Faculty of Science

Laboratory Manual

Instrumentation

Bachelor of Biotechnology (Hons.)

Edited By: Dr. Asita Elengoe

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LINCOLN UNIVERSITY COLLEGE
Wisma Lincoln, No. 12,14,16 & 18,
Jalan SS 6/12, Off Jalan Perbandaran
47301 Petaling, Jaya,
Selangor Darul Ehsan, Malaysia
Tel.: +603-7806 3478
Fax: +603-7806 3479
Toll Free: 1-300-880-111
E-mail: lucp@lincoln.edu.my
info@lincoln.edu.my
Web: www.lucp.net
www.lincoln.edu.my

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Instrumentation

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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).

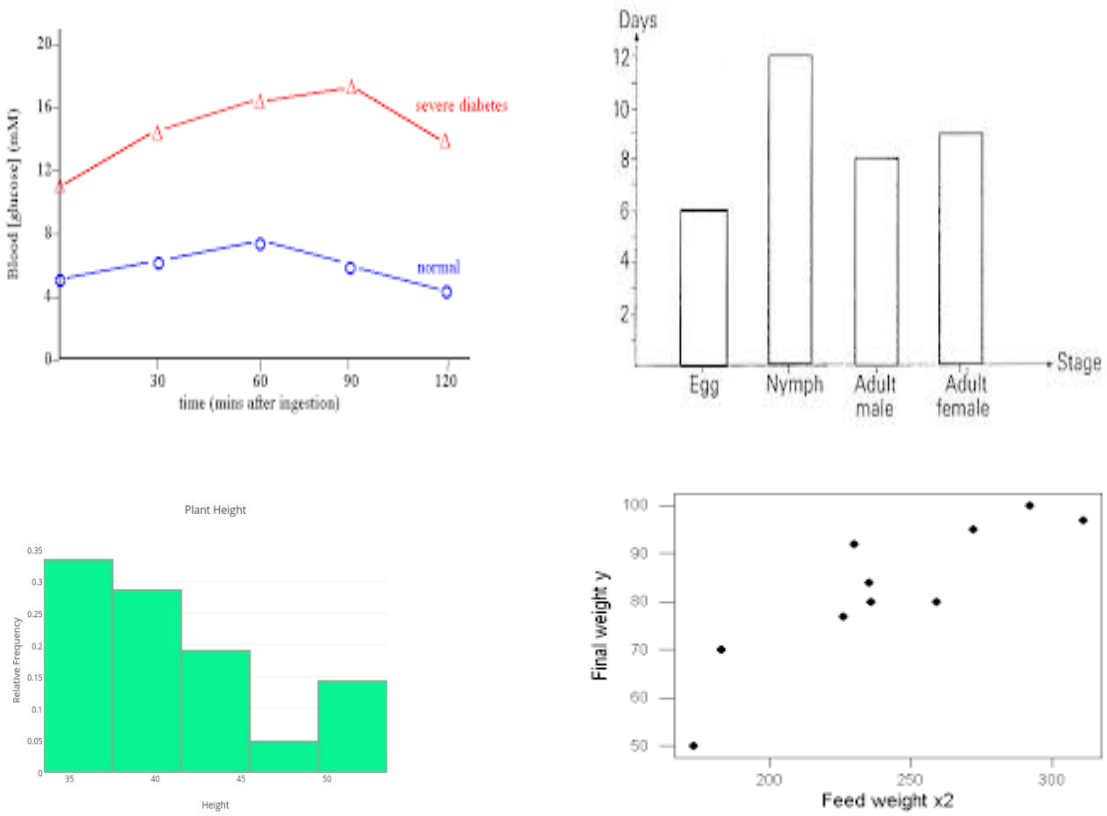


Figure 1: Line graphs, bar charts, histograms and scatter graphs

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: The Beer-Lambert law

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

1. To determine the absorption maximum of potassium permanganate
2. To verify Beer's law

Introduction:

The Beer-Lambert law relates the attenuation of light to the properties of the material through which the light is traveling. For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as Io - that's I for Intensity.

