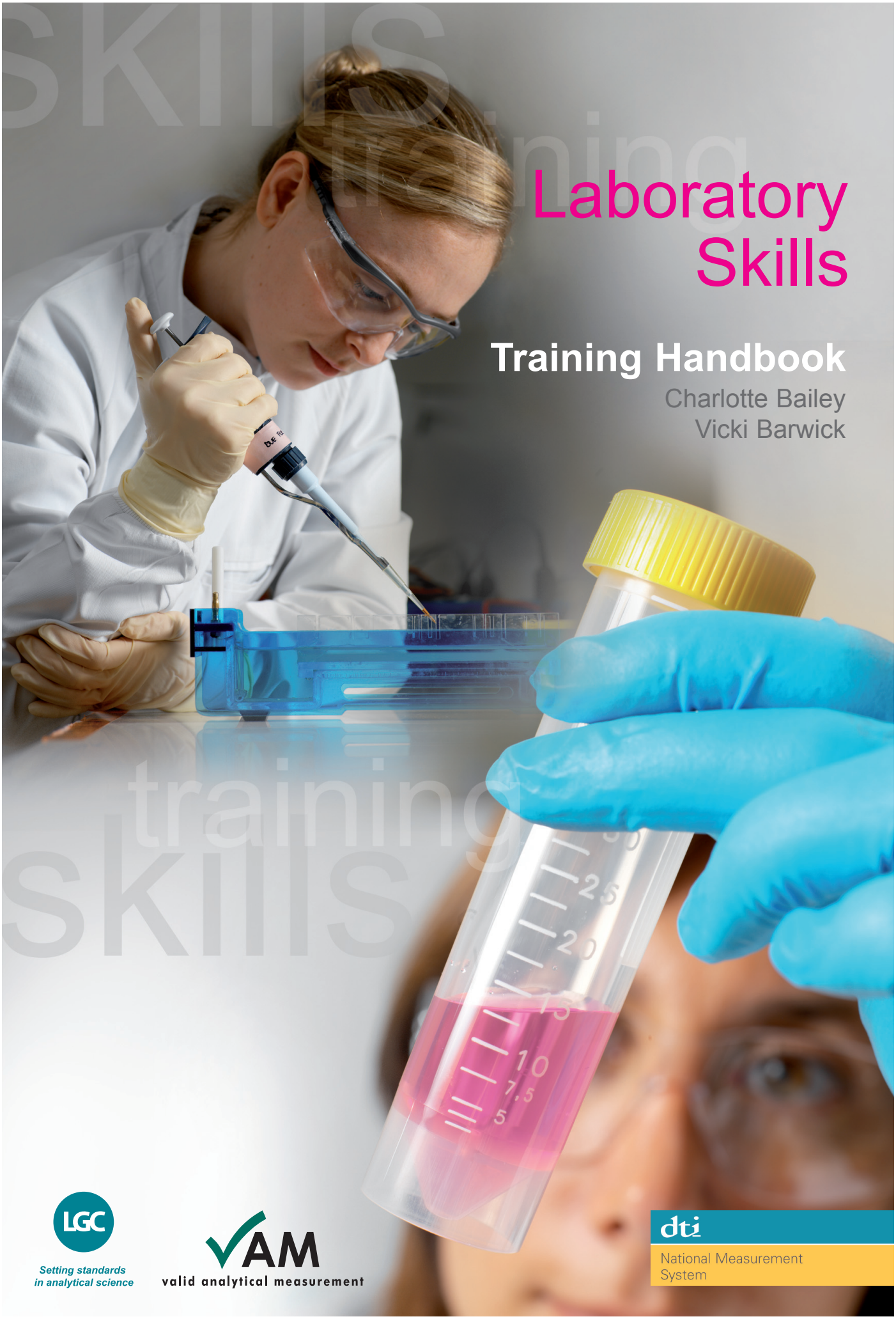

Laboratory Skills

Training Handbook

Charlotte Bailey
Vicki Barwick

LGC
Setting standards in analytical science

VAM
valid analytical measurement

dti
National Measurement System

Laboratory Skills Training Handbook

Charlotte Bailey
Vicki Barwick

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1 Introduction

1.1 The need for a laboratory skills training handbook

Although analysis of samples is frequently carried out by using sophisticated instruments, the importance of basic laboratory skills cannot be overlooked. The majority of instruments require calibration via the analysis of sets of calibration standards. It is therefore essential that analysts are able to prepare such solutions accurately. The analyst also needs to be able to handle and prepare samples correctly and be able to make 'routine' measurements, such as measurements of pH, reliably. Laboratories are increasingly being required to demonstrate the competence of their staff to carry out particular tasks and to document how competence was assessed. As well as sound practical skills, analysts should have at least a basic understanding of important quality issues such as method validation, traceability and quality control.

The aim of this handbook is to provide a basic training package in key laboratory skills and to provide an introduction to important quality topics. Those responsible for training analysts can use the handbook to help plan training programmes. Trainees can use the handbook as a guide to best practice for a range of laboratory skills and to gain a basic understanding of quality issues.

1.2 Structure of the handbook and how to use it

This handbook is divided into two sections. Part A is aimed at the trainee analyst. Chapter A1 contains the essential health and safety information that analysts should be familiar with to enable them to work safely in the laboratory. It also covers the selection of test methods and equipment plus key aspects to consider when planning and carrying out an analysis. Chapter A2 covers sample handling and storage. Chapter A3 covers the key laboratory skills that analysts need in order to be able to carry out analytical work with the required level of accuracy. There are questions relating to each 'skill' to test understanding. Chapter A4 introduces the key topics relating to quality assurance and quality control that analysts should be familiar with. Finally, Chapter A5 addresses data handling and reporting of results.

Part B of the handbook is aimed at those responsible for planning and carrying out the training of analysts. For each of the key laboratory skills covered in Chapter A3 there are key learning points, suggestions for assessing competence and observations which may indicate that retraining is required.

1.3 A note about units of volume

Volumes can be expressed in a number of different ways, e.g. cm^3, mL, dm^3, L. In this handbook we have used millilitres (mL) and litres (L) as the units of volume. However, you may encounter cm^3 and dm^3 in other texts or standard operating procedures. Remember that 1 mL is equivalent to 1 cm^3 and that 1 L is equivalent to 1 dm^3 (and that 1 L contains 1000 mL).

1.4 Acknowledgements

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Contents

Part A

Information for the analyst

Part A: Information for the analyst

A1 Working in the laboratory

The laboratory is a potentially hazardous working environment. You may well be using chemicals and/or equipment which, if not handled correctly, could cause you or your colleagues harm. However, with the proper procedures in place, work can be carried out safely. It is essential that you familiarise yourself with the general safety procedures in place in your laboratory and with any special procedures required to carry out a particular test method safely. When working in the laboratory you also need to know how to select an appropriate test method and equipment, and understand the importance of following standard operating procedures. This section covers:

- Health and safety issues (section A1.1);
- Selecting a test method and equipment (section A1.2);
- Importance of standard operating procedures (section A1.3).

A1.1 Health and safety issues

When working in the laboratory you must always:

- Wear suitable eye protection (safety glasses or goggles);
- Wear a laboratory coat;
- Wear suitable footwear (e.g. do not wear open-toed shoes or sandals).

In the laboratory you should never:

- Eat or drink;
- Smoke;
- Apply cosmetics.

You should know the meaning of common warning and hazard signs used in the laboratory. You will see different coloured signs:

- **Blue** signs are mandatory;
- **Red** signs are prohibitive (or relate to fire alarms/fire-fighting equipment);
- **Green** signs give safety instructions;
- **Yellow** signs give warnings.

Some examples of signs you may see in the laboratory are shown in Figure A1.1.

Figure A1.1: Examples of signs displayed in the laboratory

Before carrying out any laboratory work make sure:

- You are familiar with the test method;
- You know how to use all the necessary equipment/apparatus correctly and safely;
- You know of any hazards associated with the chemicals and reagents that you will be using and how to handle them correctly
    - o consult the material safety data sheet for detailed information about particular chemicals or reagents (an example is shown in Figure A1.2).

Southern Chemical Co.

Safety Data Sheet Date of issue: 1 September 2006

1.Identification of product and company

Product name:	Sodium dodecyl sulfate
Synonyms:	Dodecyl sulfate, sodium salt; sodium lauryl sulfate
Catalogue No.:	1234-ABC
Company:	Southern Chemical Co. South Park Industrial Estate Southtown Essex ES16 0PQ UK
Tel (enquiries):	+44(0)123 987 3210
Tel (emergency):	+44(0)123 100 100

2. Composition/information on ingredients

Product name:	Sodium dodecyl sulfate
CAS Number:	151-21-3
EINECS:	205-788-1
Molecular formula:	$C_{12}H_{25}OSO_3Na$
Molecular weight:	288.38 g mol^{-1}

3. Hazards identification

Highly flammable
Harmful if swallowed
Irritating to eyes and skin

Eye:	Causes moderate eye irritation.
Skin:	Causes severe skin irritation. May be harmful if absorbed through the skin.
Ingestion:	Harmful if swallowed. May cause irritation of the digestive tract. May cause nausea and vomiting.
Inhalation:	Causes respiratory tract irritation. May cause allergic respiratory reaction. May cause irritation of the respiratory tract with burning pain in the nose and throat, coughing, wheezing, shortness of breath. May be harmful if inhaled.

4.First aid measures

Eye contact:	Flush thoroughly with water for at least 15 minutes. Obtain medical attention.
Inhalation:	Remove from exposure and move into fresh air immediately. Obtain medical attention.
Ingestion:	Wash mouth out thoroughly with water and give plenty of water to drink. Obtain medical attention.
Skin contact:	Wash skin thoroughly with plenty of water. Remove contaminated clothing and wash before use. Obtain medical attention.

5. Fire fighting measures

Flammable solid, may evolve toxic fumes in a fire.
Use water spray, dry chemical, carbon dioxide, or chemical foam extinguisher.

6. Accidental release measures

Wear appropriate protective clothing.
Mix with sand, vacuum or sweep up material and transfer carefully to a suitable container. Wash site of spillage thoroughly.

7. Handling and storage

Avoid breathing dust. Avoid contact with skin and eyes.
Keep away from sources of ignition. Store in a cool dry place in a tightly sealed container.

Figure A1.2: Example of information contained in a material safety data sheet

To avoid accidents and to prevent the possible contamination of test samples it is essential to keep the laboratory area as clean and tidy as possible:

- Make sure you have sufficient space available at the laboratory bench to enable you to work safely
  - make sure that there is space available in a fume cupboard/hood if necessary;
- Work areas should be kept clean – clean up any spillages immediately. If you are unsure about how to clear up and dispose of spilled material consult your laboratory manager/supervisor;

- Take precautions to ensure that the samples cannot be contaminated by external sources or by coming into contact with other samples
  - make sure all glassware and other equipment/apparatus used is clean (see section A3.1.4 for information on cleaning glassware);
  - do not prepare calibration standards or quality control materials in the same area as the samples are prepared (this is critical for trace level analysis where the samples contain very low concentrations of the analyte);
  - avoid handling samples containing high concentrations of the analyte in the same area as samples containing trace levels of the analyte;
  - if it is possible samples received at the laboratory may contain very different levels of the analyte (but you do not know this in advance of carrying out the analysis), take special care to avoid cross-contamination when handling samples and carefully examine the results from quality control and blank samples to help identify any anomalies.
- Take precautions to ensure that any chemicals or reagents used cannot be contaminated
  - do not pipette reagents directly from the bottle – pour a suitable amount into a beaker or flask;
  - do not return any unused chemicals or reagents to the original container – dispose of any excess material correctly;
- Always tidy up after you have finished your work
  - clean apparatus and return to the correct storage place;
  - return reagents, chemicals and samples to the correct storage place.

A1.2 Method and equipment selection

A1.2.1 Method selection

Analysis is always carried out for a reason. You will analyse test samples to help answer a question or solve a particular problem. For the results of an analysis to be useful a suitable test method must be used. Once you know the reasons for carrying out the analysis, how do you decide on a suitable test method? The two key aspects to consider are:

- Method scope
  - can the test method be used to measure the analyte(s) of interest in the types of sample submitted to your laboratory at the concentrations likely to be present in the samples?
  - the method scope can be summarised as the analytes, sample matrices and analyte concentration range for which the method is applicable;
- Method performance
  - what will be the likely uncertainty in results obtained (see section A4.8) and is this acceptable?
  - are the key performance parameters, such as precision, bias and limit of detection, fit for purpose (see section A4.3)?
  - is the method performance acceptable across the required range of sample types and analyte concentrations?

Other aspects to consider when selecting a test method include:

- Cost;
- Time available to complete the analysis ('sample turnaround time');

- Availability of apparatus/equipment and staff.

There are a number of different sources of test methods:

- Methods produced and published by national or international standards organisations
  - e.g. BSI (UK), CEN (Europe), ISO (international);
- Methods produced by government bodies or agencies
  - e.g. British Pharmacopoeia (BP), United States Pharmacopoeia (USP), Environment Protection Agency (EPA);
- Methods published by professional organisations
  - e.g. Royal Society of Chemistry (RSC), Association of Official Analytical Chemists (AOAC);
- Methods supplied by trade organisations
  - e.g. Institute of Petroleum (Energy Institute);
- Methods described in regulations
  - e.g. The Fertilisers (Sampling and Analysis) Regulations 1991 (S.I. No. 973);
- Methods published in the scientific literature
  - e.g. The Analyst, Journal of AOAC International, Journal of Chromatography, Journal of Analytical Atomic Spectrometry;
- Methods developed in-house by a laboratory.

Usually, in-house method development is a last resort as developing a method to a stage where it can be used with confidence for the analysis of test samples can be costly and time-consuming.

In some situations there will not be any choice about which test method is used – it will be specified in a regulation or requested by the customer to meet a specific regulatory requirement.

In some cases there may be a method available which does not quite meet all of your requirements. For example, the method may not have been validated for the particular range of analyte concentrations or sample types that you need to analyse. In such cases, the method must be tested to ensure that it is capable of producing reliable results for the required sample types (see section A4.3). If the performance is not satisfactory then further development and revalidation of the method will be required.

Remember that the performance of all methods (including standard and regulatory methods) must be assessed before they are used for the analysis of test samples. This is discussed in section A4.3.

A1.2.2 Equipment selection

'Equipment' is everything other than the chemicals and reagents needed to carry out a particular test. The equipment required to carry out a particular test should be specified in the standard operating procedure (see section A1.3). However, before you use any item of equipment you will need to make sure that it is in an acceptable condition. Things to check include:

- Is the equipment clean? (see section A3.1.4 for information on cleaning glassware)
- Is the equipment in working order?
  - faulty equipment should be clearly labelled;

    - do not use equipment that is damaged (e.g. chipped or cracked glassware) or awaiting repair;
- Is the equipment set up correctly?
- Is the equipment correctly calibrated (see section A4.4 for information on calibration)?
    - for items calibrated by a third-party check that the calibration is still current;
    - familiarise yourself with any in-house calibration protocols;
- Are any performance checks required before use?
    - carry out any checks and ensure that the equipment is within specification;
    - do not use any equipment that fails a performance check – inform your supervisor/laboratory manager.

If you are involved with developing new test methods, or with deciding which equipment to purchase, you will need to make decisions as to which equipment is suitable for a particular purpose. Factors to consider include:

- Accuracy
    - if equipment is to be used to make measurements, will the accuracy of the measurement be sufficient? (sections A3.1.3and A3.2.3 cover the selection of suitable glassware and balances, respectively)
- Material
    - the equipment must be resistant to attack by any chemicals it may come into contact with;
- Dimensions – is there sufficient space in the laboratory?
- Environmental conditions – does the equipment require any special environmental conditions or utilities (e.g. stable power supply, water supply, piped gasses), or to be sited in a particular area, to allow it to operate correctly?
    - e.g. analytical balances need to be sited in an area that is free from vibrations (see section A3.2.4.1);
- Cost.

A1.3 The importance of standard operating procedures

All test methods should be written up as a clear and unambiguous set of instructions. The information that should be included in the description of the method is given in Table A4.2. The detailed method description is often referred to as a *standard operating procedure* (SOP). The aim of using SOPs in the laboratory is to ensure consistent application of test methods. Often analyses will be carried out by a number of different analysts; getting them all to follow the same SOP should improve the comparability of their results. Systems should be in place to control the number of copies of a particular SOP that are in circulation and to enable them to be recalled and updated if required. It is essential to ensure that only the most recent version of an SOP is being used.

When using SOPs follow these guidelines:

- Make sure you have the most recent version of the SOP
    - many laboratories have controls in place to prevent unauthorised photocopying/printing of methods – this makes it easier to manage and control the issuing of updates;
- Make sure you have read the SOP and are familiar with all steps of the method before commencing work;

- Follow the procedure exactly as it is described in the SOP
  - do not be tempted to take shortcuts or adapt the method in any way (even if you think it will speed up the analysis or 'improve' the results);
  - if you encounter any problems with the method or have any suggestions as to how the method might be improved/modified always discuss them with your supervisor/laboratory manager;
  - any changes will require the SOP to be revised and reissued. If the changes are significant then the method will require revalidation before at can be used for the analysis of test samples.

A1.4 Carrying out an analysis

Below is a checklist of things to remember when carrying out an analysis:

- Understand why you are carrying out the analysis and what the results will be used for;
- Select a suitable test method;
- Read the method carefully (especially if it is a method that you are not completely familiar with);
- Familiarise yourself with any special health and safety issues relating to the test method (e.g. are there any hazardous chemicals involved that will require special handling?);
- Always wear a laboratory coat and eye protection in the laboratory
  - wear suitable gloves if you will be handling any hazardous chemicals, reagents or samples;
- Identify the equipment you will need to carry out the method
  - make sure that all the equipment will be available when you need to carry out the analysis;
  - make sure the equipment is clean and in working order (see sections A3.1 and A3.2 for information on cleaning and maintaining glassware and balances, respectively);
  - make sure that equipment is calibrated correctly (see sections A3.1.5 and A3.2.4.2 for information on calibration of glassware and balances, respectively; see section A4.4 for general information on calibration);
- Make sure that you have received sufficient training so that you can use each piece of equipment competently and safely – if in doubt, ask your supervisor;
- Identify the chemicals and reagents that you will need to carry out the analysis
  - make sure that sufficient supplies are available and that the chemicals and reagents have not passed their 'use by' date;
- Plan the analysis carefully
  - identify any points in the procedure where it is possible to stop;
  - make sure you have enough time to complete each stage of the analysis without rushing;
  - work out how many samples you can comfortably handle at one time (i.e. how many samples can you analyse in a batch?);
- Make sure that you have adequate space at the bench/fume cupboard in which to work
  - ensure that your work area is kept clean and tidy;
  - ensure that there is no possibility of the samples being contaminated or causing cross-contamination;
- Locate the test samples

- double-check that you have selected the correct samples;
- make sure that the appearance of the samples is as expected (see section A2 for further information);

- Make sure that the samples are at the correct temperature prior to analysis
  - depending on the test method, it may not be appropriate to analyse samples straight from the refrigerator;
- Carry out the test method exactly as specified in the standard operating procedure (see section A1.3)
  - carry out any quality control procedures specified in laboratory protocols (see section A4.2 for information on quality assurance);
  - do not be tempted to take shortcuts or adjust the method in any way;
- Record all measurements, results, observations, etc. in a suitable workbook at the time that you carry out the work
  - record results in ink, not pencil;
  - if you make a mistake, do not use correcting fluid or attempt to erase/obscure the mistake – put a line through the error, write the correction nearby and initial and date the correction;
- When you have finished the analysis
  - make sure that any surplus sample is stored or disposed of correctly;
  - clean any glassware you have used (see section A3.1.4 for information on cleaning glassware) and return it to the correct storage place;
  - make sure other equipment/apparatus you have used is left clean and tidy;
  - return all chemicals and reagents to the correct storage place;
  - never return surplus chemicals or reagents to the original container – surplus material should be disposed of correctly;
  - leave your work area clean and tidy.

A2 Sample handling and storage

For test results to be of use, the composition of the sample at the time of analysis must be the same as the composition when the sample was taken. Often it is not possible for samples to be analysed immediately after receipt into the laboratory. Steps therefore have to be taken to ensure that samples are stored correctly. It is also important that samples can be uniquely identified at all points during the analytical process – from receipt to disposal – and it is essential that the correct test result is linked to the correct sample. This is not always straight forward. Many laboratories handle thousands of samples that are identical in appearance and which have to be put through a complicated series of analytical procedures. This section covers:

- Sample receipt (section A2.1);
- Sample labelling (section A2.2);
- Sample storage (section A2.3);
- Sample tracking (section A2.3).

A2.1 Receiving samples

When a sample is received at the laboratory:

- It should have a unique identification number or code
  - o ensue that samples are labelled correctly so that they can be readily identified in the future;
- Record a description of the sample, including a description of the container and closure;
- Record the appearance of the sample, highlighting any unusual features;
- Document any storage requirements and ensure that the sample is stored correctly (see section A2.3).

A2.2 Labelling

As mentioned above, all samples should be clearly labelled with a unique identifier when received into the laboratory. Factors to consider in designing a suitable label include:

- The label should be securely attached to the body of the container, not the closure;
- The label must remain legible while the sample is being stored.

It may also be helpful to label the closure of the sample container in some way, to make sure that the original closure is used once the sample container has been opened.

It is important that any subsamples or aliquots taken from the laboratory sample are also clearly labelled so that the sample can be tracked through the analytical process (see section A2.4).

Laboratories handling large numbers of samples frequently use a Laboratory Information Management System (LIMS) for tracking samples. Such systems often make use of barcode labels to help identify and track samples

A2.3 Storage

A2.3.1 Containers

All samples must be held in a suitable container. Factors to consider include:

- Container closure
  - the container mustn't leak and must prevent the possibility of contamination of the sample;
  - volatile samples require well sealed containers;
  - is a tamper-evident closure required?
- Material
  - the container must not react with the sample in any way (e.g. glass containers may adsorb or desorb elements);
  - if the sample is light-sensitive consider using amber containers or containers protected with foil.

A2.3.2 Storage conditions

Ideally, samples should be analysed as soon as possible after receipt. As this is often not practical, the samples will require storage. It is essential that samples are stored in such a way that they do not degrade or change in any way. The storage conditions must also ensure that there is no risk of contamination. For example, do not store samples in the same area as calibration standards or quality control samples. Do not store samples which are expected to contain low levels of the analyte near samples that might contain elevated levels of the analyte.

The required storage conditions will depend on the nature of the sample. Some examples are given in Table A2.1. The storage conditions and storage time should be documented. Remember that samples should normally be allowed to reach room temperature before analysis.

Table A2.1: Typical storage conditions for different sample types

Storage condition	Sample types	
	✓	✘
Deep freeze (-18 °C)	Samples with high enzymatic activity Perishable goods/products Less stable analytes	Samples which liquefy on thawing Aqueous samples
Refrigerator (4 °C)	Soils Fresh fruit and vegetables Aqueous samples	Samples with possible enzymatic activity
Room temperature (in the dark)	Dry powders and granules Minerals Stable analytes	Fresh foods
Desiccator	Hygroscopic samples	Samples which are more hygroscopic than the desiccant

Some samples require special treatment or the addition of preservatives to prevent degradation during storage. Some examples are given in Table A2.2.

Table A2.2: Examples of methods of sample preservation

Method	Examples of applications
Freeze drying[a]	Breads, biscuits etc Aqueous samples
Irradiation[b]	Aqueous samples Biological samples
Adding antioxidants[b, c]	Liquids and solutions
Adding anticoagulants[c]	Blood and clinical samples
Autoclaving[b]	Sterilising body fluids

[a] Unsuitable for volatile analytes
[b] Stability of analyte must be established
[c] Check that added compound does not interfere with the analysis

A2.4 Sample tracking

It is essential that samples can be located at any stage during the analytical process – from receipt to disposal. It is also essential that test results are related to the correct sample. The process of following a sample through the analysis and linking it to the correct test result is sometimes referred to as *traceability*. However, this can cause confusion with *metrological traceability* which is to do with ensuring that results are linked to an appropriate reference standard (see section A4.4). The terms *trackability* or *sample tracking* are therefore preferred.

The progress of the sample through the laboratory must be recorded. This includes documenting:

- Everyone who has handled the sample, when they received it and what actions they took;
- Storage conditions and time (see section A2.3);
- Any unusual features of the sample – does the sample look like other samples of the same type? If you are unsure about the condition of a sample you should consult your supervisor/laboratory manager before carrying out the analysis;
- Measurements made and results obtained at each stage of the analysis;
- Storage of samples after analysis (location and conditions);
- Disposal date of the sample when it is certain that the sample is no longer required.

All of the above must be noted against the unique sample identification number.

A3 Key laboratory skills

A3.1 Measuring volume

Many activities in the laboratory will require you to make accurate measurements of volume. These include preparing solutions of known concentration (see section A3.4) and carrying out titrations (see section A3.6). This section outlines the key points you need to remember to be able to make accurate measurements of volume:

- The different types of equipment available for handling liquids (section A3.1.1);
- Which type of equipment to use (section A3.1.3);
- How to clean the equipment (section A3.1.4);
- How to check the accuracy of the volume contained in or delivered by different items of equipment (section A3.1.5);
- How to use the equipment correctly (section A3.1.6).

A3.1.1 Types of equipment available

A3.1.1.1 Beaker

Beakers are flat-bottomed cylindrically shaped vessels, with graduation marks on the side. Beakers are not meant to be used for accurate volume measurements. They are often used as vessels for transferring and mixing solutions, before they are made up to the required volume in a volumetric flask.

A3.1.1.2 Conical/Erlenmeyer flask

Conical or Erlenmeyer flasks have a flat-bottom, with a cone shaped body and a short neck (see Figure A3.1). They have graduation marks on the side of the vessel, much like a beaker and are also not meant to be used for accurate volume measurements. Conical flasks are used as containers for samples during titrations, when mixing solutions and when transferring solutions by pipette (e.g. during the preparation of solutions of known concentration, see section A3.4.4).

Figure A3.1: Conical flasks

A3.1.1.3 Measuring cylinder

Measuring cylinders, also known as graduated cylinders, are cylindrical glass tubes which are closed at one end and fitted with a support to allow the cylinder to be freestanding (Figure A3.2). The cylinder has graduations along the length of the tube and the open end is fitted with a pouring spout or a ground glass joint. Measuring cylinders are not accurate enough for quantitative work, but they are useful for approximate volume measurements (e.g. for preparing reagents, see section A3.5).

Figure A3.2: Measuring cylinder

A3.1.1.4 Burette

Burettes are long cylindrical glass tubes of uniform bore, marked with graduations and terminating at one end with a stop-cock tap (Figure A3.3) which is usually made out of polytetrafluoroethylene (PTFE). Burettes should conform to BS EN ISO 385:2005[1] and are mainly used for dispensing variable and accurately measurable volumes of liquid, for example in a titration procedure (see section A3.6).

Figure A3.3: Burette

A3.1.1.5 Glass graduated pipette

Pipettes of this type consist of a long glass tube with no central bulb. The tube is marked with a series of graduations (as shown in Figure A3.4), enabling a range of volumes to be delivered. These pipettes are not as accurate as bulb pipettes, and are mainly used for dispensing pre-determined volumes where the highest level of accuracy is not required.

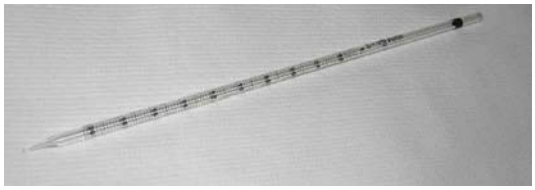


Figure A3.4: Glass graduated pipette

[1] BS EN ISO 385:2005 'Laboratory glassware – Burettes'

There are two main types of graduated pipettes:

- Type 1 delivers a measured volume from the top zero mark to the selected graduation mark;
- Type 2 delivers a measured volume from a selected graduation mark to the tip, which forms the zero mark. For this type of pipette, the residual liquid in the tip may need to be expelled, in which case the pipette will be marked 'blow-out'.

A3.1.1.6 Volumetric flask

Volumetric flasks (also known as graduated flasks) are flat-bottomed, pear-shaped glass vessels with a long narrow neck as shown in Figure A3.5. The neck of the flask has a single etched calibration mark. The top of the neck has a ground glass socket for a stopper, which may be made from polyethylene, polypropylene or glass. When filled to the calibration mark each flask will contain the specified amount of liquid. Volumetric flasks should comply with BS EN ISO 1042:2000[2]. The main use of a volumetric flask is in the preparation of a solution of known concentration (see section A3.4).

Figure A3.5: Volumetric flask

A3.1.1.7 Glass bulb pipette

As shown in Figure A3.6, these pipettes consist of a long glass tube with a central cylindrical bulb. A single calibration mark is etched around the upper end of the tube and the lower end of the tube is drawn into a fine tip. These pipettes are also referred to as transfer pipettes and one-mark pipettes. A known fixed volume of liquid is delivered through the tip under certain specified conditions, after the pipette has been filled to the calibration mark. Glass bulb pipettes should conform to BS 1583:1986[3] and should be colour coded to indicate their capacity.

Figure A3.6: Glass bulb pipette

A3.1.1.8 Automatic pipette

Figure A3.7: Air displacement automatic pipette

Automatic pipettes are available in both fixed volume and variable volume models. They are made out of plastic and metal and the variable volume models are fitted with either a mechanical or electronic volume setting device. The liquid is confined to a plastic tip, which is readily exchangeable. Two different mechanisms are used in automatic pipettes

[2] BS EN ISO 1042:2000 'Laboratory glassware - One-mark volumetric flasks'
[3] BS 1583:1986 'Specification for one-mark pipettes'

for aspirating and dispensing liquids. Figure A3.7 shows an air displacement automatic pipette. This type of pipette uses a piston to create suction to draw liquid into the pipette tip. Positive displacement pipettes have a disposable piston which sits inside the pipette tip and is in contact with the liquid. Unlike air displacement pipettes, there is no air cushion between the sample and the piston so the aspiration force is unaffected by the physical properties of the liquid, such as viscosity. Positive displacement pipettes are therefore recommended when handling viscous, dense or volatile liquids.

A3.1.2 Markings on equipment used for volumetric measurements

In addition to the graduation and calibration marks, glassware that is used for making volume measurements will carry other markings that are relevant to particular applications (see Figure A3.8). You should be aware of these markings and should check equipment before use.

Figure A3.8: Illustrations of markings on volumetric glassware

Table A3.1 lists the markings present on volumetric glassware.

Table A3.1: Markings on volumetric glassware

Marking	Information
Volume, with units	Stated volume of the equipment.
Temperature	Temperature at which the stated volume applies (usually 20 °C).
Class A or Class B	Class of glassware – defines the tolerance of the stated volume (see section A3.1.3).
Identification number	Identifies a specific item – is included on any certificate of calibration for the item (see Figure A3.9).
BS/ISO/DIN number	Indicates the item complies with the stated British (BS), international (ISO) or German (DIN) standard.
Seconds	The liquid delivery time for a pipette or burette.
Ex or In	Indicates the item either delivers (Ex) or contains (In) the stated volume.
Blow-out	Indicates (for pipettes) that the residual liquid in the tip is part of the stated volume and must be blown out.
Trade name or material	Type of glass, e.g. Pyrex, borosilicate.
Colour code	Coloured band on stem (for pipette) that indicates the capacity.

A3.1.3 Selecting a suitable piece of equipment

Your choice of equipment will depend on the level of accuracy needed to carry out the measurement you require. For graduated items of glassware, such as graduated pipettes and measuring cylinders, you should choose an appropriate sized item for the volume that you are measuring. For example, the capacity of a graduated pipette should not be more than twice the volume you want to measure.

Many items of volumetric glassware are commercially available in two classes – Class A and Class B. The distinction between the two is based on tolerance limits.

Table A3.2 shows typical tolerances for the graduations of Class A and Class B volumetric glassware such as pipettes, burettes and volumetric flasks. It is worth noting that under the British Standard there is no Class A or Class B classification for measuring cylinders; just one level of accuracy. However suppliers are able to import measuring cylinders of Class A and B levels under, for example, the DIN (Deutsches Institut für Normung) standard system.

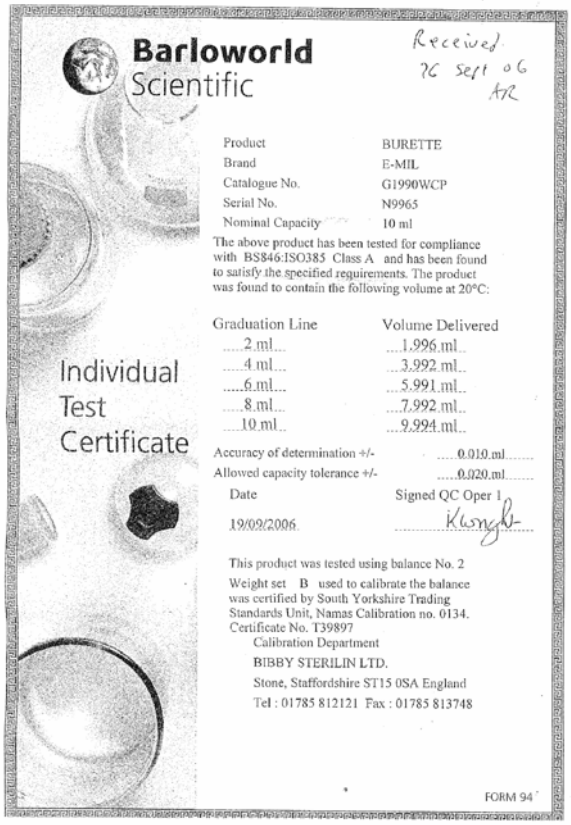
Generally, for volumetric glassware, Class A tolerances are about half those of Class B. The stated tolerance is a useful indication of the uncertainty in the measured volume, making it easier to judge which type and class of equipment is required for a particular task. Class A is generally used for quantitative work where a higher degree of accuracy is required.

Table A3.2: Tolerances for Class A and Class B volumetric glassware

Equipment	Nominal volume / mL	Graduations / mL (Class A / B)*	Tolerance / mL	
			Class A	Class B
Bulb pipette	1	-	0.008	0.015
	5	-	0.015	0.03
	10	-	0.02	0.04
	25	-	0.03	0.06
Graduated pipette	1	0.01	0.006	0.01
	5	0.05	0.03	0.05
	10	0.01/0.1	0.05	0.1
	25	0.02/0.2	0.1	0.2
Burette	5	0.05	0.01	0.02
	10	0.05	0.02	0.05
	50	0.1	0.05	0.1
	100	0.2	0.1	0.2
Volumetric flask	5	-	0.025	0.04
	10	-	0.025	0.04
	50	-	0.06	0.12
	250	-	0.15	0.3
	1000	-	0.4	0.8
Measuring cylinder	5	0.1	0.05	0.1
	10	0.2	0.10	0.2
	25	0.5	0.15	0.5
	100	1.0	0.5	1.00
	500	5.0	1.5	5.00
	2000	20.0	6.00	20.0

*For all glassware except graduated pipettes, the graduation marks are the same for both Class A and B items. For graduated pipettes, the graduation marks in the table have been noted for Class A first followed by Class B (Class A/Class B).

Class A pipettes, graduated pipettes, burettes and volumetric flasks are also available with individual calibration certificates. Figure A3.9 shows a typical calibration certificate. The certificates give an estimate of the error for an individual piece of glassware, which can be used to correct the volume for that item. Certified glassware is only needed for very high accuracy work.



Barloworld Scientific

Received 26 Sept 06 AR

Individual Test Certificate

Product	BURETTE
Brand	E-MIL
Catalogue No.	G1990WCP
Serial No.	N9965
Nominal Capacity	10 ml

The above product has been tested for compliance with BS846:ISO385 Class A and has been found to satisfy the specified requirements. The product was found to contain the following volume at 20°C:

Graduation Line	Volume Delivered
2 ml	1.996 ml
4 ml	3.992 ml
6 ml	5.991 ml
8 ml	7.992 ml
10 ml	9.994 ml

Accuracy of determination +/- 0.010 ml

Allowed capacity tolerance +/- 0.020 ml

Date 19/09/2006

Signed QC Oper 1 Kwright

This product was tested using balance No. 2

Weight set B used to calibrate the balance was certified by South Yorkshire Trading Standards Unit, Namas Calibration no. 0134. Certificate No. T39897

Calibration Department
BIBBY STERILIN LTD.
Stone, Staffordshire ST15 0SA England
Tel : 01785 812121 Fax : 01785 813748

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Figure A3.9: Typical calibration certificate for a burette

Table A3.3 shows typical manufacturers' data for the tolerances for volume measurements made using automatic pipettes. Automatic pipettes are not covered by the 'class' system used for glassware. You should consult the manufacturer's information for the exact specification for a particular pipette.

Table A3.3: Typical tolerances for automatic pipettes

Nominal volume	Tolerance
1 μL	± 0.025 μL
10 μL	± 0.1 μL
100 μL	± 0.8 μL
1000 μL (1 mL)	± 8 μL (± 0.008 mL)
10000 μL (10 mL)	± 60 μL (± 0.06 mL)

A3.1.4 Cleaning glassware

You should check the condition of all glassware before use. Take special care when using equipment such as pipettes and burettes, as the tips can be easily damaged if these items are not stored correctly.

Glassware should be clean and free from dust. To test if an item is clean:

- Allow water to drain from the item;
- The absence of discrete droplets of water on the interior surface indicates a clean, dust free interior.

