Chapter 1

Basic Laboratory Skills

Learning Objectives

- To be aware of safety aspects in the laboratory.
- To be able to record, in an appropriate style, practical information accurately.
- To be able to record numerical data with appropriate units.
- To understand the importance of sample handling with respect to both solids and liquids.
- To be able to present data effectively in tables and figures.
- To be able to perform numerical exercises involving dilution factors.

1.1 Introduction

All scientific studies involve some aspect of practical work. It is therefore essential to be able to observe and to record information accurately. In the context of environmental analyses, it should be borne in mind that not all practical work is carried out in the laboratory. Indeed it could be argued that the most important aspects of the whole practical programme are done outside the laboratory in the field, as this is the place where the actual sampling of environmental matrices (air, water, soil, etc.) takes place. It is still common practice, however, to transport the acquired sample back to the laboratory for analysis, so knowledge and implementation of the storage conditions and containers to be used are important. Both sampling and sample storage are covered in Chapters 3 and 4, respectively.

1.2 Safety Aspects

No laboratory work should be carried out without due regard to safety, both for yourself and for the people around you. While the Health and Safety at Work

Act (1974) provides the main framework for health and safety, it is the Control of Substances Hazardous to Health (COSHH) regulations of 1994 and 1996 that impose strict legal requirements for risk assessment wherever chemicals are used. Within this context, the use of the terms *hazard* and *risk* are very important. A hazardous substance is one that has the ability to cause harm, whereas risk is about the likelihood that the substance may cause harm. Risk is often associated with the quantity of material being used. For example, a large volume of a flammable substance obviously poses a greater risk than a very small quantity. Your laboratory will operate its own safety scheme, so ensure that you are aware of what it is and follow it.

The basic rules for laboratory work (and, as appropriate, for associated work outside the laboratory using chemicals) are as follows:

- Always wear appropriate protective clothing. Typically, this involves a clean laboratory coat fastened up, eye protection in the form of safety glasses or goggles, appropriate footwear (open-toed sandals or similar are inappropriate) and ensure that long hair is tied back. In some circumstances, it may be necessary to put on gloves, e.g. when using strong acids.
- Never smoke, eat or drink in the laboratory.
- Never work alone in a laboratory.
- Make yourself familiar with the fire regulations in your laboratory and building.
- Be aware of the accident/emergency procedures in your laboratory and building.
- Never mouth pipettes – use appropriate devices for transferring liquids.
- Only use/take the minimum quantity of chemical required for your work.
- Use a fume cupboard for hazardous chemicals. Check that it is functioning properly before starting your work.
- Clear up spillages on and around equipment and in your own workspace as they occur.
- Work in a logical manner.
- Think ahead and plan your work accordingly.

DQ 1.1

What is one of the first things that you should consider before starting a laboratory experiment?

Answer

You should make yourself aware of the particular safety aspects that operate in your own laboratory. This includes the position of fire safety equipment, the methods of hazard and risk assessments for the chemicals *to be used, the use of fume cupboards, fire regulations and evacuation procedures, and the disposal arrangements for used chemicals.*

1.3 Recording of Practical Results

This is often done in an A4 loose-leaf binder, which offers the flexibility to insert graph paper at appropriate points. Such binders do, however, have one major drawback in that pages can be lost. Bound books obviously avoid this problem. All experimental observations and data should be recorded in the notebook – in ink – at the same time that they are made. It is easy to forget information when you are busy!

The key factors to remember are as follows:

- Record data correctly and legibly.
- Include the date and title of individual experiments.
- Outline the purpose of the experiment.
- Identify and record the hazards and risks associated with the chemicals/equipment being used.
- Refer to the method/procedure being used and/or write a brief description of the method.
- Record the *actual* observations and not your *own* interpretation, e.g. the colour of a particular chemical test – unfortunately, colour can be subjective. In this situation, it is possible to use the *Munsell Book of Colour*. This is a master atlas of colour that contains almost 1600 colour comparison chips. The colours are prepared according to an international standard. There are 40 pages, with each being 2.5 hue steps apart. On each page, the colour chips are arranged by Munsell value and chroma. The standard way to describe a colour using Munsell notation is to write the numeric designation for the Munsell hue (H) and the numeric designation for value (V) and chroma (C) in the form H V/C.
- Record numbers with the correct units, e.g. mg, g, etc., and to an appropriate number of significant figures.
- Interpret data in the form of graphs, spectra, etc.
- Record conclusions.
- Identify any actions for future work.

