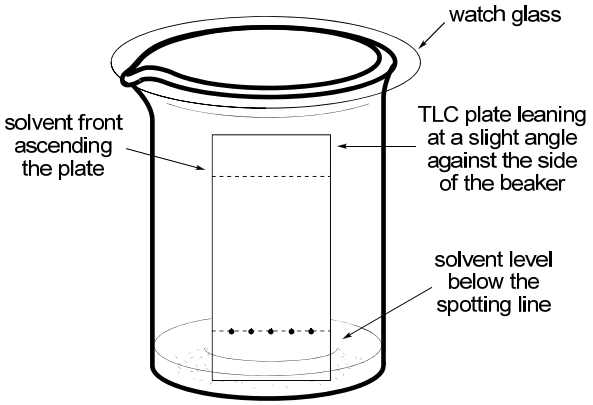
4. Follow the instructions in the experiment to load each sample onto the correspondingly labelled point on the TLC plate. The number of times each analyte needs to be spotted depends on its concentration.

Skill 10.3 Developing the TLC plate

1. When the spots are dry, place the plate into the solvent tank using a wooden peg to grip the plate at the very top. Make sure that the solvent level is below the baseline. Put the cover back on the tank.

2. Allow the plate to develop until the solvent front is about 1 cm from the top. Use a wooden peg to grasp the plate above the level of the solvent and remove it from the solvent tank.

3. Immediately mark the position of the solvent front with pencil. Place the plate on some paper towel in the fume hood until it is dry (about 1 minute).



Skill 10.4 Visualising the developed TLC plate

The spots on the developed TLC plate are usually visualised by observing them under UV light and/or by placing them into a tank containing some iodine. Coloured compounds can be observed without these techniques. The position of the analyte spots is defined by their retention factor (R_f):

R_f = distance analyte has travelled / distance solvent front has travelled

1. Rule a pencil line to indicate the solvent front.

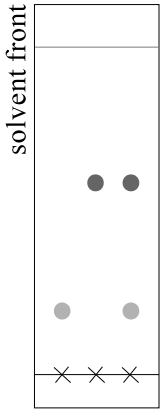
2. If visualising colourless compounds, use a large wooden test-tube holder to place the plate under the UV light. Using a long pencil, lightly outline the shape of the spots on the plate.

3. Sketch the developed TLC plate in your logbook. Include:
   - starting positions of all samples;
   - positions and shapes of all spots after development;
   - colours of spots under 356 nm and 254 nm UV light (or natural light);
   - calculation of all R_f values.

Skill 10.5 TLC Troubleshooting

Ideal Separation

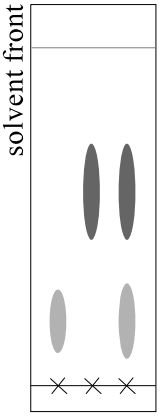
Sufficient sample has been applied in small, adequately separated spots.



Sample Streaking

Too much sample has been added, albeit in small spots causing the adsorbent to be 'overloaded'.

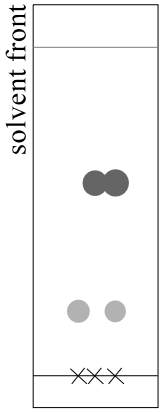
Add less sample.



Spots Overlapping Sideways

The chemist has placed the sample spots too close together on the origin.

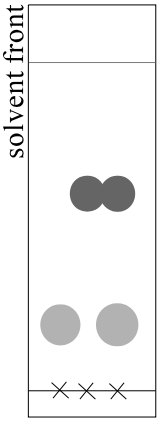
Use more space on the plate or use bigger (or several) plates.



Spots Too Big

The chemist left the spotting capillary in contact with the adsorbent for too long causing the sample to spread out.

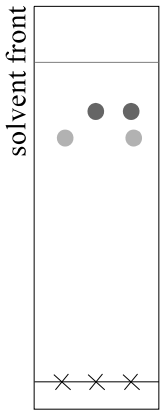
Add the sample solution with a fine capillary in short 'dabs'.



Spots Clustered Near Solvent Front

The elution solvent was too polar. The sample has migrated up the plate almost as fast as the solvent with little separation of the components.