If droplets of water remain on the interior surface of the item, the glassware should be cleaned prior to use. How to clean and dry glassware:

- Clean with a suitable detergent such as Teepol® or Decon® 90;
- Thoroughly rinse with water;
- Allow to drain at room temperature, or rinse with acetone and then allow to drain or apply a gentle stream of clean dry air;
- Volumetric glassware should not be washed in a dishwasher at high temperatures or dried by heating in an oven as this may affect the accuracy of the graduation marks.

A3.1.5 Checking the accuracy of the volume of liquid contained in / delivered by the equipment

For certain applications, when the uncertainty associated with the volume being measured needs to be minimised, it is necessary to establish the accuracy of the equipment by formal calibration, rather than to rely on the quoted tolerances shown in Table A3.2.

Formal calibration entails gravimetrically measuring the quantity of water delivered by, or contained in, the equipment. The volume can then be calculated from a simple calculation using the density of water.

Formal calibration is a complex operation, so laboratories often have volumetric glassware calibrated by a third-party that is accredited to carry out such work (e.g. accredited by UKAS in the UK). A full record of the calibration should be obtained along with a formal certificate of calibration, specifically identifying the item of equipment to which it refers (see Figure A3.9).

As a result of a formal calibration, any bias associated with a graduation mark will be detected and appropriate corrections can then be made when the equipment is used for routine work. Any bias should not exceed the manufacturer's tolerance for a particular item of equipment.

Calibrated glassware that is in regular use should be re-calibrated at specified intervals as damage, misuse or general wear and tear may affect the validity of the calibration. Automatic pipettes should be serviced and calibrated regularly (typically annually) by a recognised supplier of such equipment who is also accredited to carry out calibrations.

For those items of volumetric equipment that have not undergone a formal calibration procedure, it is useful to carry out spot checks, at regular intervals, to confirm that the accuracy is suitable for the application. Follow the check list below to carry out an accuracy check on an item of volumetric glassware or an automatic pipette:

- Select a suitable balance, bearing in mind the volume of liquid to be dispensed. For volumes above 200 mL a 2-figure top-pan balance may be used but for smaller volumes an analytical balance or micro balance will be required.
- The accuracy of the volume delivered by or contained in the equipment should be gravimetrically determined using distilled or deionised water (for information on the correct use of a balance see section A3.2.4);
- The item to be checked must be clean and dust free (if necessary it can be cleaned as outlined in section A3.1.4);
- The water used for the accuracy check needs to equilibrate to ambient temperature and this temperature should be recorded to the nearest degree centigrade;

- The item is filled to the required graduation mark;
- For pipettes and burettes the volume of water is discharged into a pre-weighed clean dry vessel. The vessel is reweighed and the mass of the water delivered by the pipette is determined;
- For volumetric flasks the mass of the water contained in the flask is determined by weighing the empty, dry flask and then weighing again after it has been filled to the graduation mark;
- The recorded mass of water is converted to a volume by dividing by the density of water at the appropriate temperature (see Table A3.4);
- If the difference between the stated volume for the item and the measured volume is more than the quoted tolerance, the procedure needs to be repeated. If it fails again then do not use the item and consult your supervisor/laboratory manager;
- For pipettes, both automatic and glass, repeatability (see section A4.3.4.2) can be checked by carrying out replicate determinations as outlined above, then calculating the mean and standard deviation (see section A5.1) of the volume delivered and comparing these to the manufacturer's specification.
- Items capable of delivering different volumes (i.e. graduated glass pipettes, burettes and variable volume automatic pipettes) should have their calibration checked when delivering a range of volumes.

Table A3.4: Density of water (based on weighing in air) at different temperatures

Temperature (°C)	Density (g mL^{-1})	Temperature (°C)	Density (g mL^{-1})
16	0.99789	21	0.99694
17	0.99772	22	0.99672
18	0.99754	23	0.99649
19	0.99735	24	0.99624
20	0.99715	25	0.99599

A3.1.6 Checklists for making measurements of volume

When using the volumetric glassware discussed in this section, it is very important to read the meniscus correctly. Figure A3.10 shows three examples of how the meniscus could be viewed. Both (a) and (c) are incorrect; viewing the meniscus from below or above will introduce parallax error. Figure (b) shows the correct position for reading the meniscus accurately. The meniscus should always be viewed at eye level.

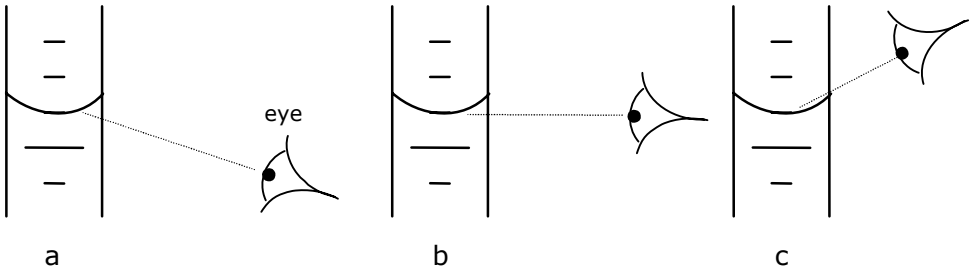


Figure A3.10: Reading a meniscus

Table A3.5 summarises the key points to remember when using a measuring cylinder.

Table A3.5: Dos and don'ts for using a measuring cylinder

Do	Don't
✓ Select an appropriate sized measuring cylinder for the volume to be measured.	✘ Use a measuring cylinder that is too large or too small.
✓ Check the measuring cylinder is clean and undamaged.	✘ Use a measuring cylinder that is dirty or damaged.
✓ Check for faded graduation marks.	✘ Use a measuring cylinder with faded graduation marks.
✓ Make sure the liquid is at ambient temperature.	✘ Measure liquids that are significantly above or below ambient temperatures.
✓ Rinse the measuring cylinder with distilled/deionised water followed by the liquid to be measured.	
✓ Dispose of the rinse solution immediately.	
✓ Line up the bottom of the meniscus with the graduation mark, making sure that your eyes are level with the calibration mark (see Figure A3.10).	✘ View the graduation mark from above or below when judging the position of the meniscus (see Figure A3.10).
✓ Clean the measuring cylinder after use by rinsing it with water and then drying it.	
✓ Store measuring cylinders in a clean dust free environment at ambient temperature and away from corrosive substances.	

Table A3.6 summarises the key points to remember when using a burette.

Table A3.6: Dos and don'ts for using a burette

Do	Don't
✓ Check the burette is clean and undamaged.	✘ Use a burette that is dirty or damaged.
✓ Check the graduation marks are not faded.	✘ Use a burette with faded graduations.
✓ Select an appropriate size burette for the volume to be delivered.	✘ Select a burette that is too large or small.
✓ Check the liquid is at room temperature before filling the burette.	✘ Fill the burette with a liquid that is much hotter or colder than the burette calibration temperature. Variations in temperature will affect the volume of the liquid delivered.
✓ Check that the burette delivers the correct volume (see section A3.1.5).	
✓ Rinse the burette twice with the solution to be measured.	✘ Use a burette that has globules of liquid adhering to the inside surface after rinsing – this indicates that the burette is not clean.
✓ Fill the burette with the liquid to be measured to about 20-30 mm above the zero graduation, using a small funnel and ensuring that the burette is held below eye level.	✘ Fill the burette with it clamped or held above eye level.
✓ Clamp the burette vertically in a suitable holder.	
✓ Remove the funnel before dispensing the liquid.	✘ Leave funnel in the top of the burette when dispensing the liquid.
✓ Operate the stop-cock by placing the fingers behind the stop-cock and the thumb in front.	
✓ Allow liquid to flow through the stop-cock until no air bubbles are present in the jet or the burette (tap the burette to remove stubborn bubbles).	✘ Allow air bubbles to remain in the burette. (Note that the presence of bubbles in the burette may indicate that it is not clean.)
✓ Line up the bottom of the meniscus with the graduation mark, making sure that your eyes are level with the graduation mark (see Figure A3.10).	✘ View the graduation mark from above or below when judging the position of the meniscus (see Figure A3.10).
✓ Read the burette scale accurately; record to the nearest half of a sub-graduation (e.g. to the nearest 0.05 mL for burettes with 0.1 mL graduations).	
✓ Clean the burette after use and store in a clean dust free environment at ambient temperature and away from corrosive substances, making sure that it is unable to roll.	✘ Leave the burette dirty and incorrectly stored.

Table A3.7 summarises the key points to remember when using a glass pipette.

Table A3.7: Dos and don'ts for using a glass pipette

Do	Don't
✓ Check the pipette is clean and undamaged.	✘ Use a pipette that is dirty or damaged.
✓ Check the calibration mark (or marks) is not faded.	✘ Use a pipette with a faded calibration mark(s).
✓ Handle the pipette holding the stem rather than the bulb.	✘ Hold the pipette by the bulb.
✓ Fill the pipette using a suitable pipette filler.	✘ Fill a pipette by mouth.
✓ Transfer liquid to be pipetted to a clean dry beaker or conical flask.	✘ Pipette directly from the stock bottle of the liquid being pipetted.
✓ Make sure the liquid is at ambient temperature.	✘ Pipette liquids that are at temperatures significantly above or below the calibration temperature of the pipette.
✓ Rinse the pipette with distilled/deionised water followed by the liquid you want to dispense.	
✓ Dispose of the rinse solution immediately.	
✓ Check the length of time it takes the liquid to drain from the pipette.	✘ Use a pipette with a delivery time that differs significantly from the expected time marked on the pipette.
✓ Carry out an accuracy check if required (see section A3.1.5).	
✓ Check that the pipette does not drip when filled.	
✓ Make sure that the tip of the pipette stays below the surface of liquid when filling.	✘ Raise the tip of the pipette above the level of the liquid when filling.
✓ Fill the pipette to just above the calibration line then remove the pipette from the liquid before adjusting the level of liquid so that the bottom of the meniscus is in line with the calibration mark, making sure that the pipette is vertical and that your eyes are level with the calibration line (see Figure A3.10).	✘ View the calibration line from above or below when judging the position of the meniscus (see Figure A3.10).
✓ Check for air bubbles after filling.	
✓ Wipe the outside of the pipette after filling and before dispensing the liquid.	✘ Forget to wipe the outside of the pipette before dispensing the liquid.
✓ Dispense the liquid with the pipette tip touching the inside of the receiving vessel at an angle.	
✓ Let the liquid drain from the pipette under gravity (unless stated otherwise).	✘ the small amount of liquid left in the tip of the pipette after emptying (unless the pipette is marked blow-out).
✓ Clean and dry the pipette and store in a clean dust free environment at ambient temperature and away from corrosive substances, making sure that it cannot roll.	✘ Leave pipettes dirty and incorrectly stored.

Table A3.8 summarises the key points to remember when using an auto pipette, however you should also consult the manufacturer's instructions on the correct operation of particular items.

Table A3.8: Dos and don'ts for using an auto pipette

Do	Don't
✓ Select a pipette with the correct capacity (consult the manufacturer's instructions).	
✓ Check the pipette is clean and undamaged.	✘ Use a pipette that is dirty or damaged.
✓ Check the pipette is within its calibration date.	✘ Use a pipette that is out of calibration.
✓ Carry out an accuracy check if required (see section A3.1.5).	
✓ Select the correct tip for the type of auto pipette and volume required.	
✓ Check that the correct volume has been set when using a variable volume auto pipette.	
✓ Hold the pipette vertically and immerse the tip to the required depth in the liquid to be pipetted.	✘ Hold the pipette at an angle while filling the tip.
✓ Pre-wet the tip by aspirating the required volume.	✘ Use a pipette tip that has not been pre-wetted.
✓ Remove excess liquid from the outside of the tip before aspirating the liquid.	✘ Leave droplets of the liquid on the outside of the tip.
✓ Aspirate the liquid at a steady rate.	✘ Operate the piston mechanism too rapidly.
✓ Dispense the liquid with the pipette tip touching the inside of the receiving vessel at an angle.	
✓ Pipette further portions of the same liquid with the same tip.	✘ Reuse a tip if any droplets of the previous portion of liquid remain in the pipette.
✓ Re-wet the tip if a different volume is selected (but make sure that the new volume is within the capacity of the tip).	
✓ Change tip if you need to pipette a different liquid.	✘ Reuse a tip if a different liquid is to be pipetted.
✓ Keep the pipette upright if the tip is filled with liquid.	✘ Place a filled pipette on its side or invert it as liquid may enter the mechanism, causing damage and contamination.
✓ Clean the pipette and store upright in a pipette stand or carousel.	✘ Leave pipettes dirty and incorrectly stored.

Table A3.9 summarises the key points to remember when using a volumetric flask.

Table A3.9: Dos and don'ts for using a volumetric flask

Do	Don't
✓ Select a flask of the required capacity.	✘ Use a flask of the incorrect capacity (take care if flasks of similar volume are available, e.g. 200 mL and 250 mL; ideally these should not be stored together).
✓ Check the flask is clean and undamaged.	✘ Use a flask that is dirty or damaged.
✓ Check that the calibration mark and other markings are not faded.	✘ Use a flask with a faded calibration mark.
✓ Carry out an accuracy check if required (see section A3.1.5).	
✓ Make sure the solution is at ambient temperature before making up to volume.	✘ Prepare solutions at temperatures that are significantly above or below the calibration temperature of the flask.
✓ Ensure that all solids have dissolved before making a solution up to volume.	
✓ Line up the bottom of the meniscus with the calibration line, making sure that the flask is vertical and that your eyes are level with the calibration mark (see Figure A3.10).	✘ View the calibration line from above or below when judging the position of the meniscus (see Figure A3.10).
✓ Avoid holding the bulb end of the flask to minimise any warming of the solution.	✘ Hold the flask by the bulb end as this may cause the solution to warm.
✓ Ensure you use the correct type of stopper for the flask.	
✓ Ensure that stoppers are 'matched' to particular flasks if a stoppered flask is to be weighed.	
✓ Rinse the flask with water and dry it after use.	
✓ Store flasks in a clean dust free environment at ambient temperature and away from corrosive substances.	

A3.1.7 Questions

Question 1

What item of glassware would you use to prepare a solution of known concentration?

Question 2

What does the marking 'blow-out' on pipettes indicate?

Question 3

What is the main distinction between Class A and Class B glassware?

Question 4

How should you read the meniscus of the liquid being measured?

Question 4

List four things you should check before using a glass bulb pipette.

A3.2 Measuring mass

When working in the laboratory you will often be required to weigh accurately a specified amount of material. This could be a portion of the sample required for analysis or a chemical required to prepare a solution of known concentration (see section A3.4). This section outlines the key points you need to know to make accurate measurements of mass:

- The different types of balance available for making mass measurements (section A3.2.2);
- Which type of balance to use (section A3.2.3);
- How to check the accuracy of the balance (section A3.2.4);
- How to use the balance correctly (section A3.2.5).

A3.2.1 Mass versus weight

Both **'mass'** and **'weight'** are used interchangeably in general conversation, however they have very different meanings.

Mass is the amount of material in an object and does not change with the environment in which the object is located. **Weight** is a force arising from the interaction of the mass with the earth's gravitational field, which varies with location.

Balances are used to determine the **mass** of an object on the basis of its **weight**; the downward force the object exerts on the balance pan.

A3.2.2 Types of balance available

There are three main types of balance used in a laboratory and they vary in their weighing capabilities, maximum capacity and readabilities.

A3.2.2.1 Micro balance

This balance typically has a readability of 0.001 mg (i.e. 0.000 001 g, also written as $1x10^{-6}$ g) and is normally used for weighing quantities of less than 0.1 g. The typical maximum capacity of a balance like this is 10 g. Micro balances are enclosed in a see-through casing with doors so dust does not collect and air currents in the room do not affect the delicate balance (see Figure A3.11).

Figure A3.11: Micro balance

A3.2.2.2 Analytical balance

An analytical balance (shown in Figure A3.12) has a readability of between 0.01 mg and 1 mg (five to three decimal places); it is normally used for weighing quantities of 0.1 to 100 g. Analytical balances are available with different maximum capacities, for example, 150 g and 500 g. Analytical balances, like micro balances, are inside a see-through enclosure with doors so dust does not collect and air currents in the room do not affect the balance.

Figure A3.12: 4-figure analytical balance

A3.2.2.3 Top-pan balance

This balance is capable of weighing quantities in excess of 1 kg with a typical readability of 0.01 to 1 g (depending on the actual balance used), and is often used for applications such as preparing large quantities of reagents and weighing bulk samples. Like the analytical balance, top-pan balances are available with different maximum capacities, normally of between 1 and 30 kg. A top-pan balance is not usually enclosed. It is normally open to the environment of the laboratory so the effect of draughts and air currents needs to be considered.

Figure A3.13: 2-figure top-pan balance

A3.2.3 Selecting a suitable balance

The choice of balance will depend on the quantity that you need to weigh and the accuracy required in the weighing. You will therefore need to consider both the readability and the capacity of the balances available in your laboratory. You should choose a balance suitable for the measurement you are making. A rule of thumb is to ensure that weighings are recorded with a minimum of four significant figures (see section A5.3.1 for information on significant figures) although more may be required for certain applications. For example, 0.012 34 g (a readability of five decimal places) and 12.34 g (two decimal places) is likely to be acceptable but values of 0.012 g (three decimal places) or 12 g may give insufficient information. The capacity of the balance is also important, for example, when weighing a mass of 2 kg it is better to use a balance with a maximum capacity of 5 kg than a balance with a capacity of 50 kg. It is important to note that a balance should not normally be used at >95% of its capacity. When selecting a balance remember to take into account the mass of the weighing vessel as well as the mass of material to be weighed.

Table A3.10 outlines some suggestions of balances that may be used for routine measurements of different masses. The suggestions in the table will give weighings with at least five significant figures which will be more that adequate for most routine laboratory operations.

Table A3.10: Balance selection for a particular weighing operation

Quantity to be weighed / g	Recommended balance	Readability
0.01	Micro (6-figure)	0.000 001 g
0.1	Analytical (5-figure)	0.000 01 g
1	Analytical (4-figure)	0.0001 g
10	Analytical (4-figure)	0.0001 g
100	Top-pan (2-figure)	0.01 g
1000	Top-pan (1-figure)	0.1 g
10 000	Top-pan (0-figure)	1 g

A3.2.4 Checking the balance accuracy and set up

A3.2.4.1 Balance location

You should pay attention to the manufacturer's recommendations regarding the environmental requirements for the location of the balance in the laboratory. You should also be aware of the important factors outlined in Table A3.11.

Table A3.11: Factors to consider when choosing the location for a balance

Factor	Advice
Draughts	The balance should be situated in a draught free location away from doors, windows, passers-by and other equipment. Air conditioning can also cause unwanted draughts.
Vibrations	For micro and analytical balances, a solid bench top of stone or slate at least 40 mm thick, free standing and isolated from any other work apart from weighing is preferred. All balances are subject to vibrations and this arrangement should help to minimise them. However, minimising the effect of vibrations is not so critical for top-pan balances which can be used on a laboratory bench top.
Level surface	The balance must be mounted on a level surface. The feet of the balance should be adjusted when the balance is set up, to ensure that it is level. Many balances have an in-built spirit level and this should be checked each time before the balance is used.
Cleanliness	The balance should be located in a clean area free from dust, water and chemical splashes, corrosive substances, organic vapours and aerosols.
Temperature	Temperature fluctuations can cause gradients in the balance mechanism, so the ambient temperature should be stable to within ±3 °C.
Humidity	Humidity is not a major concern, as it should be relatively stable in a laboratory environment, provided condensation does not appear on components of the balance. If it does, you should assess the laboratory environment immediately.
Magnetic fields	A magnetic field could cause permanent changes in the response of the balance and should be avoided. Therefore, do not locate the balance near equipment which may generate a strong magnetic field.
Electrical interference	Balances should remain on at all times. However, electronic balances are sometimes subject to electrical interference. If this becomes a problem a stable power supply (e.g. filter plug) should be used.

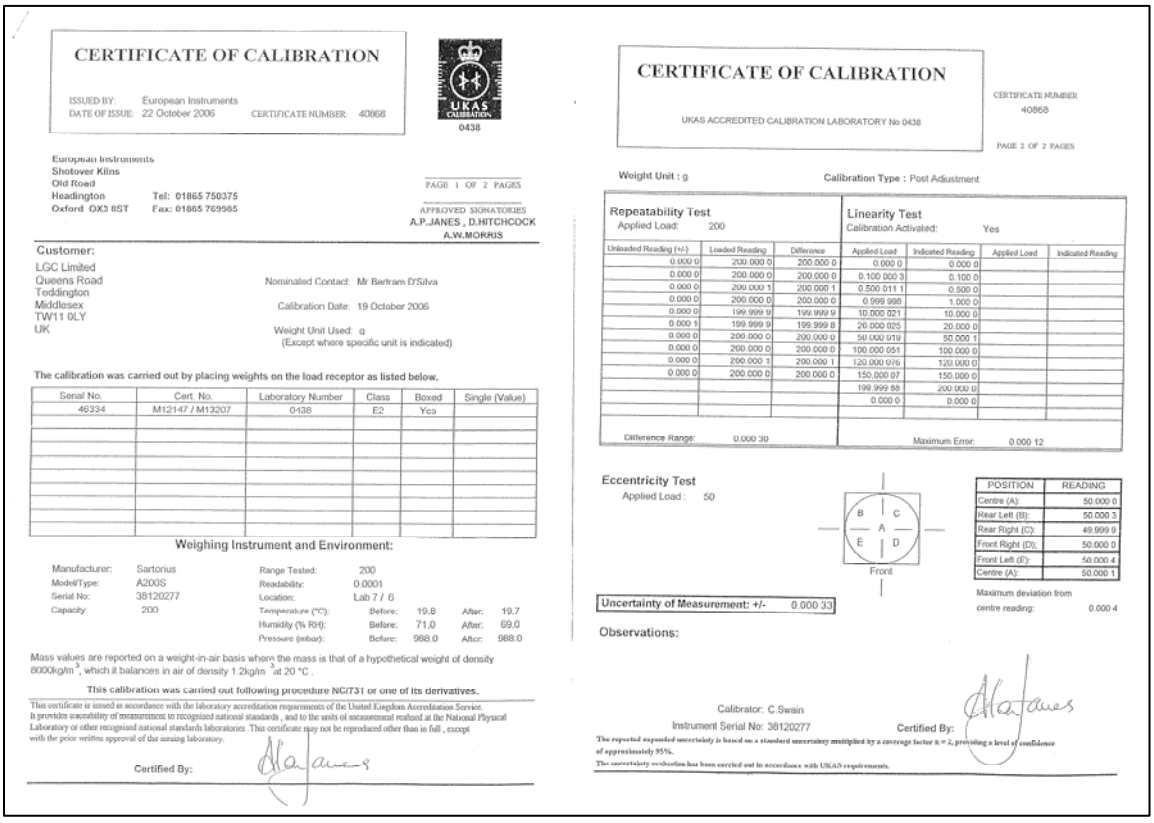
A3.2.4.2 Calibration and accuracy

Once you have selected a particular balance for a specific task, you should check that it has been correctly calibrated for the mass range required. This is easily done by reviewing the calibration certificate for the balance and checking that the interval since the last calibration is acceptable. The quality system for your laboratory should prescribe the interval between full calibrations. As a guide, 12 months is adequate for most purposes.

Full balance calibration will provide readings that are traceable to the national standard of mass (the kilogram) held in the UK by NPL (National Physical Laboratory). Through the national standard held at NPL the measurements are also traceable to the international standard kilogram, held at the International Bureau of Weights and Measures (BIPM) in Paris. Therefore, all measurements of mass carried out on a suitably calibrated balance in the UK will be comparable with readings obtained on properly calibrated balances in other laboratories in the UK and overseas (see section A4.4 and Figure A4.6 for further information on traceability and calibration).

The calibration process should be carried out by a body with suitable accreditation for such work (e.g. accredited by the United Kingdom Accreditation Service (UKAS)). The procedure should include examinations and tests of various aspects of balance performance, such as repeatability, linearity, zero and tare mechanisms and eccentric or off-centre loading. Adjustments will be made as appropriate, to ensure that the readings are within specification.

A record of the balance calibration should be kept and filed (see Figure A3.14 for an example of a calibration certificate).



CERTIFICATE OF CALIBRATION

ISSUED BY: European Instruments
DATE OF ISSUE: 22 October 2006 CERTIFICATE NUMBER: 40868

UKAS CALIBRATION 0438

European Instruments
Shotover Kilns
Old Road
Headington Tel: 01865 750375
Oxford OX3 8ST Fax: 01865 769965

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APPROVED SIGNATORIES
A.P.JANES , D.HITCHCOCK
A.W.MORRIS

Customer:
LGC Limited
Queens Road
Teddington
Middlesex
TW11 0LY
UK

Nominated Contact: Mr Bertram D'Silva

Calibration Date: 19 October 2006

Weight Unit Used: g
(Except where specific unit is indicated)

The calibration was carried out by placing weights on the load receptor as listed below.

Serial No.	Cert. No.	Laboratory Number	Class	Boxed	Single (Value)
46334	M12147 / M13207	0438	E2	Yes	

Weighing Instrument and Environment:

Manufacturer:	Sartorius	Range Tested:	200			
Model/Type:	A200S	Readability:	0.0001			
Serial No:	38120277	Location:	Lab 7 / 6			
Capacity:	200	Temperature (°C):	Before:	19.8	After:	19.7
		Humidity (% RH):	Before:	71.0	After:	69.0
		Pressure (mbar):	Before:	988.0	After:	988.0

Mass values are reported on a weight-in-air basis where the mass is that of a hypothetical weight of density 8000kg/m^3, which it balances in air of density 1.2kg/m^3 at 20 °C .

This calibration was carried out following procedure NG731 or one of its derivatives.

This certificate is issued in accordance with the laboratory accreditation requirements of the United Kingdom Accreditation Service. It provides traceability of measurement to recognised national standards , and to the units of measurement realised at the National Physical Laboratory or other recognised national standards laboratories .This certificate may not be reproduced other than in full , except with the prior written approval of the issuing laboratory.

Certified By:

CERTIFICATE OF CALIBRATION

UKAS ACCREDITED CALIBRATION LABORATORY No 0438

CERTIFICATE NUMBER 40868

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Weight Unit : g Calibration Type : Post Adjustment

Repeatability Test
Applied Load: 200

Unloaded Reading (+/-)	Loaded Reading	Difference
0.000 0	200.000 0	200.000 0
0.000 0	200.000 0	200.000 0
0.000 0	200.000 1	200.000 1
0.000 0	200.000 0	200.000 0
0.000 0	199.999 9	199.999 9
0.000 1	199.999 9	199.999 8
0.000 0	200.000 0	200.000 0
0.000 0	200.000 0	200.000 0
0.000 0	200.000 1	200.000 1
0.000 0	200.000 0	200.000 0

Difference Range: 0.000 30

Linearity Test
Calibration Activated: Yes

Applied Load	Indicated Reading	Applied Load	Indicated Reading
0.000 0	0.000 0		
0.100 000 3	0.100 0		
0.500 011 1	0.500 0		
0.999 998	1.000 0		
10.000 021	10.000 0		
20.000 025	20.000 0		
50.000 019	50.000 1		
100.000 051	100.000 0		
120.000 076	120.000 0		
150.000 07	150.000 0		
199.999 88	200.000 0		
0.000 0	0.000 0		

Maximum Error: 0.000 12

Eccentricity Test
Applied Load : 50

POSITION	READING
Centre (A):	50.000 0
Rear Left (B):	50.000 3
Rear Right (C):	49.999 9
Front Right (D):	50.000 0
Front Left (E):	50.000 4
Centre (A):	50.000 1

Maximum deviation from centre reading: 0.000 4

Uncertainty of Measurement: +/- 0.000 33

Observations:

Calibrator: C.Swain
Instrument Serial No: 38120277

Certified By:

The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor k = 2, providing a level of confidence of approximately 95%.
The uncertainty evaluation has been carried out in accordance with UKAS requirements.

Figure A3.14: Typical calibration certificate for an analytical balance

In between full calibrations, accuracy checks on all laboratory balances should take place either on a regular basis (daily, weekly, monthly) or before use, depending on your laboratory's requirements. Such checks need not be as extensive as a full calibration. Accuracy checks are most effectively done by placing (using forceps) calibrated weights of known value on the balance pan and comparing the measured mass to the true value. As long as the difference does not exceed a given critical value, the balance is believed to be performing correctly. The criteria for assessing balance accuracy are usually based on knowledge of what the balance should be able to achieve. You should record results of the accuracy checks in a suitable log book/file. Before you use a balance you should ensure that the required accuracy checks have been carried out and that the balance performance is acceptable. If the performance is not acceptable the balance should not be used and the problem should be reported to your laboratory manager/supervisor.

A3.2.5 Correct use of balances for different applications

When weighing out a substance you should always use a suitable container. The empty container should be clean, dry and free from dust. It also helps if the container is of a design that assists transfer of the weighed substance into the vessel or apparatus subsequently required for the analysis. Remember that the size and mass of the container, in relation to the amount of material that will be weighed out, also needs to be considered. For example, if you needed to weigh 1 g of material you should use a small weighing boat or weighing funnel rather than a beaker, which may weigh 50 to 100 g. Table A3.12 outlines some types of container that can be used for weighing substances.

Table A3.12: Containers and types of substance to be weighed

Substance	Type of container	Extra information
Solid substance	Weighing paper or boat*, beaker, flask or bottle, weighing funnel *	
Liquid	Weighing boat*, beaker, flask or bottle	
Volatile liquid	Stoppered weighing bottle or flask	Container must have a well-fitting stopper to minimise losses through evaporation.
Hygroscopic substance	Stoppered weighing bottle or flask	A stoppered container is used to minimise pick-up of moisture from the air, such substances may be dried in a desiccator prior to weighing.
Toxic substance	Closed container, e.g. weighing bottle or flask	Toxic substances must be weighed in a closed container. If possible this should be carried out in a fume hood/cupboard.

*As shown in Figure A3.15

The effects of static electricity can be a problem when weighing dry, finely divided materials. Static electricity can make powders difficult handle as it causes the particles to 'float' about. This, in turn, causes balance readings to drift and can introduce systematic errors. Antistatic weighing boats and antistatic 'guns' are available which can help to reduce the problem.

a) Weighing funnels b) Weighing boats

Figure A3.15: Illustration of different weighing containers

Once you have selected a suitable balance and weighing container, Table A3.13 outlines the operations required to weigh the substance correctly. It is important to note that when carrying out accurate weighings, the items being weighed should be handled as little as possible and gloves should always be worn. In addition, items should always be at ambient temperature when weighed.

There are two main approaches to weighing:

- Weighing by difference
  - o used if you need to transfer the substance into another vessel before continuing with the assay;
- Using the tare facility
  - o allows the substance to be weighed directly into the vessel used for the analytical procedure so no transfer is necessary.

Table A3.13: How to weigh a substance

Operation	Action
Setting up the balance	• Plug in the power supply and switch on at least 20 min before use to allow the balance to 'warm up' (it is, however, recommended that the balance is left on in the stand-by mode). • Check the balance is level and adjust if necessary. • Gently clean the balance pan with a brush to remove any dust or loose particulate matter.
Checking the accuracy of the balance	• Set the balance to read zero and check that zero is displayed. • Carry out an accuracy check (see section A3.2.4.2). • Check that the zero is displayed. • Ensure any calibrated weights used for the accuracy check are returned to their storage box.

Table continued

Table A3.13 continued

Operation	Action
Weighing by difference	• Place a suitable empty container on the centre of the balance pan. • Close the balance door and wait for a stable reading then record the mass (M_0). • Remove the container from the balance and transfer the substance to be weighed into the container. • Replace the container on the balance pan, close the balance door, wait for a stable reading then record the mass (M_1). • Remove the container from the balance pan. • If the substance is transferred quantitatively (i.e. by washing with water or a solvent), the mass of the substance is $M_1 - M_0$. • If the substance is transferred 'dry' to another vessel, once the transfer has been carried out, return the container to the balance pan, close the door, wait for the reading to stabilise, then record the mass (M_2). The mass of the substance transferred is $M_1 - M_2$.
Using the tare facility	• Place the empty container on the centre of the balance pan, close the door and press the tare button. • Wait for the reading to stabilise at zero. • Remove the container from the balance and transfer the substance to be weighed into the container. • Replace the container on the balance pan, close the door and wait for the reading to stabilise. • Record the reading. This is the mass of the substance in the container.
Tidying up after weighing	• Once you have finished the weighing process, gently clean the balance pan with a brush, collect any debris and discard. • Close the balance door and leave in stand-by mode. • Clean any debris from around the balance bench. • Report any problems encountered with the balance to your laboratory manager/supervisor.

A3.2.6 Checklist for making measurements of mass

Table A3.14 summarises the key points to remember when making measurements of mass.

Table A3.14: Dos and don'ts for measuring mass

Do	Don't
✓ Wear gloves where necessary.	
✓ Check the balance is switched on. If not, switch it on and leave to equilibrate for 20 min.	✘ Use a balance that has not had time to warm-up.
✓ Check the balance is level with the in-built spirit level or a separate level.	✘ Use a balance that is not level.
✓ Clean the balance pan and surrounding area before use.	✘ Use a balance that has dust or loose particulate matter on or around the balance pan.
✓ Check that the balance has been calibrated within a reasonable time period (e.g. the last 12 months).	✘ Use an uncalibrated balance.
✓ Check the zero reading.	
✓ Check the accuracy of the balance.	✘ Use a balance that has not had its accuracy checked.
✓ Use a suitable container to weigh out the material of interest.	✘ Use an unsuitable weighing container (e.g. a beaker when weighing small amounts of material, or an open container when weighing a volatile liquid, see Table A3.12 in section A3.2.4).
✓ Check items are at ambient temperature before they are weighed.	✘ Weigh items that are hotter or colder than ambient temperature.
✓ Weigh by difference if the material needs to be transferred into another vessel.	
✓ Use the tare facility if the sample can be weighed directly into the vessel used for further analytical work.	✘ Use the tare facility if weighing by difference.
✓ Close the doors (if working with a micro or analytical balance) and let the reading stabilise before recording it.	✘ Take readings without closing the balance doors and waiting for the balance to stabilise.
✓ Clean up the balance and surrounding area after use.	✘ Leave the balance dirty for the next user.
✓ Leave the balance in stand-by mode.	✘ Switch off the balance.

A3.2.7 Questions

Question 1

What is the difference between mass and weight?

Question 2

What type of vessel would you use to weigh a toxic sample?

Question 3

What type of vessel would you use to weigh a volatile sample?

Question 4

What type of balance would you use to weigh 0.005 g of material?

Question 5

What weighing technique would you use to accurately weigh 1.7 g of silver nitrate which is required to prepare a solution of known concentration?

Question 6

Read the passage below and identify the mistakes that Caroline makes when she is weighing a portion of sodium chloride to prepare 100 mL of a solution of known concentration.

Caroline has been asked to weigh out accurately 0.5 g sodium chloride to four decimal places to prepare a solution of known concentration. She decides to use a 4-figure analytical balance in the laboratory. Caroline finds a bottle of sodium chloride in the chemical cupboard. It has passed its expiry date by a couple of months but looks OK. The balance has been switched off at the mains so Caroline switches it on. She can't find the balance log book but knows that the balance is used fairly regularly so assumes that it will be OK. She opens the balance door and places a clean 100 mL beaker (which she has just taken from the drying oven) onto the centre of the balance pan. Caroline records the mass of the beaker. She then transfers some of the sodium chloride to the beaker and records the mass. Caroline removes the beaker from the balance pan and transfers as much of the material as she can from the beaker into a 100 mL volumetric flask. Caroline replaces the beaker on the centre of the balance pan and records the mass. She calculates the mass of sodium chloride transferred to the volumetric flask by subtracting the mass of the beaker after transfer of the sodium chloride from the mass of the beaker plus the sodium chloride. Caroline makes the solution in the volumetric flask up to the required volume with deionised later and stoppers the flask. She notices some of the crystals stuck to the outside of the flask and on the laboratory bench so she wipes them up before putting the flask on a shelf above the bench. She switches off the balance at the mains and gets on with the next task.

A3.3 Measuring pH

Measurements of pH are frequently made in the laboratory. Sometimes the measurement forms part of a test method (e.g. the pH of a reagent or a sample has to be adjusted to a particular value). In other cases, the pH of the sample itself may be required to determine whether it meets a particular specification. This section covers:

- Definition of pH (section A3.3.1);
- Equipment for measuring pH (section A3.3.2);
- Choosing a suitable electrode (section A3.3.3);
- Care of electrodes (section A3.3.4);
- Calibration of pH meters (section A3.3.5);
- Measuring the pH of test samples (section A3.3.6).

A3.3.1 What is pH?

The pH scale gives a measure of the acidity or alkalinity of aqueous solutions at a specified temperature (usually 20 °C or 25 °C). The pH scale is continuous, from pH=0 (very acidic) to pH=14 (very alkaline). Table A3.15 gives the approximate pH values for a range of materials.

Table A3.15: Typical pH values

pH	Acidity / alkalinity	Examples
0	Very acidic	1 mol L^{-1} hydrochloric acid
1		Battery acid
2		Stomach acid
3		Vinegar
4	Slightly acidic	Tomato juice
5		
6		Saliva
7	Neutral	Pure water
8		
9		Milk of magnesia
10	Slightly alkaline	
11		
12		Household ammonia
13		
14	Very alkaline	1 mol L^{-1} sodium hydroxide

The pH value of a solution is a measure of the activity of the hydrogen ion (a_{H^+}) in that solution:

$$\mathrm{pH} = \log_{10} \frac{1}{a_{H^+}} = -\log_{10} a_{H^+}$$

Note that the pH scale is a log scale so the hydrogen ion activity in a solution with pH=5 is ten times higher than it is in a solution with pH=6.

