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Faculty of Science

Laboratory Manual

Chemistry

Bachelor of Biotechnology (Hons.)

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Chemistry

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')

- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful
- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

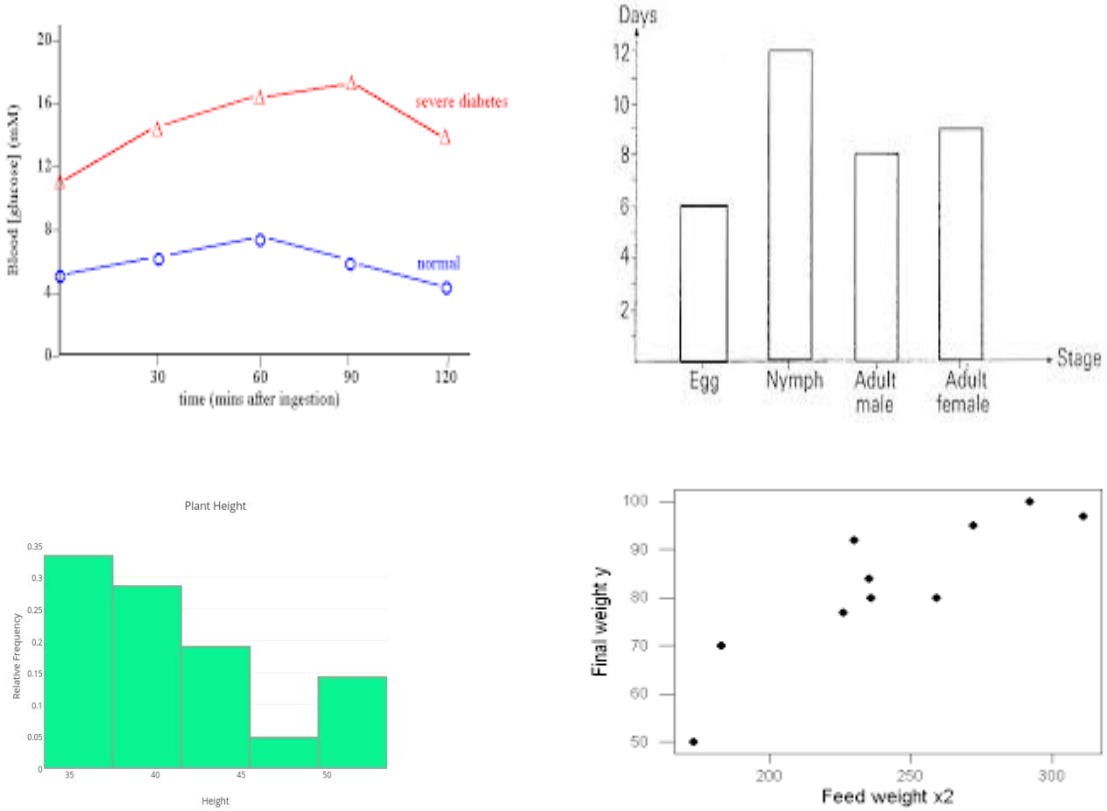


Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.

- Cite sources to substantiate background information.
- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1

Title: Preparation of standard solution

Objective:

After completing the practical, you will be able:

1. To prepare a specific volume (250 cm^3) standard solution (1M) of Na_2CO_3 and NaOH as the primary standard for volumetric analysis

Introduction

The analysis in any laboratory mainly is based on reference materials like a stock standard solution. The accuracy in the preparation of stock standard reflects the accuracy of the results. The stock standard solution is defined as a solution with a high concentration of the stable analyte(s) that can be stored at specific conditions in the laboratory for a long time and used as a standard reference material for analysis of the target analyte(s) in the daily use.

A solution is a homogeneous mixture created by dissolving one or more solutes in a solvent. The chemical present in a smaller amount, the solute, must be soluble in the solvent (the chemical present in a larger amount). Solutions with accurately known concentrations can be referred to as standard (stock) solutions. These solutions are bought directly from the manufacturer or formed by dissolving the desired amount of solute into a volumetric flask of a specific volume. Stock solutions are frequently diluted to solutions of lesser concentration for experimental use in the laboratory.

A standard solution can thus be simply defined as a solution whose concentration is known accurately. Its concentration is usually given in mol/dm^3. When making up a standard solution it is important that the correct mass of a substance is accurately measured. It is also important that all of this is successfully transferred to the volumetric flask used to make up the solution.

Preparing a Standard Solution from a Solid

A solution of known concentration can be prepared from solids by two similar methods. In the first method, the solid solute is weighed out on weighing paper or in a small container and then transferred directly to a volumetric flask. A small quantity of solvent is then added to the volumetric flask and the contents are swirled gently until the substance is completely dissolved. More solvent is added until the meniscus of the liquid reaches the calibration mark on the neck of the volumetric flask. The volumetric flask is then capped and inverted several times until the contents are mixed and completely dissolved. The disadvantage of this method is that some of the weighed solid may adhere to the original container, weighing paper, or funnel. Also, solid may be spilled when it is transferred into the slim neck of the volumetric flask.

