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Practical Note

General Biochemistry

(BCH 202)

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1. General Laboratory Guidelines

1.1 Safety

Safe practices in the biochemistry laboratory are of great significance. Students must adopt safe and skillful methods while working in the lab. To achieve this goal, the student must obtain the prerequisite knowledge of properties of materials present in the lab and must be acquainted with different hazards and harmful effects associated with their improper handling.

1.1.1 Regulations

1. Throughout your stay in the laboratory, you must wear safety goggles.
2. Immediately inform your instructor in case of any accident.
3. Do not eat, drink, chew, or smoke in the laboratory.
4. Do not depart from the lab leaving an experiment unattended. If you need to leave the lab, you must inform your instructor before leaving the lab.
5. After finishing the experiment turn off all the equipment, clean your workbench and reshelf all the equipment or chemicals.
6. Not sticking to these rules will result in instant removal from the lab.

1.1.2 Precautions

1. You must come to the lab with a serious awareness of personal liability and utmost consideration for others in the lab.
2. You must acquaint yourself with safety equipment location, acid-base neutralizing agents, eyewash, fire extinguisher, emergency shower, broom & dustpan and broken glass container.
3. You must listen carefully to all the instructions given by your instructor. If you are unsure of anything, always ask your instructor.
4. You must immediately clean all chemical spills.
5. While handling the chemicals you must wear gloves, otherwise some chemicals may result in skin irritation.
6. While handling all electrical and heating equipment extra precautions must be taken to prevent shocks and burns.
7. Do not handle broken glassware with your bare hands.
8. You must wash your hands with soap after finishing the experiment**.**

1.1.3 Personal clothing

Selection of clothing for the laboratory is generally left to the discretion of the student. However, due to the harmful nature of some chemicals, it is in the best interest of the student to wear proper and suitable clothing. You must wear a lab coat to help keep clothes protected. Open toed shoes must not be worn because they cannot protect you against chemical spills. Long hair should be tied back to avoid interference with motion or observation.

1.1.4 Equipment

Equipment must be placed in a safe and secure manner. Hot plate must be placed in safe location and kept away from the edge of the bench to reduce chances of body contact.

1.1.5 Glassware Handling

Glassware in the lab is **generally delicate** and fragile, and if not handled properly, may cause serious injuries. Do not use any chipped or broken glassware. After finishing the experiment, all glassware must be cleaned and kept back at the proper place.

1.1.6 Acids and Bases

In metabolism lab experiments, you will be using different acids and bases. Hence, care must be taken to avoid skin contact. While handling these chemicals, avoid eye and face contact. In case of acid or base contact with your skin, wash it with large amount of clean, cold water and inform your instructor immediately. For your own protection, neutralize acid or base spills before cleaning them up.

1.1.7 Laboratory Notebooks

For all laboratory experiments, use a bound notebook to keep record of all primary data and observations. You must organize your notebook every week before coming to the lab by writing the title of the experiment on a new page, with important equations or formulae from the lab manual, and all necessary calculations involving solution preparations, molar masses, etc.

Try to understand theoretical concepts and particular instructions given by your instructor before the experiment. The lab notebook must have a record of every experiment. The lab notes should be written in a manner that other people could understand them. Excellent note taking in the lab is an important skill that can be learned with little effort and practice.

1.1.8 Guidelines to be followed

1. Carry your notebook to the lab for each experiment.
2. Number all the pages in sequential order.
3. Use your notebook to record values directly and do not use loose scraps of paper.
4. Mention each measured quantity by its name and indicate the units.
5. Simply strike through the sentence and write the new sentence next to it, if you make a mistake in your notebook.
6. Tables are very useful to simplify the data entry; they should be prepared in advance before starting the experiment.
7. Do not depend on your memory and write down all observations, for example color and phase changes, etc.
8. Last but not the least, you must write a brief conclusion of your experiment. It should address the objectives of conducting the experiment.