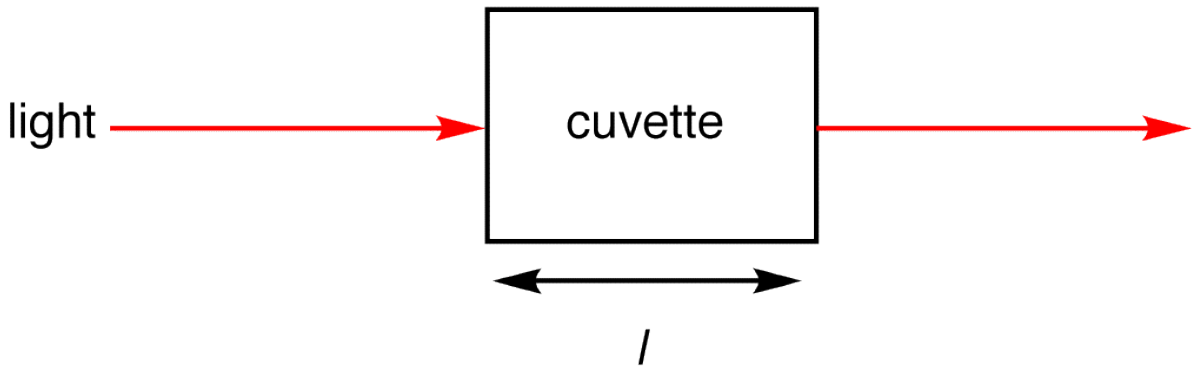


Figure 1: Light absorbed by sample in a cuvetter

The intensity of the light passing through the sample cell is also measured for that wavelength - given the symbol, I. If I is less than Io , then the sample has absorbed some of the light (neglecting reflection of light off the cuvetter surface). A simple bit of math is then done in the computer to convert this into something called the absorbance of the sample - given the symbol, A. The absorbance of a transition depends on two external assumptions.

The absorbance is directly proportional to the concentration (c) of the solution of the sample used in the experiment.

The absorbance is directly proportional to the length of the light path (l), which is equal to the width of the cuvette.

Assumption one relates the absorbance to concentration and can be expressed as

$$A \propto c \qquad (1.1)$$

The absorbance (A) is defined via the incident intensity Io and transmitted intensity I by

$$A = \log_{10}(Io/I) \qquad (1.2)$$

Assumption two can be expressed as

$$A \propto l \qquad (1.3)$$

Combining Equations 1.1 and 1.3:

$$A \propto cl \qquad (1.4)$$

This proportionality can be converted into equality by including a proportionality constant (ϵ).

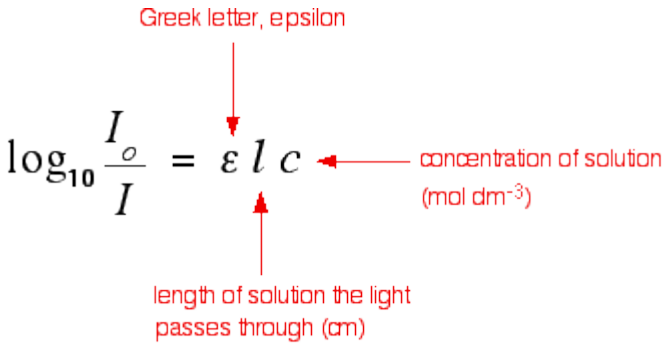
$$A = \epsilon cl \qquad (1.5)$$

This formula is the common form of the Beer-Lambert Law, although it can be also written in terms of intensities:

$$A = \log_{10}(Io/I) = \epsilon lc \qquad (1.6)$$

The constant ϵ is called molar absorptivity or molar extinction coefficient and is a measure of the probability of the electronic transition. On most of the diagrams you will come across, the absorbance ranges from 0 to 1, but it can go higher than that. An absorbance of 0 at some wavelength means that no light of that particular wavelength has been absorbed. The intensities of the sample and reference beam are both the same, so the ratio Io/I is 1 and the $\log_{10}$ of 1 is zero.

You will find that various different symbols are given for some of the terms in the equation - particularly for the concentration and the solution length.



