COLLEGE OF SCIENCE, TECHNOLOGY & APPLIED ARTS

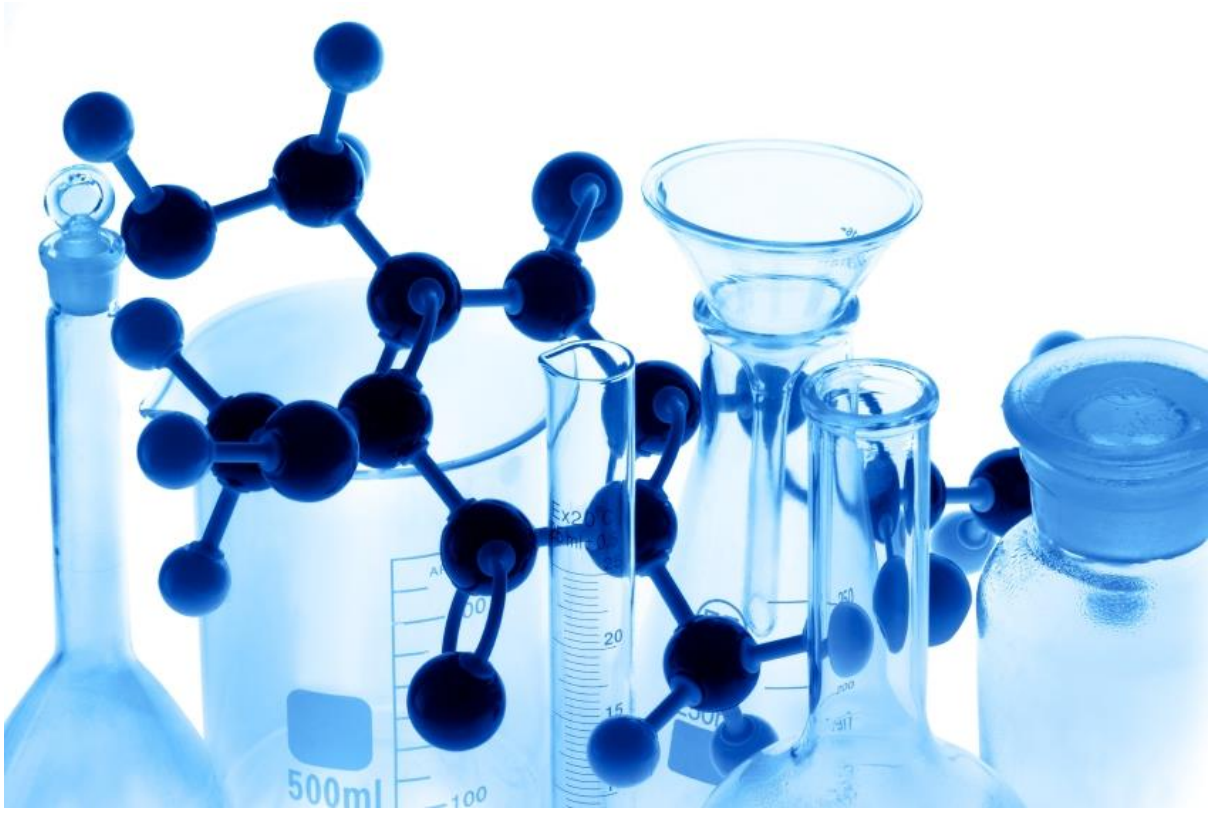
OF TRINIDAD & TOBAGO

School of Nursing Health and Environmental Sciences
Department of Natural & Life Sciences

CHEM 121 – Biochemistry for Nursing

Student Manual – Version 2 (Stream B)

Edited: 26/01/2018



A Word of Welcome

The School of Nursing Health and Environmental Sciences and the Department of Natural & Life Sciences welcome you to the practical component of the course. The experiments that form part of this course have been selected to:

- Reinforce your knowledge and understanding of the theoretical content of your first year Chemistry course.
- Develop skills such as observing, recording, measuring and reporting of results.
- Teach you special techniques, such as weighing, pipetting, titrating, filtration etc, so that you will become familiar with the basics of laboratory work.

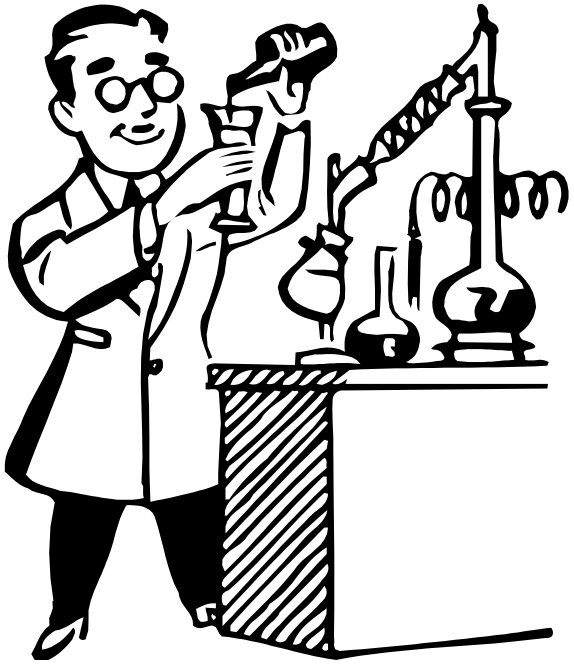


TABLE OF CONTENTS

Introduction

General

Always read and study the relevant experiment before coming to the laboratory. Check out appropriate references to help you understand the procedures you will be carrying out.

Attendance at all laboratory sessions is compulsory.

Learning lab techniques must come from *experiencing* lab techniques. Experience is gained from carrying out experiments. You will need to plan ahead for your lab session, and think about what you are doing (and why) while you are in lab.

Use past experiences to develop new skills.

You will gain new skills with new equipment and learn more about the chemistry theories and concepts you hear about in lectures, but the skills that we expect you to develop the most during these experiments are general scientific skills that should be transferable to any other scientific discipline and to your daily life. These skills developed in the laboratory are fundamental to the world of work because industries depend on the laboratory to maintain a level of quality, which enhances the company's reputation, as well as for product development.

The lab is a part of the learning process.

LABORATORY SCHEDULE:

The Lab schedules are posted at the start of the semester on notice boards, lab tech stations and on the lab doors. Please refer to them to find out the dates and times of your lab sessions.

Once the semester begins, you will have to balance your laboratory study time with all of the other demands that you have. Most of the experiments have been scheduled so that the average student can finish everything (including the report) required in the laboratory period.

You are expected to attend all scheduled laboratory periods and make-up labs will not be scheduled.

SONHES Multi-Science Laboratory Rules

Before Entering the Lab

Proper attire is required for entry into the lab*

- Lab Coat
  - Long sleeve (wrist length)
  - Knee Length
  - Drill cotton
- Long pants or long skirt (ankle length)
- Shoes that fully cover your feet
- Long hair tied back
- No dangling jewelry or accessories

Punctuality

- Students are not allowed entry into lab 15 minutes ***after*** the start of the lab session.

During the Lab Session

- No eating, No drinking, No chewing of gum, No smoking
- Mobile phones and electronic devices (laptops, tablets etc.) are not to be used in the lab.
- Students are expected to follow the lab rules and to follow the guidance of the lab technicians while in the lab.
- Students are expected to have their own lab manuals at each lab session. No new manuals will be issued to students who forgot to bring theirs.

Leaving the Lab

- Students are required to wash all glassware, return items to student cupboards as well as dispose of gloves, tissue, scrap paper etc and wipe countertops before leaving the lab.
- Lab coats are to be taken off when leaving the lab (including trips to the bathroom).