The activity, a_{H^+}, and concentration of hydrogen ions, c_{H^+}, are related via an activity coefficient, γ:

$$a_{H^+} = \gamma \times c_{H^+}$$

Obtaining a value for the activity coefficient is not straightforward so measurements of pH are not normally used to determine concentration values. You should consider pH measurements as a convenient way of making comparative measurements of acidity.

A3.3.2 Equipment for measuring pH

There are two main approaches, depending on the accuracy required:

- pH paper;
- pH meter.

pH paper

pH papers are strips of paper which are impregnated with compounds that will undergo specific colour changes at particular pH values (see section A3.6.3.1 for information on indicators). pH sticks are also available which have different indicator papers sealed along the length of the stick. pH papers/sticks provide a quick way of getting a semi-quantitative pH measurement for liquids. There are numerous different types of paper available, depending on the pH range of interest and the discrimination between pH values required. Some examples are shown in Table A3.16.

Table A3.16: Examples of pH papers/sticks

Product	pH range	Colour change
Litmus red paper	5 to 8	red to blue
Litmus blue paper	5 to 8	blue to red
Phenolphthalein paper	8.5 to 10	colourless to red
Indicator paper	2 to 12 in 2 unit steps	-
Indicator paper	0.5 to 5 in 0.5 unit steps	-
Indicator strip	0 to 14 in 1 unit steps	-
Indicator strip	1.7 to 3.8 in 0.3 unit steps	-

In all cases the pH is measured by dipping the paper/stick in the sample and comparing the colour of the paper with the reference chart supplied with the product. As there is an element of judgement involved, the readings can be somewhat subjective. Depending on the type of paper used it is possible to measure pH to an accuracy of between 0.3 and 1 pH units.

pH meter

When you require an accurate reading of pH you should use an electronic pH meter. The pH is usually determined by electrochemical measurements. The potential of a pH electrode immersed in the sample is measured with respect to a reference electrode (which is also in contact with the sample) using a pH meter. The pH electrode responds only to hydrogen ions present in the sample, even if there are other positive ions present. The response of the reference electrode is independent of the hydrogen ion concentration.

pH meters give a direct reading of pH either via a moving needle on a graduated scale or (more commonly) a digital display. Meters routinely used in the laboratory give readings to 0.1 or 0.01 pH units. Some of the controls you may find on a pH meter are shown in Table A3.17.

Table A3.17: pH meter controls

Control	Function
Set buffer	Adjusts the reading to display the pH of the standard buffer solution being measured to calibrate the pH meter (see section A3.3.5).
Temperature	pH measurements are temperature dependent so the temperature of the solution being measured must be defined (some meters have an in-built facility to measure the temperature and adjust the reading – this ensures that measurements are not affected by temperature fluctuations).
Slope	Adjusts the meter reading to the pH of a second buffer solution (after using the 'set buffer' function for the first buffer). The meter should not require a large adjustment – if it does this could indicate a fault with the electrode (see section A3.3.5).

The response of the pH electrode is calibrated using standard aqueous buffer solutions which have known reference pH values. The pH meter reading is adjusted to give the correct pH reading for the reference solution (see section A3.3.5 for information on calibration).

A pH meter may appear easy to use. However, reliable results will only be obtained if the equipment is set up, maintained and used correctly. The rest of this section discusses the correct use of electronic pH meters.

A3.3.3 Choosing a suitable electrode

To make a pH measurement two electrodes are required – the pH electrode (also known as the indicator electrode) and the reference electrode. Most pH measurements of aqueous solutions are made using a *combination electrode* in which both electrodes are contained in a single unit. In this type of electrode the reference electrode usually surrounds the glass pH electrode.

The choice of electrode will depend on the nature of the sample you are analysing. Manufacturers' websites will help you to select a suitable electrode for your application.

Glass electrode

This is the most common type of pH electrode. The end of the electrode which is immersed in the sample consists of a bulb shaped glass membrane. The bulb is filled with an acid solution such as 0.1 mol L^{-1} hydrochloric acid. A conducting wire such as silver wire coated with silver chloride is immersed in the liquid in the bulb. The other end of the wire forms a terminal at the other end of the electrode to the bulb. When the bulb comes into contact with an aqueous solution the potential developed is proportional to the hydrogen ion activity.

Reference electrode

The potential of the reference electrode must be independent of the hydrogen ion concentration of the solution being measured. Two main types of reference electrode are used:

- Silver/silver chloride (Ag/AgCl) electrode – a silver wire coated with silver chloride;
- Calomel electrode (Hg/Hg_2Cl_2) – mercury in contact with mercury (I) chloride.

The reference electrode is filled with a suitable electrolyte solution, usually potassium chloride (KCl), and fitted with a semi-porous plug so that an electrochemical contact is established with the sample solution (a liquid junction). This 'single junction' configuration is the most common configuration of a reference electrode although others are available for certain specialist applications. 'Double-junction' electrodes, containing a potassium chloride solution in an inner reservoir and a second electrolyte in an outer reservoir, are also available.

The single junction Ag/AgCl electrode is the most widely used reference electrode for routine measurements. However, this is not suitable for all types of measurement and the choice of reference electrode will depend on the sample being measured. For example, the calomel electrode is recommended for samples containing proteins, sulfides, heavy metal ions or tris buffer as these compounds can react with silver.

Aqueous samples

The factors that influence the choice of electrode when analysing aqueous solutions are summarised in Table A3.18.

Table A3.18: Choice of electrode for analysing aqueous solutions

Factor	Type of electrode
pH range	
• pH range 1 to 10	General purpose glass electrode and Ag/AgCl reference electrode.
• pH>10	General purpose glass electrodes give low readings in strongly alkaline solutions due to interference from sodium, lithium and (to a lesser extent) potassium ions. Referred to as the 'alkaline error'. Special glass may be required to minimise interferences.
• pH<1	General purpose glass electrodes give high readings for strongly acidic solutions. Referred to as the 'acid error'. Consult manufacturers' guidelines to identify suitable electrodes.
Ionic strength of solution	
• High ionic strength (>0.1 mol L^{-1})	May require modified reference electrode configuration.

Table continued

Table A3.18 continued

Factor	Type of electrode
• Very low ionic strength	May require a modified reference electrode configuration to avoid contamination of the sample by the electrolyte.
Sample composition	
• Sample contains components that react with silver (e.g. waters containing sulfur compounds or biological buffers)	Do not use Ag/AgCl chloride reference electrode. Calomel reference electrode recommended.
• Sample contains components that will react with potassium chloride	Do not use potassium chloride as the electrolyte.
Sample temperature	Calomel electrode cannot withstand temperatures >60 °C. Ag/AgCl electrode can be used at elevated temperatures.
Accuracy required for your application	Electrodes available capable of reading to between 0.01 and 0.1 pH units.

Non-aqueous samples

The pH scale is defined in terms of hydrogen activity in an aqueous solution. However, pH measurements can be made for non-aqueous samples to compare samples of similar composition. Special electrodes are often required for non-aqueous samples. Key requirements of electrodes for non-aqueous samples include:

- A low resistance glass membrane;
- A 'double junction' reference electrode rather than the porous-plug single junction electrode described previously;
- In some cases a reference electrode containing lithium chloride rather than potassium chloride is required (LiCl is soluble in many organic liquids whereas KCl has limited solubility).

Consult manufacturers' websites for information on specific applications.

Viscous samples/samples containing solids

Measuring the pH of very viscous samples or samples with high solids content (e.g. suspensions, slurries, sludges, emulsions) can cause problems. Special electrodes are often required and manufacturers' information should be consulted to identify suitable electrodes. Options for these types of sample include:

- A modified liquid junction in the reference electrode to prevent it becoming blocked/contaminated (e.g. double junction, sleeve or open junction electrode);
- Separate pH and reference electrodes (i.e. an electrode pair rather than a combined electrode);
- An electrode that can be easily cleaned.

A3.3.4 Care of electrodes

To obtain reliable pH measurements it is essential that the electrodes are properly stored and maintained. This section covers the main points to consider but you should also consult the manufacturer's instructions for information on storage and maintenance of particular electrodes. The key points are summarised in Table A3.19.

Table A3.19: Storage and cleaning of pH electrodes

Requirement	Action
Do not let the electrode membrane dry out.	Store in an appropriate storage solution: • pH 7 buffer solution for a glass pH electrode; • reference electrode filling solution (e.g. KCl) for a reference electrode; • mixture of reference electrode solution and a buffer to maintain a suitable pH for a combination electrode. Storage solutions are commercially available. Immerse the electrode(s) in sufficient storage solution to cover the glass membrane and the liquid junction. If the membrane has dried out, rehydrate by placing in the storage solution for at least 12 hours. Do not store electrodes in distilled water – this will dilute the reference electrode solution.
Check the level of the solution in the reference electrode.	From time to time the electrolyte solution in the reference electrode will require topping-up. When the electrode is not being used ensure the filling hole in the reference electrode is covered (but it should be uncovered when in use to ensure that the electrolyte flows properly through the liquid junction).
Regularly check electrode for build up of salt crystals and deposits on the membrane and liquid junction.	Always clean electrodes before use by rinsing with a gentle jet of distilled water and allowing to drain (electrodes can be blotted dry with lint-free paper but do not rub or wipe electrodes as this may cause damage). More rigorous cleaning procedure: • Soak electrode for 30 min in 0.1 mol L^{-1} hydrochloric acid or nitric acid. Rinse with distilled water. Drain and refill reference electrode. Immerse electrode in storage solution or pH 7 buffer for at least 1 hour, ensuring membrane and liquid junction are covered. If deposits have formed on the membrane special cleaning solutions may be required: • Protein deposits: soak in 1% pepsin in 0.1 mol L^{-1} hydrochloric acid for 15 min; • Inorganic deposits: soak in 0.1 mol L^{-1} tetrasodium EDTA solution for 15 min; • Grease and oil: rinse with mild detergent:methanol solution (1:10). After any of these three cleaning procedures follow the acid soaking procedure described above.
Long term storage of the electrode.	Empty the reference electrode, rinse with deionised water, fill with fresh electrolyte solution and ensure that the filling hole is securely covered. Cover the glass membrane and liquid junction with a cap containing a few drops of the electrode storage solution. Prior to use, remove the cap and immerse the electrode in storage solution for at least 12 hours.

A3.3.5 Calibration of pH meters

Calibration is required to match the reading given by the meter to the response of the electrode to solutions of a particular pH. This is achieved by measuring buffer solutions with known pH values.

There are a number of options when purchasing buffers for calibration of pH meters:

- Ready-prepared solutions with documented pH values at specified temperatures
  - each solution has a lot-specific pH value, usually quoted with a typical uncertainty of ±0.01 or ±0.02 pH units;
  - different pH solutions are available as different coloured solutions to aid identification;
- Tablets, sachets and concentrates that you make up in the laboratory to give a solution of the required pH
  - preparation instructions must be followed carefully;
- Primary pH standards
  - high purity salts used to prepare buffer solutions (e.g. a solution of potassium hydrogen phthalate with a concentration of 10.13 g L^{-1} has a pH of 4.00 at 20 °C).

Key points to remember when carrying out a calibration:

- Use at least two buffer solutions which bracket the pH of the test samples;
- Make sure that the buffer solutions are within their expiry date
  - for buffers prepared in-house the 'shelf life' should be established;
- Make sure that the buffer solutions have been stored correctly and are free from contamination, sediment or mould;
- Transfer the required amount of the buffer solution to a small, clean dry beaker
  - close the buffer solution bottle immediately after transferring the required amount of solution;
  - never return unused buffer to the buffer solution bottle;
  - never immerse the electrode in the buffer solution bottle;
- Ensure the buffer solution and samples are at ambient temperature before making measurements;
- If the pH meter does not have a temperature sensing probe, record the ambient temperature and set the temperature control on the meter to this value;
- Immerse the electrode in the first buffer solution (in a beaker)
  - make sure the electrode is held vertically and that the bulb and liquid junction are covered by the solution;
- Stir the buffer with a magnetic stirrer - do not stir solutions with the electrode
  - the optimum stirring rate will depend on the viscosity of the liquid being measured
  - choose a stirring rate that provides a homogeneous solution without forming a vortex (to avoid drawing CO_2 into the liquid from the atmosphere);
  - use the same stirring rate when calibrating the pH meter and for measuring test samples;
- When the meter reading has stabilised (to within about ±0.02 pH units) use the 'set-buffer' control to adjust the display to the pH of the buffer solution;

- Discard the buffer and refill the beaker with the same buffer solution
  - if the reading is not within ±0.02 of the reference value re-adjust the 'set buffer' control;
- Repeat the procedure until two successive readings agree to within ±0.02 pH units
  - if this is not achieved after the second or third reading you should investigate the problem;
- Transfer the second buffer to a clean dry beaker;
- Rinse the electrode with water followed by the second buffer solution and allow it to drain/blot dry with lint-free paper (do not rub or wipe electrode);
- Stir the buffer with a magnetic stirrer;
- Immerse the electrode in the buffer solution and record the reading once it has stabilised to with about ±0.02 pH units;
- The reading should not differ significantly from the reference value for the buffer but if necessary, adjust the reading using the 'slope' control;
- If a large adjustment is required (>±0.3 pH units) this could indicate that the 'slope' value of the electrode is significantly different from the expected theoretical value
  - some meters will give a warning if the slope falls outside a specified range;
  - a poor slope value can indicate a problem with the electrode – investigate the cause before using the electrode to measure test samples.

Note that some pH meters have an *auto-calibration* function which means that the meter will select the correct pH value for the buffer from a pre-programmed list. This function will also take into account temperature effects. You should always follow the manufacturer's instructions when using an auto-calibration function.

A3.3.6 Measuring the pH of the test sample

The procedure for measuring the pH of a test sample is as follows:

- Ensure that the pH meter has been calibrated;
- Ensure that the test sample is at ambient temperature (this should be the same as the temperature of the buffer solutions during the calibration procedure);
- Transfer a portion of the sample to a clean dry beaker;
- Rinse the electrode with distilled water followed by the test solution and allow it to drain/blot dry with lint-free paper (do not rub or wipe electrode);
- Immerse the electrode in the test solution following the procedure for the first buffer solution described in section A3.3.5
  - stir the test solution at the same speed as used when stirring the buffer solutions during calibration of the pH meter;
- Discard the test solution and repeat the measurement on a second portion of the sample. Depending on the nature of the sample and the type of electrode, duplicate readings should agree to within between ±0.02 and ±0.1 pH units;
- Before measuring other test samples always rinse the electrode with distilled water followed by some of the sample and allow the electrode to drain/blot dry with lint-free paper;
- If you are measuring a large number of samples re-measure at least one of the buffer solutions at regular intervals (e.g. every ten samples);
- When you have finished your measurements discard all used buffer solutions, rinse the electrode with distilled water and store as described in section A3.3.4.

A3.3.7 Checklist for making pH measurements using a pH meter

Table A3.20 summarises the key points to remember when making pH measurements using a pH meter.

Table A3.20: Dos and don'ts for making pH measurements

Do	Don't
✓ Select a suitable electrode for your sample types (see section A3.3.3).	
✓ Ensure that the electrode is clean and undamaged (see section A3.3.4).	✘ Use an electrode that has dried out without rehydrating it.
✓ Check the level of the electrolyte in the reference electrode.	✘ Use an electrode containing insufficient electrolyte.
✓ Rinse the electrode with distilled water and the test solution before use and allow to drain.	
✓ Dry electrodes by blotting with lint-free paper.	✘ Dry electrodes by wiping or rubbing.
✓ Calibrate the pH meter with at least two buffer solutions with a pH range that brackets the pH of the samples.	✘ Use a pH meter without calibrating it.
✓ Check that buffer solutions are within their expiry date, have been stored correctly and are free from mould or sediment.	✘ Use buffer solutions that have passed their expiry date or appear to be contaminated.
✓ Discard any unused buffer solutions.	✘ Return unused buffer solutions to the stock bottle.
✓ Transfer the solution to be measured to a clean dry beaker.	✘ Place the electrode directly in the buffer or sample container.
✓ Ensure that solutions to be measured are at ambient temperature prior to measurement.	
✓ Record the ambient temperature and set the temperature control (if the pH meter does not have automatic temperature compensation).	
✓ Make sure the filling hole in the reference electrode is uncovered.	✘ Use the electrode with the filling hole covered.
✓ Immerse the electrode to a sufficient depth in the solution being measured – the glass membrane and liquid junction should be covered.	
✓ Support the electrode vertically in the solution.	✘ Hold or support the electrode at an angle.

Table continued

Table A3.20 continued

Do	Don't
✓ Stir the solution at the appropriate speed with a magnetic stirrer while measuring the pH.	✘ Stir the solution with the electrode. ✘ Stir the solution too vigorously (i.e. avoid the formation of a vortex).
✓ Make sure reading has stabilised before recording the pH.	
✓ Rinse the electrode with distilled water after use.	
✓ Store electrodes in an appropriate solution (see section A3.3.4).	✘ Store electrodes in distilled water. ✘ Allow glass electrodes to dry out.

A3.3.8 Questions

Question 1

What type of reference electrode would you use when measuring the pH of water samples containing compounds of sulfur?

Question 2

Identify four things you should check before using a pH electrode and meter.

A3.4 Preparing solutions of known concentration

Solutions of known concentration are used widely in the laboratory. In some cases it may be possible to purchase a suitable solution which has the required concentration quoted with the required degree of certainty. However, many test methods require the preparation of solutions of a specified concentration. These solutions are often required for calibration purposes (see section A3.4.1) so preparing them correctly is a critical stage in the method. It is therefore important for you to be able to prepare accurately solutions of specified concentrations. This section covers:

- Uses of solutions of known concentration (section A3.4.1);
- Calculating the concentration of solutions (section A3.4.2);
- Selecting a suitable material to prepare a solution (section A3.4.3);
- Practical steps in preparing solutions of known concentration (section A3.4.4);
- Labelling and storage of solutions (section A3.4.5).

To be able to prepare solutions correctly you need to be able to make accurate measurements of mass and volume - make sure you have reviewed the relevant sections in Chapter A3.

A3.4.1 When are solutions of known concentration used?

Solutions of known concentration have a number of uses:

- In titrations (see section A3.6);
- To calibrate an instrument such as a UV spectrophotometer or a gas chromatograph (calibration is required to relate the response of the instrument to the amount of the analyte present, see section A4.4);
- To evaluate the performance of test methods both during method validation and in on-going quality control when the method is in routine use (see Chapter A4);
- To train staff and evaluate their performance.

In all of the above examples it is important that the concentration of the solution is known with a high degree of certainty. In the first two examples the stated concentration of the solution is used in the determination of the concentration of the analyte in test samples. If the actual concentration of the solution differs from the stated value (because the solution has been prepared incorrectly, for example) then the results for test samples will be in error. In the other examples, the stated concentration of the solution is being used to make a judgement about the performance of the method and/or the analyst. If the actual concentration differs from the stated value then you may conclude, incorrectly, that there is a problem with the method or the way in which the analyst has applied the method.

Different terms are used to describe solutions of known concentration, depending on the context in which they are being used. Some of the common terms are listed in Table A3.21.

Table A3.21: Terms used to describe solutions of known concentration

Terminology	Usage
Analytical standard	General term used to describe a solution of known concentration
Standard solution	As above
Calibration standard/solution	A solution used to calibrate an instrument
Stock standard/solution	A solution from which calibration standards are prepared
Working standard	Calibration standard prepared from a stock solution
Quality control standard/solution	Solution used to check the performance of a test method or analytical instrument

The terminology can be confusing so make sure you are clear about the meaning of any terms used in the standard operating procedures/method protocols in your laboratory.

A3.4.2 Calculating the concentration of solutions

To be able to prepare solutions of known concentration you need to be able to:

- Calculate the concentration of solutions from known masses and volumes;
- Work out the amount of material and volume of solvent required to prepare a solution of a specified concentration.

One of the key concepts in calculating the concentrations of solutions is the concept of *the mole*.

A3.4.2.1 Amount of substance: The mole

The internationally agreed (SI) unit for measurements of mass is the kilogram. In chemistry, the SI unit for the amount of a substance is the mole (the symbol used to represent the mole is 'mol'). 1 mol of any substance will contain the same number of particles (e.g. atoms or molecules). The number of particles is the *Avogadro constant* which is equal to 6.022×10^{23} and is the number of atoms in 12 g of carbon 12 (^{12}C). So, 1 mol of carbon will contain the same number of atoms as 1 mol of sodium which will be the same as the number of molecules in 1 mol of water. 1 mol of a material is therefore the amount of the material which contains the same number of particles as 12 g of carbon 12.

Converting from moles to mass

In the laboratory we measure out portions of solid chemicals by weighing. We therefore need to be able to convert from moles to units of mass. If you know that you need 0.5 mol of a chemical to prepare a solution, how do you determine how much material to weigh out?

We already know that 1 mol of carbon 12 weighs 12 g. The masses of all other elements are calculated relative to the mass of carbon. These masses are known as *relative atomic masses* and can readily be found in textbooks. The relative atomic mass of sodium, for example, is 23 so sodium atoms are approximately twice as heavy as carbon atoms.

The mass of 1 mol of a material is known as the *molar mass*. The molar mass of carbon is therefore 12 g mol^{-1} and the molar mass of sodium is 23 g mol^{-1}. To calculate the molar mass of a compound simply add the molar masses of the elements present. For example, the molar mass of sodium hydroxide (NaOH) is 40 g mol^{-1} (Na = 23 g mol^{-1}, O = 16 g mol^{-1}, H = 1 g mol^{-1} (using molar masses rounded to the nearest integer)). The molar mass of water is 18 g mol^{-1}.

- To convert from mol to g *multiply* by the molar mass
- To convert from g to mol *divide* by the molar mass

Examples

How many moles of potassium hydrogen phthalate molecules are contained in 5.10 g?

1) The formula for potassium hydrogen phthalate is $KCO_2C_6H_4CO_2H$. The relevant molar masses (rounded to two decimal places) are shown below.
   K = 39.10 g mol^{-1} C = 12.01 g mol^{-1} O = 16.00 g mol^{-1} H = 1.01 g mol^{-1}

   The molar mass is therefore:

   39.10 + (8x12.01) + (4x16.00) + (5x1.01) = 204.23 g mol^{-1}

   If 1 mol of $KCO_2C_6H_4CO_2H$ weighs 204.23 g then 5.10 g will contain:

   $$\frac{5.10}{204.23} = 0.025 \text{ mol}$$

2) It has been determined via a titration experiment that a solution contains 0.053 mol of sodium chloride (NaCl). How much does 0.053 mol of sodium chloride weigh?

   The molar masses of sodium ions and chloride ions are 22.99 g mol^{-1} and 35.45 g mol^{-1}, respectively (rounded to two decimal places). The molar mass of sodium chloride is therefore 22.99 + 35.45 = 58.44 g mol^{-1}.

   0.053 mol of sodium chloride would therefore weigh:

   0.053 x 58.44 = 3.10 g

A3.4.2.2 Concentrations expressed in mol L^{-1}

A 1 molar solution contains 1 mol of a substance in 1 L of solution. Note that it is 1 L of solution *not* 1 L of solvent. A 0.5 molar solution contains 0.5 mol of the substance in 1 L of solution.

1 L of a 1 molar solution of sodium chloride therefore contains 1 mol of sodium chloride. The molar mass of sodium chloride is 58.44 g mol^{-1}. To prepare 1 L of a 1 molar sodium chloride solution you would need to weigh out 58.44 g of sodium chloride, dissolve it in water and make the volume up to 1 L (see Figure A3.16 for the correct procedure). But what if you don't want 1 L of solution?

To work out how much material you need to weigh out to prepare V mL of a solution with a concentration of C mol L^{-1} use the equation:

$$m = \frac{C}{1000} \times V \times M$$

where m is the amount of material required (g), C is the required concentration (mol L^{-1}), V is the volume of solution required (mL) and M is the molar mass (g mol^{-1}) of the substance. (Note: the factor of 1000 is required to convert the concentration, C, from mol L^{-1} to mol mL^{-1}.)

To calculate the concentration of a solution in mol L^{-1} if you know the mass of the material and the volume of the solution:

$$C = \frac{m}{M \times V} \times 1000$$

(Note: the factor of 1000 is required to obtain the concentration, C, in units of mol L^{-1} rather than mol mL^{-1})

Example

How much material would you need to weigh out to prepare 500 mL of a 0.5 mol L^{-1} solution of potassium nitrate (KNO_3)?

M is 101.1032 g mol^{-1} (the relevant molar masses are K = 39.0983 g mol^{-1}, N = 14.00674 g mol^{-1}, O = 15.9994 g mol^{-1})

C is 0.5 mol L^{-1}

V is 500 mL

$$m = \frac{0.5}{1000} \times 500 \times 101.1032 = 25.2758 \text{ g potassium nitrate}$$

Sometimes you will need to know how many moles of a compound are present in a particular volume of a solution of known concentration. For example, when carrying out a titration (see section A3.6) you will need to be able to work out how many moles have been consumed in the reaction.

To calculate the number of moles (x) in V mL of a solution with a concentration of C mol L^{-1}:

$$x = \frac{C}{1000} \times V$$

Example

A titration experiment is carried out to determine the exact concentration of a solution of sodium hydroxide. In the experiment the sodium hydroxide is titrated with 25 mL of 0.1 mol L^{-1} potassium hydrogen phthalate. How many moles of potassium hydrogen phthalate are present in 25 mL?

C = 0.1 mol L^{-1} and V = 25 mL, so:

$$x = \frac{0.1}{1000} \times 25 = 0.0025 \text{ mol of potassium hydrogen phathalate}$$

A3.4.2.3 Concentrations expressed in units of mass/volume (m/v)

The concentrations of solutions are also frequently expressed in units of mass/volume such as g L^{-1} (grams per litre) or mg L^{-1} (milligrams per litre). The process of working out how much material is required to prepare a particular volume of a solution with a specified concentration is similar to that used for solutions expressed in mol L^{-1}.

To prepare V mL of a solution with a concentration of C g L^{-1} the amount of material required (m, in grams) is:

$$m = \frac{C}{1000} \times V$$

(Note that if C is expressed in mg L^{-1} then m will expressed in mg.)

Example

How much sodium hydroxide (NaOH) would you need to weigh out to prepare 500 mL of a solution with a concentration of 4 g L^{-1}?

$$m = \frac{4}{1000} \times 500 = 2 \text{ g}$$

It is also straightforward to convert between concentrations in mol L^{-1} and g L^{-1}:

- To convert from mol L^{-1} to g L^{-1} *multiply* by the molar mass
- To convert from g L^{-1} to mol L^{-1} *divide* by the molar mass

So, given that the molar mass of sodium hydroxide is 40.00 g mol^{-1}, a solution with a concentration of 4 g L^{-1} is the same as a solution with a concentration of 4/40 = 0.1 mol L^{-1}.

In some test methods, the concentrations of solutions are expressed in terms of one particular component of a compound (e.g. a particular ion). For example, in a method for the determination of nitrite in water samples, the concentrations of the calibration standards are expressed in terms of mg nitrite per L. To prepare these standards the analyst needs to identify a suitable source of nitrite ions (e.g. sodium nitrite) and dissolve an appropriate amount of the material in the required volume of water. You can calculate how much nitrite is required using the equation given above. For example, to prepare 1 L of a solution with a nitrite concentration of 1000 mg L^{-1} you would need 1000 mg (i.e. 1 g) of nitrite. If you are using sodium nitrite to provide the nitrite ions you need to work out how much to weigh out.

The formula of sodium nitrite is $NaNO_2$ so its molar mass is 68.9953 g mol^{-1}. The molar mass of nitrite ions (NO_2^-) is 46.0055 g mol^{-1}. If you weighed out 1 g of sodium nitrite you would only have 46.0055/68.9953 = 0.6668 g nitrite. To obtain 1 g of nitrite you therefore need to weigh out 68.9953/46.0055 = 1.4997 g sodium nitrite.

The general calculation for determining the mass of the compound required ($m_{compound}$) can be written:

$$m_{compound} = \frac{M_{compound}}{n \times M_{ion}} \times m_{ion}$$

where $M_{compound}$ and M_{ion} are the molar masses of the compound and the ion, respectively, n is the number of the ion of interest present in the formula of the compound and m_{ion} is the mass of the ion required. (In the case of nitrite ions obtained from $NaNO_2$, n=1.)

A3.4.2.4 Obtaining the required concentration by dilution

The practical steps in preparing a solution of known concentration by dilution are shown in Figure A3.17. Given a starting solution (sometimes called the stock solution) of known concentration, how do you decide how much of that solution to take and what volume to dilute it to? To do this you must calculate the 'dilution factor'. The dilution factor is the ratio of the concentration of the stock solution to the concentration of the diluted solution.

If a solution with a concentration of 100 mg L^{-1} is prepared from a stock solution with a concentration of 1000 mg L^{-1} the dilution factor is 1000/100 = 10. If the diluted solution has a concentration of 10 mg L^{-1} the dilution factor is 100.

Once you know the dilution factor you must select a volume of the stock solution and a final volume of the diluted solution which will give you the required dilution factor. For example, to get from a concentration of 1000 mg L^{-1} to 100 mg L^{-1} you could take 10 mL of the stock solution and dilute it to 100 mL, following the procedure shown in Figure A3.17. Alternatively, if you only required a smaller volume of the diluted solution, say 25 mL, you would need to dilute 2.5 mL of the stock solution.

If you decide on the volume of the diluted solution that you need, the equation for calculating the volume of the stock solution (V_{stock}) that you need to dilute is:

$$V_{stock} = \frac{C_{dil}}{C_{stock}} \times V_{dil}$$

where V_{dil} is the final volume of the diluted solution, C_{stock} is the concentration of the stock solution and C_{dil} is the required concentration of the diluted solution.

Sometimes it is possible to achieve the required concentration of the diluted solution in a single step. For example, a dilution factor of ten, as in the example above won't cause any problems. But what if you needed to prepare 100 mL of a solution with a concentration of 0.5 mg L^{-1} from a commercially supplied standard solution with a concentration of 1000 mg L^{-1}? This would require you to transfer 0.5 mL of the stock solution into a 100 mL volumetric flask. Table A3.2 illustrates that as the volume being measured decreases the uncertainty in the volume delivered increases. For example, the tolerance for a Class A 1 mL pipette is 0.008 mL (0.8%) compared to a tolerance of 0.02 mL for a Class A 10 mL pipette (0.2%). It can also be more difficult for the analyst to measure small volumes accurately which increases the uncertainty further. It may

therefore be necessary to carry out more than one dilution to reach the desired concentration. However, each dilution step will introduce uncertainties. You will therefore need to devise a plan which involves the minimum number of dilutions while achieving an acceptable level of uncertainty in the concentration of the diluted solution. In some cases, when the dilution factor is large, a smaller uncertainty will be achieved if one or two intermediate dilutions are carried out compared to a single dilution involving the transfer of a small volume of the stock solution.

A3.4.3 Selecting a suitable material to prepare the solution

If you consult a chemical supplier's catalogue to source a particular compound you will see that there are often a number of different 'grades' of material. The grade relates to the purity of the material and how well it has been characterised by the supplier. When preparing a solution of known concentration it is important to select a material with a suitable purity. The uncertainty associated with the purity of the material will contribute to the uncertainty in the concentration of the solution. If the solution is being used for calibration purposes, the uncertainty in its concentration will contribute to the uncertainty in the final result obtained for test samples. It is therefore good practice to use material with the highest purity available (i.e. as close to 100% as possible). Generally, the higher the purity the more expensive a material will be.

For example, if you wanted to obtain some sodium nitrite to prepare calibration standards for the determination of nitrite in water, the following options are available:

Grade	Stated purity	Information
Specified laboratory reagent/general purpose reagent	>97%	Information on a limited number of possible impurities. Information not stated for individual lots of material.
Analytical reagent	>99%	Information on a wider range of possible impurities. Analytical information for individual lots of material quoted for some impurities.

The analytical reagent would be the best choice as it has a higher purity and has been characterised more thoroughly. If you require a higher level of certainty than can be achieved using an analytical reagent it may be necessary to use a certified reference material (CRM). A CRM is a material that has been characterised very rigorously for a particular property (e.g. its purity). It is accompanied by a certificate which states the value for the property of interest plus an estimate of the uncertainty associated with the value. When calculating the concentration of a solution it will be necessary to take account of the purity of the material used as this will contribute to the uncertainty in the concentration of the solution.

A3.4.4 Practical steps in preparing solutions of known concentration

Section A3.4.2 explained how to calculate the masses and volumes required to prepare a solution of known concentration. This section describes the practical procedures for preparing a solution. Solutions of known concentration can be prepared in a number of different ways depending on the nature of the analyte and/or the concentration required:

- Weighing out a solid material of known purity, dissolving it in a suitable solvent and diluting to the required volume;
- Weighing out a liquid of known purity, dissolving it in a suitable solvent and diluting to the required volume;
- Diluting a solution previously prepared in the laboratory;

- Diluting a solution from a chemical supplier.

Remember to record all masses and volumes used in the preparation of solutions in a laboratory workbook, and to show how you calculated the concentration of the solution.

The procedure for preparing a solution by dissolving a solid material is shown in Figure A3.16. The procedure for preparing a solution by dilution of a more concentrated solution (either prepared in the laboratory or from a chemical supplier) is shown in Figure A3.17.

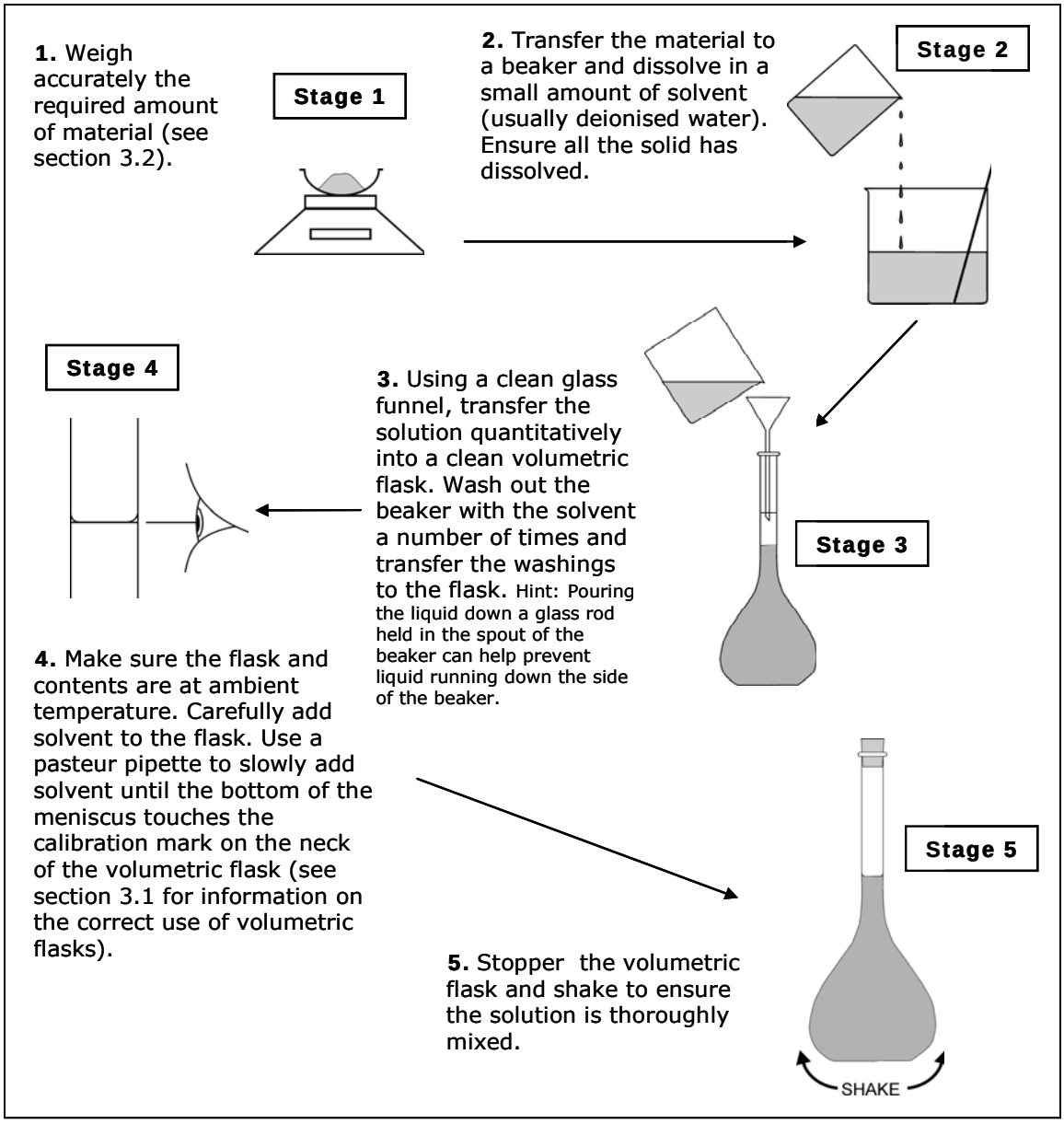


Figure A3.16: Procedure for preparing a solution of known concentration from a known amount of a solid material

Note that in some cases the solute may be a liquid rather than solid. The procedure is very similar to that shown in Figure A3.16. The required amount of the liquid is weighed accurately (see section A3.2.5). The liquid is then transferred directly to the volumetric flask containing some of the solvent.