The Greek letter epsilon in these equations is called the molar absorptivity - or sometimes the molar absorption coefficient. The larger the molar absorptivity, the more probable the electronic transition. In uv spectroscopy, the concentration of the sample solution is measured in molL^{-1} and the length of the light path in cm. Thus, given that absorbance is unitless, the units of molar absorptivity are L mol^{-1} cm^{-1}. However, since the units of molar absorptivity is always the above, it is customarily reported without units.

Materials:

- Graduated test tubes
- $KMnO_4$ solution (100mg/ml)

- Systrinics UV visible spectrophotometer
- Distilled water
- Pipette
- Volumetric flask

Procedure:

Stock solution preparation

1. Prepare a stock solution of $KMnO_4$ in water (100mg/ml).
2. From that stock solution, prepare solutions of concentrations 0.4, 0.8, 1.2, 1.6 and 2.0 mg/ml.
3. Do steps 1 and 2 with $K_2Cr_2O_7$.
4. Determine the absorbance of any concentration of $KMnO_4$.
5. Determine the Emax.
6. Determine the absorbance for all concentrations of $KMnO_4$ and $K_2Cr_2O_7$.

Instrumentation procedure

1. Switch on the computer and leave for 30 minutes to warm up.
2. Select the light source, slit width, scan speed and 1% transmittance.
3. Take two clean cuvettes with path length 1 cm.
4. Fill up one cuvette with the blank (distilled water) another with lowest concentration of $K_2Cr_2O_7$.
5. Place the blank sample and the sample in the spectrophotometer holder.
6. Run the scan.
7. Run similarly for all concentrations of $K_2Cr_2O_7$ and $KMnO_4$.
8. The wavelengths of x max, absorbance of x max for all concentrations of $K_2Cr_2O_7$ and $KMnO_4$ and record in a table.
9. Plot a graph of absorbance vs concentration for both $K_2Cr_2O_7$ and $KMnO_4$.

Observation/ Results:

Practical 2
Title: Infra-red spectrum of solid paracetamol

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

1. To prepare and measure sample: KBr pallets

Introduction:

An invaluable tool in organic structure determination and verification involves the class of electromagnetic (EM) radiation with frequencies between 4000 and 400 cm-1 (wavenumbers). The category of EM radiation is termed infrared (IR) radiation, and its application to organic chemistry known as IR spectroscopy. Radiation in this region can be utilized in organic structure determination by making use of the fact that it is absorbed by interatomic bonds in organic compounds. Chemical bonds in different environments will absorb varying intensities and at varying frequencies. Thus IR spectroscopy involves collecting absorption information and analyzing it in the form of a spectrum -- The frequencies at which there are absorptions of IR radiation ("peaks" or "signals") can be correlated directly to bonds within the compound in question. Figure 2 shows an example of IR spectrum.