*LAB LECTURERS OR LAB TECHNICIANS MAY PREVENT ENTRY OR ASK STUDENTS TO LEAVE THE LAB IN THE EVENT OF STUDENT NON-COMPLIANCE TO THE LABORATORY RULES.

- Wrist length sleeves for coverage
- Protects clothes
- Able to button at front
- Thick material for protection from spills
- Durable, long lasting protection for future labs
- Feel like a scientist!!

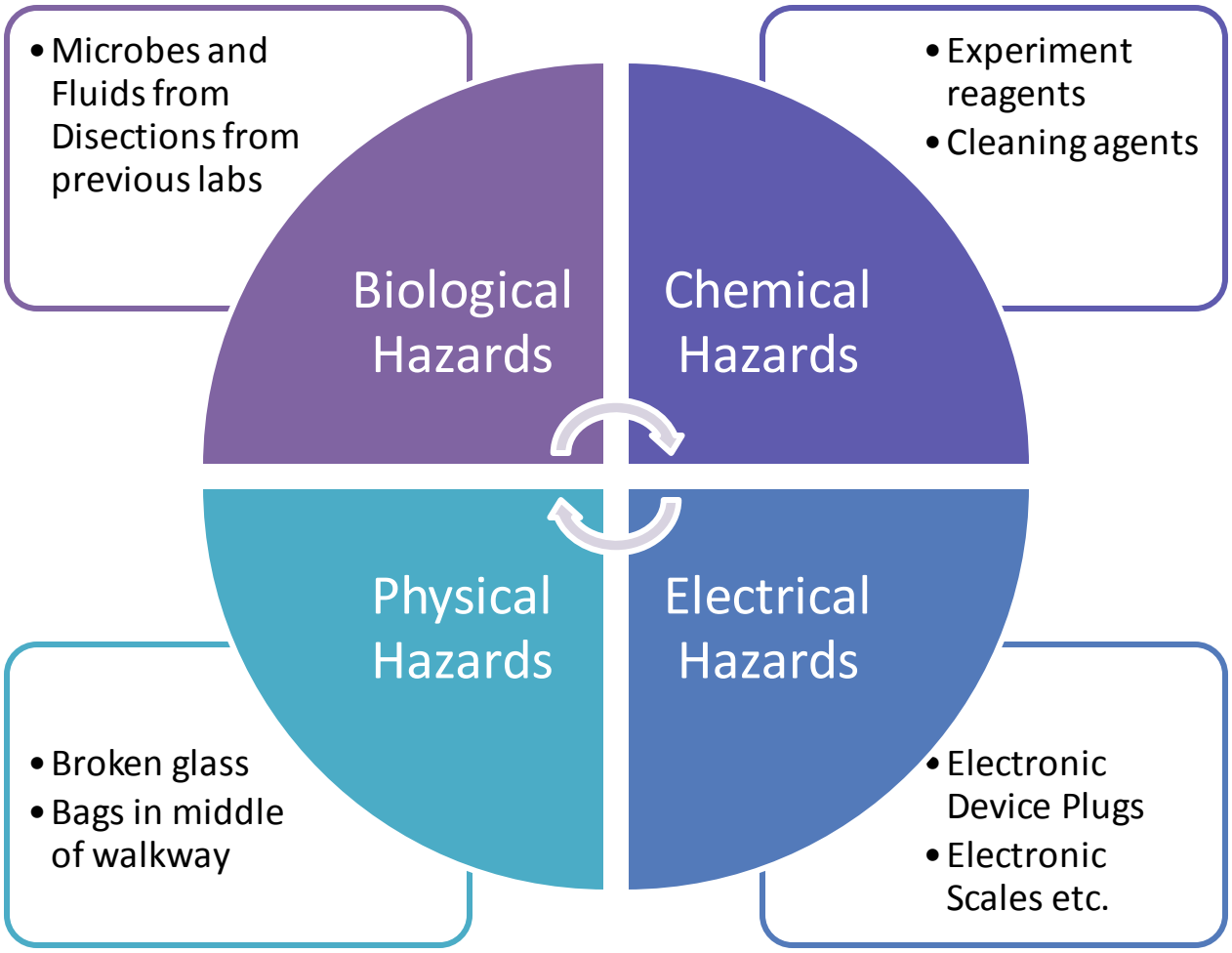
- Minimal barrier against chemicals and microbes
- Skin exposed
- Thin material allows chemicals to permeate and reach skin
- Hoodies can transmit contaminants to friends & family

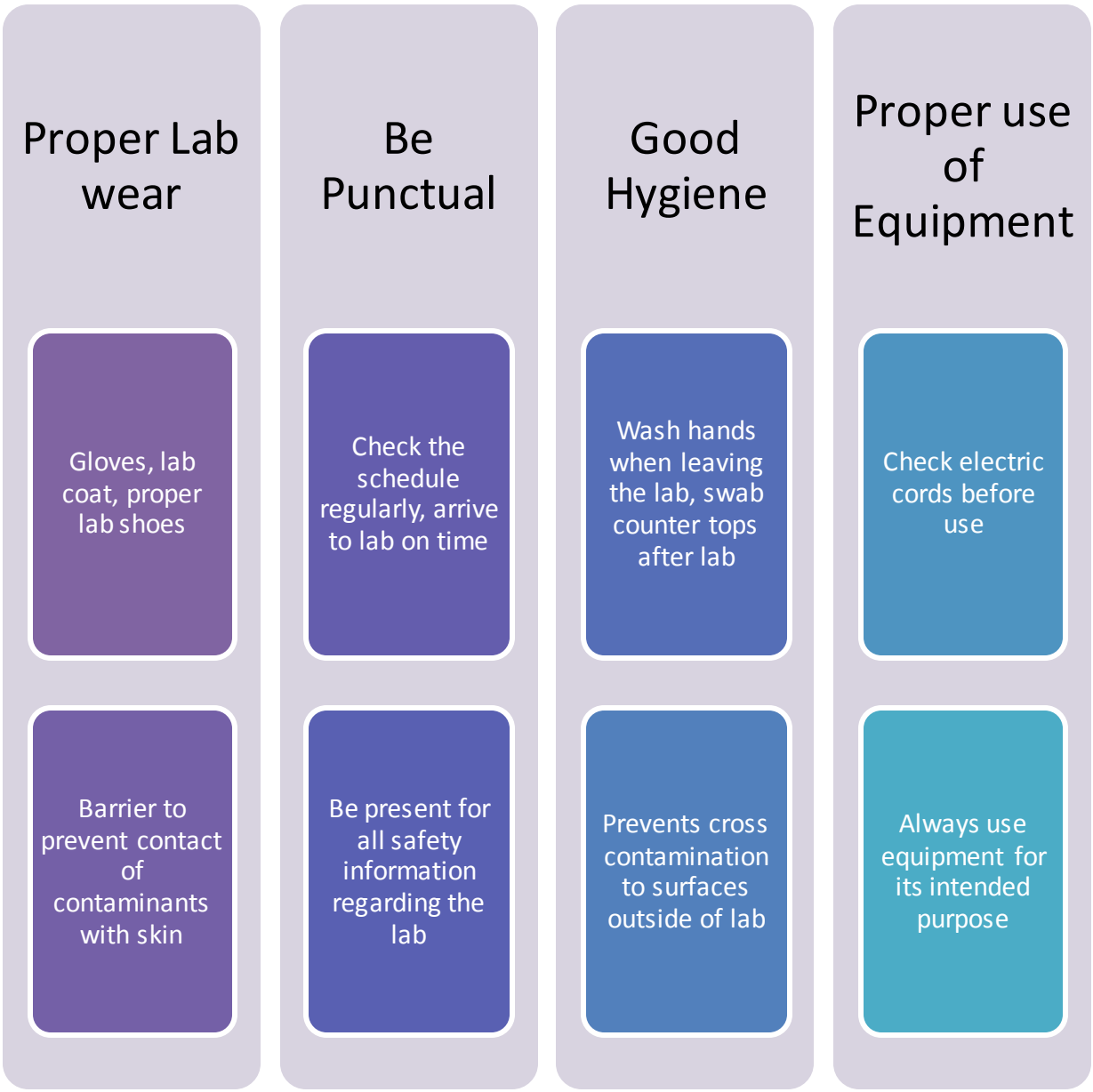
- Feet fully covered!
- Comfortable
- Ankle length Pants
- Protection from spills and broken glassware