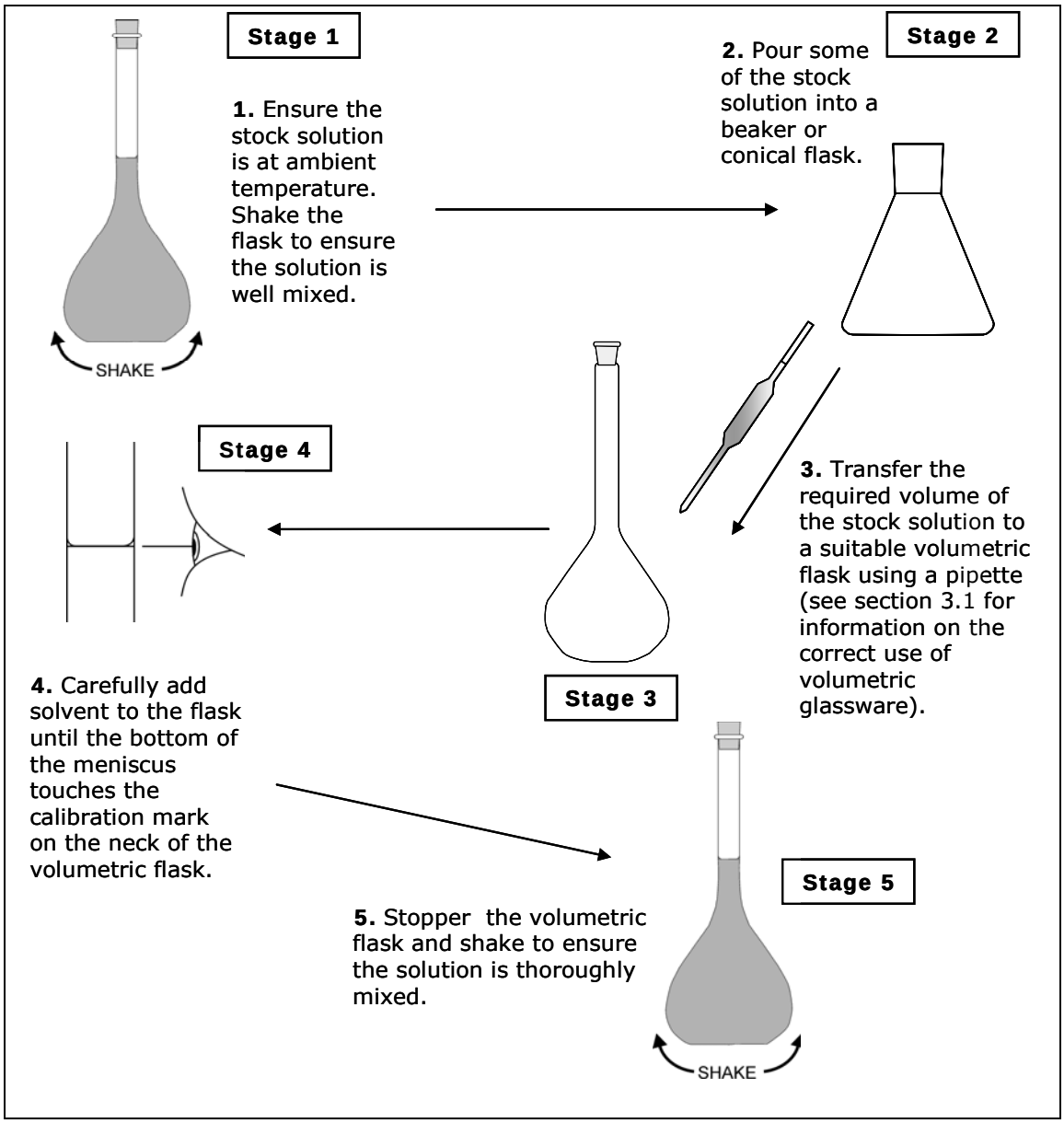


Figure A3.17: Procedure for preparing a solution of known concentration by dilution

A3.4.5 Labelling and storage of solutions

Once you have prepared the solution you need to think about how you will store it and how it will be identified in the future. Remember the following key points:

- Solutions should not be stored in volumetric flasks – transfer them to a suitable container for storage;
- Ensure that solutions are stored correctly. Some solutions will need to be stored in a refrigerator while others may be light-sensitive and need to be stored in amber bottles;
- All solutions should be clearly labelled with the following information:
  - the name and concentration of the solution;
  - date of preparation;

- name of analyst;
- review or expiry date;
- hazard information (if appropriate);

- The label must be securely attached to the container and be written in water insoluble ink.

In some cases, particularly where volatile solvents are used, it is useful to check for any changes in the mass of the solution during storage. After the solution has been prepared, it is transferred to a suitable container and the mass of the sealed container and the solution is recorded. Prior to an aliquot of the solution being used, the container is re-weighed. The mass should not be significantly different from that recorded prior to the solution being stored. After the required volume of the solution has been transferred from the storage container, the solution is reweighed before being returned to storage. If a significant change in mass is observed after the solution has been stored then it should not be used.

A3.4.6 Checklist for preparing solutions of known concentration

Table A3.22 summarises the key points to remember when preparing solutions of known concentration.

Table A3.22: Dos and don'ts for preparing solutions of known concentration

Do	Don't
Preparing a solution by dissolving a solid material	
✓ Use a material with a suitable purity (grade) and ensure that it has been stored correctly and is within its expiry date.	✘ Use material that appears to have been stored incorrectly or that has passed its expiry date.
✓ Use a clean, dry spatula to transfer the required amount of material.	✘ Return unused material to the original bottle.
✓ Make sure you have correctly calculated the amount of material required.	
✓ Weigh accurately the required amount of material (see section A3.2.5 for information on making accurate measurements of mass).	
✓ Ensure that all glassware used is clean, dust free and undamaged.	✘ Use dirty glassware or glassware that is damaged and/or has faded graduation marks.
✓ Transfer the material to a beaker and dissolve in a small amount of solvent.	✘ Transfer the material directly to the volumetric flask.
✓ Make sure that material has dissolved completely.	
✓ Use a small glass funnel to transfer the solution from the beaker to the volumetric flask.	

Table continued

Table A3.22 continued

Do	Don't
✓ Rinse the beaker with solvent and transfer the rinsings to the volumetric flask.	
✓ Make sure that the solution is at ambient temperature before making the solution up to the calibration mark with solvent (see section A3.1.6 for information on the correct use of volumetric flasks).	✘ Make the solution up to the calibration mark if its temperature is significantly different from the ambient temperature. (Note that if the ambient temperature is significantly different from the calibration temperature of the glassware used this will increase the uncertainty in the concentration of the solution.)
✓ Make sure the solution is mixed thoroughly before use.	
Preparing solutions by dilution of a stock solution	
✓ Make sure the stock solution is at room temperature.	✘ Use a stock solution straight from the refrigerator.
✓ Plan a dilution scheme to minimise the uncertainty in the concentration of the diluted solution.	✘ Carry out large dilutions in a single step.
✓ Ensure that all glassware used is clean, dust free and undamaged.	✘ Use dirty glassware or glassware that is damaged and/or has faded graduation marks.
✓ Ensure the stock solution is well mixed before use.	
✓ Transfer the stock solution to a beaker or conical flask for pipetting (see section A3.1.6 for information on the correct use of pipettes).	✘ Pipette directly from the stock solution bottle/flask. ✘ Return unused solution to the stock bottle.
✓ Make sure the diluted solution is well mixed before use.	
All solutions	
✓ Transfer the solution to a suitable container for storage.	✘ Store solutions in volumetric flasks.
✓ Clearly label containers with: the name and concentration of the solution; date of preparation; name of analyst; review or expiry data; hazard labels (if appropriate).	
✓ Store solutions correctly (e.g. in a refrigerator if necessary).	

A3.4.7 Questions

Question 1

In each case calculate the number of moles of the specified chemical in the amount of material stated:

i) Number of moles of $KCO_2C_6H_4CO_2H$ in 1 g of potassium hydrogen phthalate.

ii) Number of moles of Na_2CO_3 in 50 g of sodium carbonate.

iii) Number of moles of sodium hydroxide (NaOH) in 50 mL of a 0.5 mol L^{-1} solution.

iv) Number of moles of sodium carbonate (Na_2CO_3) in 50 mL of a standard solution prepared by dissolving 2.6500 g of sodium carbonate in water and making up to a volume of 500 mL in a volumetric flask.

Question 2

Express the concentrations of the following solutions in mol L^{-1}:

i) 2.000 g of sodium hydroxide (NaOH) dissolved in water and made up to 500 mL in a volumetric flask.

ii) 2.2822 g of potassium hydrogen phthalate ($KCO_2C_6H_4CO_2H$) dissolved in water and made up to 100 mL in a volumetric flask.

Question 3

In each case, calculate the amount of chemical required to prepare the specified volume of the solution:

i) 100 mL of a 2 mol L^{-1} sodium chloride solution.

ii) 500 mL of a 0.1 mol L^{-1} solution of potassium dihydrogen phosphate(V) (KH_2PO_4).

iii) 100 mL of a 0.1 mol L^{-1} solution of silver nitrate ($AgNO_3$).

iv) 500 mL of a solution with a sodium chloride concentration of 1000 mg L^{-1}.

v) 500 mL of a solution containing 1000 mg L^{-1} of sodium ions, prepared using sodium chloride.

vi) 500 mL of a solution containing 1000 mg of sodium ions, prepared using sodium carbonate (Na_2CO_3)

Question 4

Suggest how to prepare the following solutions:

i) 100 mL of a 0.1 mol L^{-1} solution of hydrochloric acid from a stock solution with a concentration of 1 mol L^{-1}.

ii) 500 mL of a 0.1 mol L^{-1} solution of nitric acid from a stock solution with a concentration of 0.5 mol L^{-1}.

iii) 100 mL of a 1 mg L^{-1} solution of calcium ions from a stock solution with a calcium ion concentration of 1000 mg L^{-1}.

iv) A set of calibration solutions with nickel concentrations of 1, 5, 10 and 15 mg L^{-1}, each with a volume of 100 mL, prepared using a stock solution with a concentration of 100 mg L^{-1}.

A3.5 Preparing reagent solutions

Section A3.4 explained how to prepare solutions when it is important that the concentration of the solution is known with a high degree of certainty (e.g. when a solution is used for calibration). There are often other solutions which are used when carrying out a test method that aren't involved directly in the determination of the concentration of the analyte in test samples. These solutions are known as *reagent solutions*. This section covers:

- Calculating the concentration of reagent solutions (section A3.5.1);
- Preparing reagent solutions (section A3.5.2);
- Storage and labelling of reagent solutions (section A3.5.3).

A3.5.1 Calculating the concentration of reagent solutions

There are many different types of reagent solution used in the laboratory. These include aqueous solutions of various compounds (e.g. 10% w/v sodium hydroxide solution), mixtures of liquids (e.g. a 75/25 v/v mixture of acetonitrile and water used as the mobile phase in high performance liquid chromatography) and indicator solutions used in titrations.

In some cases it will be possible to buy a suitable solution from a chemical supplier but frequently you will have to prepare reagent solutions yourself. Depending on the solution, this may involve weighing out a suitable mass of material and dissolving it in an appropriate volume of solvent, or mixing appropriate volumes of two or more liquids. Since reagent solutions are not involved with the calibration of test methods, their concentrations do not generally need to be known with the same degree of certainty as standard solutions. In other words, a larger uncertainty in the concentration of reagent solutions is usually acceptable compared to standard solutions used for calibration.

A3.5.1.1 Solution concentrations expressed as %w/v or %v/v

'% w/v' is shorthand for 'percent weight by volume'. It is the number of grams of solute in 100 mL of the solution. Note that the term '%m/v', which is shorthand for 'percent mass by volume', is also used. To prepare 1 L of a reagent solution with a concentration of 10% w/v you would therefore need to dissolve 100 g of material in 1 L of solvent.

To calculate how much material you need to weigh out (in grams), use the equation:

$$w = \frac{C}{100} \times V$$

where C is the concentration expressed as %w/v and V is the volume of solution required.

'%/v/v' is the expression used when a known volume of liquid solute is made up to a specified volume. It is therefore the number of mL of the solute in 100 mL of solution.

Some method protocols may describe the composition of a mixture of liquids as follows:

'acetonitrile and water mixture, 75/25 v/v'

This simply specifies the relative volumes of the two liquids that are required. In this case 75 mL of acetonitrile plus 25 mL of water (or 750 mL acetonitrile plus 250 mL

water) would be required. The concentrations of such solutions are approximate so it is acceptable to measure the volumes of the liquids using a measuring cylinder.

A3.5.1.2 Solution concentrations expressed as mol L^{-1}

The calculations required to prepare solutions when the concentration is expressed in mol L^{-1} are discussed in detail in section A3.4.2.

A3.5.1.3 Preparing reagent solutions by dilution

As with standard solutions you may need to prepare a reagent solution by diluting an existing solution. The principles are the same as described in section A3.4.2. However, if an approximate concentration is acceptable, measuring cylinders can be used rather than a pipette and a volumetric flask.

For example, if you needed to prepare 500 mL of approximately 0.1 mol L^{-1} sodium hydroxide from a 1 mol L^{-1} stock solution you would need to measure out:

$\frac{0.1}{1} \times 500 = 50$ mL of 1 mol L^{-1} stock solution.

To prepare the solution you could measure out 50 mL of the 1 mol L^{-1} solution using a measuring cylinder and add 450 mL of water to give a total volume of 500 mL. Remember that this approach is much less accurate than the procedure describe in Figure A3.17 and should never be used for the preparation of standard solutions.

A3.5.2 Preparing reagent solutions

The basic procedures for preparing reagent solutions are the same as those outlined in Figure A3.16 and Figure A3.17. The difference is that if a larger uncertainty in the concentration is tolerable, the solution can be prepared using equipment that has a lower level of accuracy. For example, a top pan balance rather than an analytical balance could be used and volumes could be measured using measuring cylinders rather than pipettes and volumetric flasks.

It may also be acceptable to use materials of a 'lower' grade than those used for preparing standard solutions, for example a general purpose reagent rather than an analytical reagent. However, you should check that any impurities present in lower grade materials won't interfere with the analysis you are undertaking.

It is important to be clear about the level of accuracy required. Often this can be determined from the method protocol. Statements such as, 'prepare approximately 10% w/v sodium hydroxide solution', 'prepare a water/acetonitrile mixture 50/50 v/v' indicate that a high level of accuracy is not required.

A3.5.3 Labelling and storage of reagent solutions

- All reagents should be stored in a suitable container and under suitable environmental conditions (e.g. stored in a refrigerator if temperature sensitive or protected from light if light-sensitive);
- All reagent containers must be clearly labelled with the following:
  - the name and concentration of the solution;
  - date of preparation;
  - name of analyst;
  - review or expiry date;
  - hazard information (if appropriate);

- The label must be securely attached to the container and be written in water insoluble ink.

A3.5.4 Checklist for preparing reagent solutions

Table A3.23 summarises the key points to remember when preparing reagent solutions.

Table A3.23: Dos and don'ts for preparing reagent solutions

Do	Don't
✓ Use a material with a suitable purity (grade) and ensure that it has been stored correctly and is within its expiry date.	✘ Use material that appears to have been stored incorrectly or that has passed its expiry date.
✓ Use a clean, dry spatula to transfer the required amount of material.	✘ Return unused material to the original bottle.
✓ Make sure you have correctly calculated the amount of material required.	
✓ Weigh the amount of material required using a balance with a suitable accuracy (a top pan balance is acceptable for preparing solutions of approximate concentration).	
✓ Ensure that all glassware used is clean, dust free and undamaged.	✘ Use dirty glassware or glassware that is damaged and/or has faded graduation marks.
✓ Choose glassware that will measure volumes with the required level of accuracy (measuring cylinders are acceptable for preparing solutions of approximate concentration).	
✓ Make sure the solution is mixed thoroughly before use.	
✓ Transfer the reagent solution to a suitable container for storage.	✘ Store solutions in volumetric flasks.
✓ Clearly label containers with: the name and concentration of the solution; date of preparation; name of analyst; review or expiry data; hazard labels (if appropriate).	
✓ Store solutions correctly (e.g. in a refrigerator if necessary).	

A3.5.5 Question

How would you prepare the following reagent solutions (only approximate concentrations are required)?

i) 200 mL of a 5% w/v aqueous solution of sodium chloride.

ii) 50 mL of a 1% w/v solution of phenolphthalein.

iii) 500 mL of a 2 mol L^{-1} potassium nitrate solution.

iv) 100 mL of a 0.1 mol L^{-1} solution of sodium hydroxide prepared from a stock solution with a concentration of 2 mol L^{-1}.

v) 1 L of a solution containing acetonitrile/water 5/95 v/v.

A3.6 Carrying out a titration

Titration experiments (also known as volumetric analysis) are used to make measurements of concentration and have many applications in the laboratory. When carried out correctly, a titration can yield results with a very small uncertainty. This section covers:

- Principles of titration (A3.6.1);
- Carrying out a standardisation experiment (section A3.6.2);
- Detecting the end-point of a titration (section A3.6.3);
- Carrying out a titration (section A3.6.4);
- Titration calculations (section A3.6.5).

To carry out a titration correctly you will need to know how to make accurate measurements of volume and mass, and how to prepare standard solutions – make sure you have reviewed the relevant sections in Chapter A3.

A3.6.1 Principles of titration

The concentration of a solution can be determined by measuring the volume that will react with a suitable solution of known concentration (see section A3.4 for information on preparing standard solutions). The process of adding one solution to another in a controlled manner and determining the ratio in which the two solutions react is called a *titration*. The point at which the reaction reaches completion is called the *end-point*.

There are different types of titration, depending on the chemical reactions involved:

- Acid-base titration: Involves a neutralisation reaction, e.g. the determination of the concentration of an ethanoic acid solution by titration with a solution of sodium hydroxide;
- Redox titration: A redox reaction involves the transfer of electrons. Loss of electrons is called oxidation, whilst the gain of electrons is known as reduction. In a redox reaction one compound must lose electrons (be oxidised) while another compound gains electrons (is reduced), e.g. determining the concentration of iron ions (Fe^{2+}) in a solution of iron (II) sulfate by titration with a solution of potassium manganate (VII);
- Complexometric titration: A complex is a compound in which molecules or ions (called ligands) form bonds with a metal ion. A complexometric titration is based on the formation of complexes between the analyte and the standard solution. Commonly used for the determination of the concentration of metal ions in solution, e.g. measuring the hardness of water by determining the concentration of calcium ions;
- Precipitation titration: The reaction involves the formation of a precipitate, e.g. silver ions (Ag^+) react with chloride ions (Cl^-) to form silver chloride (AgCl); a white precipitate.

The main focus of this section is acid-base titrations. For detailed information on other types of titration you should consult specialist texts.

To carry out a successful titration you will need to make accurate measurements of volume (see section A3.1) and mass (see section A3.2). A known volume of one of the solutions is transferred to a conical flask using a pipette while the other is added from a burette. The solution in the burette is known as the *titrant*. The volume of the titrant required to reach the end-point is known as the *titre*. The correct procedure for carrying out a titration is described in section A3.6.4.

A3.6.2 Carrying out a standardisation experiment

The accuracy of the determination of the concentration of the sample will depend, in part, on the accuracy with which the concentration of the other solution is known. To obtain accurate results it is therefore important that the concentration of this solution is known with a high degree of certainty. It is possible to purchase solutions specifically for volumetric analysis whose concentrations are accurately known. These are generally described in chemical suppliers' catalogues as 'volumetric solutions'. You can use other, non-volumetric, solutions but you should establish the concentration experimentally before you undertake the analysis of your samples (you should not rely on the concentrations quoted on reagent bottles). This process is known as *standardising the solution*. Standardisation is carried out by titrating the solution with a *primary standard*. The material used as the primary standard should have the following properties:

- a high level of purity and the purity must be accurately known;
- preferably be a solid and be soluble in the solvent used in the standardisation experiment;
- undergo a stoichiometric and rapid reaction with the compound in the solution being standardised, to ensure that the reaction is complete and the concentration of the solution can be accurately determined;
- be stable, e.g. does not react with carbon dioxide in the air, or gain/lose water.

Some common primary standards used for different types of titration are shown in Table A3.24. For example, sodium hydroxide used in acid-base titrations is frequently standardised using potassium hydrogen phthalate, and silver nitrate (used in precipitation titrations for the determination of the concentration of chloride ions) can be standardised using sodium chloride.

Table A3.24: Examples of primary standards

Material	Formula	Relative molecular mass	Type of titration
Anhydrous sodium carbonate	Na_2CO_3	105.9884	acid-base
Potassium hydrogen phthalate	$KCO_2C_6H_4CO_2H$	204.2212	acid-base
Sulfamic acid	NH_2SO_3H	97.0948	acid-base
Potassium dichromate	$K_2Cr_2O_7$	294.1846	Redox
Potassium iodate	KIO_3	214.0010	Redox
Sodium oxalate	$Na_2C_2O_4$	133.9985	Redox
Silver nitrate	$AgNO_3$	169.8731	Precipitation
Sodium chloride	NaCl	58.4425	Precipitation

The standardisation is carried out by following the titration procedure described in Table A3.26. The primary standard can be titrated either by weighing accurately a suitable amount of material into the conical flask (see section A3.2 for advice on making accurate measurements of mass) and dissolving it in deionised water, or by preparing a solution of known concentration (see section A3.4). The procedure for preparing a solution of the primary standard is described in Figure A3.16.

A3.6.3 Detecting the end-point

For all types of titration the analyst needs to be able to identify accurately and reliably when the reaction between the analyte solution and the solution of known concentration

is complete. The approach used will depend on the type of titration carried out. For many types of titration (particularly acid-base titrations) an 'indicator solution', which changes colour as the end-point is reached, is used. You need to choose an indicator solution that will show a clear, sharp colour change when the end-point has been reached (see section A3.6.3.1). In an acid-base titration the end-point can also be identified by measuring the pH using a pH meter (see section A3.3 for information on measurement of pH). This is a common approach when using automated titration systems. As shown in Figure A3.18, the pH changes rapidly at the end-point.

Some titrations do not need an indicator as one of the solutions changes colour at the end-point. For example, a solution of potassium manganate (VII) has an intense purple colour but turns colourless at the end-point of a redox titration to determine the concentration of iron (II) ions.

A3.6.3.1 Indicators for acid base titrations

The colour of a solution of certain compounds depends on pH. During an acid-base titration the pH changes sharply at the end-point, i.e. there is a sudden pH change on adding only a single drop of solution from the burette. The pH at which neutralisation occurs depends on the strength of the acid and the base. Remember that the *strength* of an acid or base is not the same as its *concentration*. 'Strength' relates to the degree of ionisation (dissociation) of an acid or base in solution. An acid is a compound that liberates (donates) protons (H^+ ions) in solution. The strength of an acid relates to how readily the protons are released from the compound. Hydrochloric acid is described a 'strong acid' as it is highly ionised in solution (i.e. exists in solution almost entirely as H^+ and Cl^- ions). In contrast, ethanoic acid is described as a 'weak acid' as it is only partially ionised in solution. A base is a proton 'acceptor'. A strong base, such as sodium hydroxide, is a compound which is highly ionised in solution. 'Concentration' relates to the amount of a substance present in a solution. Due to the different strengths of acids (and bases), solutions of different acids (or bases) with the same concentration may well have a different pH (see section A3.3).

Figure A3.18 shows the pH at the end-point of some different acid-base titrations.

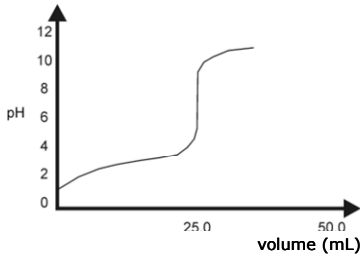
You will need to select an indicator that gives a clear colour change in the pH range shown in the vertical portion of the appropriate graph in Figure A3.18. Some common indicators, and the pH range over which they change colour, are shown in Table A3.25.

Table A3.25: Common indicators for acid-base titrations

Indicator	Colour change	pH range over which indicator changes colour
Methyl orange	red → yellow	3.2 → 4.2
Bromothymol blue	yellow → blue	6.0 → 7.0
Phenolphthalein	colourless → pink	8.3 → 10.0

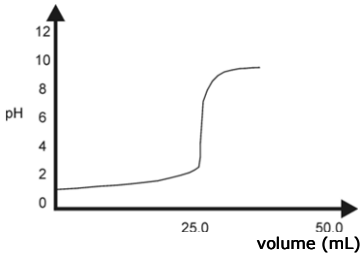
Note that indicators are often weak acids and will interfere with the neutralisation reaction. You should only add two or three drops of the indicator to the solution in the conical flask.

Graph 1:: Titration of a strong acid (hydrochloric acid) against a strong base (sodium hydroxide)



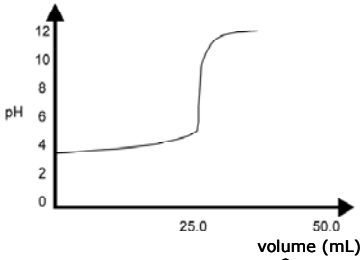
volume of 0.1 mol dm^{-3} sodium hydroxide added to 25 mL of 0.1 mol dm^{-3} hydrochloric acid

Graph 2: Titration of a strong acid (hydrochloric acid) against a weak base (ammonia)



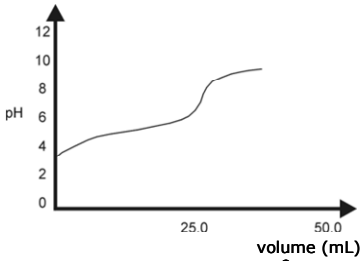
volume of 0.1 mol dm^{-3} ammonia added to 25 mL of 0.1 mol dm^{-3} hydrochloric acid

Graph 3: Titration of a weak acid (ethanoic acid) against a strong base (sodium hydroxide)



volume of 0.1 mol dm^{-3} sodium hydroxide added to 25 mL of 0.1 mol dm^{-3} ethanoic acid

Graph 4: Titration of a weak acid (ethanoic acid) against a weak base (ammonia)



volume of 0.1 mol dm^{-3} ammonia added to 25 mL of 0.1 mol dm^{-3} ethanoic acid

Figure A3.18: pH curves for different acid-base titrations

A3.6.4 Carrying out a titration

The procedure for carrying out a titration is shown in Table A3.26. You will need to decide which solution (the sample solution or the solution of known concentration) is contained in the burette. Generally an accurately measured volume of the sample solution is placed in the conical flask and the solution of known concentration is held in the burette. However, some solutions should not be placed in the burette. For example, concentrated solutions of sodium hydroxide should not be used in burettes with ground glass taps as the solution may cause the tap to stick.

The solution of known concentration and the volume or mass of the sample analysed should be chosen so that a reasonable titre volume is achieved (i.e. a volume that can be measured accurately using standard burettes).

Table A3.26: Procedure for carrying out a titration

Operation	Comments
Choose a suitable burette and pipette for measuring the required volumes and a suitable conical flask to contain the solutions.	See A3.1.3 for advice on selecting suitable equipment.
Ensure that all the glassware is clean and free from dust.	See section A3.1.4 for advice on cleaning glassware.
Rinse the burette with the titrant.	See Table A3.6 for information on using a burette correctly.
Using a funnel, fill the burette with the titrant to about 20-20 mm above the zero graduation.	
Clamp the burette in a stand making sure that the burette is vertical.	
Remove the funnel then allow the liquid to flow through the tap until there are no bubbles in the jet.	
Ensure there are no bubbles in the burette. If there are bubbles they can usually be removed by gently tapping the burette and opening the tap to allow more liquid to flow out.	
Run the liquid out of the burette until the meniscus is lined up with a calibration mark.	
Rinse the pipette with the solution to be titrated.	See Table A3.7 for information on using a pipette correctly.
Use the pipette to transfer the solution to a conical flask.[Note]	
If necessary, add a few drops of a suitable indicator and swirl the flask to mix the contents.	See section A3.6.3.1 for information on indicators.
Record the initial burette reading (V_0).	See Table A3.6 for information on using a burette correctly.
Run the liquid from the burette into the conical flask while constantly swirling the flask (alternatively a magnetic stirrer can be used to ensure the solution is well mixed).	You will see the indicator change colour as the titrant touches the solution in the flask but the colour will disperse as the flask is swirled. As the end-point is approached the colour will disperse more slowly.
As you approach the end-point, add the titrant more slowly.	It is good practice to carry out a 'rough' titration first to identify the approximate volume of titrant required to reach the end-point.

Table continued

Table A3.26 continued

Operation	Comments
Use a wash bottle filled with deionised water to rinse the sides of the conical flask and the tip of the burette with a small amount of water.	
The end-point should be obtained by the addition of a single drop (approx. 0.05 mL) of the titrant.	
Record the final burette reading (V_1).	
Calculate the titre volume (V_1-V_0).	
Repeat the titration until you obtain at least two consecutive titres that agree to within 0.1 mL.	

Note: In some cases the material to be analysed is a solid rather than a solution. In such cases, weigh accurately a suitable amount of the sample and transfer into a conical flask (see section A3.2 for advice on making accurate measurements of mass). Dissolve the solid completely in a suitable volume of deionised water. Add a few drops of a suitable indicator (if required) then proceed as described in Table A3.26.

A3.6.5 Titration calculations

To calculate the concentration of your sample from the results of a titration experiment follow these steps:

1) Write a balanced equation describing the chemical reaction that has taken place during the titration.

2) From the equation determine the ratio in which the compounds in the sample and solution of known concentration react (e.g. 1 mol of sodium hydroxide reacts with 1 mol of hydrochloric acid; 2 mol of sodium hydroxide will react with 1 mol of sulfuric acid) (see section A3.4.2.1 for information on the concept of moles).

3) Calculate the number of moles of the compound contained in the volume of the solution of known concentration used in the titration.

4) Use the ratio determined in step 2 to calculate the number of moles present in the sample solution.

5) From the answer obtained in step 4 calculate the concentration of the sample solution in terms of mol per L.

6) If necessary, convert the answer obtained in step 5 to units of m/v (e.g. g L^{-1})

Example

An experiment has been carried out to determine the concentration of a solution of hydrochloric acid (HCl) by titration with a sodium hydroxide (NaOH) solution. The sodium hydroxide has been standardised by titration with potassium hydrogen phthalate (KHP). Table A3.27 summarises the results from the standardisation experiment.

Table A3.27: Results from an experiment to standardise a solution of sodium hydroxide with a concentration of approximately 0.1 mol L^{-1}

Parameter	Symbol	Value
Mass of KHP	m_{KHP}	5.1051 g
Molar mass of KHP	M_{KHP}	204.2212 g mol^{-1}
Volume of KHP solution prepared	V_{SOL}	250 mL
Concentration of KHP solution (see section A3.4.2.2)	C_{KHP}	$C_{KHP} = \frac{m_{KHP}}{M_{KHP} \times V_{SOL}} \times 1000$ $C_{KHP} = \frac{5.1051}{204.2212 \times 250} \times 1000 = 0.1000 \text{ mol L}^{-1}$
Volume of KHP solution used in the standardisation titration	V_{KHP}	25 mL
Titre volume for NaOH (mean of 3 results)	V_{NaOH}	25.15 mL

Following the steps outlined previously the concentration of the sodium hydroxide solution is calculated as follows.

1) The equation for the reaction of potassium hydrogen phthalate with sodium hydroxide is:

   $KCO_2C_6H_4CO_2H + NaOH \rightarrow KCO_2C_6H_4CO_2Na + H_2O$

2) The equation shows that sodium hydroxide and potassium hydrogen phthalate react in a ratio of 1:1.

3) The concentration of the potassium hydrogen phthalate solution is 0.1000 mol L^{-1} (see Table A3.27). 25 mL of this solution was used in the standardisation experiment. The number of moles used (x_{KHP}) (see section A3.4.2.2) is therefore:

$$x_{KHP} = \frac{C_{KHP}}{1000} \times V_{KHP} = \frac{0.1000}{1000} \times 25 = 0.0025 \text{ mol of KHP}$$

4) As the ratio determined in step 2 is 1:1 there are 0.0025 mol of NaOH (x_{NaOH}) in 25.15 mL of the NaOH solution.

5) The concentration of the sodium hydroxide solution (in mol L^{-1}) is therefore:

$$C_{NaOH} = \frac{x_{NaOH}}{V_{NaOH}} \times 1000 = \frac{0.0025}{25.15} \times 1000 = 0.0994 \text{ mol L}^{-1}$$

The data from the experiment to determine the concentration of the hydrochloric acid solution are given in Table A3.28.

Table A3.28: Data from an experiment to determine the concentration of a solution of hydrochloric acid

Parameter	Symbol	Value
Volume of HCl taken for analysis	V_{HCl}	25 mL
Titre volume for NaOH (mean of three results)	V'_{NaOH}	25.65 mL

Following the same procedure as before, the concentration of the hydrochloric acid solution is calculated as follows.

1) The equation for the reaction of hydrochloric acid with sodium hydroxide is:

   $HCl + NaOH \rightarrow NaCl + H_2O$

2) The equation shows that NaOH and HCl react in a ratio of 1:1.

3) The concentration of the sodium hydroxide solution was determined as 0.0994 mol L^{-1} in the standardisation experiment. 25.65 mL of the solution were used in the titration. The number of moles used (x'_{NaOH}) is therefore:

$$x'_{NaOH} = \frac{C_{NaOH}}{1000} \times V'_{NaOH} = \frac{0.0994}{1000} \times 25.65 = 0.00255 \text{ mol of NaOH}$$

4) As the ratio determined in step 2 is 1:1 there are 0.00255 mol of HCl (x_{HCl}) in 25 mL of the HCl solution.

5) The concentration of the hydrochloric acid solution (in mol L^{-1}) is therefore:

$$C_{HCl} = \frac{x_{HCl}}{V_{NHCl}} \times 1000 = \frac{0.00255}{25} \times 1000 = 0.102 \text{ mol L}^{-1}$$

A3.6.6 Checklist for carrying out a titration

Table A3.29 summarises the key points to remember when carrying out a titration.

Table A3.29: Dos and don'ts when carrying out a titration

Do	Don't
✓ Make sure that all glassware is clean, dust free and undamaged.	✘ Use dirty or damaged glassware, or glassware with faded graduation marks.
✓ Make sure you are familiar with best practice guidelines for using volumetric glassware (see section A3.1).	
✓ Standardise the solution of known concentration by titrating against a primary standard, unless you are using a 'volumetric solution' from a chemical supplier (see section A3.6.2).	✘ Rely on the concentration stated on the reagent bottle.
✓ Choose a suitable indicator which will give a clear sharp colour change at the end-point.	
✓ Add only a few drops of the indicator to the solution in the conical flask.	✘ Add too much indicator as this may interfere with the end-point.
✓ Constantly swirl the flask as the titrant is added or use a magnetic stirrer.	
✓ Carry out a rough titration to establish the approximate titre volume required to reach the end-point.	
✓ Add the titrant very slowly (drop wise) as the end-point is approached and wash the walls of the conical flask and the jet of the burette with a small volume of deionised water from a wash bottle.	✘ Add too much solution at the end-point and 'overshoot' it.
✓ Repeat the titration until two consecutive titre volumes agree to within 0.1 mL.	✘ Take the average of titre volumes that differ by more than 0.1 mL.

A3.6.7 Questions

Question 1

You have been asked to carry out a titration to determine the concentration of a solution of ethanoic acid by titration with a 0.1 mol L^{-1} solution of sodium hydroxide. Which of the indicators listed in Table A3.25 would be suitable for detecting the end-point? Explain your choice.

Question 2

A titration experiment has been carried out to determine the purity of a sample of sodium carbonate (Na_2CO_3). The experiment involved transferring an accurately measured amount of the sodium carbonate into a conical flask, dissolving it in deionised

water and titrating with 0.1 mol L^{-1} hydrochloric acid using bromocresol green as the indicator (the concentration of the hydrochloric acid solution has been determined via a standardisation experiment). The experiment was repeated three times. The results from the first experiment are given in Table A3.30.

Table A3.30: Results from a titration experiment to determine the purity of a sample of sodium carbonate

Parameter	Symbol	Value
Mass of sodium carbonate	m	0.1332 g
Initial burette reading	V_0	0.00 mL
Final burette reading	V_1	23.95 mL

i) Use the data in Table A3.30 to calculate the number of moles of Na_2CO_3 in 0.1332 g of the sample.

ii) Convert the number of moles of Na_2CO_3 calculated in i) into a mass (g). (Hint: The molar mass of sodium carbonate is 105.9884 g mol^{-1}.)

iii) Calculate the purity of the sample of sodium carbonate.

A3.7 Centrifugation

This section outlines the things that you will need to know when using a centrifuge:

- When to use a centrifuge (section A3.7.1);
- The difference between revolutions per minute (rpm) and *g* (acceleration due to gravity) (section A3.7.2);
- The different types of centrifuge available (section A3.7.3);
- How to use a centrifuge correctly (section A3.7.4).

A3.7.1 What is centrifugation and when is it used?

Centrifugation is a separation process which uses the action of centripetal (centre seeking) force to promote accelerated settling of particles in a solid/liquid mixture. Particles in a solution are separated according to their size and shape, the density and viscosity of the medium and rotor speed. Two particles of different masses will separate at different rates in response to gravity (inertia).