1.4 Units

The Système International d'Unitès (SI) is the internationally recognized system for measurement. This essentially uses a series of base units (Table 1.1) from which other terms are derived. The most commonly used SI derived units are shown in Table 1.2. It is also common practice to use prefixes (Table 1.3) to denote multiples of 10^3. This allows numbers to be kept between 0.1 and 1000. For example, 1000 ppm (parts per million) can also be expressed as 1000 $\mu g\,ml^{-1}$, 1000 $mg\,l^{-1}$ or 1000 $ng\,\mu l^{-1}$.

Table 1.1 The base SI units

Measured quantity	Name of unit	Symbol
Length	Metre	m
Mass	Kilogram	kg
Amount of substance	Mole	mol
Time	Second	s
Electric current	Ampere	A
Thermodynamic Temperature	Kelvin	K
Luminous intensity	Candela	cd

Table 1.2 SI derived units

Measured quantity	Name of unit	Symbol	Definition in base units	Alternative in derived units
Energy	Joule	J	$m^2\,kg\,s^{-2}$	N m
Force	Newton	N	$m\,kg\,s^{-2}$	$J\,m^{-1}$
Pressure	Pascal	Pa	$kg\,m^{-1}\,s^{-2}$	$N\,m^{-2}$
Electric charge	Coulomb	C	A s	$J\,V^{-1}$
Electric potential difference	Volt	V	$m^2\,kg\,A^{-1}\,s^{-3}$	$J\,C^{-1}$
Frequency	Hertz	Hz	s^{-1}	—
Radioactivity	Becquerel	Bq	s^{-1}	—

Table 1.3 Commonly used prefixes

Multiple	Prefix	Symbol
10^{18}	exa	E
10^{15}	peta	P
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a

SAQ 1.1

The prefixes shown in Table 1.3 are frequently used in environmental science to represent large or small quantities. Convert the following quantities by using the suggested prefixes.

Quantity	m	μm	nm
6×10^{-7} m			
Quantity	mol l^{-1}	mmol l^{-1}	μmol l^{-1}
2.5×10^{-3} mol l^{-1}			
Quantity	μg ml^{-1}	mg l^{-1}	ng $\mathrm{\mu l}^{-1}$
8.75 ppm			

1.5 Sample Handling: Liquids

The main vessels used for measuring out liquids in environmental analyses can be sub-divided into those used for quantitative work and those used for qualitative work. For the former, we frequently use volumetric flasks, burettes, pipettes and syringes, and for the latter, beakers, conical flasks, measuring cylinders, test tubes and Pasteur pipettes.

The nature of the vessel may be important in some instances. For example, some plasticizers are known to leach from plastic vessels, especially in the presence of organic solvents, e.g. dichloromethane. This is particularly important in organic analyses. In inorganic analyses, contamination risk is evident from glass vessels that may not have been cleaned effectively. For example, metal ions can adsorb to glass and then leach into solution under acidic conditions, thereby causing contamination. This can be remedied by cleaning the glassware prior to use by soaking for 24 h in 10% nitric acid solution, followed by rinsing with deionized water (three times). The cleaned vessels should then either be stored upside down or covered with Clingfilm® to prevent dust contamination.

1.6 Sample Handling: Solids

The main vessels used for weighing out solids in environmental analyses are weighing bottles, plastic weighing dishes or weighing boats. These containers are used to accurately weigh the solid, using a four-decimal-place balance, and to transfer a soluble solid directly into a volumetric flask. If the solid is not totally soluble it is advisable to transfer the solid to a beaker, add a suitable solvent, e.g. deionized or distilled water, and stir with a clean glass rod until all of the solid has dissolved. It may be necessary to heat the solution to achieve complete dissolution. Then quantitatively transfer the cooled solution to the volumetric flask and make up to the graduation mark with solvent. NOTE – volumetric flasks are accurate for their specified volume when the solution itself is at a particular temperature, e.g. 20°C.

1.7 Preparing Solutions for Quantitative Work

Solutions are usually prepared in terms of their molar concentrations, e.g. $\text{mol}\,\text{l}^{-1}$, or mass concentrations, e.g. $\text{g}\,\text{l}^{-1}$. It should be noted that both of these refer to an amount per unit volume, i.e. concentration = amount/volume. It is important to use the highest (purity) grade of chemicals (liquids or solids) for the preparation of solutions for quantitative analysis, e.g. AnalaR® or AristaR®. For example, consider the preparation of a 1000 ppm solution of lead from its metal salt.

NOTE: the molecular weight of $Pb(NO_3)_2 = 331.20$; the atomic weight of $Pb = 207.19$.

$$\frac{331.20}{207.19} = 1.5985\ g\ of\ Pb(NO_3)_2\ in\ 1\ litre$$

Therefore, dissolve 1.5985 g of $Pb(NO_3)_2$ in 1 vol% HNO_3 (AnalaR® or equivalent) and dilute to one litre in 1 vol% HNO_3. This will give you a 1000 ppm solution of Pb.