1.1.9 Equipment mostly used in biochemistry lab

Volumetric Flask

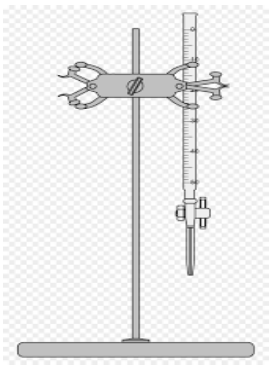
Conical Flask

Beaker

Reagent Bottle

Wash Bottle

Measuring Cylinder



Burette

Automatic Pipette

Plastic Pasture

Test tubes in a Rack

1.2 Guidelines for preparing Laboratory reports

The laboratory reports are major written assignments and should be written in the form of a scientific paper. The laboratory reports should contain the following sections:

- Title Page
- Brief Introduction
- Materials and Methods
- Results/Discussion
- References

All laboratory reports are expected to be well written, typed in English. Follow the following guidelines for each section to write a lab report.

1.2.1 Abstract section:

The abstract is a short and yet thorough summary of the report so that one can get an idea about the experiment without reading your whole report. It should include the purpose of your experiment, the procedures you used to carry out the experiment, results you obtained from the experiments, and your conclusions. The abstract should be no longer than a small paragraph (10-12 lines).

1.2.2 Introduction section:

This part should consist of any theoretical background information pertinent to understand your report. This section should be around 30-40 lines.

1.2.3 Materials and Methods section:

In this section you will write the materials and methods that you used, you must also mention exact volumes, amounts, incubation times, and any modifications from the procedure mentioned in the manual.

1.2.4 Results section:

In this section of your lab report, you should report all your results that you get from your experiment such as calculations, exact volumes, amounts, incubation times, etc.). You should present them in a tabulated form so it will be easy for quick reference. You must number and label all the tables and figures (graphs, diagrams). This way it will be easy for you to refer to them in your discussion section. You should also include your sample calculations (if any) in the result section.

1.2.5 Discussion section:

In this section you are required to give a thorough description of what happened in the experiment. The discussion section is also where you interpret your results and make conclusions. You should refer to your tables and diagrams while explaining your results. You should compare your results to expected values (calculated or from the literature). Even if you obtained unexpected results, the discussion section is the section to justify or explain the reasons why you have obtained such results. Please remember how you interpret your results carries more weight than the results themselves.

1.2.6 Conclusion section:

The conclusion section is just a quick overview of what was done and how. However, more importance is given to the results.

1.2.7 References section:

In this section, you will provide an alphabetical listing (by first author's last name) of the references which have been used in the report

2. Buffer Solutions

2.1 Objectives

1. To understand the nature of buffer solution
2. Understanding the buffer capacity
3. To learn how to prepare buffer solution

2.1.1 To understand the nature of buffer solution:

All biochemical reactions occur under strict conditions of the concentration of hydrogen ion. Biological life cannot withstand large changes in hydrogen ion concentrations which we measure as the pH. Those solutions that have the ability to resist changes in pH are called buffers. A buffer is a solution that resists changes in pH upon the addition of limited amounts of acid or base. A buffer is made up of a weak acid and its conjugate base. It resists pH changes when it's two components are present in specific proportions. A buffer is best used close to its pK_a ($pK_a = - \log K_a$).

The Henderson-Hasselbalch equation is an equation that is often used to perform the calculations required in preparation of buffers for use in the laboratory. It relates the Ka of a weak acid, HA and the pH of a solution of the weak acid.

The Henderson–Hasselbalch equation is derived from the acid dissociation constant equation by the following steps:

$$K_a = [H^+][A^-] / [HA]$$

Rearranging the equation to solve for $[H^+]$;

$$1/[H^+] = 1/[K^a] * [A^-]/[HA]$$

By definition, $\log 1/[H^+] = pH$, and $\log 1/Ka = pK_a$, so that by taking the log of the equation above, we get the Henderson–Hasselbalch equation;

$$pH = pK_a + log \frac{[A^-]}{[HA]}$$

Buffers resist pH changes because they use up excess hydrogen ion or hydroxide ion. If we have a solution with both weak acid and its salt, and we add some H+ then the following reaction occurs:

$$A^- + H^+ \leftrightarrow HA$$

Conversely, if we added OH- the following occurs:

$$HA + OH^- \leftrightarrow A^- + H_2O$$

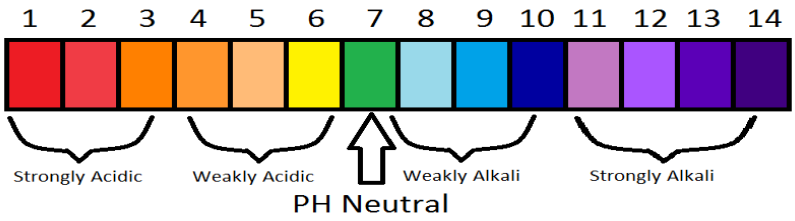
Thus a buffer can protect against pH changes from added H^+ or OH^- ion as long as there is sufficient basic and acidic forms respectively. As soon as you run out of one of the forms you no longer have a buffer. To act as a good buffer, the pH of the solution must be within one pH unit of the pKa. The proper choice and preparation of a buffer is paramount to your success in a biochemistry lab.

2.1.1.1 Hydrogen number pH

The scientist Sorensen had suggested a formula that describe the acidity of certain solutions by using hydrogen number pH which can be defined as: The negative logarithm of the hydrogen ion concentration.

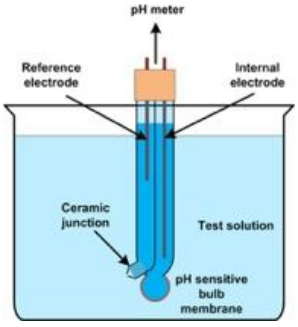
$$pH = -\log [H^+]$$

*Notes that, the negative logarithm of hydrogen ion number increase when the concentration of hydrogen ion decrease and vice versa.



2.1.1.2 Measuring of hydrogen number

To measure the hydrogen number in certain solution in very accurate way, we use a special instrument called pH meter. It's consist of glass electrode which contain a very thin bulb, blown onto a hard glass tube. The bulb is made of high conductivity glass which is sensitive to pH. The bulb contains a solution of hydrochloric acid (0.1N) and is connected to a platinum lead via silver - silver chloride electrode which is reversible with respect to hydrogen ions. The glass electrode is very sensitive and readily responds to changes in hydrogen ion concentration.



2.1.2 Buffer Capacity

Buffer solutions are solutions that can resist changes in pH upon addition of small amounts of acid/base. Common buffer mixtures contain two substances, a conjugate acid and a conjugate base. Together the two species (conjugate acid and conjugate base) resist large changes in pH by absorbing the H+ ions or OH- ions added to the system. When H+ ions are added to the system they will react with the conjugate base in the buffer as follows,

$$H^+ + A^- \; HA$$

When OH^- ions are added they will react with the conjugate acid in the buffer as follows,

$$OH^- + HA \quad A^- + H_2O$$

Thus the buffer is effective as long as it does not run out of one of its components.

Quantitative measure of this resistance to pH changes is called buffer capacity. Buffer capacity can be defined in many ways; it can be defined as the number of moles of H^+/OH^- ions that must be added to one liter of the buffer in order to decrease /increase the pH by one unit respectively.

2. 1.3 Buffer solution preparation:

2.1.3.1 Preparation of phosphate buffer:

Example: Prepare 500ml from phosphate buffer with concentration 0.25M and pH= 7.4, if you know that (pK_a=7.2)

1. Buffer solution content, which are
   * Monosodium dihydrogen phosphate NaH_2PO_4
   * Disodium hydrogen phosphate Na_2HPO_4
2. Calculation of the Acid and conjunct base percentage by using Henderson equation:

$$pH = pKa + \mathbf{log}\ \frac{[A^-]}{[HA]}$$

$$7.4 = 7.2 + \mathbf{log}\ \frac{[A^-]}{[HA]}$$

$$7.4 - 7.2 = \mathbf{log}\ \frac{[A^-]}{[HA]}$$

$$0.2 = \mathbf{log}\ \frac{[A^-]}{[HA]}$$

$$Na_2HPO_4 \; / \; NaH_2PO_4 = 1.6$$

The percentage is 1.6 : 1 which means these component mixed as per as this percentage will have buffer solution with pH=7.4 which the pH we want.