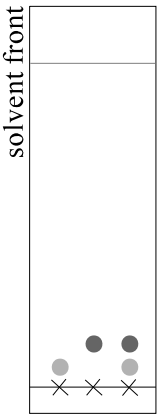
Use a less polar solvent.



Spots Clustered Near Origin

The elution solvent was insufficiently polar. The sample has not moved far up the plate with little separation because it is too strongly bound to the adsorbent.

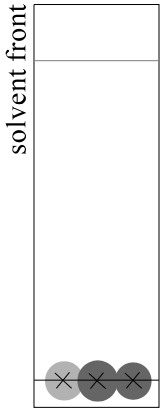
Use a more polar solvent.



Spots Have Spread - no elution

The origin was below solvent level so no elution occurred. The spots have become smeared by diffusion.

Use less solvent in the jar or spot the samples higher up the plate.



Skill 11: Centrifugation

The technique of centrifugation is used to separate substances suspended in a liquid base. A centrifuge spins liquid suspensions within a sample tube (centrifuge tube) around a central axis at high speed. This creates a centrifugal force, which forces all suspended particles towards the bottom of the tube. The velocity with which particles settle depends on many factors, including their size and shape, the speed of the centrifuge and the relative densities between the particles and the liquid. By an appropriate choice of the rate of spinning (which determines the magnitude of the centrifugal force) and the total spinning time, suspended particles will thus separate out within the centrifuge tube, with heavier particles at the bottom of the centrifuge tube and lighter particles near the top.

A very important practical point regarding the use of a centrifuge is that it **must always be balanced before it is switched on**. This means that the test-tubes being used must be placed in the centrifuge opposite each other with the same amount of fluid in each. If unbalanced there is a danger that the centrifuge rotor won't keep spinning about its central axis, *i.e.* the rotor could fly off its axis, through the instrument casing, and destroy the instrument and possibly cause physical damage to someone nearby. The danger of this happening increases with the spinning rate.

There are a number of important safety aspects that must be observed when using a centrifuge.

1. Make sure the centrifuge is balanced before you turn it on. Tubes must be placed opposite each other and must have the same amount of liquid in them.
2. Never leave a spinning centrifuge unattended. They have a tendency to "wander" across benches and fall off the edge.
3. N**ever touch a centrifuge rotor until it has come to a complete stop.** You risk breaking or losing a finger. Allow it to come to a complete stop by itself.

Skill 12: Flame tests

Flame tests are a very quick and simple way of determining the presence of certain metal ions. A platinum wire is cleaned by dipping it in hydrochloric acid and rinsed with deionised water. The wire is dipped into the solution or powder to be tested and then inserted into the luminous blue part of a Bunsen burner flame. The colour of the flame produced is indicative of the metal ion present. Conversely, the absence of any colour can be used to show that some particular ions are not present.

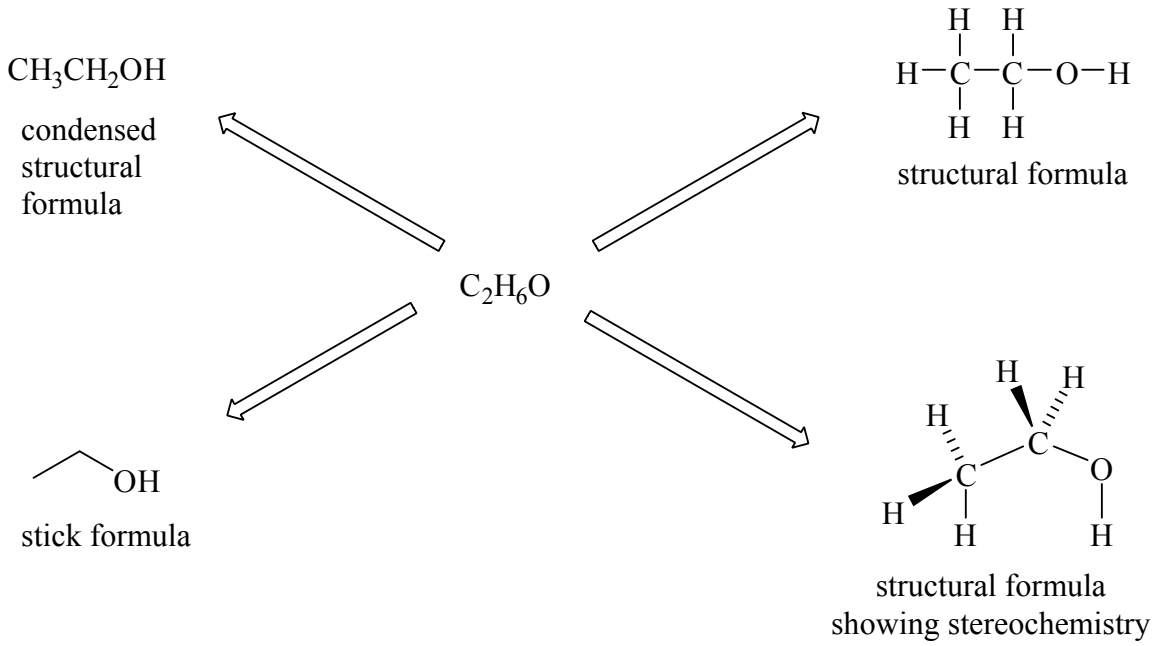
The following table lists the characteristic colours observed for a number of common ions.