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample rather than specifying the rotational speed such as revolutions per minute (rpm). However, in chemical analysis rpm are frequently quoted. The acceleration is often quoted in multiples of *g*, the acceleration due to gravity. This distinction is important because two centrifuge rotors with different diameters running at the same rotational speed will subject samples to different accelerations. The higher the rotational speed, the higher the *g* force exerted on the solid phase and the faster the solids will accumulate at the bottom of the centrifuge tube. Acceleration can be calculated as the product of the radius of the rotor and the square of the angular velocity (see section A3.7.2). The speed and time of the run will depend on the type of centrifuge that is being used and the nature of the samples.

Centrifugation is an important mechanical means of separating the components of a mixture and is a widely applied technique for general use, for example to separate liquids from solids, drainage of liquids from solid particles and stratification of liquids according to density. It is utilised widely in both chemical and biological analysis. For example it is used in food science, during sample treatment, to separate insoluble components such as fats and oils (in meat products) from the liquid phase extraction solvent. A similar procedure is undertaken for separating insoluble wax materials from the liquid extracting phase used in the treatment of cosmetic products such as mascara. In biological analysis centrifugation is commonly used for extraction and clean up protocols for DNA analysis and cell culture.

A3.7.2 rpm versus *g*

As discussed in section A3.7.1, it is important to understand the difference between the speed of a centrifuge in revolutions per minute (rpm) and the acceleration or force applied in multiples of *g*. The following equations show their relationship.

$$RCF = 0.000\,011\,18 \times r \times \omega^2$$

$$\omega = \sqrt{\frac{RCF}{0.000\,011\,18 \times r}}$$

Where: RCF is the relative centrifugal force or number of *g*, i.e. acceleration
ω is the speed in revolutions per minute
r is the centrifugation rotor radius in cm

There are a number of websites where these calculations and more can be carried out at the press of a button, for example:

http://www.beckmancoulter.com/resourcecenter/labresources/centrifuges/rotorcalc.asp
http://www.msu.edu/~venkata1/gforce.htm

A3.7.3 Different types of centrifuge

There are many different types of centrifuge (some are shown in Figure A3.19), here they have been categorised by speed and capacity.

1. **Ultracentrifuge**
    a. Bench top
    b. Floor standing (see Figure A3.19 a))
2. **High-speed**
    a. Micro (see Figure A3.19 b))
    b. Bench top
    c. Floor standing
3. **Low-speed**
    a. Small
    b. Bench top (see Figure A3.19 c))
    c. Floor standing.

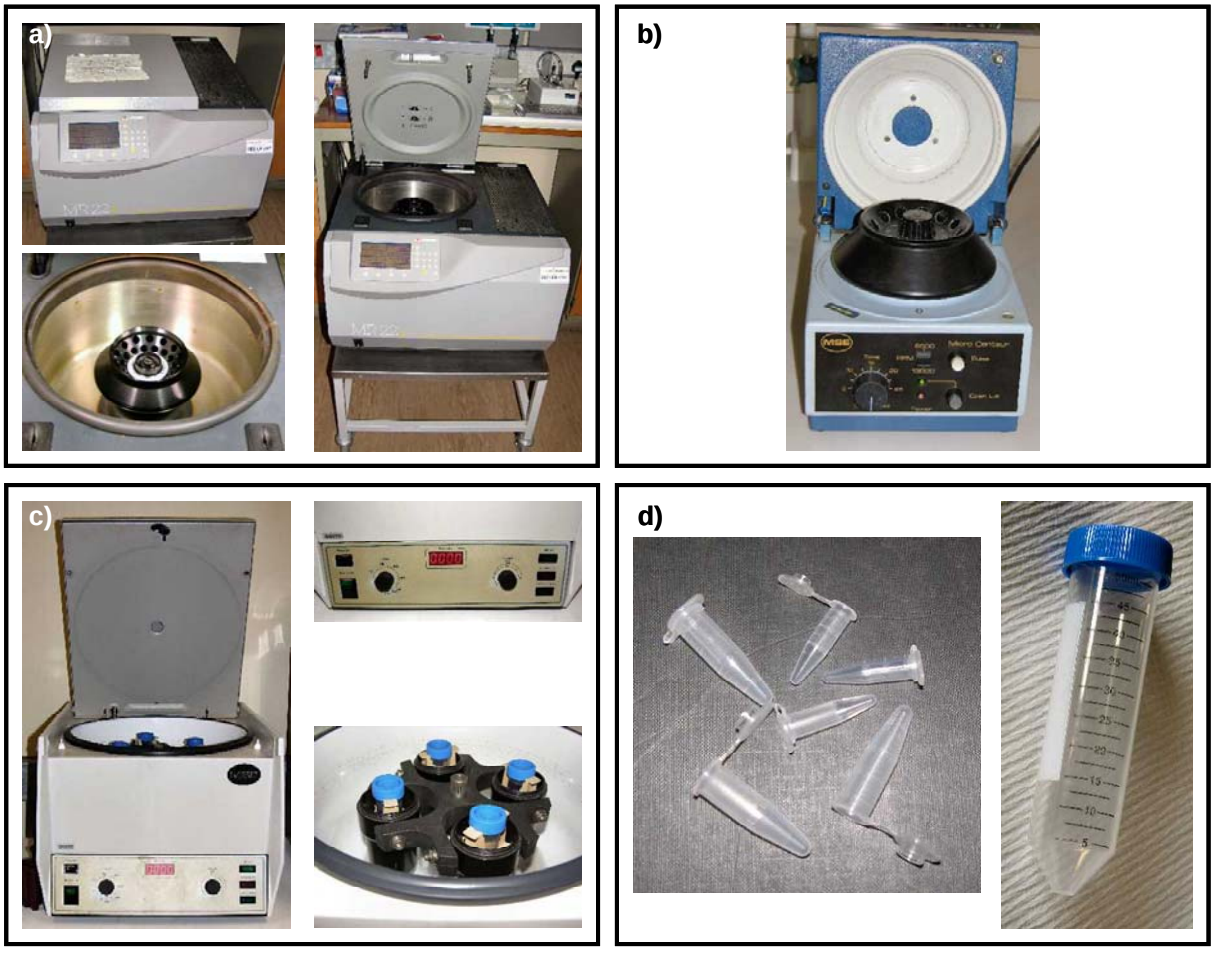


Figure A3.19: Different types of centrifuge, (a) to (c), and centrifuge tubes (d)

A3.7.3.1 Rotors

The two main types of centrifuge rotor are a fixed angle rotor (where the sample is held at a specific fixed angle) or swinging bucket mechanisms (where the sample swings out on a pivot). Other rotors are available for particular applications – consult manufacturers' websites for details.

Fixed angle rotors generally achieve separation more quickly, as the substance under centrifugation will have an increased relative centrifugal force applied to it for a given rotor speed and radius. One other significant advantage is that fixed rotor centrifuges have very few moving parts and therefore have virtually no major mechanical failures.

Swinging bucket rotors (also known as horizontal rotors), although slower to achieve separation than the fixed rotor mechanism, have advantages due to their centrifugal action. Unlike the fixed rotor mechanisms, particles for separation have to travel the full distance of the centrifuge tube and are not forced against the side of the tube. Therefore particles separated in this way will stay intact. The only major disadvantage of this type of rotor is that they have a number of moving parts which are prone to failure with extended use.

Table A3.31 outlines the differences between the main types of centrifuge and summarises some of the more common applications. This has been collated using information from a number of different manufacturers. When selecting a suitable centrifuge you will need to consider its capacity and the speed/force required. For the majority of centrifuges both fixed angle and swinging bucket rotors can be used, depending on the application and the capacity required. Centrifuges generate heat due to the friction of the spinning rotor. Many models (especially those that operate at the highest speeds) are therefore available with a refrigeration unit. Refrigeration mechanisms maintain low temperatures (typically 4 °C at maximum speed) to protect heat sensitive samples and heat labile compounds. Refrigerated centrifuges are commonly used in the isolation of DNA, RNA and protein cell viruses. Some models are also available with a heating element.

Table A3.31: Examples of centrifuge specifications and applications

Type of centrifuge	Typical specifications	Applications
Ultracentrifuge		
a) Bench top	a) Maximum RCF (x *g*): 280 000 – 1 019 000 Typical capacity: 20 – 108 mL (e.g. 8 tubes each with a capacity of 13.5 mL)	• Final stage isolation of sub-cellular organelles; protein precipitation and proteomics; virus isolation; genetic analysis such as DNA, RNA and plasmid preps; lipoprotein work; • Equilibrium centrifugation; • Liquid-liquid extraction.
b) Floor standing	b) Maximum speed (rpm): 50 000 – 100 000 Maximum RCF (x *g*): 302 000 – 802 000 Typical capacity: 50 – 500 mL (e.g. 12 tubes x 40 mL or 8 tubes x 7 mL)	

Table A3.31 continued

Type of centrifuge	Typical specifications	Applications
High-speed		
a) Micro	a) Maximum speed (rpm): 13 000 – 14 000 Maximum RCF (x *g*): 12 000 – 18 000 Typical capacity: 12/24 tubes x 1.5-2 mL	a) Fast pelleting; PCR post-reaction clean-up; Cell culture; Plasma and general purpose separations; DNA sample preparation; Sub-cellular fractionation and protein identification; Liquid-liquid extraction of emulsions.
b) Bench top	b) Maximum speed (rpm): 10 000 – 30 000 Maximum RCF (x *g*): 17 700 – 100 000 Typical capacity: 4 tubes x 100 mL, 24 tubes x 0.5 mL or 10 x microplates (1-10 µL per well)	b) Separation or preparation of sub-cellular components, proteins, precipitates, viruses nucleic acids, mammalian/insect cells and blood components; Liquid-liquid extraction of emulsions; Liquid-solid extraction of fatty substances from meat products during sample preparation; Liquid-solid extraction of wax substances from cosmetic products (e.g. mascara) during sample preparation; Solvent extraction.
c) Floor standing	c) Maximum speed (rpm): 20 000 Maximum RCF(x *g*): 42 037 Maximum capacity: 6 tubes x 290 mL	

Table A3.31 continued

Type of centrifuge	Typical specifications	Applications
Low-speed		
a) Small	a) Maximum speed (rpm): 5000 Maximum RCF (x *g*): 2500 Typical capacity: 10 tubes x 20 mL	
b) Bench top	b) Maximum speed (rpm): 6000 Maximum RCF (x *g*): 4750 Typical capacity: 4 tubes x 50 mL or 8 tubes x 15 mL	b) Separation of emulsions and suspended matter during sample preparation of cosmetic products; Separation of fat from milk, often with heating up to 37 °C.
c) Floor standing	c) Maximum speed (rpm): 8000 Maximum RCF (x *g*): 9320 Typical capacity: 4 tubes x 750 mL	c) Liquid/solid extractions; High weight precipitates.

A3.7.4 Correct operation of a centrifuge: safety and quality issues

The safe use of a centrifuge is important for both the operator and the instrument. Centrifuges should be serviced regularly (at least every 12 months) by an expert service engineer to identify any problems, and to check the rotors for corrosion and strain.

It is also important to ensure that the centrifuge is operated in such a way as to minimise any possibility of cross-contamination of samples. The key issues to consider are discussed in the following sections.

A3.7.4.1 Types of container

It is important to use the correct type of container for a particular rotor. Follow the manufacturer's guidelines on which containers are most suitable for the different types of rotor. Centrifuges have adaptable rotor plates and buckets to take different tubes and plates. Tube capacities range from 50 mL to 0.5 mL (see Figure A3.19 d)).

In chemical assays tubes of between 15 – 40 mL are typically used to centrifuge samples but volumes of up to 250 mL are not uncommon. With biological/clinical techniques the volumes will often be much smaller. Therefore, the sample may be spun in 0.5 – 1.5 mL tubes, or even in a 384 well plate (each well can contain up to 5 μL of sample). Figure A3.19 d) shows some examples of containers used in centrifugation.

A3.7.4.2 Operating a centrifuge

When using a centrifuge it is very important that you follow the correct protocol. You must always consult the manufacturer's instruction manual before use. Table A3.32 summarises the step by step actions needed to carry out the correct operation of a centrifuge.

Table A3.32: Operating a centrifuge

Operation	Action
Open the lid	The lid of the centrifuge can only be lifted if the centrifuge is plugged in and the rotor has stopped moving. Otherwise, for safety, the lid will remain in the locked position.
Load the centrifuge rotor	The centrifuge rotor should be loaded with appropriate sample tubes and balanced as discussed in section A3.7.4.3.
Close the lid	Once the centrifuge rotor has been loaded, close the lid and push down firmly so that it 'clicks'. This indicates that it is locked (often a light will illuminate to show the lid is locked).
Set the speed	Different centrifuges will have different speed setting mechanisms. Some will have digital dials that you use to adjust the speed to the required rpm, others will have set speeds which can be selected as appropriate.
Set the run time	As with the speed settings, different types of centrifuge will have different mechanisms for setting the run time. A specific time can be selected using digital buttons or pre-selected options. Many centrifuges also have the option to 'pulse' for a few seconds by holding the pulse button down.
Press start	Once both the speed and time have been set, the start button can be pressed to begin centrifugation. The centrifuge will spin at the specified speed until the set time has elapsed.
Wait for process to finish	Once the centrifuge has spun for the allocated time, the rotor will slow down and stop. Many centrifuges will display 'end' when the run has finished.
Open lid and remove tubes	The lid light will be illuminated so as to alert the operator that the run has finished and the rotor is stationary. The lid locking mechanism will then unlock and the lid can be lifted and the sample tubes removed.

A3.7.4.3 Balancing the load

The load in the centrifuge must be carefully balanced. Small differences in the mass of the load at different positions on the rotor can result in a large unbalanced force when the rotor is at high speed. This force can put strain on the equipment, which eventually can lead to failure of the rotor. This will cause serious damage to the equipment and may lead to personal injury. Therefore, the minimum number of centrifuge tubes you can use is two as this will allow the load to be evenly balanced. All tubes should contain approximately the same amount of liquid. If you need to centrifuge more than two tubes they should be evenly spaced around the rotor plate so as to keep the centrifuge balanced. If you do not have sufficient samples to balance the rotor you should use 'balance tubes' containing a liquid similar to the sample (or of similar density). If using a swinging bucket rotor, the buckets should be balanced. This is easily achieved by matching the weight of each bucket using balance tubes containing water and/or adding additional solvent to sample tubes. Figure A3.20 shows some examples of balanced rotors with different numbers of samples.

Some centrifuges are fitted with 'imbalance detectors' and will shut down if an unbalanced rotor is detected. The main symptoms of an imbalance are excessive noise and vibration, and possibly movement of the centrifuge. If an imbalance does occur, stop the run immediately and rebalance the rotor plate or buckets before repeating the run.

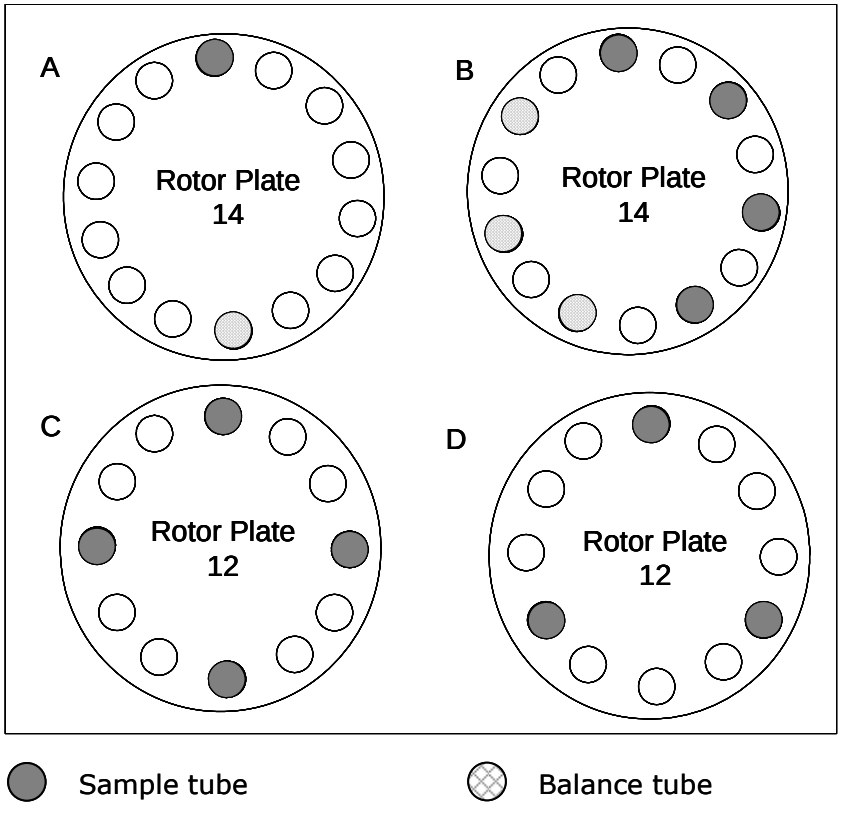


Figure A3.20: Some examples of balanced rotor plates

A) Represents a balanced 14 position rotor plate with one sample and one balance tube. B) Shows a 14 position rotor plate with four samples and three balance tubes. C) Represents a 12 position rotor plate with four balanced samples. D) illustrates a 12 position rotor with three balanced samples.

A3.7.4.4 Touching moving parts

Centrifuge rotors should never be touched while they are moving, as they can cause serious injury. Modern centrifuges have safety features that prevent accidental contact with the rotor, e.g. locking lids (see section A3.7.2).

A3.7.4.5 Spills

Liquid that has spilled in or on the centrifuge should be wiped up immediately, this will help prevent corrosion or damage to the rotor and other parts of the centrifuge. You shouldn't clean the rotor with a wire brush or any corrosive (e.g. alkaline) detergents as this may cause damage to the mechanism.

It is also essential to keep centrifuges clean to avoid any possibility of cross-contamination of samples.

A3.7.4.6 Corrosive / infectious materials

Extra care should be taken when using corrosive or infectious material in a centrifuge. If an infectious material is being used, tubes should be sterilised immediately after use and if spillages occur, the rotor should be autoclaved.

A3.7.5 Checklist for using a centrifuge

Table A3.33 summarises the key points to remember when using a centrifuge.

Table A3.33: Dos and don'ts for centrifugation

Do	Don't
✓ Ensure that the correct rotor is in place and properly fitted.	
✓ Make sure the rotor is correctly balanced.	✘ Use an unbalanced rotor. ✘ Use a swinging bucket rotor with missing buckets.
✓ Check that the centrifuge tubes are undamaged otherwise they may break under the force of centrifugation. Use suitable sample tubes for the rotor you have chosen.	
✓ If there are insufficient samples to balance the rotor, use 'balance tubes'.	✘ Overload the centrifuge.
✓ Fill the sample tubes correctly.	✘ Use tubes with too much or too little sample.
✓ For swinging bucket rotors use bucket lids/covers if available.	
✓ Make sure that the centrifuge lid is closed and locked.	✘ Try to use the rotor if the lid is not fully closed.
✓ Set the speed and time you require to centrifuge your samples.	
✓ Stop the centrifuge immediately if there is excessive noise or vibration.	✘ Leave a centrifuge running if it is making excessive noise and vibrating.

Table continued

Table A3.33 continued

Do	Don't
✓ Wait until the centrifuge has reached its full operating speed and you are sure that it is operating correctly before you leave it.	✘ Leave the centrifuge until you are sure it is operating correctly.
✓ Wait for the centrifugation process to stop completely before opening the lid.	✘ Open the lid while the rotor is still running. ✘ Touch moving parts while the centrifuge/rotor is working.
✓ Remove samples carefully so as to not disturb any particles.	
✓ Clean up any spills or leaks in the centrifuge rotor.	✘ Leave spilled substances to cause corrosion to the rotor or contamination of future samples.
✓ Leave the lid open for a short time after use if the centrifuge has been heated or cooled to avoid condensation.	
✓ Record the centrifuge usage – rotors are subject to significant stresses and may need to have a reduced maximum speed as they age and eventually be retired.	

A3.7.6 Questions

Question 1

Calculate the relative centrifugal force (RCF) of a centrifuge with a centrifugation rotor radius of 9.65 cm and a speed of 5300 rpm.

Question 2

What type of centrifuge would you use to isolate sub-cellular organelles?

Question 3

How would you balance three samples in a rotor plate containing 12 positions?

Question 4

What would you do if the centrifuge you had just started began to vibrate and make excessive noise?

A4 Quality issues

Chapter A3 describes the steps you should take when carrying out some key laboratory operations to help make sure that the results you produce in the laboratory are reliable. Sound practical skills are a key part of ensuring the quality of analytical results. But what do we actually mean when we talk about 'quality' or 'quality assurance'? In addition to staff training, what other activities should laboratories carry out to ensure the quality of the results they produce? This section provides a brief introduction to a number of key topics:

- Definition of quality and why it is important (section A4.1);
- Quality management, quality assurance and quality control (section A4.2);
- Method validation (section A4.3);
- Precision, bias, accuracy and other method performance parameters (sections A4.3.4.1 to A4.3.4.5);
- Calibration and traceability (section A4.4);
- Documenting test methods (section A4.5);
- Control charts (section A4.6);
- Proficiency testing and external quality assessment (section A4.7);
- Errors and uncertainty (section A4.8).

A4.1 Definition of quality

A dictionary definition of quality is, "the degree of excellence of a thing." In relation to a company offering a service or manufacturing a product, the concept of quality is about providing customers with a service or item that meets their needs, i.e. it is 'fit for purpose'. Laboratories can be considered to be both offering a service (the ability to carry out particular tests) and delivering a product (the results obtained from the analysis of test samples). The measurements you make in the laboratory will always have a customer – the person that will use the data. Your customers may be external to your organisation or they may be people from other departments within your company – both are equally important. Measurements are always made for a reason; the customer will use the measurement results to help them solve a problem or answer a particular question. It is important to understand why you are carrying out analyses and to know what the data produced will be used for. Without this information, it is impossible to judge whether results are fit for purpose as you do not know what the purpose is.

Measurement results are considered fit for purpose if the measurement uncertainty (see section A4.8) is acceptable. In some situations (e.g. the analysis of pesticide residues in foodstuffs) an uncertainty of 10 or 20% might be acceptable and the customer would still be able to use the data. In another situation - the analysis of precious metals for example - such a large uncertainty would be totally unacceptable as there are large amounts of money at stake. However, knowing the measurement uncertainty and demonstrating that it is acceptable is not the whole story. Results that are known with a high degree of certainty will be of no use to the customer if you have measured the wrong thing. For example, if you needed to measure the concentration of lead in a sample of paint you could digest the paint in acid, then measure the total concentration of lead present in the sample using a spectroscopic technique such as flame atomic absorption spectroscopy or inductively coupled plasma–optical emission spectroscopy. But if the sample of paint had come from a toy, and the customer wanted to check that the toy complied with European toy safety standards, it's the amount of lead that is extracted into a stomach-acid simulant that is important rather than the total lead concentration. The end measurement might be the same but measuring total lead instead of 'extractable' lead would not meet the customer's requirements. To be of use

to the customer results also need to be reported within a reasonable timescale. If the customer needs the measurement results before they can release a batch of a product onto the market they may not want (or be able) to wait several weeks for the data.

Laboratories need to have systems in place to give them confidence that the results they deliver to their customers on a daily basis are fit for purpose – it cannot be left to chance! This is where the concepts of quality management, quality assurance and quality control come into play.

A4.2 Quality management, quality assurance and quality control

You may have come across the term 'quality management system'. A quality management system is a set of procedures put in place by an organisation to ensure that staff have the facilities, equipment and resources to carry out their work effectively. A quality management system will include quality assurance and quality control activities. The terms 'quality control' and 'quality assurance' are often used interchangeably but they actually have quite different meanings.

- **Quality control** is a planned programme of activities designed to ensure that the product (e.g. analytical data) is fit for purpose. Quality control is *what you do* on a day to day basis to ensure that laboratory systems are working correctly and that results produced are of the required quality.
- **Quality assurance** is a planned set of documented activities designed to ensure that the quality control programme is carried out effectively, to demonstrate that it has been done and that it is appropriate. If quality control is *what you do* then quality assurance tells you *how to do it* and provides the infrastructure within your organisation to enable it to be done.

Many organisations base their quality management system on international standards such as ISO/IEC 17025:2005 and have the system audited by a third-party. This enables the organisation to demonstrate to their customers that they take quality seriously and have sound systems in place (see section A4.2.1).

Day to day quality control activities include:

- Analysis of blanks
  - check for contamination or interferences;
- Analysis of standards and reference materials
  - calibration of instruments (see section A4.4);
- Analysis of QC samples (see section A4.6)
  - check the method is working consistently;
  - plot QC results on control charts;
- Replicate analysis of samples
  - gives greater confidence in the result;
- Participation in proficiency testing schemes (external quality assessment) (see section A4.7)
  - an independent check of laboratory performance.

When thinking about the reasons for carrying out quality control and quality assurance, it is worth remembering that it usually costs less to prevent a problem than it does to correct it!

A4.2.1 International quality standards

There a number of international standards available that cover the quality of service that organisations offer to their customers. The standards most commonly used in laboratories are:

- ISO 9001:2000, Quality management systems – Requirements;
- ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories;
- ISO 15189:2003, Medical laboratories - Particular requirements for quality and competence;
- Good Laboratory Practice Regulations.

Different standards are appropriate for different organisations, depending on the nature of their work; some laboratories work to more than one standard. The main features of the standards are summarised below.

ISO 9001:2000

- A general standard that applies to all organisations;
- Specifies the requirements for a quality management system where the organisation has to:
  - demonstrate its ability consistently to provide a service that meets customer and applicable regulatory requirements;
  - aim to enhance customer satisfaction through the effective application of the system, including processes for continual improvement of the system;
- Does not test the competence of organisations (e.g. the ability of a laboratory to produce reliable test results) but checks how the organisation controls its processes;
- Organisations are *certified* by a third-party (e.g. BSI) against the requirements of the standard.

ISO/IEC 17025:2005

- Applies to all laboratories carrying out tests and/or calibrations;
- Laboratories have to operate a quality management system that meets the principles of ISO 9001;
- Tests the competence of the laboratory and its staff to carry out particular tests/calibrations;
- Laboratories are *accredited* to the requirements of the standard by the national accreditation body (e.g. United Kingdom Accreditation Service (UKAS));
- Accreditation is usually awarded for a particular combination of analyte, matrix and test method (the 'scope' of the accreditation).

ISO 15189:2003

- Developed to cover the specific needs of medical laboratories;
- Incorporates the requirements of ISO 9001 and ISO/IEC 17025;
- Effectively a customised version of ISO/IEC 17025 for medical laboratories;
- Accreditation is carried out in the UK by Clinical Pathology Accreditation (UK) Ltd.

Good Laboratory Practice (GLP)

- Legal requirement that regulatory studies undertaken to demonstrate the health or environmental safety of new chemical or biological substances must be conducted in *compliance* with the principles of GLP;
- The principles set out a quality system dealing with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported
  - there must to be sufficient information available for the study to be reconstructed at a future date;
- Relates to a *study* and not specific tests;
- Each country has a monitoring authority that assesses studies to ensure they meet the requirements of the GLP principles (e.g. GLP Monitoring Authority in the UK which is part of the Department of Health).

An organisation may be accredited to ISO/IEC 17025 for a number of its test methods but may also be certified to ISO:9001 for the 'non-technical' areas of its business (e.g. finance, human resources) and the technical areas not covered by ISO/IEC 17025. This ensures that all areas of the organisation are working under the same management system.

A4.3 Method validation

A4.3.1 Definition of validation

The discussion in section A4.2 assumes that the laboratory has a suitable test method up and running but needs to ensure that the methods are operating (and being operated) correctly. But how do we get to that stage? How does a laboratory decide that the test method it would like to use is fit for purpose and ready to be used for the analysis of test samples? The process of evaluating method performance and demonstrating that it meets a particular performance requirement is call *method validation*.

The definition of validation given in ISO/IEC 17025 is, 'the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.'

The definition has three important parts which help to outline the validation process:

- *Specific intended use*: Measurements are made to help answer a specific question or solve a particular problem. To be able to choose a suitable test method you need to understand why you are analysing the samples and what the resulting data will be used for.
- *Objective evidence*: This is the set of results generated from planned experimental validation studies which are used to evaluate the performance of the chosen test method.
- *Confirmation*: This is the final step of the validation process. The information on method performance, obtained from the experimental studies, is compared with the performance that is required to meet the customer's requirements. If the comparison shows that the method performance is satisfactory, then the method is declared fit for purpose and the validation process is complete. If the performance is unsatisfactory then further refinement/development of the method is required.

A4.3.2 When is method validation required?

The performance of all test methods should be evaluated before they are used in a laboratory for the first time. The amount of validation required will depend on the history of the method and the availability of any existing validation data. For a new method that has been developed in-house by the laboratory, a very detailed validation study will be required to demonstrate that the method is fit for purpose. In contrast, if you are using a published standard test method that has previously been validated via an inter-laboratory study then a more limited study is required to demonstrate that you can get the method to work satisfactorily in your laboratory for your test samples. The process of checking performance against previously established limits is sometimes known as *verification.*

Revalidation is required if changes are made to existing test methods or if samples outside the scope of the original validation are to be analysed. For example, if you start receiving samples that typically contain less than 50 mg kg^{-1} of the analyte but the test method was validated for samples containing 100 – 1000 mg kg^{-1} you cannot assume that the method performance will be acceptable at the lower concentrations. Some additional validation should therefore be carried out to demonstrate that the method performance is fit for purpose across the extended concentration range.

A4.3.3 How much validation is required?

The amount of effort required to validate a method will depend on the criticality of the measurement, the scope of the method and the level of information and experience already available. Table A4.1 lists the most important performance parameters and the types of analysis for which they are relevant. Descriptions of the parameters are given in section A4.3.4.

Table A4.1: Performance parameters required for the validation of different types of analysis

	Type of analysis			
		Quantitative		
Parameter	**Qualitative**	**Major component[1]**	**Trace analysis[2]**	**Physical property**
Selectivity/specificity	✓	✓	✓	✓
Precision		✓	✓	✓
Bias		✓	✓	✓
Limit of detection	✓		✓	
Limit of quantitation			✓	
Linearity/working range		✓	✓	✓
Ruggedness	✓	✓	✓	✓

[1]Major component: analyte concentration in the range 1 % to 100 %
[2]Trace analysis: analyte concentration less than 100 mg kg^{-1}

Not all of the performance parameters listed in Table A4.1 will be relevant for every test method. Validating a new method for trace analysis would require a detailed study of all of the parameters highlighted in the table. However, if you were planning to implement a method for the measurement of a major component, which had been previously validated by an inter-laboratory study, a limited study of precision and bias to confirm that you are applying the method correctly, may be all that is required.

A4.3.4 Method performance parameters

Method validation involves studying a number of aspects of method performance. Brief descriptions of the parameters are given in this section.

A4.3.4.1 Selectivity and specificity

It is important to establish during method validation that the test method is measuring only what it is intended to measure. In other words, the method must be free from interferences which could lead to an incorrect result. Selectivity is defined as the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour.[4] In some fields of measurement, specificity is used as an alternative term for selectivity and is described as the ability of a method to assess unequivocally the analyte in the presence of components which may be expected to be present.

A4.3.4.2 Precision, bias and accuracy

There is often some confusion about the exact meaning of these terms. The relationship between precision, bias and accuracy is illustrated in Figure A4.1.

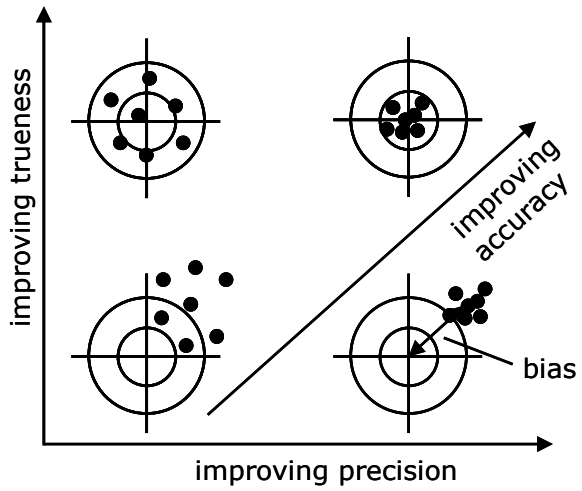


Figure A4.1: Precision, bias and accuracy – the aim is to get all the 'results' as close together as possible in the centre of the target

Precision

Precision describes the closeness of agreement between the results obtained from replicate measurements made on the same sample under particular conditions. The measurement conditions determine the nature of the precision estimate. It is important to understand the differences between different types of precision estimate to be able to plan suitable validation experiments and to interpret data correctly.

- **Repeatability:** variation in results from replicate measurements made in one laboratory, by a single analyst using the same equipment over a short timescale. Repeatability gives an indication of the short-term variation in measurement

[4] 'Selectivity in Analytical Chemistry', J. Vessman, R. I. Stefan, et al., Pure Appl. Chem, 2001, Vol. 73, No. 8, 1381-1386.

results and is typically used to estimate the likely difference between replicate measurement results obtained in a single batch of analysis.

- **Reproducibility:** variation in results from replicate measurements carried out in different laboratories (by different analysts using different pieces of equipment). Reproducibility therefore has to be evaluated by carrying out an inter-laboratory study.
- **Intermediate precision:** variation in results obtained from replicate measurements made in one laboratory under differing conditions (e.g. different analysts making measurements on different days using different pieces of equipment). Intermediate precision is used to give an estimate of the likely variation in results when the method is used routinely over an extended time period. This type of precision estimate is also known as *within-laboratory reproducibility*.

Precision is generally expressed as the *standard deviation* or *relative standard deviation* (see section A5.1) of the results obtained from replicate measurements of an appropriate sample. The sample should be stable and sufficiently homogeneous, and representative of test samples in terms of the matrix and analyte concentration. The replicate measurements must be *independent*. In other words, each replicate result should be obtained from the application of the entire test method, including any sample preparation and/or pre-treatment. If the method being validated is to be applied to the analysis of a range of analyte concentrations and/or sample types the precision should be studied for a representative range of samples. The precision of methods often gets worse (i.e. the standard deviation of results increases) as the concentration of the analyte in the sample decreases.

Figure A4.2 illustrates that as the number of parameters that vary from one measurement to the next increases, the spread of results (the standard deviation) increases. The variation in results produced by different laboratories is usually greater than the variation in results produced by a single laboratory.

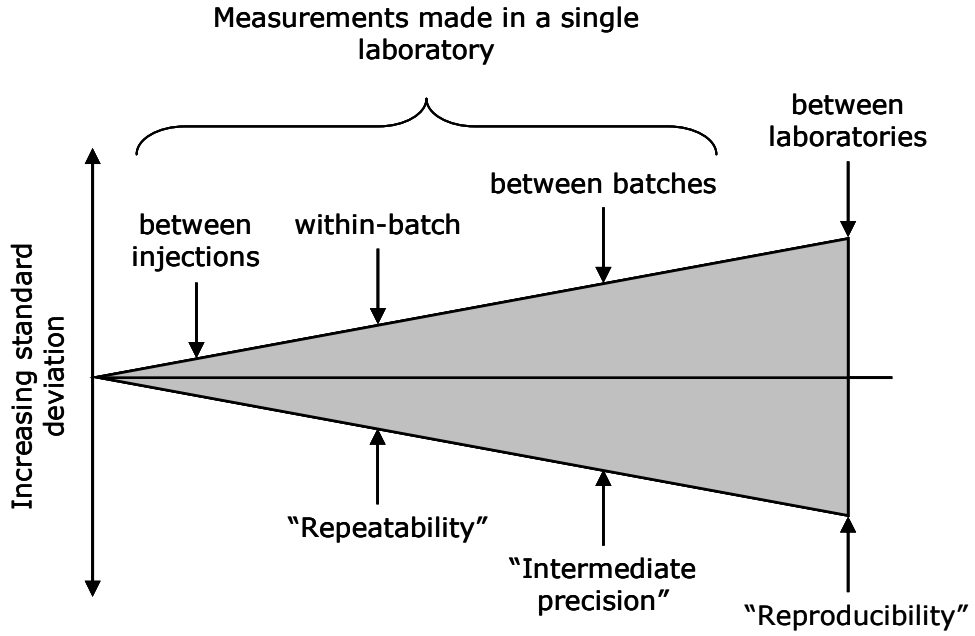


Figure A4.2: Illustration of the relationship between different precision estimates

Bias

Precision describes the variation in results obtained from the replicate analysis of a sample but it gives no information as to how close the results are to the actual concentration of the analyte in the sample. It is possible for a test method to produce results that are in very close agreement with one another (i.e. very precise) but that are consistently wrong (e.g. significantly lower (or higher) than they should be)!

Bias is the difference between what you observe (i.e. the test results you obtain in the laboratory) and the right answer (i.e. the true value or actual amount of analyte present in the test sample). Figure A4.3 illustrates how bias is evaluated.

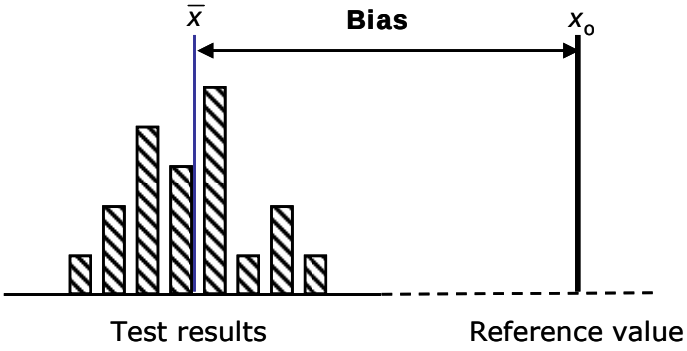


Figure A4.3: Illustration of bias

To evaluate bias you need to carry out replicate analysis of a suitable material containing a known amount of the analyte of interest. There are a number of different ways of expressing the bias in measurement results.