3. Calculate the weight for both compounds: We calculate the weight by using equation:

$$\text{Wight} = \frac{\text{Number of mols} \times \text{Molecular weight} \times 100}{1000}$$

for both Monosodium dihydrogen phosphate and Disodium hydrogen phosphate.

4. Dissolve both component in 250ml distilled water in beaker
5. pH will measure by pH meter which will be around 7.4 however to get the exact pH by adding those amounts of acid (HCl) or base (NaOH)
6. All of these amounts will placed in volumetric flask there we complete to the final volume 500ml by adding distilled water.

2.1.3.2 Studying the properties of buffer solutions

Main idea

We are going to exanimate if the buffer solution pH will change tremendously or it will reset the change in pH by adding week base or week acid and compare it with distilled water by adding the amount from the acid and the base.

Materiel

- Two beakers 50ml and glass spatula for mixing
- pH meter to measure the pH
- Phosphate buffer pH=7.4, that we prepare previously
- Hydrochloric acid with 0.1M (HCl)
- Sodium hydroxide with 0.1M (NaOH)

Method

1. Put 40ml of distilled water in beaker (A) and in another Beaker (B) put 40ml from phosphate buffer that we are previously prepared.
2. Both beaker consonant will be measured the will be measured the pH by using pH meter
3. Add to both particular amount of hydrochloric acid or sodium hydroxide and mix it gently
4. Measure the hydrogen number pH for both beakers again.
5. Write down your observation in the table below.

Results:

Volume of HCl 0.1M	pH value for **Phosphate buffer** after the addition	pH value for **Water** after the addition
0.5mL		
1ml		
2ml		
pH average after the additions		

Volume of NaOH 0.1M	pH value for **Phosphate buffer** after the addition	pH value for **Water** after the addition
0.5ml		
1ml		
2ml		
pH average after the additions		

3. Qualitative Analysis of Carbohydrates

3.1 Carbohydrates (1)

The term carbohydrates applied generally to the group of Polyhydroxy Aldehydes or Ketones commonly known as Sugars. Carbohydrates are produced from carbon dioxide and water by plants through the process of photosynthesis. Carbohydrates are the major food supply and energy source for the people of the world. Depending on the dietary habits, 50-90% of the carbohydrates consumed comes from grain, starchy vegetables and legumes. Despite the major utilization of carbohydrates for energy only a small amount is stored in the body. The average adult reserve is about 370 g stored mainly as liver and muscle glycogen.

Carbohydrates can be classified as:

1) **Simple Carbohydrates**, often called monosaccharides or simple sugars, contain one saccharide unit.
2) **Complex Carbohydrates** are those containing more than one saccharide group.

Also, it can be classified as:

- **Monosaccharides** contain one monosaccharide unit.
- **Disaccharides** contains two monosaccharide units.
- **Oligosaccharides** contains 3 - 6 monosaccharide units.
- **Polysaccharides** can contain over 7 or more monosaccharide units. Complex carbohydrates can be broken down into smaller sugar units through a process known as hydrolysis.

Monosaccharides can be classified in a number of ways:

1. They can be classified by the number of carbon atoms they contain; pentoses (5 carbons) and hexoses (6 carbons) are the most common.
2. Monosaccharides can also be classified as ketoses or aldoses. A ketose contains a carbonyl group attached to two R groups having one or more hydroxyl groups. An aldose contains terminal aldehyde group in addition to R group containing -OH.

3.1.1 Physical Properties:

Monosaccharide and Disaccharide can be dissolved freely in water because water is a polar substance, while polysaccharide cannot be dissolved easily in water, because, it has high molecular weight, which give colloidal solutions in water-soluble.