Metal ion		Colour of the flame
Na^+	sodium	intense yellow
K^+	potassium	lilac
Ca^{2+}	calcium	dull red
Sr^{2+}	strontium	crimson
Ba^{2+}	barium	pale green
Cu^{2+}	copper(II) (non-halide)	emerald green
Cu^{2+}	copper(II) (halide)	blue-green
Zn^{2+}	zinc	bluish green

The lilac flame which indicates the presence of K^+ is often masked by the intense yellow sodium flame due to minor contamination.

Skill 13: Representing organic structures

Many organic structures are very complex and can easily contain lots of different atoms. An efficient way to represent the structures of the organic compounds is therefore essential. For example, ethanol always has the molecular formula C_2H_6O, but its actual structure can be represented in a number of different ways, each of which has a particular level of sophistication.



Ethanol is a very simple molecule, but as soon as any complexity is introduced, it is very difficult to comprehend a molecule's structure if all the H atoms are included. So in practice, the stick representation is always used for all but the simplest molecules.

In a stick structure:

1. Lines represent bonds - 1, 2 or 3 lines for single, double or triple bonds respectively.

2. Carbon atoms are not shown - they are assumed to be at intersections and ends of lines (bonds).

3. C–H bonds are omitted - the number of hydrogens bonded to each carbon can be calculated, as carbon always has a valence of 4.

4. All the other types of atoms (heteroatoms) are shown, and so are the hydrogens bonded to them.

5. When drawing neutral molecules:

C always has valence of 4 (4 bonds, 0 non-bonding electron pairs)

N always has valence of 3 (3 bonds, 1 non-bonding electron pair)

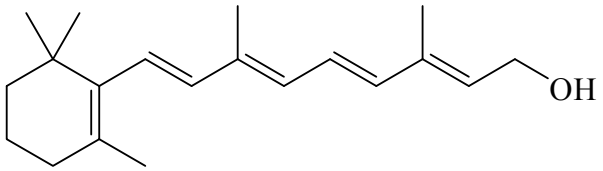
O always has valence of 2 (2 bonds, 2 non-bonding electron pairs)

F, Cl, Br, I always have valence of 1 (1 bond, 3 non-bonding electron pairs)

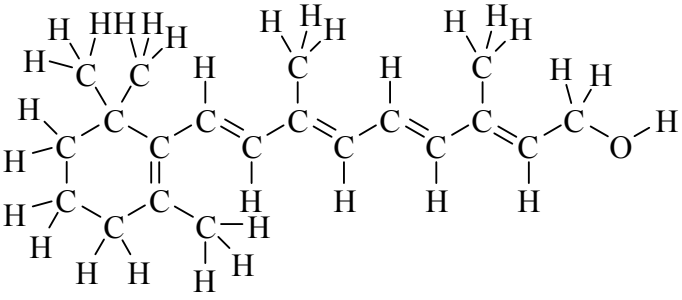
6. The bond angles around carbons, nitrogens and oxygens are usually drawn as 120° or 90°, though the actual bond angle is closer to 109.5° unless double or triple bonds are involved. Otherwise, bond angles are drawn to be as realistic as possible.