In the second method, the solid is weighed out first in a small beaker. A small amount of solvent is added to the beaker and the solution is stirred until the solid is dissolved. The solution is then transferred to the volumetric flask. Before adding additional solvent to the flask, the beaker, stirring rod, and funnel must be rinsed carefully and the washings added to the volumetric flask making sure all remaining traces of the solution have been transferred. Finally, the volumetric flask is diluted to volume (additional solvent is added to the flask until the liquid level reaches the calibration mark). The flask is capped and inverted as before until the contents are thoroughly mixed. The disadvantage to this method is that some of the solutions may adhere to the beaker, stirring rod, or funnel if not washed thoroughly. Also, a possibility of contamination exists from the beaker, rod, or funnel if they have not been washed carefully.

In general chemistry, **molarity** is the most commonly used concentration unit.

$$\text{Molarity} = \frac{\text{moles of solute}}{\text{liters of solution}} = \frac{\text{grams of solute}}{\text{molar mass solute x liters of solution}}$$

Materials:

- Volumetric flask
- Watch glass
- Glass funnel
- Reagent bottle
- Graduated cylinder
- Conical flask
- Analytical balance

Procedure:

1. All the apparatus should be thoroughly washed by de-ionized water before the experiment.
2. Weigh out the specific amount of the anhydrous sodium carbonate (Na_2CO_3) and NaOH using the weighing machine.

Background calculations

Remember that you are to prepare 250 cm^3 of the 1M solution. It implies that the concentration is 1 mol/dm^3.

Work out the mass of the solutes needed to make up a solution with the required volume and concentration. Show your working in the space below:

Molar concentration (mol/dm^3) = mass concentration (g/dm^3)/ molar mass (g/mol)

3. Transfer it to a beaker and add about 100 cm^3 of de-ionized water to dissolve it with stirring of the glass rod.
4. After dissolving, transfer the solution to a 250.00 cm^3 volumetric flask.
5. Add water to just below the line on the volumetric flask.
6. Add the final drops with a teat pipette to ensure that the bottom of the meniscus is on the line.
7. Put the lid on the flask and turn the flask over a couple of times to mix the solution.
8. Label your solution with your name, the date, and the contents, e.g. 1.0M NaOH. Then tidy up!

Question:

Differentiate between primary and secondary standard solutions.

Practical 2
Title: Titration-volumetric analysis

Objective:

After completing the practical, you will be able:

1. To determine the molarity (concentration) of an acid using the standard base solutions prepared in experiment 1.

Introduction

Volumetric analysis is a well-established and versatile form of quantitative chemical analysis. The purpose of this type of analysis is to use an accurately known volume and concentration of one solution to find the accurate concentration of a second. The experimental procedure which allows us to do this is called a titration or titrimetric analysis because the volume is an important factor in the titration. It's a laboratory method of quantitative chemical analysis which is used to determine the unknown concentration of an analyte.

Titration is a volumetric analysis because it's based on the measurement of volumes. One standard solution is used which is a solution of a reagent, also called titrant or titrator. A known volume of titrant reacts with a solution of analyte or titrand to determine its concentration. Thus, the process involves measuring the volumes of two solutions which react to each other (Gros, Bruttel, & von Kloeden, 2005).

The basic principles of volumetric analysis are given as below:

1. The one solution to be analyzed contains an unknown amount of chemicals.
2. The reagent of known concentration reacts with a chemical of an unknown amount in the presence of an indicator to show the end-point. This is the point which shows the completion of the reaction.
3. The volumes are measured by a titration which completes the reaction between reagent and solution.
4. The volume and concentration of reagent used in the titration give the amount of reagent in moles.
5. The amount of unknown chemical in the measured volume of solution is calculated by using the mole ratio of the equation.
6. The amount of unknown chemical in the original sample is calculated by the amount of unknown chemical in the measured volume.

Materials:

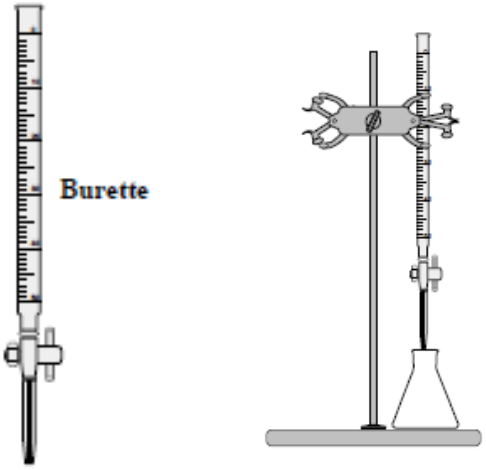
- Conical flask
- Glass funnel
- Reagent bottle
- Graduated cylinder
- Burette
- Pipette
- Acid-base indicator – methyl orange

Procedure:

Filling the burette

1. Clean the burette, rinse it and dry the outside.

2. Rinse it with the solution it is going to contain (acid).
3. Fill the burette to above the 0 ml mark.
4. Check for air bubbles and invert to remove any, if required.
5. Open the tap and run out some of the liquid until the tap connection is full of acid and no air remains (air bubbles would lead to an inaccurate result as they will probably dislodge during the titration).
6. Remove the funnel (stops dripping while you read the meniscus).
7. Release the liquid until the bottom of the meniscus is on the 0 ml.