3.1.2 Chemical Properties:

3.1.2.1 Molisch test:

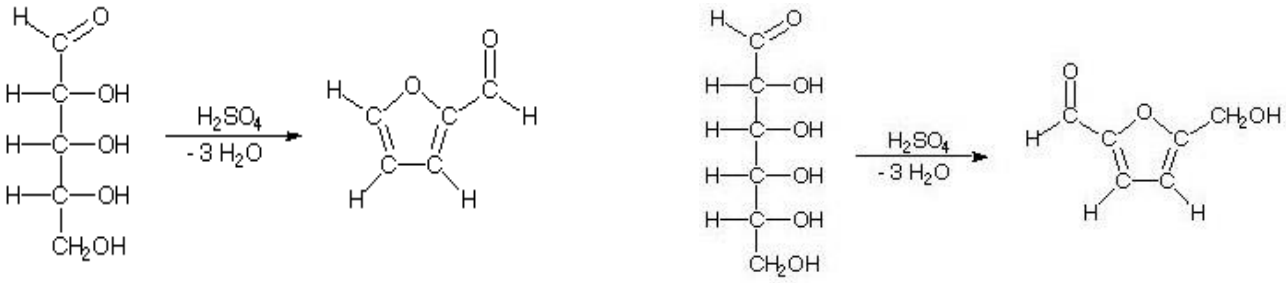
This test is specific for all carbohydrates, Monosaccharide gives a rapid positive test, Disaccharides and Polysaccharides react slower.

Objective:

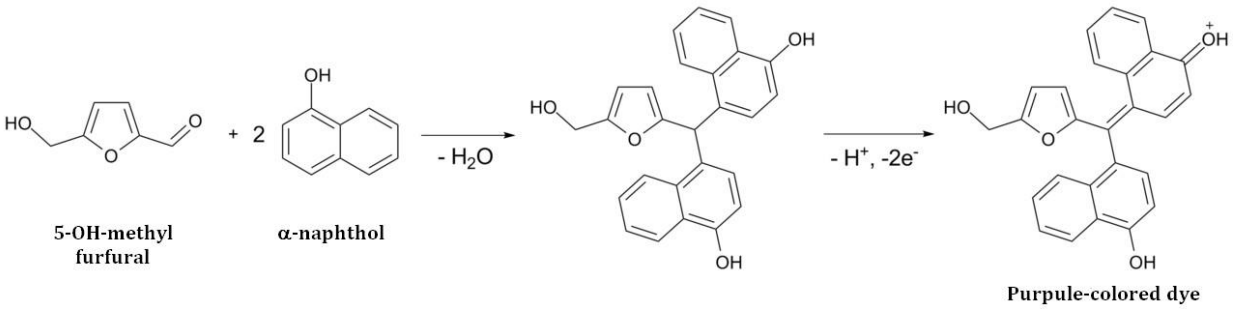
To identify the carbohydrate from other macromolecules Lipids and Proteins.

Principle:

The Conc. sulfuric acid dehydrate pentose to form furfural and dehydrates hexoses to form 5-hydroxymethyl furfural. The furfurals further react with α-naphthol present in the test reagent to produce a purple product.



Ribose (C_5) Furfural Glucose (C_6) 5-OH-methylfurfural



5-OH-methyl furfural α-naphthol Purpule-colored dye

Materials:

- α-Naphthol in 95% ethanol (50gm of α-Naphthol in 1000 ml ethanol)
- Different sugar solutions 1% w/v
- Concentrated sulfuric acid

Method:

1. Place 1ml of a sample solution in a test tube.
2. Add 2 drops of the Molisch reagent (a solution of α-naphthol in 95% ethanol).
3. Add 2ml of concentrated sulfuric acid with care on tube wall so that two layers form, producing violet ring appear as liaison between the surface separations.

Results:

Tube	Observation	Result
1		
2		
3		
4		

3.1.2.2 Benedict's test:

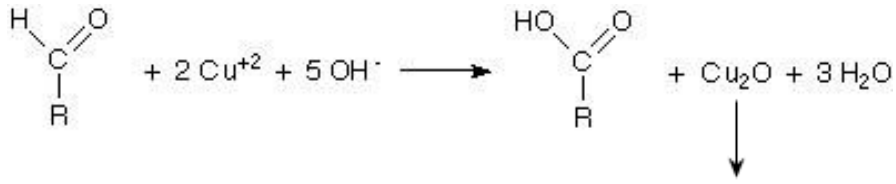
Benedict's reagent is used as a test for the presence of reducing sugars. All monosaccharides are reducing sugars; they all have a free reactive carbonyl group. Some disaccharides have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution. Starches are also non-reducing sugars.