- Feet exposed
- Limited protection from spills
- Possiblity of cuts from broken glassware
- Possible exposure to pathogens

SAFETY IN THE CHEMISTRY LABORATORY

The laboratory is a hazardous environment in which to work. The hazards are often unavoidable since scientists regularly have to use hazardous materials and equipment. However, with sensible precautions the laboratory is probably no more dangerous than your home. You MUST AT ALL TIMES OBSERVE ALL RULES stated in this manual.




Proper Lab wear
Gloves, lab coat, proper lab shoes
Barrier to prevent contact of contaminants with skin
Be Punctual
Check the schedule regularly, arrive to lab on time
Be present for all safety information regarding the lab
Good Hygiene
Wash hands when leaving the lab, swab counter tops after lab
Prevents cross contamination to surfaces outside of lab
Proper use of Equipment
Check electric cords before use
Always use equipment for its intended purpose

Essential Rules for Laboratory Safety

The essential rules for laboratory safety can be expressed under two simple headings:

ALWAYS AND NEVER

ALWAYS

- Familiarize yourself with the laboratory safety procedures.
- Dress appropriately.
- Wash your hands before leaving the laboratory.
- Read the instructions for the laboratory protocol **carefully** before starting any experiment.
- Handle all chemicals with great care.
- Keep your working area tidy.
- Immediately inform your instructor about spills.

NEVER

- Eat or drink in the laboratory. CHEWING OF GUM IS STRICTLY PROHIBITED!
- Smoke in the laboratory
- Inhale, taste or sniff chemicals.
- Fool around or distract neighbours.
- Carry out unauthorized experiments.

Laboratory Safety Procedures

Your laboratory will have certain procedures, which you must be familiar with. Make sure you know where all the exits from the laboratory are, in the event of an evacuation because of fire or other incident. Locate the SHOWERS and eyewash stations and know the type the fire extinguishers that are in the laboratory and how to operate them.

Dress Code

- Wear a Lab coat.
- Wear shoes that cover your entire feet (sneakers) Avoid open footwear & high heels. So sandals, slipper and ballet flats are unacceptable.
- Wear approved safety goggles or safety glasses when in the lab.
- Avoid floppy garments; avoid things that dangle. These get tangled up in equipment or glassware and cause accidents.
- Avoid long, loose hair styles for the same reason. When Bunsen burners are in use, long hair sometimes catches on fire.

Laboratory Techniques

Handling of Acids or Bases

- In addition to the dress code above, personal protective equipment, such as Vinyl" gloves or "nitrile" GLOVES SHOULD be worn.
- For even more dangerous liquids, thick "nitrile" gloves should be used. These are very safe.
- **DO NOT add *WATER* to *CONCENTRATED ACID*.** The heat generated will cause splattering. If necessary to prepare certain solutions, **DO** add ACID to WATER (instead of the reverse order of addition). The heat generated will be less, but splattering still may occur. A good practice in all lab operations is to keep things at arm's length.

Become acquainted with handling laboratory safety equipment e.g.

- Fire Extinguishers
- Fire Blanket
- Eye-wash Fountain
- First-Aid Kit

Become acquainted with using of the FUME HOOD

- Use the HOOD for reactions that give off vapours, especially smelly vapours.
- The draft of the HOOD will sweep away vapours so that the lab itself maintains reasonable air quality.

Locate the Safety Shower and understand how it works

- Shower should be used for ***dire EMERGENCY*** only!
- If you are ***ON FIRE***, or suffer a ***massive spill*** of a **dangerous chemical**, and need to get it off rapidly.

Position yourself under the safety shower and pull the handle — a deluge of water will result.

Immediately clean up Broken Glass

- Sweep it up right away
- Place the broken glass in a "SHARP'S CONTAINER.
- This is a thick walled carton that will be sealed and discarded as such.

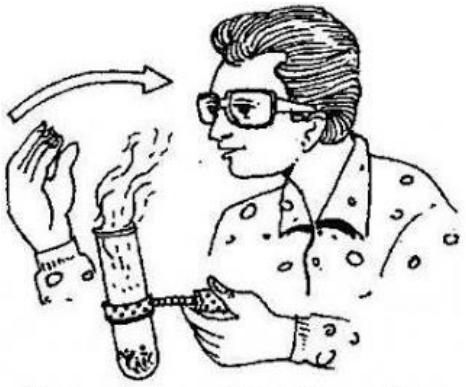
Student Use of the Laboratory

- No UNAUTHORIZED experiments! These are terrible dangers in unskilled hands
- No EATING or DRINKING in the LAB. A good practice is to assume everything in the lab is toxic.
- DO NOT drink from lab equipment. NOT EVEN distilled water.
- When using pipettes, **DO NOT** suck the liquid into the pipette directly by mouth.
- Frequent mistakes, even by skilled lab workers, lead to the liquid reaching the mouth.
- Do NOT smell the flask directly.

Housekeeping:

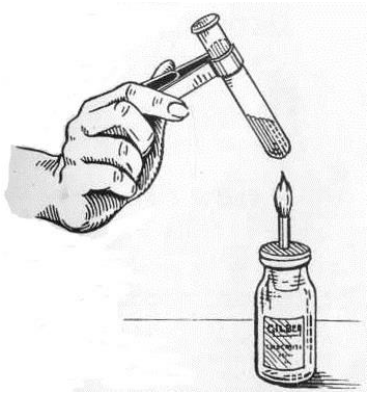
- **STUDENTS MUST CLEAN UP AFTER THEMSELVES**.
- Paper and trash must not be left in the room, thrown into drawers, sinks, on the floor, etc.
- When the equipment boxes are on carts, students who take a box form the cart to do an experiment must put everything back into the box neatly and put the box back on the cart.

If you absolutely must test the odour, carefully waft the vapour from the flask toward your nose with your hand, keeping the flask quite distant from your face.



Correct way of wafting odors.

Make sure that reaction tubes, e.g. test tubes, are not directed toward yourself or other persons. The chemicals may splatter out the tube.



WASTE DISPOSAL

We all have a responsibility towards a clean and safe environment. The Chemistry Department's policy dealing with the disposal of laboratory waste is in accordance with the Occupational Health and Safety Act, which is a law of our country. We do not expect you to understand every aspect of this law, but as responsible citizen and laboratory user you must be aware of the correct procedures for disposal of the different classes of laboratory waste. You will be given specific instructions on how to dispose of your chemical wastes during each pre-lab briefing. Here are also some general rules that you must take note of:

1. Place broken glass into the specially marked bins.
2. Drain all harmless chemicals in solution into the sink followed by plenty of running water.
3. Pour toxic inorganic waste solutions into the specially labelled container in the fume cupboard.
4. Discard paper and any other solid waste into the bin.
5. Ensure that matches are extinguished before disposing of them in this way.
6. Shut off all gas and water lines when not in use.