Bias expressed as a difference:

$\text{Bias} = \bar{x} - x_o$ or as a percentage: $\%\text{Bias} = \frac{\bar{x} - x_o}{x_o} \times 100$

Bias expressed as a ratio (when it is usually referred to as recovery):

$\%\text{Recovery} = \frac{\bar{x}}{x_o} \times 100$

where $\bar{x}$ is the mean of the measurement results and x_o is the reference value (the actual concentration of the analyte in the sample).

One of the main problems when planning experiments to study bias is choosing a suitable material to analyse. There are a number of options:

- Certified reference material (CRM);
- Spiked test samples;
- Reference method.

A CRM is a material that has been produced and characterised to high standards and that is accompanied by a certificate stating the value of the property of interest (e.g. the concentration of a particular analyte) and the uncertainty associated with the value. If a suitable CRM is available (i.e. one that is similar to test samples in terms of sample form, matrix composition and analyte concentration) then it should be your first choice material

when planning a bias study. Unfortunately, compared to the very large number of possible analyte/matrix combinations, the number of CRMs available is relatively limited so a suitable material may not be available. An alternative is to prepare a reference sample in the laboratory by spiking a previously analysed sample with an appropriate amount of the analyte of interest. With this type of study you must take care to ensure that the spike is in equilibrium with the sample matrix before any measurements are made, for example by leaving the spiked samples for several hours before analysis. You should also add the spike in a different solvent to the one that will be used to extract the analyte from the sample. Taking these precautions will help to ensure that the behaviour of spiked samples is as similar as possible to the behaviour of real test samples.

Bias can also be evaluated by comparing results obtained from a reference method with those obtained using the method being validated. If this approach is taken, the evaluation can be carried out using test samples rather than a CRM or spiked sample – the mean of the results obtained from the reference method provides the reference value x_o. Alternatively, a range of different samples can be analysed using both methods and the differences between the pairs of results obtained evaluated statistically to determine whether there is a significant difference between the results produced by the two methods.

Accuracy

Accuracy is defined as the closeness of the agreement between the result of a measurement and the true value of the quantity being measured. It describes how close a *single measurement* result is to the true value and therefore includes the effect of both precision and bias, as illustrated in Figures A4.1 and A4.3.

A4.3.4.3 Detection capability

In many situations it is useful to know the lower 'operating limit' of a test method, for example, the minimum concentration of the analyte that can be detected and/or quantified with a reasonable degree of certainty.

The limit of detection (LoD) is the minimum concentration of the analyte that can be detected with a specified level of confidence. It can be evaluated by obtaining the standard deviation of results obtained from replicate analysis of a blank sample (containing none of the analyte of interest) or a sample containing only a small amount of the analyte. The resulting standard deviation is multiplied by a suitable factor (3*s* is frequently used as the basis for LoD estimates). The multiplying factor is based on statistical reasoning and is specified so that the risk of false positives (wrongly declaring the analyte to be present) and false negatives (wrongly declaring the analyte to be absent) is kept to an acceptable level (a 5% probability is usually specified for both types of error). Note that the LoD determined during method validation should be taken only as an indicative value and the approach described is adequate if results for test samples will be well above the LoD. If you expect the sample concentrations to be close to the limit of detection then you should monitor the LoD after validation and a more statistically rigorous approach may be required.

In some sectors (particularly clinical measurements) the limit of detection is referred to as the *sensitivity* of the method.

The limit of quantitation (LoQ) is the lowest concentration of analyte that can be determined with an acceptable level of uncertainty. A value of 10*s* is typically used (where *s* is the standard deviation of the results from replicate measurements of a blank or low concentration sample).

A4.3.4.4 Linearity, working range and calibration

The working range of a method is the concentration range over which the method has been demonstrated to produce results that are fit for purpose. The lower end of the working range is defined by the LoD and LoQ (see section A4.3.4.3). The upper end is usually signified by a change in sensitivity, for example a 'tailing-off' or 'plateauing' in the response, as illustrated in Figure A4.4.

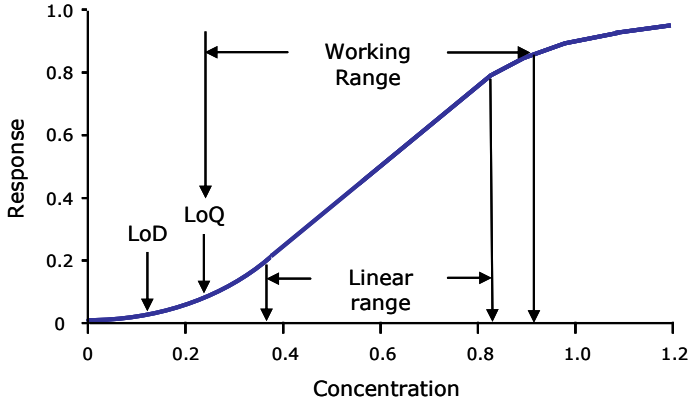


Figure A4.4: Linearity and working range

The linear range of a method is the concentration range over which the change in response is directly proportional to the amount of analyte present. The determination of linearity and working range at the method validation stage is important, because it allows you to demonstrate the suitability of the method over the range required by the analytical specification. It also helps in the design of the calibration strategy for the method, specifically:

- How frequently the method needs to be calibrated during normal routine testing (between every run, once a day, once a week, etc.);
- How many calibration levels are required (single level, bracketing, six different concentrations, etc.);
- The number of replicates which need to be determined at each concentration level.

See section A4.4 for further information on calibration.

In order to assess the working range, and confirm its fitness for purpose, standards whose concentrations cover the expected concentration range ±10% (or even ±20%) should be analysed. The standards should be evenly spaced across the concentration range. Establishing linearity during method validation normally requires more standards (and more replication at each concentration) than is typical for calibration of a validated method in regular use. It is recommended that at least seven different concentration levels are studied during method validation.

Many test methods require the test sample to be treated in some way so as to get the analyte into a form suitable for measurement. During method validation it is sensible to carry out an initial study to evaluate the response of the instrument to the analyte across the required concentration range. This can be done by analysing standards containing known concentrations of the analyte in a suitable solvent. This study will allow you to establish the calibration function for the instrument. Once the instrument performance has been demonstrated to be satisfactory the linearity of the whole method should be studied. This requires the analysis of CRMs, spiked samples or matrix-matched standard

solutions (i.e. solutions containing a known amount of the analyte plus the sample matrix). If the instrument response has been demonstrated to be linear then any non-linearity observed in the second study may indicate problems such as the presence of interfering compounds or incomplete extraction of the analyte from the sample matrix.

A4.3.4.5 Ruggedness

When a method is used routinely, the operating and environmental conditions often vary much more than during the method development and validation studies. Sometimes a method will perform perfectly well during method validation but when it is used routinely in the laboratory, by a number of different analysts, the variation in results is much greater than expected. This can happen if the critical parameters which affect method performance have not been identified or are not being controlled adequately.

Ruggedness testing evaluates how small changes in the method conditions affect the measurement result, e.g. small changes in temperature, pH, flow rate, composition of extraction solvents etc. The aim is to identify and, if necessary, better control method conditions that might otherwise lead to variation in results when measurements are carried out at different times or in different laboratories. It can also be used to identify factors which need to be addressed to improve precision and bias.

Ruggedness testing can be carried out by considering each effect separately, by repeating measurements after varying a particular parameter by a small amount (say 10%) and controlling the other conditions appropriately. However, this can be labour intensive as a large number of effects may need to be considered. Since for a well-developed method most of the effects can be expected to be small, it is possible to vary several parameters at the same time according to a well-defined, statistically based experimental design. Any stable and homogeneous sample within the scope of the method can be used for ruggedness testing experiments.

A4.4 Calibration and traceability

All measurements involve making a comparison, as shown in Figure A4.5.

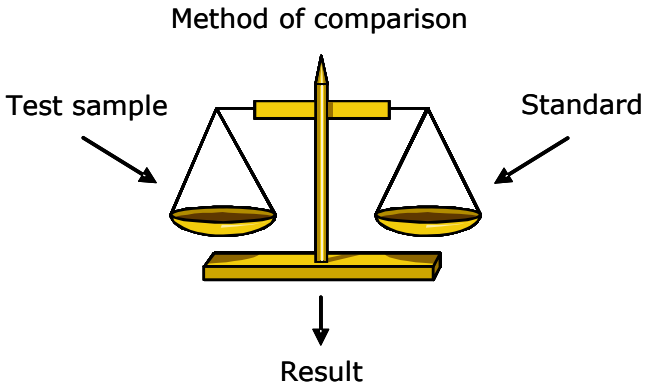


Figure A4.5: The measurement process

The test sample is compared with a standard which provides a link to the measurement scale being used. The method of comparison can be straightforward, e.g. using a ruler to measure the dimensions of an object, but in the laboratory it often involves complex analytical equipment, such as a gas chromatograph (GC) used to determine the concentration of pesticide residues in samples of apples or an inductively coupled plasma-optical emission spectrometer (ICP-OES) used to measure the concentrations of

metals in water samples. All measuring devices - whether it's a ruler, an analytical balance or an ICP-OES - will give a reading (or a 'response') but how does that response relate to the property being measured? When you weigh out 1 g of a chemical on one particular analytical balance, how can you be sure that if you used a different balance to weigh 1 g of the chemical you would have the same amount of material? *Calibration* is the process of establishing the relationship between the response of a measuring device and the value of a standard. So, calibrating a balance involves weighing standard weights of known mass and adjusting the balance until it gives the correct reading; calibrating a gas chromatograph for the analysis of pesticides would involve analysing a number of standard solutions (see section A3.4) containing known amounts of the pesticides of interest and plotting a calibration curve to establish the relationship between the response from the GC and the amount of pesticide present. From this description it should be clear that the nature of the standards used for calibration is critical. For measurement results to have any meaning they need to relate to internationally recognised scales of measurement and the standards used for calibration need to be linked in some way to a recognised reference point. The international system of units (SI) defines seven base units of measurement:

- The *metre* (m) – the unit of length;
- The *kilogram* (kg) – the unit of mass;
- The *second* (s) – the unit of time;
- The *mole* (mol) – the unit of amount of substance (see section A3.4.2.1);
- The *ampere* (A) – the unit of electric current;
- The *kelvin* (K) – the unit of thermodynamic temperature;
- The *candela* (cd) – the unit of luminous intensity.

So for measurements of mass made at different times and in different locations to be comparable they should all relate to the same reference point, that is the international standard kilogram held at the International Bureau of Weights and Measures (BIPM) at Sèvres near Paris. In other words, measurements of mass should be *traceable* to the standard kilogram. How is traceability achieved in practice? This is illustrated in Figure A4.6.

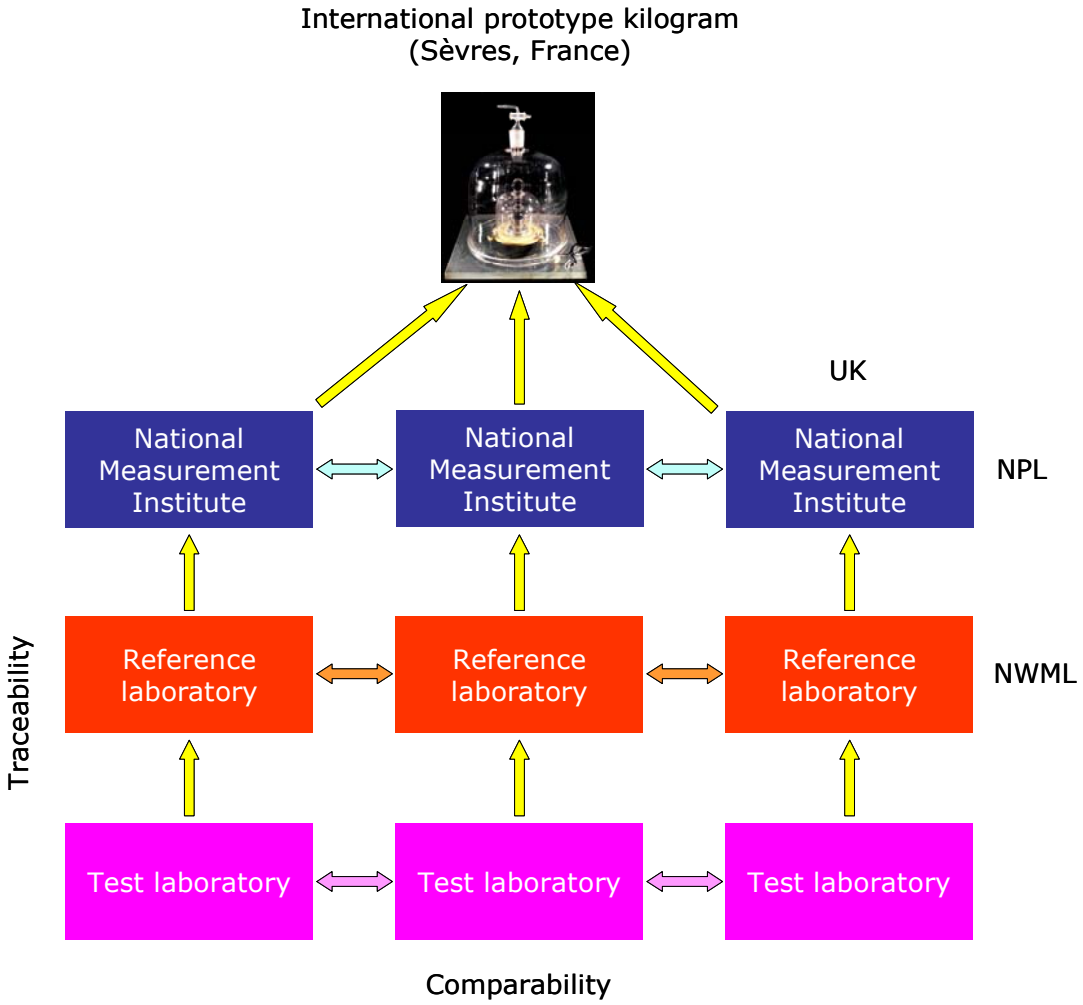


Figure A4.6: Establishing traceability

An analytical balance used in a test laboratory should be calibrated periodically with a set of calibrated weights (see section A3.2.4.2). The weights used to calibrate balances will be regularly calibrated against a set of weights held at a reference laboratory (e.g. National Weights and Measures Laboratory (NWML) in the UK). The weights held by reference laboratories are regularly calibrated against the national standard kilogram. This is a copy of the international prototype kilogram and is held by the National Measurement Institute (the National Physical Laboratory (NPL) in the UK). The national kilogram is periodically calibrated against the international prototype kilogram. Measurements of mass made in test laboratories are therefore traceable to the international standard of mass through an unbroken chain of comparisons. If the same system is in place in all countries, measurements of mass made using properly calibrated and maintained balances should be comparable.

For results produced by different laboratories to be comparable it is essential that all measurements are traceable to appropriate reference values. This includes all the measurements of mass, volume, temperature, time, amount of substance etc. that are required to carry out a particular test method. Establishing the traceability of measurements of 'physical' properties such as mass and volume is relatively straightforward. You need to select a suitable measuring device which will measure the property of interest with the required level of uncertainty. The choice of equipment for measuring volume and mass is discussed in sections A3.1.3 and A3.2.3, respectively.

Relating measurements of concentration (i.e. amount of substance) involves obtaining and preparing suitable chemical standards. This is discussed in section A3.4.

Calibration of an analytical instrument, such as a UV/visible spectrophotometer or a gas chromatograph, usually involves preparing and analysing a set of chemical standards covering the concentration range of interest and preparing a plot of the instrument response vs. the concentration of the standards, as shown in Figure A4.7.

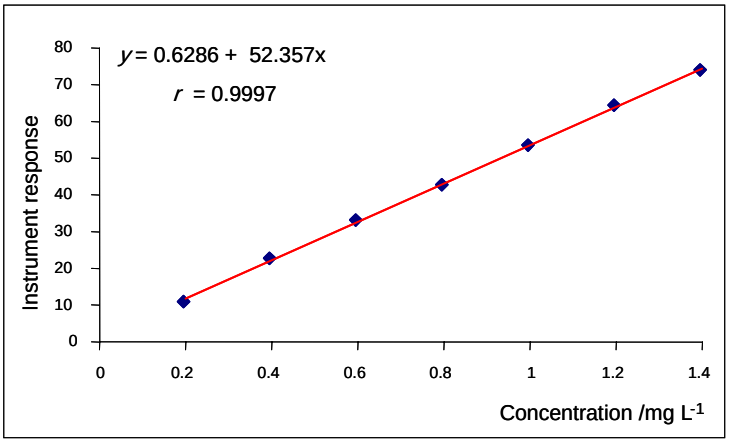


Figure A4.7: Typical calibration curve

The relationship between response and concentration is established mathematically (using linear least squares regression if the relationship is expected to be linear).

A linear relationship is described by the equation:

$$y = mx + c$$

where m is the gradient of the line and c is the intercept with the *y*-axis.

For measured values of *y*, the value of *x* is predicted by rearranging the above equation:

$$x = \frac{y - c}{m}$$

A4.5 Documenting test methods

Once method validation is complete and the method is ready to be used for the analysis of test samples, a clear and detailed description of the method should be prepared. This will help to ensure that the method is applied consistently in the future, especially if it is to be used by different analysts (possibly working in different laboratories). There are a number of formats available for documenting methods. A standard format is described in ISO 78-2[5]. Table A4.2 summarises the sections which commonly appear in descriptions of test methods. The formal method description is sometimes referred to as a *standard operating procedure* (SOP).

[5] ISO 78-2:1999 Chemistry - Layouts for standards - Part 2: Methods of chemical analysis.

Table A4.2: Key headings for documenting test methods

Section	Comments
0 Update and review	Record any minor changes to the method. It is good practice to periodically review methods to ensure they remain fit for purpose. Record the review date and the outcome of the review.
1 Title	Brief description including analyte(s), sample type(s) and principle.
2 Warning & safety precautions	Give detailed precautions in the relevant sections but draw attention here to any hazards and the need for precautions.
3 Scope	Allows the user to quickly identify whether the method is likely to be suitable. Include information on the analyte(s), sample type(s), concentration range, known interferences that prevent the method from working.
4 Normative references	References where compliance with their requirements is essential.
5 Definitions	Define any unusual terms.
6 Principle	Outline the principle by which the method operates.
7 Reagents & materials	A numbered list of all the reagents, materials, blank samples, QC samples, reference materials and standards required to carry out the method.
8 Apparatus & equipment	A numbered list describing the individual items of equipment required and how they are connected (if applicable).
9 Sampling & samples	Describe how to select a test sample from the sample received in the laboratory. Include storage conditions. Note that the initial selection of samples is generally covered by a separate sampling plan.
10 Calibration	Identify the items of equipment that need to be calibrated. Describe the calibration procedure and the frequency of calibration.
11 Quality control	Describe the quality control procedures required to monitor the performance of the method (see section A4.6).
12 Procedure	Describe the analytical procedure in detail, cross-referencing previous sections (including numbered reagents & equipment).
13 Calculation	Give the equations for calculating the results ensuring that all terms are clearly defined.
14 Special cases	Include any modifications to the method necessitated by the presence or absence of specific components in the samples to be analysed.
15 Reporting procedures	Indicate how results should be reported, including: rounding of numbers; final units; uncertainty; level of confidence.
16 Method validation (Appendix)	Include a summary of the validation (e.g. key performance parameters) and/or cross-reference a separate validation file.
17 Measurement uncertainty (Appendix)	Include a summary of the uncertainty estimate and/or cross-reference a separate file.
18 Bibliography	Any references that give background information on the development and validation of the method.

Changes to methods may occur due to changes in applications or technological advances. Any changes need to be formally implemented and recorded (after appropriate revalidation). A system of document control, including restrictions on unofficial photocopying/printing of methods, is required to ensure that only the current versions of properly authorised test methods are in use (see also section A1.3).

A4.6 Introduction to control charts

Once a method is put into routine use, it is important to continue to monitor its performance to ensure that results are fit for purpose. As mentioned in section A4.2 the aim of quality control is to spot problems and take corrective actions before results are released to the customer.

When a method is used on a regular basis, it is good practice to regularly analyse quality control samples and plot the results on a control chart. A typical control chart (Shewhart chart) is shown in Figure A4.8.

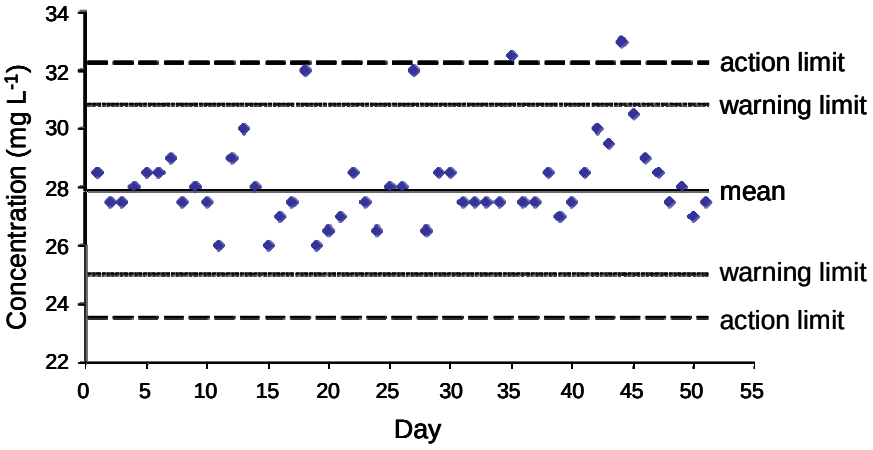


Figure A4.8: Typical Shewhart control chart

Quality control samples should have a similar composition to test samples in terms of the analyte concentration and sample matrix. They must also be homogeneous and stable and be available in sufficient quantities to enable portions of the QC sample to be analysed over an extended time period. QC samples are included with batches of test samples and should be treated in exactly the same way as the test samples.

Plotting the data, as in Figure A4.8, allows you to visualise how the results obtained for the QC sample are varying. You should always expect to see some variation in replicate measurement results due to the effect of random errors (see section A4.8.1). But how do you know when the variation in results is bigger than what would be considered 'normal' or not only due to random errors? 'Warning' and 'action' limits are used to help judge when the variation in measurement results is bigger than expected. These are illustrated in Figure A4.8. The central line is the mean of the first 20 results obtained from the analysis of the QC sample. This becomes the 'target value'. The warning and action limits are used to determine whether individual results differ from this target by an unusually large amount.

- The warning limits are generally set at $2s/\sqrt{n}$ either side of the mean;
- The action limits are set at $3s/\sqrt{n}$ either side of the mean.

where s is the standard deviation and n is the number of measurements of the QC sample used to produce each point on the chart (often $n=1$) (in Figure A4.8 the standard deviation used to set the limits was calculated from the first 20 results obtained from the QC sample and $n=1$).

The limits correspond approximately to the 95% and 99.7% confidence levels, respectively (see section A5.1).

For normally distributed data there is a 5% chance of obtaining a result outside of the warning limit but only a 0.3% chance of obtaining a result outside of the action limits.

If a result falls outside of the action limits, the measurement process should be stopped immediately and the cause of the problem investigated. The procedure for dealing with results which fall outside of the warning limits should be detailed in your laboratory's quality procedures, for example, repeat the measurement and take action if the second result is also outside of the warning limit.

In addition to using the warning and action limits, it is important to look for any trends in the results. If a method is operating correctly, the results would be expected to be randomly scattered about the mean value. A significant drift in data, a step change or a sequence of results all on the same side of the mean could indicate a problem, and the cause should be investigated.

A4.7 Proficiency testing and external quality assessment

As discussed in section A4.6, control charts are used to monitor the performance of methods and spot when things have gone wrong. In addition to the activities that analysts carry out on a daily basis to ensure the quality of their results (sometimes referred to as Internal Quality Control (IQC)), it is useful for laboratories to get an independent check on their performance. This is achieved by participating in *proficiency testing (PT)* schemes, also known as *External Quality Assessment (EQA)*. The aim of PT schemes is to provide an infrastructure for laboratories to monitor and improve the quality of their routine analytical measurements.

The main features of a PT scheme are as follows:

- Homogeneous samples are distributed simultaneously to participants by the scheme organiser (this is often referred to as a 'round' of the PT scheme)
  - samples are representative of the types of samples laboratories analyse routinely;
- Participants analyse the PT samples using a method of their choice and submit their results to the scheme organiser
  - PT samples should be handled in exactly the same way as routine test samples – they shouldn't be given special treatment!
- The scheme organiser carries out a statistical analysis of the results submitted by the participants;
- Each participant receives a score which indicates how they have performed in that round of the scheme (see section A4.7.1);
- Confidentiality is maintained
  - participants are identified by a code number known only to themselves and the scheme organiser;

- reports from the scheme identify participants only by their code numbers so each laboratory can identify their own performance and compare it with the performance of other laboratories without knowing their identities;
- Technical advice is available from the scheme organiser;
- Rounds of the PT scheme are repeated at defined intervals.

As samples are distributed by the PT scheme several times a year, participating in a scheme provides laboratories with a regular snapshot of their performance.

A4.7.1 Scoring systems in PT schemes

A key feature of PT schemes is that participants receive a score which enables them to readily asses their performance in a particular round of the scheme. There are a number of different scoring schemes in use but they generally have these common features:

- The difference between the laboratory's result (x) and a target or 'assigned value' (X) is calculated;
- The difference is compared with a target range such as a standard deviation ($\hat{\sigma}$).

The scheme organiser will set the assigned value and target range; there are a number of different approaches that can be used. The assigned value is the accepted value for the property being measured for a particular PT sample. The target range is often chosen to reflect the variation in results that would be considered acceptable for a particular type of analysis.

When calculating the performance score the participants are effectively being judged on whether their result differs from the assigned value by more than would be considered fit for purpose.

One of the most commonly used scoring systems is the z-score.

$$z = \frac{x - X}{\hat{\sigma}}$$

The z-score is based on the properties of the normal distribution (see section A5.1). In a normal distribution approximately 95% of values are expected to lie within ±2 standard deviations of the mean and 99.7% of values are expected to lie within ±3 standard deviations of the mean. There is therefore only a 0.3% chance of finding a value that is a member of a particular population of data more than three standard deviations from the mean.

Bearing in mind the properties of the normal distribution, you should interpret z-scores as follows:

$\|z\| \leq 2$	acceptable performance
$2 < \|z\| \leq 3$	questionable performance
$\|z\| > 3$	unsatisfactory performance

A plot of z-scores from a round of a PT scheme for the determination of nickel in soil samples by aqua regia extraction is shown in Figure A4.9.

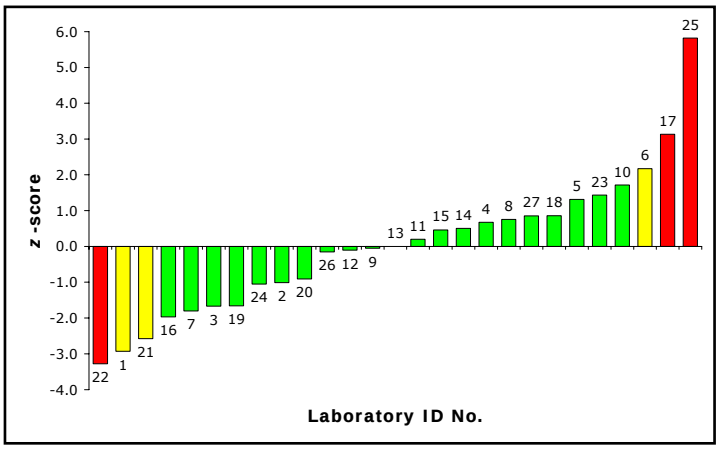


Figure A4.9: Plot of *z*-scores from one round of a PT scheme for the determination of nickel in soils by aqua regia extraction

The results from laboratories 17, 22 and 25 would be considered unsatisfactory as in each case $|z|>3$. The performance of laboratories 1, 6 and 21 is questionable as $2<|z|\leq 3$. The performance of all the other participants is acceptable as $|z|\leq 2$.

A4.7.2 Evaluation of performance scores

What should the laboratory do when it receives its score for a particular round? If the score indicates acceptable performance this generally means that the laboratory's systems are operating correctly for that particular test. The laboratory will need to take action if any of the following occur:

- An unsatisfactory result is obtained (an investigation of the cause of an unsatisfactory result is mandatory for laboratories accredited to ISO/IEC 17025);
- Two consecutive questionable results have been obtained for the same test method;
- Nine consecutive results with the same bias against the assigned value, for the same test method, have been obtained (e.g. nine consecutive negative *z*-scores indicates that the laboratory's results are consistently lower than the assigned value).

Laboratories should view participation in proficiency testing schemes as an educational activity. The whole concept of participation in PT is to learn from the experience, regardless of whether the performance has been unsatisfactory. Positive as well as negative feedback is valuable to laboratory staff.

A4.8 Errors and uncertainty

Whenever you make a measurement there will always be some *uncertainty* or doubt about the result obtained. This is not because you have done anything wrong in your application of the test method; all measurements are subject to errors which will contribute to the uncertainty in the result.

Error is defined as the *difference* between a measurement result and the true value of the property being measured.

Uncertainty is a *range* of values within which the true value is believed to lie.

There are two types of error: random errors and systematic errors. It is the presence of these errors that causes the uncertainty in measurement results. The aim of an uncertainty estimate is to combine the effect of all the sources of error in a measurement process to give a single value which indicates how sure the analyst is about the result reported. Note that in this context 'error' does not mean a mistake. Measurement uncertainty should reflect the variation in results when the method is being operated correctly (i.e. is operating under statistical control) so the effect of mistakes or 'gross errors' is not included in the uncertainty estimate.

A4.8.1 Random errors

Random errors cause results to vary in an unpredictable way from one measurement to the next. They are caused by chance variations in the measurement process that are outside of your control. Random errors are present when any measurement is made but due to their unpredictable nature, they cannot be corrected for. The effect of random errors can be reduced, however, by making more measurements and reporting the mean. The magnitude of the random errors will determine the *precision* of the measurement results (see section A4.3.4.2).

When using volumetric glassware there will always be slight variation in judging the position of the meniscus relative to the graduation marks, and when using pipettes and burettes there will be small variations in the actual amount of liquid delivered. These are examples of random errors which will contribute to the precision of the volume of liquid measured by a particular piece of equipment.

A4.8.2 Systematic errors

Systematic errors cause results to differ from the true value by the same amount (and in the same direction) each time a measurement is made. Systematic errors are not reduced by making repeat measurements as each measurement will be in error by the same amount. In some cases it may be possible to correct results to remove systematic errors. The magnitude (and direction) of systematic errors will determine the *bias* in the measurement results (see section A4.3.4.2).

For example, the manufacturing tolerance permitted for an item of volumetric glassware (see section A3.1.3) means that a volumetric flask may not contain the stated volume of liquid, even when it is filled exactly to calibration line. The tolerance for a Class A 250 mL volumetric flask is 0.15 mL. A particular flask may contain, for example, 250.1 mL when it is filled to the calibration mark (ignoring the effect of any random errors). This will introduce a small systematic error as the flask will always contain 0.1 mL more than the nominal value when it is filled exactly to the calibration mark.

A4.8.3 Evaluating measurement uncertainty

The examples in sections A4.8.1 and A4.8.2 illustrate that there will nearly always be more than one source of error that will influence measurement results. The aim when evaluating measurement uncertainty is to combine the effects of all of the errors into a single value which can be quoted with the measurement result. The uncertainty represents the range within which the analyst believes the true value lies, with a specified level of confidence.

For example, a titration experiment is carried out to determine the concentration of a solution of hydrochloric acid. The analyst reports the result as 0.102 ± 0.0016 mol L^{-1}.

The actual concentration of the acid is therefore somewhere between 0.1004 and 0.1036 mol L^{-1}.

A measurement result has three components:

- the result obtained from the measurement;
- the units of measurement;
- an estimate of the uncertainty in the measurement result.

How is the uncertainty estimate obtained? Even for a relatively simple experiment, such as a titration to determine the concentration of a hydrochloric acid solution, there are a number of factors which will influence the result obtained. Some examples are listed in Table A4.3 (see section A3.6 for information on the procedure for carrying out a titration).

Table A4.3: Sources of uncertainty for the determination of the concentration of HCl by titration with NaOH which has been standardised against a potassium hydrogen phthalate (KHP) primary standard

Operation	Source of uncertainty
Preparing KHP standard solution for standardisation of NaOH	
Weighing out KHP	Purity of KHP Calibration of balance Linearity of balance Precision of weighing
Making solution up to volume	Calibration of volumetric flask Precision of filling the flask to the calibration mark Effect of laboratory temperature differing from flask calibration temperature
Standardisation of NaOH by titration against KHP standard solution	
Volume of standard solution used	Calibration of the pipette Precision of filling the pipette to the calibration mark and emptying the pipette Effect of laboratory temperature differing from pipette calibration temperature
Titre volume of NaOH	Calibration of the burette Readability of the burette (judging the position of the meniscus in relation to the calibration marks) Precision of judging the end-point
Titration of HCl with standardised NaOH	
Volume of HCl analysed	Calibration of the pipette Precision of filling the pipette to the calibration mark and emptying the pipette Effect of laboratory temperature differing from pipette calibration temperature
Titre volume of NaOH	Calibration of the burette Readability of the burette (judging the position of the meniscus in relation to the calibration marks) Precision of judging the end-point

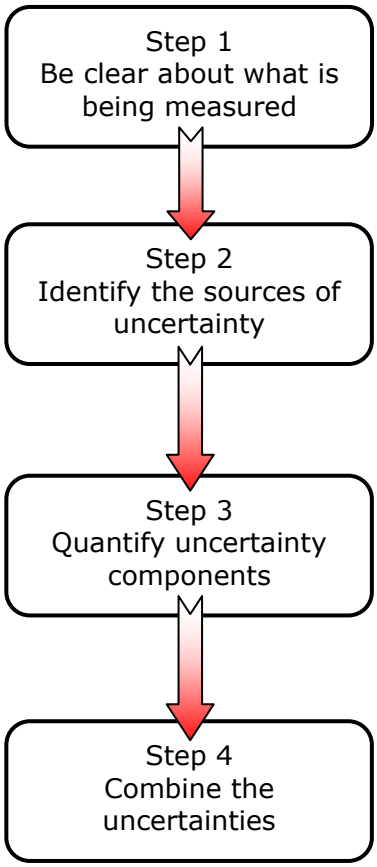
An estimate of the uncertainty in the concentration of the acid solution should include a contribution from all of the sources of uncertainty listed in Table A4.3.

The general sources of uncertainty that affect the results from the analysis of test samples are shown in Table A4.4.

Table A4.4: Examples of sources of uncertainty

Source	Examples
Analyst	Small differences in application of the method
Sample	Homogeneity and stability Matrix – interferences and influence of the matrix on analyte recovery
Test method	Sample pre-treatment – drying, grinding, blending Analyte extraction from sample – extraction conditions, incomplete recovery Sample clean-up – possible loss of analyte Measurement of the analyte concentration – instrument effects and calibration
Laboratory	Environmental conditions
Computational effects	Calibration model – uncertainty associated with predicted values obtained from a calibration curve
Random effects	Always present for all stages of the method – variation in extraction efficiency, variation in filling volumetric glassware to the calibration mark, variation in instrument response

The general procedure for evaluating uncertainty is shown in Figure A4.10.



At the beginning of the process of evaluating uncertainty it is important to be clear about what is being measured. Write down the equation that will be used to calculate the result. The parameters in the equation (masses, volumes, instrument response, etc) will contribute to the uncertainty in the measurement result. (Step 1)

A critical step in evaluating uncertainty is compiling a list of all the possible sources of uncertainty. This will include the sources that contribute to the parameters identified in step 1, but there are likely to be other sources of uncertainty as well. It is useful to consider each stage of the test method in turn and think about the things which could cause results to vary. These will be sources of uncertainty. (Step 2)

The next step is to estimate the size of each uncertainty component identified in step 2. This can be done by using published data, or method performance data from validation studies or ongoing quality control. Where such data are not available additional experimental studies will be required. It is often possible to plan experiments that can account for a number of different sources of uncertainty. (Step 3)

Step 3 will result in a list of a number of sources of uncertainty together with an estimate of their magnitude. This is often referred to as the 'uncertainty budget'. The individual contributions must be expressed as standard deviations and are combined using mathematical rules for the combination of variances. This results in a combined uncertainty estimate for the result of the measurement. This is known as the combined standard uncertainty. (Step 4)

Figure A4.10: Steps in the uncertainty evaluation process

The basic mathematical rule for combining uncertainties is shown in Figure A4.11. Standard uncertainties are denoted by the symbol u.

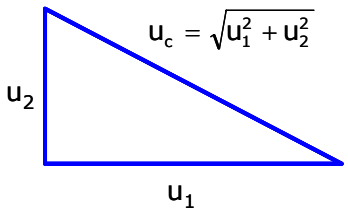


Figure A4.11: Combining standard uncertainties

Standard uncertainties are combined using the mathematical rule for combining variances. This is sometimes referred to as the 'root sum of squares' rule and is illustrated in Figure A4.11. u_1 and u_2 are independent uncertainty components, expressed as standard deviations and u_c is the combined uncertainty. Due to the way the standard uncertainties are combined, if u_1 is much greater than u_2 then u_c will be approximately equal to u_1.