1.8 Presentation of Data: Tables

A useful method of recording numerical data is in the form of a table. All tables should have a title that adequately describes the data presented (they may need to be numbered so that they can be quoted in the text). It is important to display the components of the table such that it allows direct comparison of data and to allow the reader to easily understand the significance of the results. It is normal to tabulate data in the form of columns and rows, with columns running vertically and rows horizontally. Columns contain, for example, details of concentration and units, sampling sites or properties measured, while rows contain numerical or written descriptions for the columns. The first column often contains the independent variable data, e.g. concentration or site location, while subsequent columns may contain numerical values of concentrations for different metals or organics. A typical tabulated set of data obtained from an experiment to determine the level of lead in soil by using atomic absorption spectroscopy is shown in Table 1.4.

Table 1.4 Calibration data obtained for the determination of lead in soil by using atomic absorption spectroscopy

Concentration (ppm)	Absorbance
0	0.000
2	0.015
4	0.032
6	0.045
8	0.062
10	0.075

It is important when tabulating or graphing (see below) data to not quote values to more significant figures than is necessary.

1.9 Presentation of Data: Graphs

The common usage of computers means that graphs are now most frequently produced by using computer-based graphics packages. However, irrespective of the mode of preparation, it is important to ensure that the graph is correctly presented. All graphs should have a title that adequately describes the data presented (they may need to be numbered so that they can be quoted in the text). Most graphs are used to describe a relationship between two variables, e.g. x and y. It is normal practice to identify the x-axis as the horizontal (abscissa) axis and to use this for the independent variable, e.g. concentration. The vertical (or ordinate) axis (y-axis) is therefore used to plot the dependent variable, e.g. concentration response. Each axis should contain a descriptive label indicating what is being represented, together with the appropriate units of measurement.

The mathematical relationship most commonly used for calibration is of the following form:

$$y = mx + c$$

where y is the signal response, e.g. absorbance or signal (mV), x is the concentration of the working solution (in appropriate units, e.g. $\mu g\,ml^{-1}$ or ppm), m is the slope of the graph, and c is the intercept on the x-axis.

A typical graphical representation of the data obtained from an experiment to determine the level of lead in soil by using atomic absorption spectroscopy is shown in Figure 1.1 (also tabulated in Table 1.4 above).[†]

[†] R, in this figure (and also in Figure 1.2), is known as the *correlation coefficient*, and provides a measure of the quality of calibration. In fact, R^2 (*the coefficient of determination*) is used because it is more sensitive to changes. This varies between -1 and $+1$, with values very close to -1 and $+1$ pointing to a very tight 'fit' of the calibration curve.

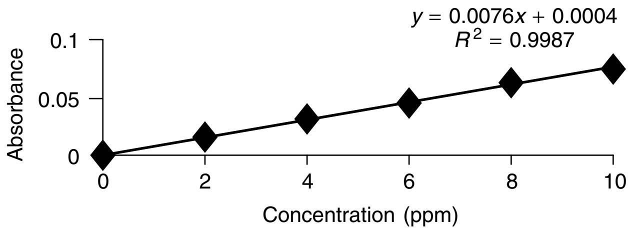

Figure 1.1 Determination of lead in soil: direct calibration approach.

SAQ 1.2

Graphically plot the following data, ensuring that you correctly identify and label the axes. These data were obtained for the analysis, by gas chromatography with flame ionization detection, of lindane in waste water. The signal for lindane has been ratioed to the internal standard. This acts to compensate for any variations in injection volume that are inherent in injecting 1 μl of sample solution.

Concentration (ppm)	Lindane/internal standard
0	0.00
2	0.39
4	0.71
6	1.15
8	1.54
10	1.84

An alternative approach to undergoing a *direct calibration*, as has just been described, is the use of the *method of standard additions*. This may be particularly useful if the sample is known to contain a significant portion of a potentially interfering matrix. In standard additions, the calibration plot no longer passes through zero (on both the x- and y-axes). As the concept of standard additions is to eliminate any matrix effects present in the sample, it is not surprising to find that the working standard solutions all now contain the same volume of the sample, i.e. the same volume of the sample is introduced into a succession of working solutions. Each of the working solutions, containing the same volume of the sample, is then introduced into the instrument and the response is again recorded as before. However, 'graphing' the signal response (e.g. absorbance, signal (mV), etc.) against analyte concentration in this case produces a very different type of plot. In this situation, the graph no longer passes through zero on either axis, but if correctly drawn, the graph can be extended towards the x-axis (extrapolated) until it intercepts it. By maintaining a constant concentration x-axis, the unknown sample concentration can be determined (Figure 1.2). It is