MANAGING YOUR TIME DURING THE PRACTICAL

1. It is recommended that you arrange your workbench according to the diagram below. Keeping your workspace organized and free of clutter will save you time and frustration, and contributes greatly to laboratory safety.

BENCH SOLUTIONS		
USED APPARATUS AND DIRTY GLASSWARE		SOLUTIONS AND DRY CHEMICALS
PAPERWORK (PRAC MANUAL, REPORT SHEET AND FLOW DIAGRAM)	EXPERIMENTAL SET-UP	CLEAN GLASSWARE

2. When collecting chemicals:

   - Choose the size of your container according to the volume of chemical that you will be collecting, for instance: for 100 cm^3 of solution, use a 250 cm^3 beaker and for 10 cm^3 use a test tube.
   - Before collecting chemicals and/or solutions from the dispensary, mark each container with the name or formula of the chemical to be collected. This will prevent any mix-ups later on.

3. Use a small notebook for jotting down masses, measurements and observations. Writing these bits of information on slips of scrap paper that can easily be lost is not only unprofessional, but also risky because it means that your whole afternoon's practical work can be wasted because of lost data.

GETTING STARTED

For each laboratory period, including the first, you will be required to:

- Study the entire experimental outline in the **Laboratory Manual**. It includes specific directions concerning laboratory philosophy and protocols.
- Prepare your **Laboratory Notebook**. Include a summary and a procedural outline for your experiment (see "Rules for Keeping Your Laboratory Notebook" and Instruction in the experiment outline).
- Bring your safety goggles or use the safety goggles provided. These offer adequate protection against accidental splashing of corrosive chemicals.
- Come to lab dressed appropriately

A GOOD PRACTICE:

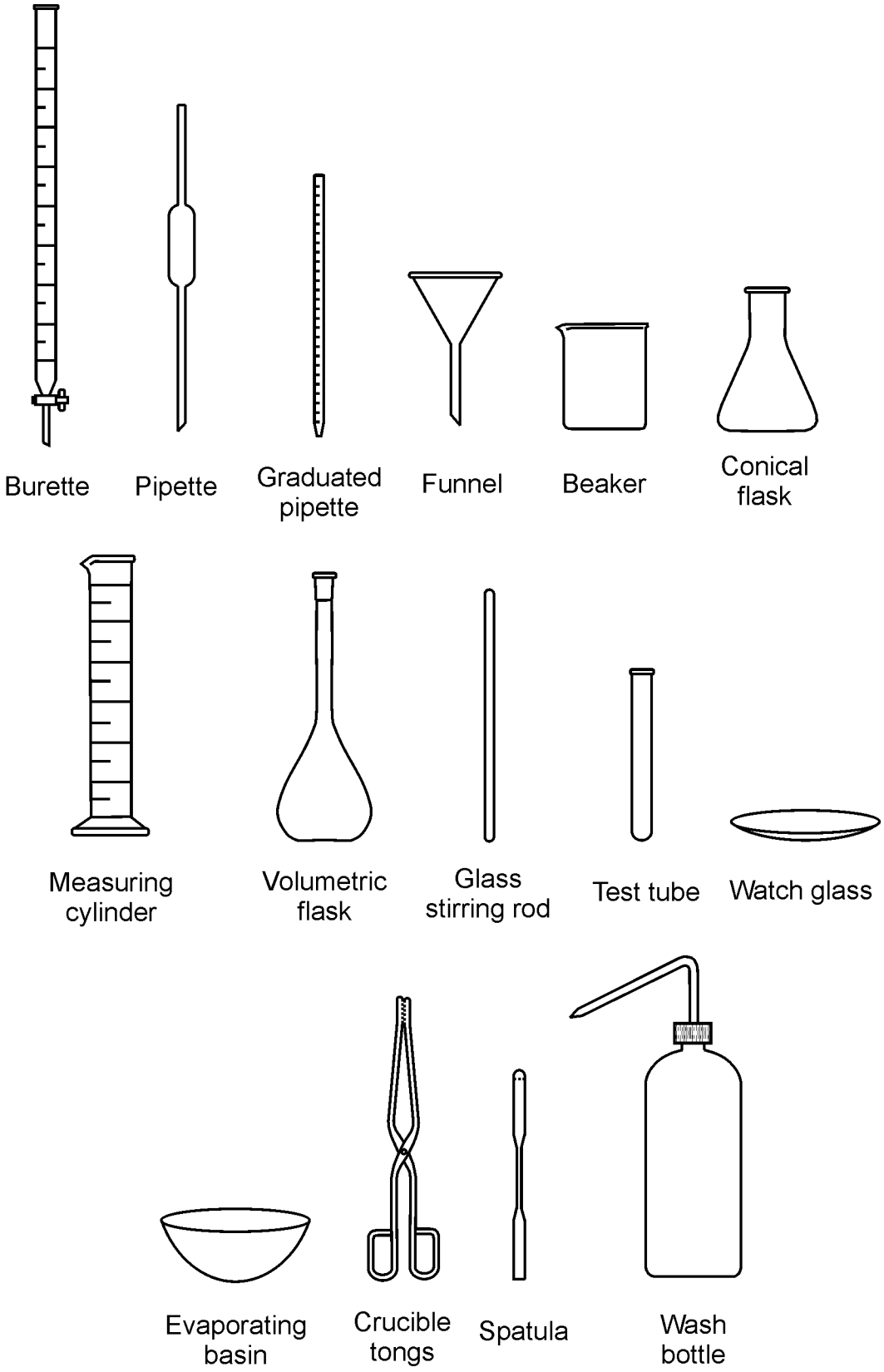
Read the experimental procedure ahead of lab.