Preparing the pipette

1. Wash and rinse well.
2. Rinse with the solution it is to contain.
3. Suck up a solution with a pipette filler, above the grad mark.
4. Release the solution until the bottom of the meniscus is on the grad line.
5. Tip-off any hanging drop (this should not be counted).
6. Allow to drain under gravity (do not blow).
7. When drained touch the tip off the side, any drops which should be included will drain in. Leave the rest.

Preparing the conical flask

1. Rinse several times with deionized water.
2. Dry outside.
3. Add base solution as described above, from the pipette.

4. Rinse down walls of the flask with deionized water (you know exact volume added of base)

Titration procedure

1. Add an indicator to the flask, 2 or 3 drops are enough because all indicators are weak acids or bases.
2. Rinse down the sides with water.
3. Run the solution into the flask from the burette, slowly.
4. Rinse the sides of the flask regularly.
5. Swirl the flask constantly, to ensure thorough mixing of reagents.
6. As the end point nears, add the solution drop by drop.
7. When the end-point is reached the indicator will change color suddenly.
8. At this point, the acid will have exactly neutralized the base.
9. Now read the meniscus of the burette, from the bottom, at eye level.
10. Record your result and repeat the titration several times as below:

Burette reading (cm^3)	Rough titer	First titer	Second titer	Third titer
Final reading				
Initial reading				
Vol. of acid used				

Questions

1. What is the average value of acid used?
2. Write the balanced equation of the reaction.
3. Work out the concentration of the acid.
4. Define an indicator and state the conditions on which the choice of indicator depends.

Practical 3
Title: Buffer preparation

Objective:
After completing the practical, you will be able:

1. To prepare acetate buffer from a mixture of acetic acid and sodium acetate and to examine the effect of adding acid and base to the prepared buffer.

Introduction

A buffer solution is one in which the pH of the solution is "resistant" to small additions of either a strong acid or strong base. Buffers usually consist of a weak acid and its conjugate base, in relatively equal and "large" quantities.

A buffer solution is prepared as a combination of weak acids and their salts (sodium salts, etc.) or of weak alkalis and their salts.

Common preparation methods include: 1) dripping an acid (or alkali) into an aqueous solution of a salt while measuring the pH with a pH meter and 2) making an aqueous solution of an acid with the same concentration as the salt and mixing while measuring the pH with a pH meter.

By preparing a buffer to a randomly chosen pH value and comparing the theoretical pH to the actual pH, students apply their theoretical understanding of the Henderson-Hasselbalch equation, activity coefficients, and the effect of adding acid or base to a buffer. This experiment gives students experience in buffer preparation for research situations and helps them in advanced courses such as biochemistry where a fundamental knowledge of buffer systems is essential (Buckley, 2001).

Materials:

- Conical flask
- Reagent bottle
- Graduated cylinder
- Acetic acid solution
- Sodium acetate
- NaOH solution
- HCl solution
- pH meter

Procedure:

1. Mix equal volumes of acetic acid and sodium acetate solution in beaker or flask.
2. Divide the solution in step 1 above into three flasks, A, B & C.
3. Add 1 ml of NaOH solution to one of the flasks.
4. Add 1 ml of HCl solution to one of the flasks.

Record your observation in the table below:

Flask	Action	pH
A	Buffer only	
B	Buffer + 1 ml of NaOH	
C	Buffer + 1 ml of HCl	

Questions:

1. What is the pH of the prepared buffer? Justify your observation
2. What factors affect buffering capacity?

Practical 4
Title: Separation techniques

Objective:

After completing the practical, you will be able:

1. To explore relevant separation techniques in purifying some crude extracts

Introduction

Separation techniques are used to separate mixtures into its constituent elements and/or compounds. A mixture generally contains elements and/or compounds which are not chemically combined together.

By separating the constituents of the mixtures, then, the properties of the known/unknown substances can be found out from the mixtures and possibly use them for the production of useful substances such as medicines.

Depending on the physical and chemical properties of the substances in the mixture, we can choose the most appropriate separation technique to isolate them from the mixture. Generally, mixtures come in many forms and phases. Most of them can be separated, and the kind of separation method depends on the kind of mixture it is (Burtis, Ashwood, & Bruns, 2012).

Materials:

- Beaker
- Funnel
- Filter paper
- Reagent bottle
- Conical flask
- Analytical balance
- Water bath

Procedure:

You will be provided with some crude extracts from different solvents

1. Use filter paper to filter the crude extracts
2. Evaporate the filtrate to determine the yields

Record your observation in the table below:

Crude extracts	Mass of the residue (mg)	Volume of the filtrate (ml)	Yield (mg)
A			
B			
C			

Questions:

1. Differentiate between simple distillation and fractional distillation with examples.
2. Identify the factors determining the rate of evaporation.

References

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