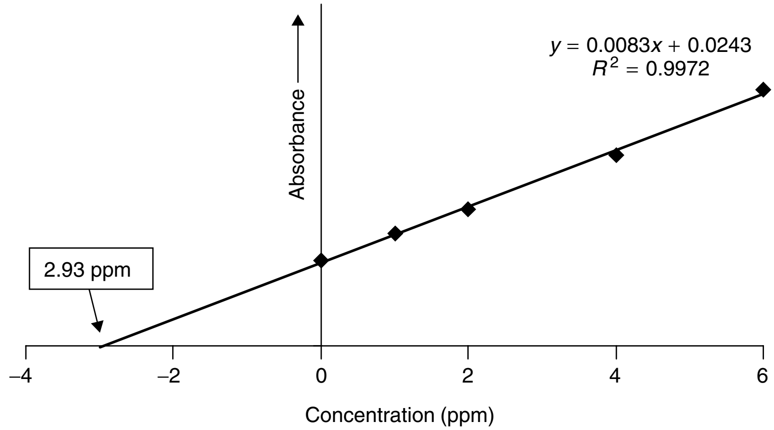

Figure 1.2 Determination of lead in soil: standard additions method.

essential that this graph is linear over its entire length or otherwise considerable errors can be introduced.

DQ 1.2

You have successfully determined the levels of lindane in waste water using gas chromatography. Should you graph the data or record the data in the form of a table?

Answer

The simple answer is that you are trying to convey to the reader information on what you have observed experimentally in the most effective manner. You may decide that the most effective means to convey your message is to plot a graph; this is particularly useful if you have a lot of numerical data based on, for example, responses to different concentrations. On the other hand, if you have more than two variables (remember you are plotting a graph in two dimensions only) it is probably easiest to tabulate the data, for example, responses to different concentrations at different sampling sites. As you can probably guess, even the latter can be represented in graphical form by simply plotting more then one trend line on one graph using different colours (for clarity).

1.10 Calculations: Dilution Factors

1. Calculate the concentration (in $\mu g\,g^{-1}$) of *lead* in the soil sample described above. An accurately weighed (2.1270 g) soil sample is digested in 25 ml of concentrated nitric acid, cooled and then quantitatively transferred to a 100 ml

volumetric flask and made up to the mark with distilled water. This solution is then diluted by taking 10 ml of the solution and transferring to a further 100 ml volumetric flask where it is made up to the mark with high-purity water. What is the dilution factor?

$$\frac{100\ ml}{2.1270\ g} \times \frac{100\ ml}{10\ ml} = 470\ ml\ g^{-1}$$

If the solution was then analysed and found to be within the linear portion of the graph (see Figure 1.1), the value for the dilution factor would then be multiplied by the concentration from the graph, thus producing a final value representative of the element under investigation.

SAQ 1.3

What is the concentration of lead in the original soil sample? If the absorbance from the digested sample was 0.026, calculate the concentration of lead from the graph and then apply the dilution factor.

2. Calculate the concentration (in $\mu g\, ml^{-1}$) of lindane in the waste water sample discussed in SAQ 1.2 above. A waste water sample (1000 ml) was extracted into dichloromethane (3 × 10 ml) using liquid–liquid extraction. The extract was then quantitatively transferred to a 50 ml volumetric flask and made up to the mark with dichloromethane. What is the dilution factor?

$$\frac{50\ ml}{1000\ ml} = 0.05\ ml\, ml^{-1}$$

If the solution was then analysed and found to be within the linear portion of the graph (see SAQ 1.2), the value for the dilution factor would then be multiplied by the concentration from the graph, thus producing a final value representative of the element under investigation.

SAQ 1.4

What is the concentration of lindane in the waste water sample? If the ratio of lindane to internal standard from the extracted sample was 0.26, calculate the concentration of lindane from the graph and then apply the dilution factor.

Summary

A good set of practical notes should provide the following:

- A brief indication of what you hope to achieve by carrying out the work, i.e. the aims of the experiment.

- A record of all of the chemicals/reagents (and their purities/grades) used in the work.
- A record of all of the equipment/apparatus used in the work and their experimental settings. For example, when using flame atomic absorption spectroscopy it is important to record the make and model of instrument used, the metal to be determined, the wavelength used, and the flame constituents and their flow rates. If using gas chromatography, for example, it is important to identify the make and model of instrument being used, the type of detector being employed, the column and its dimensions, the carrier gas and its flow rate, and the retention time(s) of your peak(s) of interest.

In addition, it is important to:

- Record all data immediately. At this stage, a simple table can be used to accurately record the data.
- Note any immediate conclusions and possible suggestions for future work.

Further Reading

Dean, J. R., Jones, A. M., Holmes, D., Reed, R., Weyers, J. and Jones, A., *Practical Skills in Chemistry*, Prentice Hall, Harlow, UK, 2002.