To increase the level of confidence that the uncertainty quoted includes the true value, the combined standard uncertainty is usually *expanded* to include 95% of values. This is achieved by multiplying the combined standard uncertainty by a *coverage factor, k*. To obtain a confidence level of approximately 95%, a coverage factor of 2 is generally used. Expanded uncertainties are denoted by the symbol U.

A4.8.4 Why is measurement uncertainty important?

Without knowledge of measurement uncertainty it is difficult to make a meaningful interpretation of a measurement result. If you do not know the reliability of results, how can you tell whether the results produced by different laboratories are genuinely different or whether a legislative limit has definitely been exceeded? Measurement uncertainty allows you to judge whether results are likely to be fit for a particular purpose. In some cases a relatively large uncertainty may be acceptable, while in others this would be totally unacceptable.

A5 Data handling and reporting results

The preceding sections of this handbook have outlined the steps you should take when working in the laboratory to help ensure that the results you produce are valid and fit for purpose. Once you have completed the practical aspects of the analysis of your test samples manipulation of the analytical data is often required. Finally you have to decide how to report your results. Even if the analytical work has been carried out perfectly, problems will arise if mistakes are made in calculations, if data are incorrectly transcribed or if results are reported in the wrong units. This section covers:

- Essential statistical terms;
- Units of measurement;
- Reporting results.

A5.1 Essential statistical terms

If you carry out replicate analyses of a test sample it is useful to be able to summarise the results and obtain information about the *distribution* of the results. The information you may require includes:

- An estimate of the true value – usually the average of the results;
- An estimate of the spread of the results;
- An indication of how estimates of the true value might vary from one experiment to the next.

In analytical science, where measurements are subject to several different effects (environment, reagent variation, instrument 'noise' etc.), the results will generally follow a *normal* distribution (also known as a *Gaussian* distribution) as shown in Figure A5.1. Most of the results are clustered around the central value with a decreasing number appearing at a greater distance from the centre (in the 'tails' of the distribution). Note that the distribution has an infinite range so it is possible, although not very likely, for results to appear at great distances from the centre of the distribution.

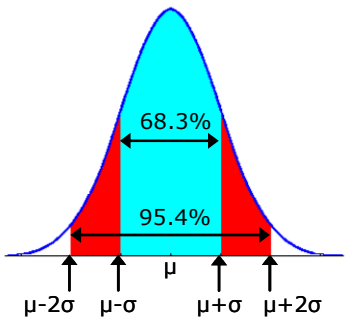


Figure A5.1: The normal distribution

The distribution can be characterised by its central value (the mean, μ) and its dispersion or spread (the standard deviation, σ). For any normal distribution, approximately 68% of the population values lie within ±1 standard deviation of the mean; approximately 95% of the population values lie within ±2 standard deviations of the mean; and approximately 99.7% of the population values lie within ±3 standard deviations of the mean (see Figure A5.1).

When carrying out analysis in the laboratory, you generally make a relatively small number of measurements and use the information obtained to estimate the properties of the underlying population of data from which the measurement results are drawn. The Greek letters μ and σ used in Figure A5.1 refer to the properties of the population (its mean and standard deviation, respectively). The corresponding parameters for a sample of data are $\overline{x}$ (the sample mean) and *s* (the sample standard deviation).

Mean

The mean is the sum of all the measurement results (x_i) divided by the number of results (n):

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Sample standard deviation

Indicates the extent to which results differ from each other:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \overline{x})^2}{n-1}}$$

The ***variance*** is the square of the standard deviation.

Relative standard deviation

A measure of the spread of data in comparison to the mean of the data:

$$\text{RSD} = \frac{s}{\overline{x}}$$

Coefficient of variation

The relative standard deviation expressed as a percentage:

$$\text{CV} = \text{RSD\%} = \frac{s}{\overline{x}} \times 100$$

Standard deviation of the mean

The variation in estimates of the mean value (contrast with *s* which represents the variation in *single* results):

$$s(\overline{x}) = s / \sqrt{n}$$

Confidence interval for the mean

The range of values within which the true mean is likely to lie, at a stated level of confidence:

$$\bar{x} \pm t \times \frac{s}{\sqrt{n}}$$

t is the Student t-value (obtained from statistical tables). The value will depend on n-1 (the degrees of freedom) and the required level of confidence. For a 95% confidence interval when n is relatively large (say ten or more) t is approximately equal to 2.

Degrees of freedom (ν)

A measure of the number of independent pieces of data that have been used to evaluate a particular parameter. In general the degrees of freedom is the number of data points (n) less the number of parameters already estimated from the data. In the case of the sample standard deviation, for example, the degrees of freedom is equal to n-1 as the mean, $\bar{x}$, has to be calculated from the data as part of the calculation of the standard deviation.

A5.2 Units of measurement

In Chapter A3 you will have encountered a number of different units of measurement used to express the concentration of an element or a compound in a standard or reagent solution. The same units are used to express the concentration of the analyte in a sample.

For any particular sample you could report the actual amount of the analyte present (in grams or moles, for example). However, this is not generally very useful as another portion of the sample may have a slightly different mass (or volume) and therefore contain a slightly different amount of the analyte. The amount of analyte present is therefore nearly always expressed in terms of a fixed mass (or volume) of the sample. The units commonly used are described in the following sections.

To avoid misinterpretation of data it is essential that results are reported with the correct units. The units used should be unambiguous.

A5.2.1 Concentration expressed as a percentage

Do not report concentrations as a percentage without specifying whether the results are in terms of:

- Percent mass by mass (%m/m) (also referred to as weight by weight (%w/w) or wt%) – number of grams of analyte in 100 g of sample;
- Percent mass by volume (%m/v) (also referred to as weight by volume (%w/v)) – number of grams of analyte in 100 mL of sample;
- Percent volume by volume (%v/v) – number of mL of analyte in 100 mL of sample.

In food analysis, results are often reported in terms of grams per 100 grams of sample which is abbreviated as g/100 g or g $(100\ g)^{-1}$).

A5.2.2 Concentration expressed as m / m or m / v

The concentration of the analyte in a test sample is frequently reported in terms of grams per kilogram (abbreviated g/kg or g kg^{-1}) or grams per litre (abbreviated g/L or g L^{-1}). In food analysis, results are often reported in terms of grams per 100 grams of sample (abbreviated g/100 g or g $(100\ g)^{-1}$).

When analysing samples you may be dealing with low concentrations of the analyte. In such cases, it is not convenient to use grams to express the amount of analyte present as the numbers get very small. Results are therefore expressed in terms of, for example, milligrams per litre (mg L^{-1}) or micrograms per kilogram (µg kg^{-1}). Table A5.1 gives some common prefixes used for SI units.

Table A5.1: Prefixes for SI units

Prefix	Symbol	Fraction	Examples
deci	d	10^{-1}	decimetre (dm^3 = 1000 mL = 1 L)
centi	c	10^{-2}	centimetre (cm)
milli	m	10^{-3}	milligram (mg = 0.001 g) millilitre (mL = 0.001 L)
micro	µ	10^{-6}	microgram (µg = 0.000 001 g) microlitre (µL = 0.000 001 L = 0.001 mL)
nano	n	10^{-9}	nanogram (ng = 0.000 000 001 g)
pico	p	10^{-12}	picogram (pg = 0.000 000 000 001 g)
femto	f	10^{-15}	femtogram (fg = 0.000 000 000 000 001 g)

Results can also be expressed in term of the number of moles of the analyte present (see section A3.4.2.1), e.g. mol L^{-1}. The procedure for converting between moles and units of mass is discussed in section A3.4.2.1.

A5.2.3 Concentration expressed as parts per million or parts per billion

The use of parts per million (ppm) or parts per billion (ppb) is discouraged as it can cause confusion. Without further explanation you do not know whether the results are being reported on a mass by mass basis or mass by volume. It is better to use the units described in section A5.2.2.

1 ppm is equivalent to 1 mg kg^{-1} or 1 mg L^{-1}
1 ppb is equivalent to 1 µg kg^{-1} or 1 µg L^{-1}

Figure A5.2 illustrates some of the different units that are used. In each example one lump of sugar (6 g) is dissolved in the amount of water specified.

teapot  0.6 L	1% = 1 per cent	10 g L^{-1}	10 mg mL^{-1}
bucket  6 L	0.1% = 1 per thousand	1 g L^{-1}	1 mg mL^{-1}
Tanker lorry  6000 L	1 ppm = 1 part per million	1 mg L^{-1}	1 µg mL^{-1}
super-tanker  6 million L	1 ppb = 1 part per billion	1 µg L^{-1}	1 ng mL^{-1}
reservoir  6 billion L	1 ppt = 1 part per trillion	1 ng L^{-1}	1 pg mL^{-1}
bay  6 trillion L	1 ppq = 1 part per quadrillion	1 pg L^{-1}	1 fg mL^{-1}

Figure A5.2: Units of measurement – one lump of sugar (6 g) dissolved in differing volumes of water

A5.3 Reporting results

The previous section discussed the different units of measurement that are used to report the concentration of the analyte in the test sample. In addition to using appropriate units you will also need to decide how to report the actual value. When a reading is obtained from an instrument, or data are manipulated using a calculator or a spreadsheet, you may obtain results with a large number of decimal places. A calculator may give an answer such as 3.239 871 532 but in the vast majority of cases it would not be sensible to report all of the digits after the decimal point. How do you decide how many digits to report and, once you have decided, how should you 'round' the result?

A5.3.1 Decimal places and significant figures

There is often some confusion as to whether results should be rounded according to the number of digits after the decimal point (the number of decimal places) or rounded to a specified number of significant figures. It is important to be clear about the difference between 'decimal places' and 'significant figures' as rounding a number to two decimal places, for example, will not necessarily give the same result as rounding to two significant figures.

The concept of *decimal places* is straightforward – it is simply the number of digits after the decimal point. So 3 mg kg^{-1} is reported with no decimal places, 3.1 mg kg^{-1} is reported with one decimal place, 3.10 mg kg^{-1} is reported with two decimal places, and so on.

When dealing with *significant figures*, there are two types of number we need to consider:

- Numbers with a decimal part indicated (i.e. of the form: 12.345);
- Numbers with no decimal part indicated (i.e. of the form: 1234).

Numbers with a decimal part

Significant figures rule 1:	All non-zero digits (1-9) are, by definition, significant figures.
Significant figures rule 2:	Zeros having a non-zero digit anywhere to their LEFT are significant figures.
Significant figures rule 3:	All other zeros not covered in rule 2 are NOT significant figures.

Examples

Example 1: 0.007 100 0 µg L^{-1}

The '7' and '1' are significant (Rule 1), but what about the zeros? The last three zeros would all be considered significant, since each of them has a non-zero digit (two in fact) somewhere to their left (Rule 2). The first three zeros however are not significant, since they do not have a non-zero digit anywhere to their left (Rule 3).

The number therefore has a total of five significant figures (but seven decimal places).

The non-significant zeros in this example are simply 'place holders'; they are necessary in order to fix the position of the decimal point.

Example 2: 120.003 40 mg kg^{-1}

The digits 1, 2, 3, and 4 are all considered significant (Rule 1). All the zeros are considered significant since each of them has non-zero digits somewhere to their left (Rule 2). There are therefore a total of eight significant figures (but five decimal places).

Numbers without a decimal part

Rule 1 still applies. However, for measurement values such as 1200 or 10000 there is an ambiguity about whether the zeros are significant or not. Consider, for example, the measurement value 1200. This can be interpreted in three ways as follows:

1. The measurement instrument was capable of reading to the nearest 100 units. The 1 is certain but there is uncertainty about the 2, as the reading displayed by the instrument will have been rounded to the nearest 100 units. The last two zeros have been inserted as place holders for the tens and unit positions.

   In this case there are two significant figures.

2. The measurement instrument was capable of reading to the nearest 10 units. The 1 and the 2 are certain but there is uncertainty about the first zero, as the reading

displayed by the instrument will have been rounded to the nearest 10. The last zero has been inserted as a placeholder for the units position.

In this case there are three significant figures.

3. The measurement instrument was capable of reading to the nearest 1 unit. The 1, 2 and first zero are certain but there is uncertainty about the second zero as the reading displayed by the instrument will have been rounded to the nearest unit.

   In this case there are four significant figures.

Without further information it is impossible to know which scenario is correct. When interpreting significant figures (and deciding the number of significant figures that are appropriate) you therefore need to know about the capabilities of the measuring device used. This is discussed in section A5.3.3.

A5.3.2 Rounding rules

When rounding off numbers it is important to be consistent and to avoid introducing bias. The following rules implement the standard convention and are easy to remember.

Locate the position of the last significant digit you wish to retain. To make them easier to see, it can be helpful to enclose the remaining, insignificant, digits in brackets.

Look at the insignificant digits and think of them as a fraction (e.g. 678 becomes 678/1000; 27 becomes 27/100, and so forth).

Rounding Rule 1: If the fraction is less than 0.5, then round down.

Rounding Rule 2: If the fraction is greater than 0.5, round up.

Rounding Rule 3: If the fraction is exactly 0.5 and the preceding significant figure is even, round down; if it is odd, round up.

Examples

Example 1: Round 12.3456 to three significant figures.

The three significant figures are 12.3. We can place the insignificant figures in brackets to highlight them: 12.3(456).

Now 456, as a fraction, is 456/1000 = 0.456
Since 0.456 is less than 0.5, we round down and the required result is 12.3.

Example 2: Round 12.3456 to four significant figures.

The four significant figures are 12.34. Placing the insignificant figures in brackets, we get 12.34(56).

Here, 56 as a fraction is 0.56 which is greater than 0.5 so we round up to get 12.35.

Example 3: Round 12.3450 to four significant figures.

The four significant figures are 12.34. Placing the insignificant figures in brackets gives 12.34(50).

This time 50 as a fraction is exactly 0.5.

Since the last significant figure is even we leave this as it is and get 12.34.

Example 4: Round 12.3750 to four significant figures.

Placing the insignificant figures in brackets gives 12.37(50).

Again, 50 as a fraction is exactly 0.5.

Since the last significant figure is odd we round up to get 12.38.

Remember: You should only round results at the end of a series of calculations. For intermediate calculations retain as many decimal places as possible. Rounding values too early in a sequence of calculations can introduce errors.

A5.3.3 Deciding the number of significant figures to report

When reporting the result obtained from a measuring device you should report all the figures you are certain about plus the first figure which is uncertain. Consider the situation shown in Figure A5.3.

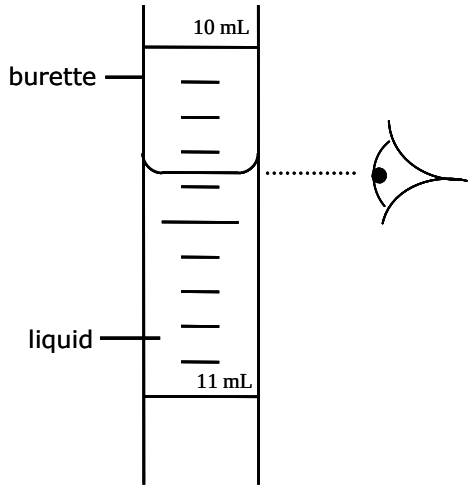


Figure A5.3: Estimating the volume reading shown by a burette

If you had to read the burette shown in Figure A5.3 you would be certain that the volume is somewhere between 10.3 mL and 10.4 mL. However, you would need to estimate the second decimal place. You might record the reading as 10.36 mL but a colleague might record 10.35 mL. There is therefore uncertainty in the fourth figure. The burette reading can therefore be recorded to four significant figures - all the digits in a number that are known with certainty (i.e. 10.3) plus the first that is uncertain.

There are also a couple of useful rules to remember when deciding how many significant figures to report when values have been added or subtracted, or multiplied or divided.

When adding or subtracting values: The result should have the same number of decimal places as the input value with the least number of *decimal places*.

When multiplying or dividing values: The result should have the same number of *significant figures* as the input value with the least number of significant figures.

Examples

Example 1: 873.123 + 37.9 = 911.023
The result should be reported as 911.0 (i.e. rounded to one decimal place)

Example 2: $1234.5 \times 3.142 = 3878.799$

1234.5 has five significant figures, 3.142 has four significant figures. The result should therefore be reported as 3879 (rounded to four significant figures).

Reporting means, standard deviations and relative standard deviations

When reporting the mean, standard deviation and relative standard deviation of a set of results, a judgement needs to be made about a sensible number of figures to report. As mentioned in section A5.1, a sample standard deviation is an estimate of the standard deviation of the entire population. Sample standard deviations are therefore inherently variable, and the fewer the number of data points used to estimate the standard deviation, the more variable the estimate will be. For this reason, standard deviations are generally reported to not more than two significant figures. The uncertainty in any additional digits will be so great as to make them meaningless. Therefore, when considering how to report summaries of data, you should start with the standard deviation. Once this has been rounded to two significant figures (using the rules given in section A5.3.2), the reporting of the mean and relative standard deviation can be considered. These should be rounded to be consistent with the standard deviation. The relative standard deviation – calculated from the unrounded standard deviation and the unrounded mean – is reported to the same number of *significant figures* as the rounded standard deviation. Obviously it would be unreasonable to quote the mean to five decimal places say, if the standard deviation indicates that there is actually variability in the data in the first decimal place. The AOAC[6] recommends that the mean is rounded to the same number of *decimal places* as the standard deviation. It should be noted though that a problem can arise with this policy when data having a low dispersion (very low standard deviation) are encountered. To allow for this case, the following procedure is recommended:

Step 1
Round the mean so that it contains no more decimal places than the measurement observation having the least number of decimal places.

Step 2a
If the rounded mean (from step 1) contains the same or fewer decimal places than the rounded standard deviation then do nothing further.

Step 2b
If the rounded mean (from step 1) contains more decimal places than the rounded standard deviation then reject it and, instead, round the mean so that it contains the same number of decimal places as the rounded standard deviation.

The ASTM[7] also provides useful guidance on how to arrive at an appropriate number of significant figures for test results. The ASTM provides the rule that results should be rounded to not greater than $0.5s$ and not less than $0.05s$ (where s is the standard deviation).

[6] AOAC – Association of Official Analytical Chemists
[7] American Society for Testing and Materials

Example

10 replicate measurements have been carried out to determine the concentration of cholesterol in a sample of oil. The results are shown in Table A5.2. The mean, standard deviation and relative standard deviation shown are the values obtained using a statistical calculator.

Table A5.2: Results from the determination of cholesterol in a sample of oil

Cholesterol concentration (mg (100 g)$^{-1}$)	
271.4	272.5
266.3	269.5
267.8	270.1
269.6	269.7
268.7	268.6
mean	269.42
sample standard deviation	1.749 158 528
relative standard deviation	0.006 492 311

Following the AOAC rules:

The individual results are all reported to one decimal place. The mean is therefore rounded to one decimal place: 269.4 mg (100 g)$^{-1}$.

The sample standard deviation is rounded to two significant figures: 1.7 mg (100 g)$^{-1}$. This has the same number of decimal places as the rounded mean so no further treatment of the mean value is required.

The relative standard deviation is also rounded to two significant figures: 0.0065.

Reporting measurement uncertainty

When an estimate of measurement uncertainty has been obtained, as described in A4.8.3, it should be reported with a sensible number of significant figures. In general, uncertainty estimates should be reported with no more than two significant figures. The result to which the uncertainty applies is rounded to be consistent with the quoted uncertainty. For example, if the standard uncertainty in the concentration of a solution of cadmium in acid has been estimated as ±0.8 mg L^{-1}, the concentration of the solution should be quoted to no more than one decimal place.

A5.3.4 Checking data

Before results are reported to the customer it is good practice to have your calculations checked by a colleague. Errors can creep in if data are transcribed incorrectly, the wrong units are used or mistakes are made in the calculations (e.g. forgetting that a sample was diluted prior to analysis). Spreadsheets should be checked to make sure that the calculations are correct (e.g. that the correct cell references are being used) and that data have been entered/imported correctly.

A5.4 Questions

The data shown in Table A5.3 were obtained from the replicate analysis of a soil sample for total molybdenum.

Table A5.3: Results from the determination of molybdenum in a sample of soil

Molybdenum concentration (mg kg^{-1})	
33.92	34.70
33.20	34.63
35.66	34.70
34.28	35.06
34.55	34.74
34.82	

i) Calculate the mean, standard deviation, relative standard deviation, standard deviation of the mean and number of degrees of freedom for the data in Table A5.3.

ii) Round the mean, standard deviation, relative standard deviation and standard deviation of the mean to an appropriate number of digits.

Part B

Information for the supervisor

Part B: Information for the supervisor

To be able to work effectively in the laboratory all analysts need to be competent in a set of key laboratory skills. These skills underpin all analyses, regardless of the instrumentation that is used to make the final measurement of the amount of analyte present. Even the most sophisticated instruments will require calibration, which generally relies on an analyst being able to prepare a set of calibration standards accurately. Part A of this guide is aimed at the analyst and describes how to carry out a range of laboratory operations correctly and safely. Part A also introduces some of the key 'background' topics that analysts should be aware of to enable them to work safely and produce reliable results that are fit for purpose.

Part B of the guide is aimed at the supervisor/trainer. It provides check lists of things that analysts should be aware of if they are to carry out the various laboratory operations correctly. It also includes suggestions as to how competence can be assessed and summarises 'points to look for' that might indicate that the analyst needs retraining.

B1 Accreditation requirements

The laboratory accreditation standards ISO/IEC 17025[8] and ISO 15189[9] place significant emphasis on ensuring that staff are fully trained and on demonstrating their competence to carry out a particular task. ISO/IEC 17025 requires that:

- 'The laboratory management shall ensure the competence of all who operate specific equipment, perform tests and/or calibrations……Persons performing specific tasks shall be qualified on the basis of appropriate education, training, experience and/or demonstrated skills, as required.' (Clause 5.2.1)
- 'The management of the laboratory shall formulate goals with respect to the education, training and skills of the laboratory personnel……The effectiveness of the training actions taken shall be evaluated.' (Clause 5.2.2)
- 'The laboratory shall maintain records of the relevant ….competence, educational and professional qualifications, training, skills and experience of all technical personnel.' (Clause 5.2.5)

ISO 15189 requires that:

- 'Laboratory management shall maintain records of the relevant educational and professional qualifications, training and experience, and competence of all personnel.' (Clause 5.1.2)
- 'Personnel shall have training specific to quality assurance and quality management for services offered.' (Clause 5.1.6)
- 'The competency of each person to perform assigned tasks shall be assessed following training and periodically thereafter.' (Clause 5.1.11)

In addition, ISO 9001[10] (which is a normative reference in ISO 15189) has the following requirements in Clause 6.2.2:

- The organisation shall:
  - determine the necessary competence for personnel performing work affecting product quality,
  - provide training or take other actions to satisfy these needs,

[8] ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories
[9] ISO 15189:2003 Medical laboratories – Particular requirements for quality and competence
[10] ISO 9001:2000 Quality management systems

- evaluate the effectiveness of the actions taken,
- maintain appropriate records of education, training, skills and experience.

It is therefore essential that effective training programmes are put into place and that achievements against appropriate performance targets are documented.

B2 General approach to assessing competence

Assessing the competence of an analyst to carry out a particular task requires a judgement as to whether the performance produces results that are fit for purpose. Fitness for purpose ultimately relates to the uncertainty that is acceptable in the results produced. There are a number of different ways that performance criteria which are used to assess competence can be set. Examples include:

- For individual items of equipment refer to the manufacturer's specification;
- Use quality control logs for equipment used by experienced operators to determine the typical acceptable variability in results;
- Use the warning and action limits established for quality control charts;
- Use data from method validation studies to establish acceptable precision and bias for test results.

Before the analyst is trained in carrying out particular test methods, competence in carrying out key laboratory skills should be assessed. These skills underpin the successful execution of virtually all analyses carried out in the laboratory. Part A of this handbook summarises best practice for carrying out a number of key laboratory tasks. Suggestions for how to assess competence in carrying out the activities are given in section B3.

Once the analyst is proficient in the core skills their ability to use more sophisticated pieces of equipment and to carry out particular test methods needs to be assessed. For competence to be assessed the trainee needs to carry out an operation a number of times. Both the precision and bias of results should be assessed; the latter requires the analysis of a sample of known composition. In general, at least six results should be obtained to give a reasonable estimate of precision and bias. The types of materials that could be analysed as part of a training programme include:

- Certified reference materials;
- Quality control materials/reference materials produced in-house;
- Samples previously analysed by an experienced analyst;
- Samples from proficiency testing/external quality assessment schemes.

Training should always be documented and the targets against which performance has been assessed should be recorded (this is essential for accredited laboratories). See Appendix 3 for a template of a laboratory skills training record.

B3 Key laboratory skills

B3.1 Measuring volume

B3.1.1 Key learning points for measuring volume

The analyst should:

- Be able to identify different items of equipment used for measuring volume;
- Understand the relative accuracies of volume measurements made with different items of equipment;
- Understand the difference between Class A, Class B and individually certified glassware;
- Understand the difference between calibrating an item and verifying that the correct volume is being measured by the item;
- Be able to select a suitable item of equipment for a particular application;
- Know how to use different items of equipment correctly (see below);
- Know that the liquid being measured should be at a temperature close to the calibration temperature of the item;
- Know how to clean and store glassware.

When using a burette the analyst should:

- Know how to check that the burette is clean and undamaged, paying particular attention to the jet and tap;
- Remember to check that the graduation marks are clear and unfaded;
- Remember to make sure that the solution is at ambient temperature before filling the burette;
- Never fill a burette with it held or clamped above eye level;
- Know how to clamp a burette correctly, ensuring that it is vertical;
- Know how to read the burette correctly (ensuring that the meniscus is read at eye level);
- Record readings with an appropriate number of significant figures;
- Know how to operate the tap correctly;
- Know how to check whether the burette is delivering the correct volume of liquid;
- Remember to rinse the burette with the solution to be delivered before use;
- Know how to remove air bubbles from the jet and body of the burette;
- Remember to remove the funnel from the top of the burette before dispensing the liquid;
- Know how to clean and store burettes correctly.

When using glass pipettes the analyst should:

- Be able to identify the different types of pipette (e.g. bulb and different types of graduated pipettes);
- Understand the relative accuracies of the different types of pipette;
- Be able to select a suitable combination of pipettes to deliver the required volume as accurately as possible;

- Know how to check that the pipette is clean and undamaged, paying particular attention to the tip;
- Know how to handle pipettes correctly;
- Know how to fit and use a pipette filler;
- Know that liquids should never be pipetted by mouth;
- Know that the liquid to be pipetted should always be transferred to a clean dry beaker or conical flask – never pipette from the stock container;
- Remember to make sure that the liquid to be pipetted is at ambient temperature;
- Remember to rinse the pipette with distilled water followed by the liquid to be pipetted before use;
- Remember to ensure that the tip of the pipette remains below the level of the liquid as it is being filled;
- Know how to line up the meniscus of the liquid with the calibration mark;
- Remember to check for air bubbles when the pipette has been filled;
- Remember to wipe the outside of the pipette after filling and before dispensing the liquid;
- Know how to dispense the liquid correctly (with the pipette tip touching the inside of the receiving vessel at an angle);
- Know that the liquid should be allowed to drain under gravity and not 'forced' from the pipette;
- Know that the liquid contained in the tip should not be dispensed unless the pipette is marked 'blow-out';
- Remember to check that the pipette does not drip when filled;
- Know how to check the length of time it takes to drain the liquid from the pipette;
- Know how to check the volume delivered by a pipette;
- Know how to clean and store pipettes.

When using automatic pipettes the analyst should:

- Be aware of the different types of automatic pipette available in the laboratory;
- Be aware of the accuracy with which different volumes of liquid can be delivered;
- Be able to select a suitable automatic pipette for a particular application, taking into account the volume of liquid to be measured and the nature of the liquid;
- Be able to select a suitable pipette tip and know how to fit it to the pipette;
- Know how to select the volume when using a variable volume pipette;
- Remember to pre-wet a new tip with the liquid to be dispensed;
- Know how to draw the liquid into the tip, making sure that the pipette is vertical and the tip remains below the level of the liquid while it is being filled;
- Know how to prevent liquid being drawn into the body of the pipette;
- Remember to remove excess liquid from the outside of the tip after filling and before dispensing the liquid;
- Know how to dispense the liquid correctly (with the pipette tip touching the inside of the receiving vessel at an angle);
- Know how to check the volume delivered by a pipette;
- Know how to clean and store automatic pipettes.

When using volumetric flasks the analyst should:

- Remember to check that the flask is clean and undamaged;

- Remember to check that the liquid is at ambient temperature before filling the flask to the calibration mark;
- Know how to judge the position of the meniscus against the calibration mark;
- Know how check the accuracy of the volume of liquid contained in a flask.

B3.1.2 Assessing competence in using volumetric glassware

Competence in using volumetric glassware can be assessed by carrying out a series of 'fill and weigh' experiments. This will assess the precision with which the item is being used and also determine whether there is a significant difference between the observed volume and the stated volume of the item of equipment. You should also observe the trainee using the equipment to check that best practice is being followed. The procedures for the fill and weigh experiments are outlined below.

Volumetric flasks

1. Select a clean dry flask;
2. Record the temperature of the deionised water that will be used to fill the flask;
3. Select a suitable balance and weigh the flask;
4. Following the correct procedure (see Table A3.9), fill the flask with deionised water to the calibration mark;
5. Make sure the outside of the flask is dry;
6. Reweigh the flask;
7. Calculate the mass of the water contained in the flask and convert from mass to volume by dividing by the density of water at the appropriate temperature (see Table A3.4);
8. Empty the flask and make sure that it is completely dry;
9. Repeat steps 2 to 8 until at least six results have been obtained;
10. Calculate the mean and standard deviation of the volumes obtained.

Glass and automatic pipettes

1. For glass pipettes, make sure the item being used is clean and dry;
2. Select a suitable balance and a suitable stoppered weighing container;
3. Weigh the stoppered container;
4. Record the temperature of the deionised water to be dispensed;
5. Following the correct procedure, fill the pipette with the deionised water (see Table A3.7 and Table A3.8);
6. Following the correct procedure dispense the water into the weighed container;
7. Stopper the container and reweigh;
8. Calculate the mass of the water in the container and convert from mass to volume by dividing by the density of water at the appropriate temperature (see Table A3.4);
9. Repeat steps 3 to 8 until at least six results have been obtained;
10. Calculate the mean and standard deviation of the volumes obtained.

Burettes

1. Ensure that the burette being used is clean and dry;
2. Select a suitable balance and a suitable stoppered conical flask;
3. Set up the burette correctly and fill with deionised water;
4. Run the water from the burette until the meniscus is aligned with a calibration mark;

5. Weigh the stoppered conical flask;
6. Record the temperature of the deionised water to be dispensed;
7. Following the correct procedure (see Table A3.6), deliver a specified volume of water (e.g. 10 mL) into the conical flask;
8. Reweigh the conical flask;
9. Calculate the mass of the water contained in the flask and convert from mass to volume by dividing by the density of water at the appropriate temperature (see Table A3.4) ;
10. Repeat steps 5 to 10 until at least six results have been obtained;
11. Calculate the mean and standard deviation of the volumes obtained.

B3.1.3 Observations indicating that retraining may be required

- Damaged glassware being used (e.g. pipettes with chipped tips, glassware with faded graduation marks);
- Evidence that glassware has not been cleaned properly (e.g. droplets stuck to the interior surface);
- Using pipette fillers incorrectly;
- Liquid inside the pipette filler;
- Using pipettes or burettes with air bubbles present;
- Pipetting directly from the stock bottle;
- Pipetting solutions that are not at ambient temperature;
- Forcing the last drop of liquid from the tip of a bulb pipette;
- Forgetting to dry the outside of a pipette before dispensing the liquid;
- Drying volumetric glassware in an oven;
- Filling a burette with it clamped or held above eye level;
- Forgetting to remove the funnel from the top of the burette after filling;
- Using a burette with a leaking tap;
- Reading the meniscus incorrectly (i.e. reading the meniscus from above or below rather than at eye level);
- Liquid present in the barrel of an automatic pipette;
- Wrong tip fitted to an automatic pipette;
- Pipettes held an angle while liquid is aspirated;
- Pipettes left lying on the bench rather than being stored correctly;
- Using inappropriate equipment to deliver the required volume (e.g. using a graduated pipette or automatic pipette with too large a capacity).

B3.2 Measuring mass

B3.2.1 Key learning points for measuring mass

The analyst should:

- Be able to identify the different types of balance in use in the laboratory;
- Understand the relative accuracy of measurements made using different types of balance;
- Be able to select a suitable balance for a particular weighing application (taking into account the readability and capacity of the balance);
- Be aware of the key aspects to consider when siting a balance in the laboratory;

- Know how to check that the balance is level;
- Know that electronic balances should not be switched off but should be left in 'stand-by' mode (but if a balance has been switched off, remember to let it equilibrate before use);
- Remember to check the calibration status of a balance before use;
- Understand the difference between calibrating a balance and verifying the performance of a balance;
- Understand the importance of using check weights as a part of quality control procedures;
- Know how to carry out a balance check;
- Remember to record the results of balance checks in the appropriate log book;
- Be familiar with laboratory policies on checking balances before use;
- Know what action to take if the results from the balance check are out of specification;
- Know how to clean the balance before use;
- Be able to select a suitable weighing vessel;
- Remember to make sure that items are at ambient temperature before weighing;
- Know how to place the weighing vessel in the correct position on the balance pan;
- Remember to close the doors of an analytical balance/micro balance before taking a reading;
- Remember to let the balance stabilise before recording the reading;
- Understand the difference between 'taring' the balance and 'weighing by difference'; be able to carry out both procedures and be able to select a suitable procedure for a particular application.

B3.2.2 Assessing competence in making measurement of mass

Competence in making measurements of mass can be assessed by carrying out replicate weighings of different check weights on different types of balance. For each check weight carry out a minimum of six weighings. Calculate the mean and standard deviation of each set of weighings to assess the bias and precision of the measurements. Observe the trainee carrying out different weighing techniques for different types of material (e.g. taring the balance and weighing by difference).

B3.2.3 Observations indicating that retraining may be required

- Messy working area;
- Material spilled on or under the balance pan;
- Balance not level;
- Balance does not read zero when there is nothing on the balance pan;
- Using an inappropriate balance for the application (e.g. using a top pan balance when a high degree of accuracy is required; weighing items that are too heavy for the balance capacity);
- Failure to carry out appropriate balance checks before use;
- Failure to record the results from balance performance checks;
- Using unsuitable weighing vessels;
- Material added to the weighing vessel while it is on the balance pan;
- Weighing items that are not at ambient temperature;
- Items placed off-centre on the balance pan;
- Balance door not closed when weighing;

- Balance not allowed to stabilise before reading is taken;
- Using the tare facility when weighing by difference would be more appropriate;
- Balance switched off rather than being left in stand-by mode.

B3.3 Measuring pH

B3.3.1 Key learning points for measuring pH

The analyst should:

- Understand the meaning of pH and the pH scale;
- Know the different techniques for measuring pH (e.g. pH paper and pH meters) and know their relative accuracies;
- Know how to set up a pH meter;
- Be able to select a suitable electrode(s) for a particular sample type;
- Know how to clean and store electrodes;
- Know how to refill a reference electrode with the appropriate electrolyte solution;
- Understand the effect of temperature on pH readings;
- Know how to calibrate a pH meter correctly using appropriate buffer solutions;
- Know how to prepare buffer solutions (e.g. from tablets, powders or concentrates);
- Remember to check that buffer solutions are within their expiry date;
- Understand the importance of stirring solutions at the appropriate speed when taking pH readings;
- Remember to remover the cover of the reference electrode filling hole when the electrode is being used;
- Know how to immerse the electrode in the liquid being measured (i.e. holding electrode vertically with the glass membrane and liquid junction covered);
- Remember to let the meter stabilise before recording the pH value;
- Remember to rinse electrodes with distilled water and the solution being measured between measurements;
- Remember to check the calibration periodically by reanalysing at least one buffer solution if a large number of samples are being measured.

B3.3.2 Assessing competence in making measurement of pH

- Observe the trainee calibrating the pH meter;
- Make at least six repeat measurements of the pH of a buffer solution (different to the buffers used in calibration) and a sample solution (use a sample that has been tested previously if available);
- Calculate the mean and standard deviation of each set of readings;
- Compare the mean with the specified pH value for the buffer and previous data from the sample (if available).

B3.3.3 Observations indicating that retraining may be required

- pH meter not calibrated before use;
- Buffer solutions incorrectly prepared or being used outside of their expiry date;
- Electrodes not cleaned properly between measurements and after use;
- Insufficient electrolyte solution in the reference electrode;
- Using an unsuitable electrode for the type of sample;

- Reference electrode used with filling hole cover in place;
- Temperature adjustment not made (if pH meter does not have automatic temperature compensation);
- Liquid not stirred while reading is being taken;
- Different stirring rates used during calibration of the pH meter and measurement of test samples;
- Solutions stirred to vigorously so that a vortex forms;
- Electrode being used to stir liquid;
- Electrode immersed in liquid to insufficient depth;
- Electrode immersed in bulk sample/buffer solution rather than transferring a portion to a beaker/flask;
- Electrode not held vertically in the liquid;
- Electrodes stored incorrectly (e.g. left to dry out or stored in distilled water rather than the appropriate storage solution).