7. Some aspects of stereochemistry (the relative positions of the atoms in space) may be shown if relevant to the discussion at hand.

Note in the following example how the structure of vitamin A, a relatively simple molecule, is much tidier and comprehensible when shown as a stick formula.



stick formula



full structural formula

Vitamin A

Skill 14: Using the TPS temperature probe

The temperature probe comes in two parts. The first part is the blue TPS Aqua-pH meter. The second part is the temperature probe. The cable from the probe plugs into the 'CXA' socket. After connecting the probe, rotate the plastic base of the plug so that the connection remains secure. To measure a temperature you need to place the metallic end of the probe into the solution.

But before you use the probe to measure temperatures, you need to be sure that it's working properly. This is done by calibrating it with a solution of known temperature - a mixture of ice and water which has a temperature of 0 °C.

1. Press the ON/OFF button to switch the meter on.
2. Press the MODE button until you get to CM or until a temperature (in °C) is displayed; this is the temperature mode.
3. Press and hold the CAL button for 2 seconds until "Enter Temp" and two temperatures are displayed. The reading from the probe is now displayed on the top line, and the value you are going to set is on the bottom line.
4. Immerse the probe in a beaker containing a mixture of ice and water.
5. When the reading has stabilised, press the down (∨) button until the bottom line shows 0.0.
6. Press the CAL button again. The probe is now calibrated and ready for use. No further calibration is required for the rest of the session.
7. Subsequent measurements are made by immersing the probe in the liquid to be tested and reading the temperature displayed.

Names and Formulae of Some Common Ions and their Colours in Aqueous Solution

CATIONS			ANIONS		
Hg^{2+}	mercury(II) ion		OH^-	hydroxide ion	
Ag^+	silver ion		O^{2-}	oxide ion	
Cu^{2+}	copper(II) ion	blue	F^-	fluoride ion	
Pb^{2+}	lead(II) ion		Cl^-	chloride ion	
Sn^{4+}	tin(IV) ion		Br^-	bromide ion	
Sn^{2+}	tin(II) ion		I^-	iodide ion	
Ni^{2+}	nickel(II) ion	green	ClO_4^-	perchlorate ion	
Co^{2+}	cobalt(II) ion	pink	NO_3^-	nitrate ion	
Fe^{3+}	iron(III) ion	brown-yellow	NO_2^-	nitrite ion	
Fe^{2+}	iron(II) ion	pale green	SO_4^{2-}	sulfate ion	
Cr^{3+}	chromium(III) ion	blue-green	SO_3^{2-}	sulfite ion	
Cd^{2+}	cadmium ion		S^{2-}	sulfide ion	
Zn^{2+}	zinc ion		PO_4^{3-}	phosphate ion	
Mn^{2+}	manganese(II) ion	pale pink	CN^-	cyanide ion	
Al^{3+}	aluminium ion		CO_3^{2-}	carbonate ion	
Ca^{2+}	calcium ion		$CH_3CO_2^-$	acetate ion	
Sr^{2+}	strontium ion		$C_2O_4^{2-}$	oxalate ion	
Ba^{2+}	barium ion		$S_2O_3^{2-}$	thiosulfate ion	
Na^+	sodium ion		NCS^-	thiocyanate ion	
K^+	potassium ion		MnO_4^-	permanganate ion	purple
NH_4^+	ammonium ion		CrO_4^{2-}	chromate ion	yellow
			$Cr_2O_7^{2-}$	dichromate ion	orange
			HCO_3^-	hydrogencarbonate ion	
			HSO_4^-	hydrogensulfate ion	
			HPO_4^{2-}	hydrogenphosphate ion	
			$H_2PO_4^-$	dihydrogenphosphate ion	
			HSO_3^-	hydrogensulfite ion	
			HS^-	hydrogensulfide ion	
			$[Fe(CN)_6]^{3-}$	hexacyanidoferrate(III) ion	brown
			$[Fe(CN)_6]^{4-}$	hexacyanidoferrate(II) ion	yellow

Notes

- Ions are colourless in solution unless otherwise stated.
- Where more than one ion of an element is listed (*e.g.* Fe^{2+} and Fe^{3+}), no indication of the relative stability of the ions is given.