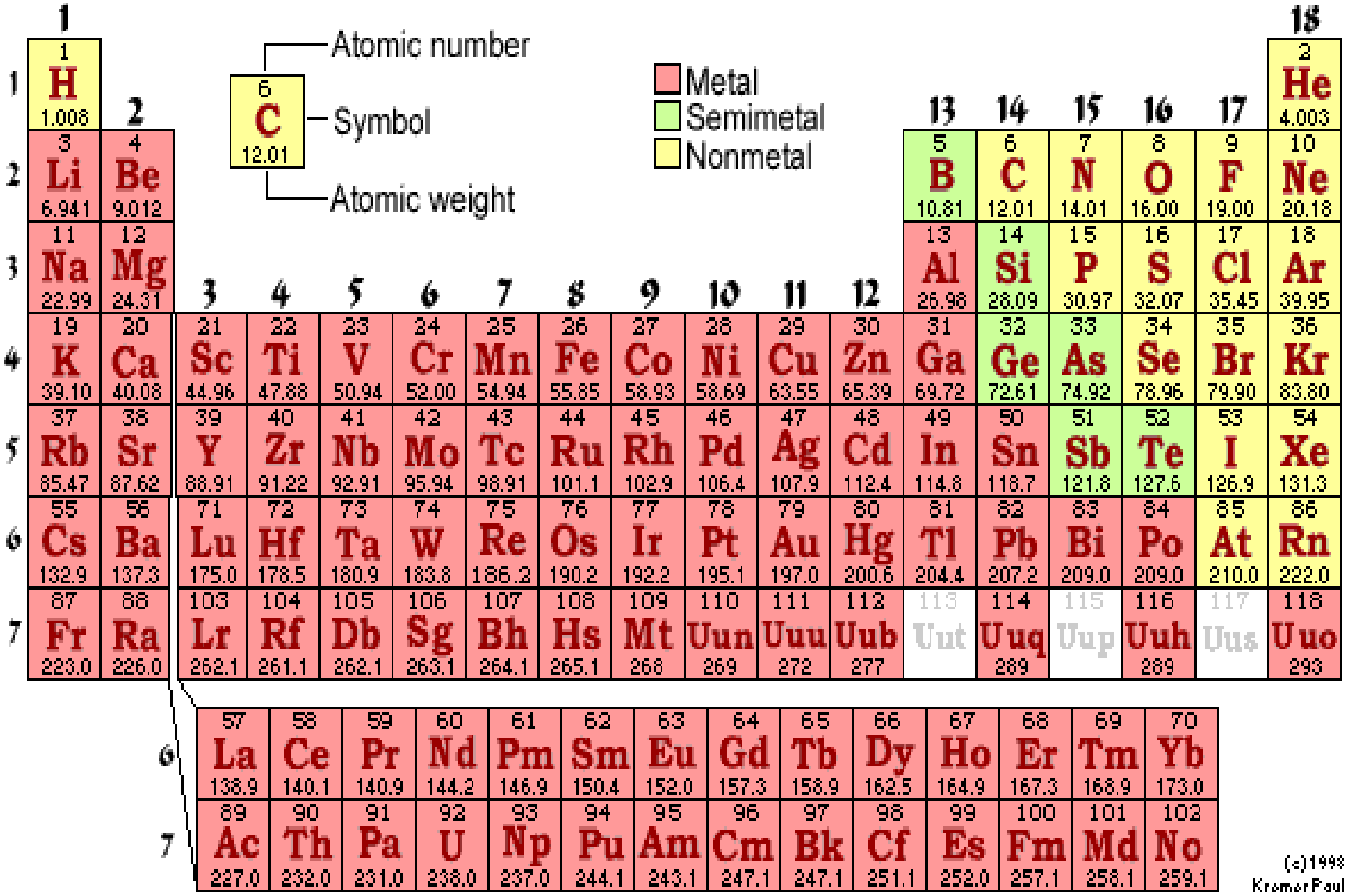
Avoid horseplay. In a laboratory setting, horseplay, even if good-natured, is absolutely unacceptable.

- **No pushing!**
- **No shoving!**

At the end of the lab period: Exit the lab in an orderly manner. Again: no running, no pushing, AND NO shoving.

Figure 1: Apparatus that you will be using in the laboratory




Atomic number
Symbol
Atomic weight
Metal
Semimetal
Nonmetal
(c)1998
Kremer Paul

Experiment 1: Test for Carbohydrates

Aim: To use se physical and chemical tests to distinguish among monosaccharides, disaccharides, and polysaccharides; to identify an unknown carbohydrate.

Background:

A. Benedict's Test for Reducing Sugars

All of the monosaccharides and most of the disaccharides can be oxidized. When the cyclic structure opens, the aldehyde group is available for oxidation. Benedict's reagent contains Cu^{2+} ion that is reduced. Therefore, all the sugars that react with Benedict's reagent are called *reducing sugars*. Ketoses also act as reducing sugars because the ketone group on carbon 2 isomerizes to give an aldehyde group on carbon 1.When oxidation of a sugar occurs, the Cu^{2+} is reduced to Cu^{+}, which forms a red precipitate of cuprous oxide, $Cu_2O(s)$. The color of the precipitate varies from green to gold to red depending on the concentration of the reducing sugar.

Sucrose is not a reducing sugar because it cannot revert to the open-chain form that would provide the aldehyde group needed to reduce the cupric ion.

B. Seliwanoff's Test for Ketoses

Seliwanoff's test is used to distinguish between hexoses with a ketone group and hexoses that are aldehydes. With ketoses, a deep red color is formed rapidly. Aldoses give a light pink color that takes a longer time to develop. The test is most sensitive for fructose, which is a ketose.

C. Fermentation Test

Most monosaccharides and disaccharides undergo fermentation in the presence of yeast. The products of fermentation are ethyl alcohol (CH_3CH_2OH) and carbon dioxide (CO_2). The formation of bubbles of carbon dioxide is used to confirm the fermentation process.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} \underset{\text{Ethanol}}{2C_2H_5OH} + 2CO_2(g)$$

Although enzymes are present for the hydrolysis of most disaccharides, they are not available for lactose. The enzymes needed for the fermentation of galactose are not present in yeast. Lactose and galactose give negative results with the fermentation test.

D. Iodine Test for Polysaccharides

When iodine (I_2) is added to amylose, the helical shape of the unbranched polysaccharide traps iodine molecules, producing a deep blue-black complex. Amylopectin, cellulose, and glycogen react with iodine to give red to brown colors. Glycogen produces a reddish-purple color. Monosaccharides and disaccharides are too small to trap iodine molecules and do not form dark colors with iodine.

E. Hydrolysis of Disaccharides and Polysaccharides

Disaccharides hydrolyze in the presence of an acid to give the individual monosaccharides.

$$\text{Sucrose} + H_2O \xrightarrow{H^+} \text{Glucose} + \text{Fructose}$$

In the laboratory, we use water and acid to hydrolyze starches, which produce smaller saccharides such as maltose. Eventually, the hydrolysis reaction converts maltose to glucose molecules. In the body, enzymes in our saliva and from the pancreas carry out the hydrolysis. Complete hydrolysis produces glucose, which provides about 50% of our nutritional calories.

Experimental Procedures:

A. Fermentation Test

Materials: Fermentation tubes (or small and large test tubes), baker's yeast, 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch, and an unknown

Fill fermentation tubes with a solution of glucose, fructose, sucrose, lactose, starch, water, and unknown. Add 0.2 g of yeast to each and mix well. If fermentation tubes are not available, use small test tubes placed upside down in larger test tubes. Cover the mouth of the large test tube with filter paper or cardboard. Place your hand firmly over the paper cover and invert. When the

small test tube inside has completely filled with the mixture, return the larger test tube to an upright position. See **Figure 2.**

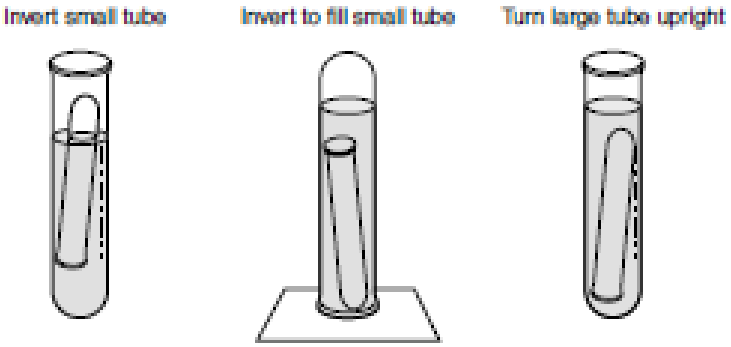


Figure 2 Test tubes used as fermentation tubes

Set the tubes aside. At the end of the laboratory period, and again at the next laboratory period, look for gas bubbles in the fermentation tubes or inside the small tubes. Record your observations. See Figure 3.

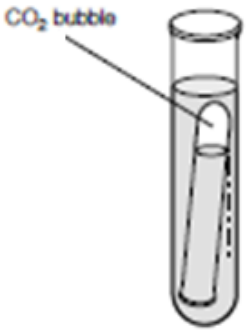


Figure 3 Fermentation tubes with CO_2 bubbles

B. Benedict's Test for Reducing Sugars

Materials: Test tubes, 400-mL beaker, droppers, hot plate or Bunsen burner, 5- or 10-mL graduated cylinder, Benedict's reagent, 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch, and an unknown.