B3.4 Preparing solutions of known concentration

B3.4.1 Key learning points for preparing solutions of known concentration

The analyst should:

- Be aware of the uses of solutions of known concentration in the laboratory;
- Understand the importance of preparing solutions in such a way that the uncertainty in the concentration of the solution is minimised;
- Understand the meaning of units such as mg L^{-1} and %v/v;
- Be able to calculate the masses and volumes required to prepare solutions of a specified concentration;
- Know how to calculate the volumes required to prepare solutions by dilution;
- Be able to select appropriate equipment to prepare solutions with concentrations that have the required level of uncertainty;
- Be aware of the different grades of chemical available and be able to select chemicals of the required grade to prepare solutions of known concentration;
- Remember to check that all chemicals used are within their expiry date and have been stored correctly;
- Know how to weigh the amount of chemical required to prepare a solution accurately and how to quantitatively transfer the material to a volumetric flask;
- Know how to use the volumetric glassware required to prepare solutions of known concentration;
- Remember that solutions must be at ambient temperature before making up to volume;
- Know how to store solutions correctly;
- Understand the importance of labelling solutions and the information that should be recorded on a label;
- Understand the importance of mixing solutions thoroughly before use.

B3.4.2 Assessing competence in preparing solutions of known concentration

The approach to assessing competence in preparing solutions of known concentration will depend on the use to which the solutions will be put. You should observe the trainee preparing a solution to check that best practice is being followed and assess competency in the use of the relevant volumetric glassware (see section B3.1.2) and balances (see section B3.2.2). Check that solutions are labelled and stored correctly. Some

approaches to assessing that the solutions prepared have the correct concentration are listed below:

- Ask an experienced analyst to perform a titration to check the concentration of the solution;
- If the solutions are calibration standards:
  - analyse the new standards alongside a set of existing standards and compare the instrument response;
  - analyse a sample/quality control sample using both the new and existing set of standards and compare the results.

B3.4.3 Observations indicating that retraining may be required

- Incorrect use of volumetric glassware/analytical balances (see sections B3.1.3 and B3.2.3);
- Inappropriate equipment used (e.g. top-pan balances and/or measuring cylinders used for high accuracy work);
- Carrying out large dilutions in a single step;
- Using the wrong grade of chemical to prepare the standard solution;
- Attempting to transfer a chemical directly into a volumetric flask rather than dissolving in solvent in a beaker and carrying out a quantitative transfer;
- Chemical not completely dissolved in the solvent;
- Solutions made up to volume when not at ambient temperature;
- Solutions labelled incorrectly;
- Solutions stored incorrectly (e.g. in volumetric flasks rather than transferred to a suitable container);
- Forgetting to mix the solution before use.

B3.5 Preparing reagent solutions

B3.5.1 Key learning points for preparing reagent solutions

The analyst should:

- Be aware of the different types of reagent solutions used in the laboratory;
- Understand the meaning of units such as mg L^{-1} and %v/v;
- Be able to calculate the masses and volumes of chemicals required to prepare reagent solutions of a specified concentration;
- Know how to calculate the volumes required to prepare solutions by dilution;
- Understand the difference between reagent solutions and standard solutions and appreciate that the concentrations of the former do not always need to be known with the highest level of confidence;
- Be able to select the appropriate equipment to prepare solutions with concentrations that have the required level of uncertainty;
- Be aware of the different grades of chemical available and be able to select chemicals of the required grade to prepare reagent solutions;
- Remember to check that all chemicals used are within their expiry date and have been stored correctly;
- Know how to weigh the amount of chemical required to prepare a reagent solution with the required degree of accuracy and how to transfer the material to the container used to prepare the reagent;
- Know how to use the glassware required to prepare reagent solutions;

- Remember that solutions should be at ambient temperature before making up to volume;
- Know how to store reagents correctly;
- Understand the importance of labelling reagents and the information that should be recorded on a label;
- Understand the importance of mixing reagents thoroughly before use.

B3.5.2 Assessing competence in preparing reagent solutions

Generally, reagent solutions do not have to be prepared with same degree of accuracy as solutions of known concentration. However, it should be established that reagent solutions can be prepared within the concentration range specified in the method procedure. Ensure that the trainee is competent in the use of the relevant volumetric glassware (see section B3.1.2) and balances (see section B3.2.2). Check that solutions are labelled and stored correctly. Use the questions in A3.5.5 to verify that the trainee is familiar with the calculations and procedures required for preparing reagents.

B3.5.3 Observations indicating that retraining may be required

- Incorrect use of volumetric glassware/balances (see sections B3.1.3 and B3.2.3);
- Inappropriate equipment used;
- Using the wrong grade of chemical(s) to prepare reagent solution(s);
- Chemical not completely dissolved in the solvent;
- Solutions made up to volume when not at ambient temperature;
- Solutions labelled incorrectly;
- Solutions stored incorrectly;
- Forgetting to mix the reagent solution before use.

B3.6 Carrying out a titration

B3.6.1 Key learning points for carrying out a titration

- Understand the principles of different types of titration (e.g. acid-base, redox, etc.);
- Be able to select suitable glassware to carry out a titration;
- Be able to use pipettes, burettes, volumetric flasks and analytical balances correctly;
- Be able to select a suitable indicator to identify the end-point of the titration;
- Be able to select an appropriate solution of known concentration for titration with the test sample;
- Understand the importance of standardising the solution of known concentration;
- Be able to select a suitable primary standard for the standardisation;
- Be able to prepare a primary standard solution of the required concentration;
- Know how much indicator to add and know how to judge the end-point;
- Know how to carry out a 'rough' titration to determine the approximate titre volume required to reach the end-point;
- Remember to swirl the flask gently as the solution is added from the burette;
- Remember to add the solution dropwise as the end-point is approached;
- Remember to use a wash bottle filled with deionised water to rinse the sides of the conical flask and the tip of the burette as the end-point is approached;

- Understand the different units that can be used to express the concentration of the sample solution, e.g. mol L^{-1} and mg L^{-1}, and know how to convert from one to the other;
- Know how to use the data from a titration experiment to calculate the concentration of the sample solution.

B3.6.2 Assessing competence in carrying out a titration

- Assess competency in the use of the relevant volumetric glassware (see section B3.1.2) and balances (see section B3.2.2);
- Observe the analyst carrying out titrations to standardise the solution of known concentration;
- Carry out at least six replicate titrations on a solution of known concentration (or a previously analysed sample/proficiency testing sample);
- Calculate the mean and standard deviation of the results and use to assess bias and precision.

B3.6.3 Observations indicating that retraining may be required

- Incorrect use of volumetric glassware/analytical balances (see sections B3.1.3 and B3.2.3);
- Using a burette with an unsuitable capacity;
- Failure to standardise the solution of known concentration before analysing test samples;
- Placing concentrated alkali solutions in the burette;
- Forgetting to remove the funnel from the burette;
- Using an unsuitable indicator/adding too much indicator;
- Forgetting to swirl the solution while the liquid is being added from the burette;
- Forgetting to rinse the walls of the conical flask and jet of the burette as the end-point is approached;
- Recording burette readings with an inappropriate number of significant figures;
- Large variability in titre volumes.

B3.7 Centrifugation

B3.7.1 Key learning points for using a centrifuge

The analyst should:

- Be aware of the different types of centrifuge available in the laboratory;
- Be aware of the different types of rotor that can be used with each centrifuge;
- Know how to operate each type of centrifuge correctly and safely;
- Be able to select a suitable centrifuge/rotor for a particular application;
- Know which sample containers can be used with each type of rotor;
- Understand the difference between rpm and *g* and be able to convert between the two;
- Understand the importance of ensuring that a centrifuge is balanced and know how to balance it correctly;
- Understand the importance of logging centrifuge use (especially for ultacentrifuges);
- Know how to transfer samples from the sample tubes after centrifugation.

B3.7.2 Assessing competence in using a centrifuge

- Observe the trainee using each type of centrifuge available in the laboratory;
- Ensure that the correct rotor and sample tubes are used for each application;
- Check that the rotor is balanced correctly.

B3.7.3 Observations indicating that retraining may be required

- Centrifuge set to incorrect speed;
- Sample tubes with inappropriate capacity used;
- Inappropriate sample tubes used for the rotor type;
- Excessive noise/vibration indicating centrifuge rotor may not be balanced correctly.

Appendix 1: Glossary of Terms

Accreditation	Third-party statement following a review that competence to carry out a task has been demonstrated (e.g. accreditation to ISO/IEC 17025 for particular combinations of analyte, sample and test method). *See section A4.2.1*.
Accuracy	The difference between a single test result and the accepted reference value. *See section A4.3.4.2*.
Analyte	The substance subject to analysis.
Balance capacity	The maximum mass a balance can weigh. *See section A3.2.2*.
Balance readability / resolution	The minimum difference in mass that can be detected by a balance. *See section A3.2.3*.
Bias	The difference between the expectation of the test results and an accepted reference value. *See section A4.3.4.2*.
Buffer solution	A solution of known pH which resists a change in hydrogen ion concentration (i.e. pH) on the addition of an acid or an alkali. Used to control the pH of a solution or for the calibration of a pH meter (*see section A3.3.5*).
Calibration	Operation that establishes the relationship, obtained by reference to one or more measurement standards, between the response of an instrument and the values of the standards. *See section A4.4*.
Certification	Third-party statement based on a decision following review that fulfilment of specified requirements have been demonstrated (e.g. certification to ISO 9001). *See section A4.2.1*.
Certified reference material (CRM)	A reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that states the value of the specified property, its associated uncertainty, and a statement of metrological traceability.
Coefficient of variation	The ratio of the standard deviation to the mean expressed as a percentage. *See section A5.1*.
Confidence interval	The range about the mean within which a stated percentage of values would be expected to lie. *See section A5.1*.
Control charts	Routine charting of data obtained from the analysis of quality control materials to check that the results lie within predetermined limits. *See section A4.6*.
Error	The difference between a result of a measurement and the true value of the measurand. *See section A4.8*.
Expanded uncertainty	A quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand. *See section A4.8.3*.
Fitness for purpose	A formal process of assessing that a method is suitable for a given application.
Limit of detection	The lowest amount of an analyte that can be measured with reasonable statistical certainty. *See section A4.3.4.3*.

Limit of quantitation	The lowest concentration of an analyte that can be determined with an acceptable level of uncertainty under the stated conditions of the test. *See section A4.3.4.3*.
Linearity	The ability of the method to obtain test results proportional to the concentration of the analyte. *See section A4.3.4.4*.
Matrix	All components of the test sample excluding the analyte.
Material safety data sheet (MSDS)	A detailed description of any hazards associated with a substance (e.g. a solid chemical, solution or solvent). *See section A1.1*.
Mean	The (arithmetic) mean of a set of values. *See section A5.1*.
Measurand	A particular quantity subject to measurement.
Measurement uncertainty	A parameter, associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand. *See section A4.8.3*.
Mole	The SI unit of amount of substance. 1 mol is the amount of a material which contains the same number of entities (e.g. atoms or molecules) as 12 g of carbon 12. *See section A3.4.2.1*.
Precision	The closeness of agreement between independent test results obtained under stipulated conditions. *See section A4.3.4.2*.
Primary standard	A standard that is designated as having the highest metrological qualities and whose value is accepted without reference to other standards of the same quantity. *See section A3.6.2*.
Proficiency Testing	Determination of laboratory testing performance by means of inter-laboratory comparisons (also know as external quality assessment (EQA)). *See section A4.7*.
Quality Assurance	Part of quality management focused on providing confidence that quality requirements will be fulfilled. *See section A4.2*.
Quality Control	Part of quality management focused on fulfilling quality requirements. *See section A4.2*.
Quality control material	A material that is fully characterised in-house or by a third-party, similar in composition to the types of samples normally examined, stable, homogeneous and available in large quantities so that it can be used over a long period of time for monitoring method performance. *See section A4.6*.
Random error	Errors that cause results to differ in an unpredictable way. *See section A4.8.1*.
Recovery	Fraction of the analyte that is recovered from the sample by a particular test method.
Relative standard deviation	The ratio of the standard deviation to the mean value. *See section A5.1*.
Repeatability	Precision under repeatability conditions, i.e. independent test results obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. *See section A4.3.4.2*.
Reproducibility	Precision under reproducibility conditions, i.e. independent test results obtained with the same method on identical test items in different laboratories by different operators using

	different equipment. *See section A4.3.4.2*.
Relative centrifugal force (RCF)	Acceleration delivered by a centrifuge. Depends on the speed and the radius of the centrifuge rotor (expressed in multiples of *g*). *See section A3.7.2*.
Revolutions per minute (rpm)	Used to express the speed of a centrifuge rotor. *See section A3.7.2*.
Ruggedness	Test of the extent to which the results of an analytical procedure are affected by slight changes in the procedure. *See section A4.3.4.5*.
Selectivity	The extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour. *See section A4.3.4.1*.
Sensitivity	The change in the response of a measuring instrument divided by the corresponding change in the stimulus. Note that in some sectors 'sensitivity' is used to describe the limit of detection.
SI	International system of units (e.g. kilogram, metre, mole)
Spiked sample	A sample prepared by adding a known quantity of analyte to a matrix which is as close to or identical to that of the sample of interest. *See section A4.3.4.2*.
Standard deviation	A measure of the dispersion of a set of values. *See section A5.1*.
Standard deviation of the mean	A measure of the dispersion of a set of mean values. *See section A5.1*.
Standard operating procedure	A detailed written instruction to achieve uniformity in the performance of a specific function. *See sections A1.3 and A4.5*.
Standard solution	A solution of known concentration (generally used for calibration purposes). *See section A3.4.*
Standard uncertainty	Uncertainty of a measurement expressed as a standard deviation. *See section A4.8.3*.
Stock solution	A solution from which standard/calibration solutions are prepared. *See section A3.4.*
Systematic error	Errors that cause results to differ from the expected result in a predictable way, either always higher or always lower. *See section A4.8.2*.
Traceability	A property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties. *See section A4.4*.
Validation	Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled. *See section A4.3.*
Variance	The square of the standard deviation. *See section A5.1.*
Verification	Confirmation that the method performance parameters established during method validation can be met. *See section A4.3.*

Within-laboratory reproducibility / Intermediate precision	Precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by different operators using different equipment on different days. *See section A4.3.4.2*.
Working range	The interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of uncertainty. A4.3.4.4.
***z*-score**	Statistical score used in proficiency testing schemes to describe a participant's performance. *See section A4.7.1*.

Appendix 2: Answers to Questions

Section A3.1: Measuring volume

Question 1

What item of glassware would you use to prepare a solution of known concentration?

A volumetric flask should be used to prepare a solution of known concentration.

Question 2

What does the marking 'blow-out' on pipettes indicate?

The marking 'blow-out' on pipettes indicates that the residual liquid in the tip is part of the stated volume and must be blown out.

Question 3

What is the main distinction between Class A and Class B glassware?

The main distinction between Class A and Class B glassware is based on the tolerance of the stated volume. Generally, for volumetric glassware, Class A tolerances are about half those of Class B.

Question 4

How should you read the meniscus of the liquid being measured?

The meniscus of the liquid being measured should always be viewed at eye level to avoid parallax error that is introduced when viewing the meniscus from below or above.

Question 5

List four things you should check before using a glass bulb pipette.

Things you may have identified include:

- Check the pipette is clean;
- Check the pipette is undamaged (especially the tip);
- Check the calibration mark is not faded;
- Check the pipette does not drip when filled;
- Check the delivery time for the pipette and compare it with the value etched on the pipette. If the delivery time is faster then expected this can indicate that the tip is damaged. If the delivery time is slower than expected this can indicate that the tip is partially blocked;
- Check the accuracy of the volume delivered by the pipette.

Section A3.2: Measuring mass

Question 1

What is the difference between mass and weight?

Mass is the amount of material in an object and does not change with the environment in which the object is located. Weight is a force arising from the interaction of the mass with the earth's gravitational field which varies with location.

Question 2

What type of vessel would you use to weigh a toxic sample?

A toxic sample should be weighed in a closed container, such as a weighing bottle, preferably in a fume hood/fume cupboard.

Question 3

What type of vessel would you use to weigh a volatile sample?

A volatile sample should be weighed in a container with a well-fitting stopper to minimise evaporation.

Question 4

What type of balance would you use to weigh 0.005 g of material?

Using a 5-figure analytical balance would allow the mass to be recorded with three significant figures (i.e. 0.005 00 g). A 6-figure micro balance would allow the mass to be recorded with four significant figures. A micro balance would therefore be the first choice for weighing 0.005 g of material.

Question 5

What weighing technique would you use to accurately weigh 1.7 g of silver nitrate which is required to prepare a solution of known concentration?

Weighing by difference should be used to accurately weigh 1.7 g of silver nitrate which is required to prepare a solution of known concentration

Question 6

Read the passage below and identify the mistakes that Caroline makes when she is weighing a portion of sodium chloride to prepare 100 mL of a solution of known concentration.

Caroline has been asked to weigh out accurately 0.5 g sodium chloride to four decimal places to prepare a solution of known concentration. She decides to use a 4-figure analytical balance in the laboratory. Caroline finds a bottle of sodium chloride in the chemical cupboard. It has passed its expiry date by a couple of months but looks OK. The balance has been switched off at the mains so Caroline switches it on. She can't find the balance log book but knows that the balance is used fairly regularly so assumes that it will be OK. She opens the balance door and places a clean 100 mL beaker (which she has just taken from the drying oven) onto the centre of the balance pan. Caroline records the mass of the beaker. She then transfers some of the sodium chloride to the

beaker and records the mass. Caroline removes the beaker from the balance pan and transfers as much of the material as she can from the beaker into a 100 mL volumetric flask. Caroline replaces the beaker on the centre of the balance pan and records the mass. She calculates the mass of sodium chloride transferred to the volumetric flask by subtracting the mass of the beaker after transfer of the sodium chloride from the mass of the beaker plus the sodium chloride. Caroline makes the solution in the volumetric flask up to the required volume with deionised later and stoppers the flask. She notices some of the crystals stuck to the outside of the flask and on the laboratory bench so she wipes them up before putting the flask on a shelf above the bench. She switches off the balance at the mains and gets on with the next task.

The following list contains the problems associated with Caroline's procedure that you may have identified:

- Out of date chemical used;
- Balance was not in stand-by mode and insufficient time was allowed for the balance to equilibrate before use;
- Does not check that the balance is level
- Balance accuracy not verified – should check the log book to see if the required accuracy check has been carried out. If the check hasn't been completed this should be completed before the balance is used. Should also check that the balance calibration is current;
- Uses a 100 mL beaker to weigh a relatively small amount of material, better to use a weighing boat or weighing funnel;
- Uses a warm beaker from the oven – all objects/materials should be at ambient temperature when weighed;
- Does not zero the balance before use;
- Does not close the balance door or wait for the reading to stabilise before recording the mass;
- Does not remove the container from the balance pan when transferring the material;
- Tries to transfer the material from the beaker directly into the flask. Material is less likely to be lost if it is first transferred to a beaker and dissolved in a small amount of solvent before transferring to the volumetric flask;
- The material stuck on the outside of flask/on the bench implies that some of the material has been spilled, so the mass of the material calculated from the weighings won't accurately reflect what is actually in the volumetric flask;
- Flask containing solution not labelled;
- Solutions should not be stored long term in a volumetric flaks;
- The balance shouldn't be switched off at the mains; it should be left in the stand-by mode.

Section A3.3: Measuring pH

Question 1

What type of reference electrode would you use when measuring the pH of water samples containing compounds of sulfur?

Sulfur containing compounds can react with silver so a calomel electrode is recommended rather than a silver/silver chloride electrode.

Question 2

Identify four things you should check before using a pH electrode and meter.

Things you may have identified include:

- Check the electrode is clean;
- Check the electrode has been stored correctly (e.g. the membrane hasn't dried out);
- Check the level of electrolyte solution in the reference electrode;
- Check the filling hole in the reference electrode is uncovered before use;
- Measure the temperature of buffer solutions and the samples;
- Check the condition of buffer solutions before calibrating the pH meter.

Section A3.4: Preparing solutions of known concentration

Question 1

i) Number of moles of $KCO_2C_6H_4CO_2H$ in 1 g of potassium hydrogen phthalate.

The molar mass of potassium hydrogen phthalate is 204.23 g mol^{-1} (rounded to two decimal places). 1 g contains:

$$\frac{1}{204.23} = 0.0049 \text{ mol}$$

ii) Number of moles of Na_2CO_3 in 50 g of sodium carbonate.

The molar mass of sodium carbonate is 105.99 g mol^{-1} (rounded to two decimal places). 50 g contains:

$$\frac{50}{105.99} = 0.47 \text{ mol}$$

iii) Number of moles of sodium hydroxide (NaOH) in 50 mL of a 0.5 mol L^{-1} solution.

The number of moles is $\frac{0.5}{1000} \times 50 = 0.025$

iv) Number of moles of sodium carbonate (Na_2CO_3) in 50 mL of a standard solution prepared by dissolving 2.6500 g of sodium carbonate in water and making up to a volume of 500 mL in a volumetric flask.

The molar mass of sodium carbonate is 105.9884 g mol^{-1}.

Therefore 2.6500 g of sodium carbonate contains $\frac{2.6500}{105.9884} = 0.025 \text{ mol of } Na_2CO_3$

0.025 mol are dissolved in 500 mL and 50 mL of this solution are used. Number of moles of sodium carbonate in 50 mL solution:

$$\frac{0.025}{500} \times 50 = 0.0025 \text{ mol}$$

Question 2

Express the concentrations of the following solutions in mol L^{-1}.

i) 2.000 g of sodium hydroxide (NaOH) dissolved in water and made up to 500 mL in a volumetric flask.

The molar mass of sodium hydroxide is 39.9971 g mol^{-1}.

2.000 g contains $\frac{2}{39.9971} = 0.050$ mol of NaOH

The concentration in mol L^{-1} is therefore:

$$\frac{0.05}{500} \times 1000 = 0.10 \text{ mol L}^{-1}$$

ii) 2.2822 g of potassium hydrogen phthalate ($KCO_2C_6H_4CO_2H$) dissolved in water and made up to 100 mL in a volumetric flask.

The molar mass of potassium hydrogen phthalate is 204.2212 g mol^{-1}.

2.2822 g contains $\frac{2.2822}{204.2212} = 0.0112$ mol of potassium phthalate

The concentration in mol L^{-1} is therefore:

$$\frac{0.0112}{100} \times 1000 = 0.1118 \text{ mol L}^{-1}$$

Question 3

In each case, calculate the amount of chemical required to prepare the specified volume of the solution.

i) 100 mL of a 2 mol L^{-1} sodium chloride solution.

The molar mass of sodium chloride is 58.4425 g mol^{-1}.

The amount of material required is therefore:

$$\frac{2}{1000} \times 100 \times 58.4425 = 11.6885 \text{ g}$$

ii) 500 mL of a 0.1 mol L^{-1} solution of potassium dihydrogen phosphate(V) (KH_2PO_4).

The molar mass of potassium dihydrogen phosphate(V) is 136.0855 g mol^{-1}.

The amount of material required is therefore:

$$\frac{0.1}{1000} \times 500 \times 136.0855 = 6.8043 \text{ g}$$

iii) 100 mL of a 0.1 mol L^{-1} solution of silver nitrate ($AgNO_3$).

The molar mass of silver nitrate is 169.8731 g mol^{-1}.

The amount of material required is therefore:

$$\frac{0.1}{1000} \times 100 \times 169.8731 = 1.6987 \text{ g}$$

iv) 500 mL of a solution with a sodium chloride concentration of 1000 mg L^{-1}.

The amount of sodium chloride required is:

$$\frac{500}{1000} \times 1000 = 500 \text{ mg}$$

v) 500 mL of a solution containing 1000 mg L^{-1} of sodium ions, prepared using sodium chloride.

The amount of sodium ions required is:

$$\frac{500}{1000} \times 1000 = 500 \text{ mg}$$

The molar mass of sodium chloride is 58.4425 g mol^{-1}.

The molar mass of sodium ions is 22.9898 g mol^{-1}.

The amount of sodium chloride required is:

$$\frac{58.4425}{1 \times 22.9898} \times 500 = 1271.1 \text{ mg} = 1.2711 \text{ g}$$

vi) 500 mL of a solution containing 1000 mg of sodium ions, prepared using sodium carbonate (Na_2CO_3).

The amount of sodium ions required is:

$$\frac{500}{1000} \times 1000 = 500 \text{ mg}$$

The molar mass of sodium carbonate is 105.9884 g mol^{-1}

The molar mass of sodium ions is 22.9898 g mol^{-1}.

$$\frac{105.9884}{2 \times 22.9898} \times 500 = 1152.6 \text{ mg} = 1.1526 \text{ g}$$

Question 4

Suggest how to prepare the following solutions:

i) 100 mL of a 0.1 mol L^{-1} solution of hydrochloric acid from a solution with a concentration of 1 mol L^{-1}.

The volume of the stock solution required is:

$$\frac{0.1}{1} \times 100 = 10 \text{ mL}$$

Transfer 10 mL of 1 mol L^{-1} hydrochloric acid to a 100 mL volumetric flask using a pipette and make up to volume with water.

ii) 500 mL of a 0.1 mol L^{-1} solution of nitric acid from a stock solution with a concentration of 0.5 mol L^{-1}.

The volume of the stock solution required is:

$$\frac{0.1}{0.5} \times 500 = 100 \text{ mL}$$

Transfer 100 mL of 0.5 mol L^{-1} nitric acid to a 500 mL volumetric flask using a pipette and make up to volume with water.

iii) 100 mL of a 1 mg L^{-1} solution of calcium ions from a stock solution with a calcium ion concentration of 1000 mg L^{-1}.

The volume of the stock solution required is:

$$\frac{1}{1000} \times 100 = 0.1 \text{ mL}$$

However, this volume is rather small and may be difficult to measure accurately. It is therefore better to prepare an 'intermediate' standard and dilute from that. For example, prepare 100 mL of an intermediate standard with a concentration of 50 mg L^{-1}, which requires a volume of:

$$\frac{50}{1000} \times 100 = 5 \text{ mL}$$

The 1 mg L^{-1} dilute solution is prepared by taking:
$\frac{1}{50} \times 100 = 2$ mL of the intermediate (50 mg L^{-1}) solution.

Another alternative would be to prepare 100 mL of an intermediate standard with a concentration of 10 mg L^{-1} by diluting 1 mL of the stock solution. Diluting 10 mL of the intermediate standard to 100 mL would give a final concentration of 1 mg L^{-1}.

iv) A set of calibration solutions with nickel concentrations of 1, 5, 10 and 15 mg L^{-1}, each with a volume of 100 mL, prepared using a stock solution with a concentration of 100 mg L^{-1}.

The volume of stock solution required in each case is:

1 mg L^{-1}: $\frac{1}{100} \times 100 = 1\,\text{mL}$

5 mg L^{-1}: $\frac{5}{100} \times 100 = 5\,\text{mL}$

10 mg L^{-1}: $\frac{10}{100} \times 100 = 10\,\text{mL}$

15 mg L^{-1}: $\frac{15}{100} \times 100 = 15\,\text{mL}$

Section A3.5: Preparing reagent solutions

In all cases, as only an approximate concentration is required, it would be acceptable to weigh materials using a top-pan balance and measure volumes using measuring cylinders.

i) 200 mL of a 5% w/v aqueous solution of sodium chloride.

Weigh out: $\frac{5}{100} \times 200 = 10\,\text{g}$ sodium chloride, transfer to a beaker and gradually add 200 mL water from a measuring cylinder while stirring the solution to dissolve the sodium chloride.

ii) 50 mL of a 1% w/v solution of phenolphthalein.

Weigh out: $\frac{1}{100} \times 50 = 0.5\,\text{g}$ phenolphthalein, transfer to a beaker and gradually add 50 mL water from a measuring cylinder while stirring the solution to dissolve the phenolphthalein.

iii) 500 mL of a 2 mol L^{-1} potassium nitrate solution.

The molar mass of potassium nitrate is 101.11 g mol^{-1}.

Weigh out: $\frac{2}{1000} \times 500 \times 101.11 = 101.11\,\text{g}$ potassium nitrate, transfer to a beaker and gradually add 500 mL water from a measuring cylinder while stirring the solution to dissolve the potassium nitrate.

iv) 100 mL of a 0.1 mol L^{-1} solution of sodium hydroxide prepared from a stock solution with a concentration of 2 mol L^{-1}.

The volume of 2 mol L^{-1} sodium hydroxide required is: $\frac{0.1}{2} \times 100 = 5\,\text{mL}$.

Measure out 5 mL of 2 mol L^{-1} sodium hydroxide using a measuring cylinder, transfer to a beaker and add 95 mL water from a measuring cylinder.

v) 1 litre of a solution containing acetonitrile/water 5/95 v/v.

Measure out 50 mL of acetonitrile and 950 mL of water (using measuring cylinders) and mix.

Section A3.6: Carrying out a titration

Question 1

You have been asked to carry out a titration to determine the concentration of a solution of ethanoic acid by titration with a 0.1 mol L^{-1} solution of sodium hydroxide. Which of the indicators listed in Table A3.25 would be suitable for detecting the end-point? Explain your choice.

Bromothymol blue or phenolphthalein would be a suitable indicator.

Ethanoic acid is a weak acid and sodium hydroxide is a strong base. Graph 3 in Figure A3.18 shows the pH curve when 0.1 mol L^{-1} sodium hydroxide is added to 25 mL 0.1 mol L^{-1} ethanoic acid. The vertical portion of the graph (i.e. where there is a rapid change in pH with only a very small increase in the amount of sodium hydroxide added) occurs between about pH5 and pH10. Bromothymol blue and phenolphthalein are suitable because their colour will change within the required pH range. Methyl red is unsuitable as its colour changes over a pH range that falls on the curved part of the pH graph before the end-point.

Question 2

A titration experiment has been carried out to determine the purity of a sample of sodium carbonate (Na_2CO_3). The experiment involved transferring an accurately measured amount of the sodium carbonate into a conical flask, dissolving it in deionised water and titrating with 0.1 mol L^{-1} hydrochloric acid using bromocresol green as the indicator (the concentration of the hydrochloric acid solution has been determined via a standardisation experiment).

i) Calculate the number of moles of Na_2CO_3 in 0.1332 g of the sample.

The equation for the reaction of hydrochloric acid and sodium carbonate is:

$2HCl + Na_2CO_3 \rightarrow 2NaCl + H_2O + CO_2$

Hydrochloric acid and sodium carbonate therefore react in a ratio of 2:1.

The concentration of the hydrochloric acid solution is 0.1 mol L^{-1}. 23.95 mL of this solution was used in the titration. The number of moles used is therefore:

$$\frac{0.1}{1000} \times 23.95 = 0.002\,395 \text{ mol of HCl}$$

As 2 mol of HCl react with 1 mol of Na_2CO_3 there must be 0.5 x 0.002 395 = 0.001 197 5 mol of Na_2CO_3 in 0.1332 g of the sample.

ii) Convert the number of moles of Na_2CO_3 calculated in i) into a mass (g).

The molar mass of sodium carbonate is 105.9884 g mol^{-1}. 0.001 197 5 mol of sodium carbonate therefore weighs 0.001 197 5 x 105.9884 = 0.1269 g.

iii) Calculate the purity of the sample of sodium carbonate.

0.1332 g of sample was analysed. The results from the titration indicate that there is 0.1269 g sodium carbonate in the sample. The purity (expressed as a percentage) is:

$$\frac{0.1269}{0.1332} \times 100 = 95.27\%$$

Section A3.7: Centrifugation

Question 1

Calculate the relative centrifugal force (RCF) of a centrifuge with a centrifugation rotor radius of 9.65 cm and a speed of 5300 rpm.

The relative centrifugal force (RCF) of a centrifuge with a centrifugation rotor radius of 9.65 cm and a speed of 5300 rpm is:

0.000 011 18 x 9.65 x 5300^2 = 3031 *g*

Question 2

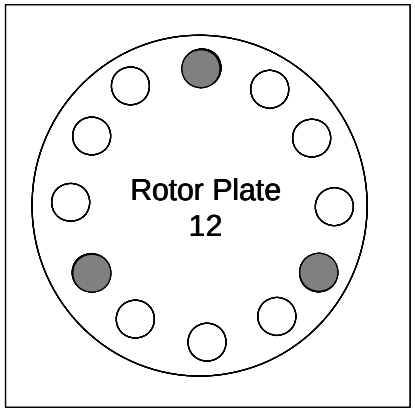
What type of centrifuge would you use to isolate sub-cellular organelles?

To isolate sub-cellular organelles you would use an ultracentrifuge.

Question 3

How would you balance three samples in a rotor plate containing 12 positions?

Three samples in a rotor plate containing 12 positions would be balanced by placing the tubes three positions away from each other, as shown in the figure below.



Question 4

What would you do if the centrifuge you had just started began to vibrate and make excessive noise?

If the centrifuge you had just started began to vibrate and make excessive noise you should switch it off immediately and make sure it is balanced before beginning the centrifugation process again.

Section A5: data handling and reporting results

i) Calculate the mean, standard deviation and relative standard deviation, relative standard deviation, standard deviation of the mean and number of degrees of freedom.

Molybdenum concentration (mg kg^{-1})	
33.92	34.70
33.20	34.63
35.66	34.70
34.28	35.06
34.55	34.74
34.82	

The values shown were obtained using a statistical calculator.

mean: 34.569 090 91 mg kg^{-1}
standard deviation: 0.627 765 163 mg kg^{-1}
relative standard deviation: 0.627 765 13/34.569 090 91 = 0.018 159 724
standard deviation of the mean = 0.627 765 163/√11 = 0.189 278 318 mg kg^{-1}

ii) Round the mean, standard deviation, relative standard deviation and standard deviation of the mean to an appropriate number of digits.

Initially the mean is rounded to the same number of decimal places as the individual results: 34.57 mg kg^{-1}.

The standard deviation is rounded to two significant figures: 0.63 mg kg^{-1}. This has the same number of decimal places as the rounded mean so no further treatment of the mean value is required.

The relative standard deviation and standard deviation of the mean are rounded to have the same number of significant figures as the standard deviation:

relative standard deviation: 0.018
standard deviation of the mean: 0.19 mg kg^{-1}

Appendix 3: Training Record

Laboratory Skills Training Record

Name:		Team:		Supervisor:	

Training requirement	Assessment activity	Performance target	Target achieved?	Supervisor's signature	Trainee's signature	Date

Appendix 4: Additional resources

Further information on the topics covered in this handbook can be found on the VAM website (www.vam.org.uk) which contains a wide range of information and resources relating to quality in measurement.

The following resources, which were produced with support from the Valid Analytical Measurement (VAM) programme, may be of particular interest. Further information is available at www.vam.org.uk:

Analytical measurement terminology, E. Prichard, 2001, RSC, ISBN: 0 85404 443 4.

Applications of reference materials in analytical chemistry, V. Barwick, S. Burke, R. Lawn, P. Roper and R. Walker, 2001, RSC, ISBN: 0 85404 448 5.

A review of volumetric laboratory glassware available in the UK with guidance on differences in specifications, P. Roper, 2003, LGC, ref: LGC/VAM/2002/033. Available to download from www.vam.org.uk.

Errors and uncertainty, C. Bailey, 2005, LGC. Leaflet available free of charge from vam@lgc.co.uk.

In-house method validation: A guide for chemical laboratories, B. King, 2003, LGC, ISBN: 0 948926 18 X.

Introducing measurement uncertainty, V. Barwick and E. Prichard, 2003, LGC, ISBN: 0 948926 19 8.

Introduction to measurement terminology, E. Prichard, 2004, LGC, ISBN: 0 948926 21 X.

Meeting the traceability requirements of ISO 17025: An analyst's guide, 3rd Edition, V. Barwick and S. Wood (Ed), 2005, LGC, ISBN: 0 948926 23 6. Available to download from www.vam.org.uk.

mVal: Software for analytical method validation, 2003, LGC.

Practical Laboratory Skills Training Guides: Measurement of Mass, R. Lawn, E. Prichard, 2003, RSC, ISBN 0 85404 463 9.

Practical Laboratory Skills Training Guides: Measurement of pH, R. Lawn, E. Prichard, 2003, RSC, ISBN 0 85404 473 6.

Practical Laboratory Skills Training Guides: Measurement of Volume, R. Lawn. E. Prichard, 2003, RSC, ISBN 0 85404 468 X.

*Practical Laboratory Skill*s, Two CD-ROM set, 2003, RSC.

Preparation of calibration curves: A guide to best practice, V. Barwick, 2003, LGC, ref: LGC/VAM/2003/032. Available to download from www.vam.org.uk.

Quality assurance in analytical chemistry, E. Prichard and V. Barwick, 2007, Wiley, ISBN: 978 0 470 01204 8.

Quantifying uncertainty in analytical measurement, 2nd Edition, S. Ellison, M. Rösslein, A. Williams (Ed), 2000, EURACHEM/CITAC, ISBN: 0 948926 15 5. Available to download from www.eurachem.org.

The importance of valid analytical measurements; *Introduction to method validation*; *What is measurement uncertainty?*; *Traceability in chemical measurements*, C. Bailey and V. Barwick, 2006, LGC. A set of four leaflets available free of charge from vam@lgc.co.uk.

Traceability in chemical measurement, S. L. R .Ellison, B. King, M. Rösslein, M. Salit, A. Williams (Ed), 2003, EURACHEM/CITAC. Available to download from www.vam.org.uk.

VAMSTAT II: A computer-aided learning package for statistics, 2001, LGC.

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Queens Road, Teddington, Middlesex TW11 0LY, UK
Tel: +44 (0)20 8943 7000 Fax: +44 (0)20 8943 2767

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