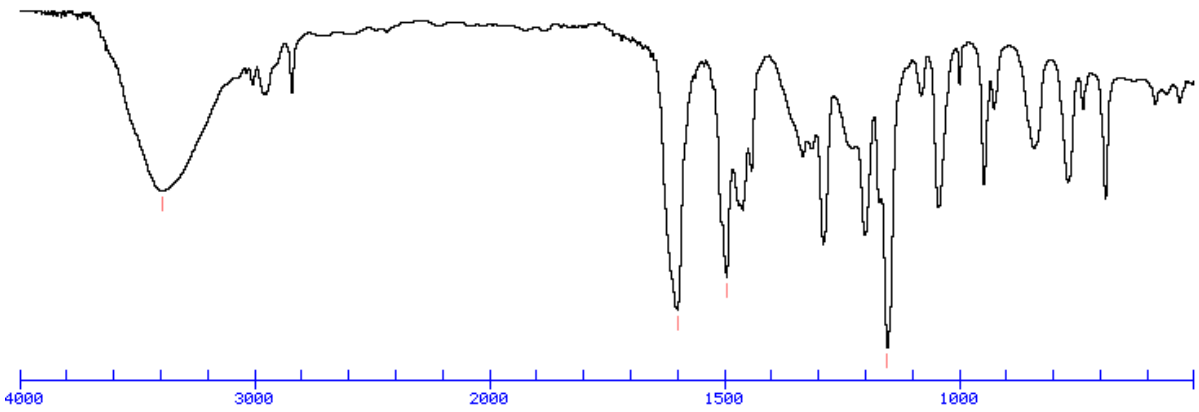


Figure 2: An example of IR Spectrum

Because each interatomic bond may vibrate in several different motions (stretching or bending), individual bonds may absorb at more than one IR frequency. Stretching absorptions usually produce stronger peaks than bending, however the weaker bending absorptions can be useful in differentiating similar types of bonds (e.g. aromatic substitution). It is also important to note that symmetrical vibrations do not cause absorption of IR radiation. For example, neither of the carbon-carbon bonds in ethene or ethyne absorb IR radiation.

Materials:

- Spatula
- Agate
- Mortar & pestle
- Die set
- Paracetamol
- KBr powder

Procedure:

Preparation of blank

1. Grind roughly 100 mg of KBr in mortar until fine particles.
2. Place one 7 mm bottom anvil place on table surface.
3. Slid 7 mm die over anvil column. DO NOT TOUCH OR SCRATCH the top SURFACE OF THE anvil column.
4. Fill the well formed by die and anvil with ground KBr, about ¼ full of the surface of column.
5. Position the second the anvil on top the assembly & pushed gently.
6. Place the die in barrel of 'hand press & adjust 'Platen positions dial'.
7. Squeeze the handle gently down until the end and pause for 15 minutes.
8. Release and remove the die set.
9. Die holder is used to attach die into sample compartment.

Preparation of sample

1. Weigh about 3 mg of sample (paracetamol) with 300 mg of KBr.
2. Mix and grind it finely with mortar and pestle.
3. Repeat steps 2-10 of preparation of blank.

Sample scanning

1. Click 'Measurement'.
2. Key the scan parameters in the Data according to the sample requirement/SOP.
3. All the instrument status displayed in the 'Status Monitor' is ensured to be OK (in green light).
4. Create data file name, comments and folder location that it will be saved is set.
5. Attach the blank die (without sample, only KBr) into the sample compartment.
6. Run a background by clicking BKG under Measurement.
7. Take out the blank die and replace with the die loaded with sample.
8. Click sample at the Measurement to scan the sample.

When the required numbers of scans are completed, the spectrum is viewed in the View.

Observation/ Results:

Practical 3

Title: Quantitative analysis by high performance liquid chromatography

Objective:

After completing the practical, you will be able:

1. To determine the samples by high performance liquid chromatography

Introduction:

High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

Sample retention time will vary depending on the interaction between the stationary phase, the molecules being analyzed, and the solvent, or solvents used. As the sample passes through the column it interacts between the two phases at different rate, primarily due to different polarities in the analytes. Analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

Instrumentation:

Main components in an HPLC system include the solvent reservoir, or multiple reservoirs, a high-pressure pump, a column, injector system and the detector. The reservoir holds the solvent, which is referred to as the mobile phase because it moves. There are usually a minimum of two reservoirs in a system, with each holding up to 1000 cc of solvent and usually fitted with a gas diffuser through which helium can be bubbled. A pump is used to generate a specified flow of the mobile phase. Although manual injection of samples is still possible, most HPLCs are now fully automated and controlled by computer. The injector, or auto sampler, introduces the solvent into a phase stream that carries the sample into the high pressure (up to 400 bar) column, which contains specific packing material needed to effect separation. The packing material is referred to as the stationary phase because it is held in place by the column hardware.

A detector is needed to see the separated compound bands as they elute from the high pressure column. The information is sent from the detector to a computer which generates the chromatogram. The mobile phase exits the detector and is either sent to a waste, or collected, as desired.

Helium sparging is an effective method of degassing the mobile phase to avoid unstable baselines caused by dissolved air. Nitrogen is used as a nebulisation gas in Evaporative Light Scattering Detector (ELSD) where the solvent is evaporated from the sample leaving a mist as is measured.