Place 10 drops of solutions of glucose, fructose, sucrose, lactose, starch, water, and unknown in separate test tubes. Label each test tube. Add 2 mL of Benedict's reagent to each sample. Place the test tubes in a boiling water bath for 3–4 minutes. Record your observations. Classify each as a reducing or nonreducing sugar.

C. Seliwanoff's Test for Ketoses

Materials: Test tubes, 400-mL beaker, droppers, hot plate or Bunsen burner, 5- or 10-mL graduated cylinder, Seliwanoff's reagent, 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch, and an unknown.

Place 10 drops of solutions of glucose, fructose, sucrose, lactose, starch, water, and unknown in separate test tubes. Add 2 mL of Seliwanoff's reagent to each. ***The reagent contains concentrated HCl. Use carefully.*** Place the test tubes in ***a boiling hot water bath*** and note the time. After 1 minute, observe the colors in the test tubes. A rapid formation of a deep red color indicates the presence of a ketose. Record your results as a fast color change, slow change, or no change.

D. Iodine Test for Polysaccharides

Materials: Spot plate or test tubes, droppers, iodine reagent, 2% carbohydrate solutions in dropper bottles: glucose, fructose, sucrose, lactose, starch, and an unknown.

Using a spot plate, place 5 drops of each solution of glucose, fructose, sucrose, lactose, starch, water, and unknown in the wells. (If you do not have a spot plate, use small test tubes.) Add 1 drop of iodine solution to each sample. A dark blue-black color is a positive test for amylose in starch. A red or brown color indicates the presence of other polysaccharides. Record your results. Complete the table to identify your unknown.

E. Hydrolysis of Disaccharides and Polysaccharides

Materials: Test tubes, 10-mL graduated cylinder, 400-mL beaker (boiling water bath), hot plate or Bunsen burner, spot plate or watch glass, 10% HCl, 10% NaOH, red litmus paper, iodine reagent, Benedict's reagent, 2% starch and sucrose solutions in dropper bottles.

Place 3 mL of 2% **starch** in two test tubes and 3 mL of 2% **sucrose** solution in two more test tubes. To one sample each of sucrose and starch, add 20 drops of 10% HCl. To the other samples of sucrose and starch, add 20 drops of H_2O. Label the test tubes and heat in a boiling water bath for 10 minutes.

Remove the test tubes from the water bath and let them cool. To the samples containing HCl, add 10% NaOH (about 20 drops) until one drop of the mixture turns litmus paper blue, indicating the HCl has been neutralized. Test the samples for hydrolysis as follows:

Iodine Test Place 5 drops of each solution on a spot plate or watch glass. Add 1 drop of iodine reagent to each. Record observations. Determine if hydrolysis has occurred in each.

Benedict's Test Add 2 mL of Benedict's reagent to each of the samples and heat in a boiling water bath for 3–4 minutes. Determine if hydrolysis has occurred in each.

F. Testing Foods for Carbohydrates

Materials: Sugar samples (refined, brown, "natural," powdered), honey, syrups (corn, maple, fruit), foods with starches: cereals, pasta, bread, crackers, potato, Benedict's solution, Seliwanoff's reagent, iodine reagent.

Obtain TWO carbohydrate samples to test. Perform the Benedict's, Seliwanoff's, and iodine tests on each. Describe the kinds of carbohydrates you identify in each sample.

Results:

Table 1: Results of Carbohydrate Tests

	Benedict' s Test	**Seliwanoff's Test**	**Fermentation Test**	**Iodine Test**
Glucose				
Fructose				
Sucrose				
Lactose				
Starch				
Water				
Unknown				

Table 2: Identifying an unknown carbohydrate

	Results with Unknown	**Possible Sugars present**
Benedict's		
Seliwanoff's Test		
Fermentation		
Iodine		

What carbohydrate (s) is/are in your unknown? _____

Table 3: Hydrolysis of disaccharides and polysaccharides

Results	**Sucrose + H_2O**	**Sucrose + HCl**	**Starch + H_2O**	**Starch + HCl**
Iodine				
Benedict's				
Hydrolysis products present				

Table 4: Testing Food for Carbohydrates

	Food Item 1	**Food Item 2**
Benedict's		
Seliwanoff's Test		
Fermentation		
Iodine		

Discussion

As part of your discussion you should include the answers to the following questions:

1. Which sugars gave a positive fermentation test?
2. From the results in Part B, identify the sugars that are reducing sugars and those that are not.
3. Which sugars are ketoses?
4. Which carbohydrates gave a blue-black colour in the iodine test?
5. How do the results of the Benedict's test indicate that hydrolysis of sucrose and starch occurred?
6. How do the results of the iodine test indicate that hydrolysis of starch occurred?
7. What carbohydrate (s) is/are in your unknown?
8. What types of carbohydrates are present in the two food items you tested?

Post Lab Questions

What carbohydrate(s) would have the following results?

(a) Produces a reddish-orange solid with Benedict's and a red colour with Seliwanoff's reagent in 1 minute

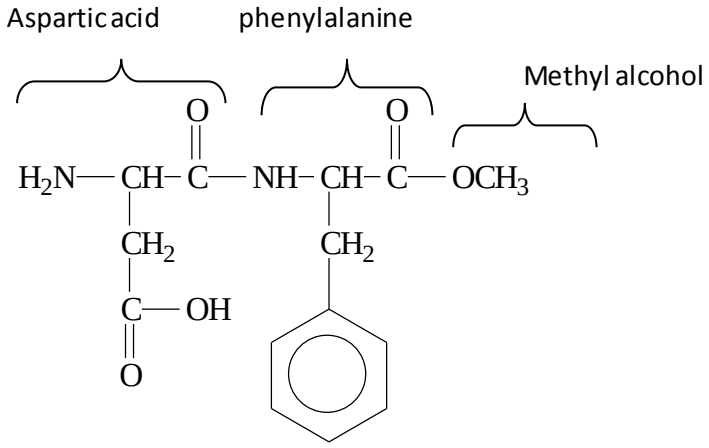
(b) Gives a colour change with Benedict's test, a light orange colour with Seliwanoff's reagent after 5 minutes, and produces no bubbles during fermentation

(c) Gives no colour change with Benedict's or Seliwanoff's test, but turns a blue-black color with iodine reagent

Experiment 2: Aspartame – The Study of a Peptide Bond

Aim: To identify aspartame and its hydrolysis products using thin-layer chromatography.

Background: Aspartame, commonly known as NutraSweet or Equal, is the most popular artificial, low-calorie sweetener available to consumers today. If you look closely at the structure of aspartame, you will notice that the terminal acid group is not a carboxylic acid, but rather the methyl ester of carboxylic acid. This type of variation in protein structure is very common throughout all types of amino acids. Therefore, upon hydrolysis, aspartame will yield aspartic acid, phenylalanine, and methyl alcohol (methanol).



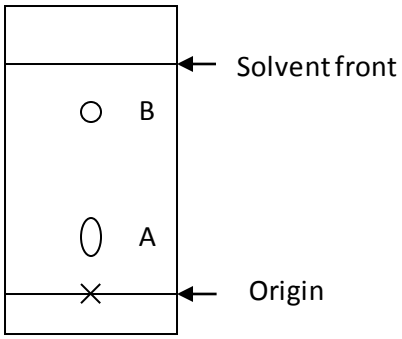
ASPARTAME

Weight for weight, aspartame is almost 200 times sweeter than sucrose (table sugar). The use of aspartame does have some drawbacks. For example, it has a relatively short shelf-life since it can be converted to a tasteless cyclic form in solution in as little as 3 months. In addition, it appears to decompose in the presence of heat or strongly acidic/basic media.

Thin-layer chromatography is a useful analytical technique that allows various components of a mixture to be separated based on their polarity. Generally, a thin sheet of plastic or glass is used that has been coated with silica gel. Silica gel is a polar substance that will attract other polar substances. As a result, non-polar substances will not have a strong affinity for the gel. A small

drop of the mixture to be separated is applied near one end of the plate. The spotted end of the plate is then dipped into a developing solvent, called the *mobile phase*, which flows up the plate by capillary action. As the developing solvent flows up the plate, it can carry along the components of the mixture. The more soluble the component is in the solvent, the faster it travels up the plate. If it is not very soluble in the solvent, it will remain adsorbed on the surface of the silica gel, or *stationary phase*, and travel at a much slower rate.

The rates of flow are measure in terms of R_f values. An R_f is the relative distance that a sample component has moved relative to the distance moved by the developing solvent. The following illustration will demonstrate how the R_f is calculated. R_f is measured by dividing the distance the component travelled by the distance the solvent travelled. Therefore, an R_f value can never be greater than 1.



$$R_f\,(A) = \frac{\text{distance A travelled}}{\text{Distance solvent travelled}}$$

The R_f value for a particular component is characteristic for that component in that particular solvent. Therefore, it will always be the same and it can be identified in other mixtures. In this experiment you will first be determining the R_f value of pure substances and then comparing the data with the experimental data observed from the hydrolysis products of aspartame.

Materials

- 0.12% solutions of aspartic acid, phenylalanine, serine, and aspartame
- Diet Coca-Cola, aged and fresh
- 1 mL 3M HCl
- 0.2% ninhydrin spray
- 10 mL 12:3:5 butanol:acetic acid:water solution
- Equal artificial sweetner
- two 9 x 6 cm TLC plates
- Capillary tubes
- Heat gun
- 400-mL beaker
- Ruler

Procedure:

1. Prepare a boiling water bath in a 250 mL beaker.
2. Obtain a TLC plate. Handle it carefully by the edges and do not bend. Using a lead pencil (not a pen), lightly draw a line across the plate about 1 cm from the bottom. Lightly mark 5 positions where your samples will be spotted. You may want to number the spots to number the spots to keep them straight. Repeat the procedure for a second TLC plate.
3. Pour 10 mL of the butanol:acetic acid: water solvent mixture into a large (400 mL) beaker. Place a piece of filter paper in the mixture to get it wet and prop the filter paper up along the side. Cover the beaker with a watch glass or parafilm.
4. Dissolve about 10 mg of Equal in 1 mL of 3 M HCl in a small test tube. Heat the tube in the boiling water bath for 5 min. Make sure the liquid does not completely evaporate! Cool the test tube and label it as **hydrolyzed aspartame**.
5. You are provided with the following substances: phenylalanine, serine, aspartame, aspartic acid, fresh Diet Coca-Cola and aged Diet Coca-Cola. You will use these samples for spotting your plates.
6. On one of the plates, spot samples of phenylalanine, aspartic acid, serine, aspartame, and hydrolyzed aspartame. Using capillary tubes, apply the sample to the plate until it spreads about 1 mm in diameter. Be sure to keep a log of which spot contains which sample! Allow the spots to dry.

7. Place the spotted TLC plate in the chamber you prepared in step 3. Make sure that the spots applied to the plate are above the surface of the solvent.
8. On the second plate, spot samples of fresh Diet Coca-Cola on lane 1, aspartic acid on lane 2, aspartame on lane 3, phenylalanine on lane 4, and aged Diet Coca-Cola on lane 5. Multiple spotting will be required for the Diet Coca-Cola samples (about 12-15 times.) Allow the spot to dry each time before reapplying in order to prevent the spotting area from getting too large. Make sure you keep track of which spot is which and allow the spots to dry.
9. This TLC plate should be placed in the same chamber as the first one. The developing process will take a long time. Watch the plates carefully. When the solvent is about 1 cm from the top, remove from the chamber IMMEDIATELY mark the solvent front with a pencil. When finished, the drying process can be accelerated by using an air gun.
10. Using gloves, spray the plates with ninhydrin solution holding the solution about 6 inches away from the plate. Dry the plates.
11. Calculate your R_f values. Be sure to record your data.

Results

Plate I	Distance traveled (mm)		Solvent front (mm)	R_f	R_f
Phenylalanine					
Aspartic acid					
Serine					
Aspartame					
Hydrolyzed aspartame					

Plate II	Distance traveled (mm)		Solvent front (mm)	R_f	R_f
Aspartame					
Phenylalanine					
Aspartic acid					
Fresh Diet Coca-Cola					
Aged Diet Coca-Cola					

Discussion

As part of your discussion you should include the answers to the following questions:

1. Show the hydrolysis of aspartame, using structural formula.

2. Name the amino acids you found in the hydrolysate of the sweetener Equal.

3. Compare the R_f values for aspartame, aspartic acid and phenylalanine on plates I and II. What do you notice?

4. Which of the aspartame samples, hydrolyzed or non-hydrolyzed, does the aged Diet Coca-cola best represent? Give a reason for your answer.

5. The shelf- life of Diet Coca-Cola is approximately 3 months. Based on your results explain why do you think this is?

Experiment 3: Lactase Enzyme Lab

Aim: To investigate enzyme specificity and the factors that affect enzyme activity.

Background: Lactose, the sugar found in milk, is a disaccharide composed of glucose and galactose (both six-sided sugars). **Lactase** is an enzyme that breaks lactose down into galactose and glucose. Lactase can be purchased in pill form by people who are lactose intolerant. These people lack the enzyme, lactase, and cannot break down the sugar lactose into its component parts.

Although lactose is similar to sucrose (table sugar), lactase will break down only lactose because of the shape of the sugar.

In this lab, you will observe the breakdown of lactose into galactose and glucose by the enzyme, lactase. You will also observe the effect of heating on an enzyme-catalyzed reaction.

Materials

- Lactase tablets
- Skimmed Milk
- Sucrose
- Clinitix® glucose tape (or Diastix®)
- 100-ml measuring cylinder
- 400 ml beaker
- Boiling tube
- 5 Test tubes and test tube rack
- Hot plate
- Micropipettes
- Stop watch

Procedures

Solution Preparation

1. Enzyme solution (groups can share): Add one lactase tablet to 200 ml of distilled water. Stir until the tablet has dissolved.
2. Skimmed milk: this solution contains the lactose.
3. Sucrose solution (groups can share): Add five grams of sugar to 100 ml of water. Stir until the sugar has dissolved.
4. Boiled enzyme solution (groups can share):
    - Place 20 ml of enzyme solution into a boiling tube.
    - Add 200 ml of water to a 400-ml beaker.
    - Place the boiling tube in the beaker (gently laying the boiling tube so it rests on the side of the beaker) and boil the water in the beaker for 30 minutes on a hot plate.
    - Let the solution cool to room temperature before use.

Test for enzyme activity

1. Label 5 test tubes, A through E
2. In tube A, add 2.0 ml of skimmed milk and 1.0 ml of enzyme solution.
3. Time for 2 minutes and then test for glucose with the glucose test tape. Record this data in Table 1. If there was glucose present mark a '+' in the table. If glucose was absent, mark a '-' in the table.
4. Set up tube B and repeat step 3.
5. Set up tube C and repeat step 3.
6. Set up tube D and repeat step 3.
7. Set up tube E and repeat step 3.