Procedure:

HPLC Conditions:

Colum: C18 250 x 4.6 mm (5 mm)
Mobile phase: 85 volume of methanol and 15 volume of 0.1 M ammonium acetate
Flow rate: 2.0 ml/min
Injection volume: 20 µl
Wavelength: 322 nm

(A)Preparation of solutions

1. Stock: Completely dissolve approximately 112 mg of sample in 100 ml of mobile phase.
2. Standard solution: Dilute 10 ml of stock solution to 100 ml with mobile phase in volumetric flask.
3. Test sample: Weigh and powder 20 tablets. Dissolve about 195 mg of powder with 50 ml of mobile phase, filter and dilute to 100 ml with mobile phase in volumetric flask. Dilute 10 ml of this solution to 100 ml with mobile phase.

(B) Analysis:

1. Inject the 20 µl of standard solution in the HPLC system and run the system following instructor's directions.
2. Print chromatogram and integration information for each sample.
3. Similarly, do the same with test sample.

Observation/Results:

Questions:

1. Which solvent mixture is normally used in HPLC?
2. How AR reagents differ from HPLC grade.
3. Compare the gas chromatography and HPLC.

Practical 4
Title: Quantitative analysis by gas chromatography

Objective:

After completing the practical, you will be able:
1. To determine the samples by gas chromatography

Introduction:

In early 1900s, Gas chromatography (GC) was discovered by Mikhail Semenovich Tsvett as a separation technique to separate compounds. In organic chemistry, liquid-solid column chromatography is often used to separate organic compounds in solution. Among the various types of gas chromatography, gas-liquid chromatography is the method most commonly used to separate organic compounds. The combination of gas chromatography and mass spectrometry is an invaluable tool in the identification of molecules. A typical gas chromatograph consists of an injection port, a column, carrier gas flow control equipment, ovens and heaters for maintaining temperatures of the injection port and the column, an integrator chart recorder and a detector.

To separate the compounds in gas-liquid chromatography, a solution sample that contains organic compounds of interest is injected into the sample port where it will be vaporized. The vaporized samples that are injected are then carried by an inert gas, which is often used by helium or nitrogen. This inert gas goes through a glass column packed with silica that is coated with a liquid. Materials that are less soluble in the liquid will increase the result faster than the material with greater solubility. The purpose of this module is to provide a better understanding on its separation and measurement techniques and its application.

In GLC, the liquid stationary phase is adsorbed onto a solid inert packing or immobilized on the capillary tubing walls. The column is considered packed if the glass or metal column tubing is packed with small spherical inert supports. The liquid phase adsorbs onto the surface of these beads in a thin layer. In a capillary column, the tubing walls are coated with the stationary phase or an adsorbant layer, which is capable of supporting the liquid phase. However, the method of GSC, has limited application in the laboratory and is rarely used due to severe peak tailing and the semi-permanent retention of polar compounds within the column. Therefore, the method of gas-liquid chromatography is simply shortened to gas chromatography and will be referred to as such here. The purpose of this module is to provide a better understanding on its separation and measurement techniques and its application.

Procedure:

Instruments setting:

Column	: 30m x 0.25mm ID-BPX5 0.25 m
Colum Temperature	: 175°C
Carrier gas	: Hydrogen gas
Split ratio	: 1:50
Ancillary gases	: Hydrogen gas and air
Detector	: Flame ionization detector (FID)

Analysis:

1. Inject the 20 µl of standard solution in the HPLC system and run the system following instructor's directions.
2. Print chromatogram and integration information for each sample.
3. Similarly, do the same with test sample.

Observation/Results:

Question:

1. Describe the components and its functions of the gas chromatography.

LINCOLN UNIVERSITY COLLEGE

Wisma Lincoln, No. 12, 14, 16 & 18,
Jalan SS 6/12,47301 Petaling Jaya,
Selangor Darul Ehsan, Malaysia.
Tel.: +603-7806 3478 Fax: +603-7806 3479
Toll Free: 1-300-880-111
E-mail: lucp@lincoln.edu.my
Web.: www.lucp.net

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