Table 1: Reaction mixtures for testing lactase activity

Tube	Reaction mixture	Presence of glucose
A	2.0 ml of skimmed milk and 1.0 ml of enzyme	
B	2.0 ml of skimmed milk and 1.0 ml of distilled water	
C	2.0 ml of skimmed milk and 1.0 ml of boiled enzyme	
D	2.0 ml of sucrose and 1.0 ml of enzyme	
E	2.0 ml of sucrose and 1.0 ml of water	

Discussion

As part of your discussion you should include the answers to the following questions:

1. Diagram and describe the lactose and lactase reaction.
2. Why did the enzyme react to lactose but not to sucrose?
3. What happened when the enzyme was boiled?
4. Another way to affect the enzyme is by lowering the pH of the solution. However, lactase is supposed to be able to work in the stomach. Would lowering the pH of the enzyme solution affect the enzyme? Why or why not?
5. What type of reaction is this? Dehydration or hydrolysis?

APPENDIX A- Lab Format

OLD FORMAT	NEW FORMAT	INFORMATION IN THIS SECTION	CORE COMPETENCY BEING DEVELOPED
Aim	**Introduction**	*Gives the purpose of the lab and its theoretical background.*	EFFECTIVE COMMUNICATION
Theory			KNOWLEDGE
Apparatus	**Materials and Methods**	*Details what materials and equipment were/should be* used to carry out the experiment, and the way in which they were/will be* used. Also clarifies how potential sources of error can be avoided by the choice of suitable methods and materials.*	KNOWLEDGE*
Method			EFFECTIVE COMMUNICATION
Precautions			TECHNICAL EXPERTISE
Drawing	**Results**	*Provides raw (i.e., uninterpreted) data collected and (perhaps) expresses the data in table form, as percentages/ratios, charts, tables, graphs, drawings. Data may also be used to perform calculations.*	TECHNICAL EXPERTISE
Results			
Treatment of results			EFFECTIVE COMMUNICATION
Discussion and Conclusion	**Discussion**	*Considers how the data you obtained is linked to the purpose of the lab and explores the applications of the experiment and the conclusions that can be made. Judges any unavoidable limitations of your experimental design and assesses their effect on the results.*	ANALYTICAL ABILITY
Sources of error			

*Refers to Planning and Design Labs ONLY

APPENDIX B- Lab Rubric

SECTION	COMPETENCY	PERFORMANCE RATING			
		Excellent **4**	**Average** **3**	**Fair** **2**	**Poor** **1**
Introduction	Knowledge	The theoretical discussion is complete, factual and relevant.	The theoretical discussion is incomplete or not factual or irrelevant.	The theoretical discussion is either incomplete or not factual, and irrelevant.	The theoretical discussion is incomplete, not factual and irrelevant.
	Effective Communication 1	The aim and/or hypothesis is clearly stated in a testable form.	The aim and/or hypothesis is slightly unclear.	The aim and/or hypothesis is quite vague.	The aim and/or hypothesis is not stated.
	Effective communication 2 Lab Format	Lab neatly written with all required sections included in the correct order including date, title and all post lab questions.	Lab tidily/ untidily written with most/all required sections included in the correct order including date, title and all/most post lab questions.	Lab tidily/ untidily written with few/most required sections included in the correct order including date, title and most post lab questions.	Lab untidily written with few required sections included in the correct order. Few post lab questions answered.
Materials and Methods	Knowledge **(Plan & Design only)**	The student chooses an appropriate method, and includes a complete, factual and relevant theoretical discussion.	The student chooses an inappropriate method, or includes an incomplete or non- factual or irrelevant theoretical discussion.	The student chooses an inappropriate method, and includes either an incomplete or non-factual, and irrelevant theoretical discussion.	The student chooses an inappropriate method, and includes an incomplete, non-factual and irrelevant theoretical discussion.

	Effective Communication	Method is correct, logical, complete and written in appropriate tense. Materials list is complete.	Method is incorrect, or illogical, or incomplete or written in inappropriate tense, or materials list is incomplete.	Method has two - three deficiencies and/or materials list is incomplete.	Method is incorrect and illogical and incomplete and written in inappropriate tense and materials list is incomplete.
	Technical expertise	Method and type of materials chosen so as to eliminate all potential sources of error.	Method and type of materials chosen so as to eliminate most potential sources of error.	Method and type of materials chosen so as to eliminate few potential sources of error.	No effort made to choose method and materials chosen so as to eliminate potential sources of error.
Results	Effective Communication	All results are included in appropriate/ suitable format.	All/Most results are included in inappropriate/ appropriate format.	Most results are included in inappropriate format.	Few results are recorded in inappropriate format.
	Technical Expertise 1	Guidelines for each different mode of data presentation used strictly adhered to. All results correct.	Guidelines for each/most different mode of data presentation used generally/strictly adhered to. All/most results correct.	Guidelines for most different mode of data presentation used generally adhered to and most results correct.	No effort made to adhere to guidelines for each different mode of data presentation used or most results incorrect.

	Technical Expertise 2 Laboratory skills **(marked during the lab session)**	All lab equipment used and techniques performed correctly. All general and safety rules strictly adhered to.	Most lab equipment used and techniques performed correctly. All general and safety rules strictly adhered to.	Most lab equipment used and techniques performed correctly. Most general and safety rules strictly adhered to.	Few lab equipment used and techniques performed correctly. Few general and safety rules strictly adhered to.
Discussion	Analytical Ability	1. Evaluates the results obtained in the context of the aim/ hypothesis. 2. Advances possible explanations of the results with reference to the theoretical discussion in the Introduction. 3. Deduces which procedures may have introduced errors into the results and assesses their effects. 4. Draws appropriate, relevant conclusions from the results.	Fulfills any three requirements of this section completely and one only partially/ not at all.	Fulfills any two requirements of this section completely and two only partially/ not at all.	Fulfills any one requirement of this section completely and three only partially/ not at all.
Post lab questions	Analytical Ability	All questions answered correctly.	Most questions answered correctly.	Few questions answered correctly.	No questions answered correctly.

APPENDIX C- LAB ASSESSMENT SHEET

Students please submit **one sheet per lab with Sections i-iv filled out**.

i. Student name:						
ii. Course:						
iii. Lab Title:						
iv. Date:						
v. FOR LECTURER'S USE ONLY- DO NOT WRITE IN THIS SECTION						
Section	**Competency**	**SCORE**				**COMMENTS**
Introduction	Knowledge	**4**	**3**	**2**	**1**	
	Eff. Comm. 1	**4**	**3**	**2**	**1**	
	Eff. Comm. 2	**4**	**3**	**2**	**1**	
	TOTAL					
Materials and Methods	Knowledge	**4**	**3**	**2**	**1**	
	Eff. Comm.	**4**	**3**	**2**	**1**	
	Tech. Expertise	**4**	**3**	**2**	**1**	
	TOTAL					
Results	Eff. Comm.	**4**	**3**	**2**	**1**	
	Tech. Exp. 1	**4**	**3**	**2**	**1**	
	Tech. Exp. 2	**4**	**3**	**2**	**1**	
	TOTAL					
Discussion	Anal. Ability	**4**	**3**	**2**	**1**	
	TOTAL					
Post Lab questions	Anal. Ability	**4**	**3**	**2**	**1**	
	TOTAL					
TOTAL LAB MARK (%):						
Lecturer Signature:						