Objective:

To distinguish between the reducing and non-reducing sugars.

Principle:

The copper sulfate ($CuSO_4$) present in Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar. Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.



Aldehyde Carboxyl Red precipitate

Materials:

- Solution of different Carbohydrate (1%)
- Benedict's reagent (a solution of sodium citrate and sodium carbonate mixed with a solution of copper sulfate)
- Boiling water bath

Method:

1. Place 1 ml of a sample solution in a test tube.
2. Add 2 ml of Benedict's reagent
3. The solution then heated in a boiling water bath for 3 minutes.

A positive test is indicated by: The formation of a reddish or orange precipitate within 3 minutes.

Results:

Tubes	Observation	Result
1		
2		
3		
4		
5		

Questions:

Why sucrose gives negative Benedict test?

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Explain, although starch has free hemiacetal bond it gives negative Benedict test?

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Why glucose (monosaccharide) and maltose (disaccharide) give positive Benedict test?

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3.1.2.3 Barfoed's test:

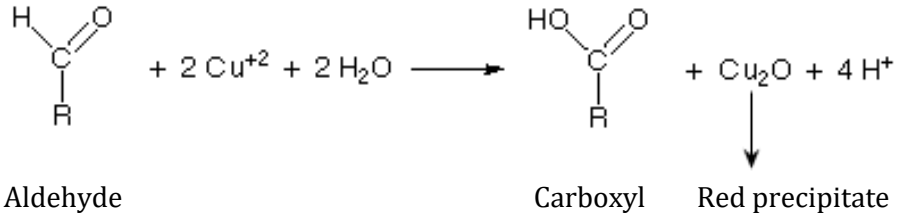
This test performed to distinguish between reducing monosaccharides, reducing disaccharides and non-reducing disaccharides.

Objective:

To distinguish between mono-, di- and poly saccharides.

Principle:

Braford's test used copper (II) ions in a slightly acidic medium reducing monosaccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide within three minutes. Reducing disaccharides undergo the same reaction, but do so at a slower rate. The non-reducing sugars give negative result.



Aldehyde Carboxyl Red precipitate

Materials:

- Brafords' reagent (a solution of cupric acetate and acetic acid) 13.3gm. of cupric acetate in 100ml dis. H2O + 1.8 ml conc. acetic acid
- Glucose, Sucrose
- Boiling water bath.

Method:

1. Place 1ml of a sample solution in a test tube.
2. Add 2ml of Braford's reagent.
3. Heat the solution in a boiling water bath for 3 minutes.
4. Note your observation

Results:

Tubes	Observation	Result
1		
2		
3		
4		

Questions:

What is the difference between Benedict and Braford's reaction?

……

……

Why should be avoid boiling more than 5 minutes?

……

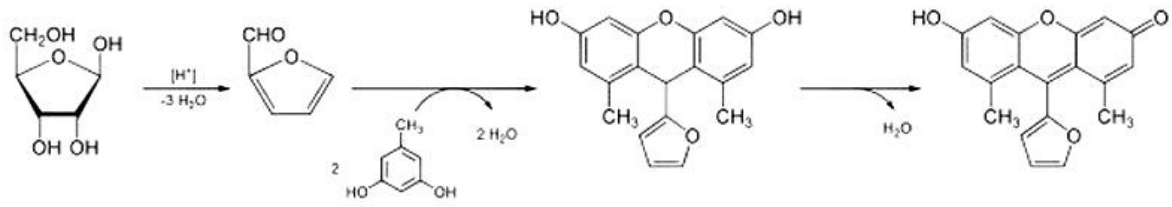
……

3.1.2.4 Bial's test:

Objective: To distinguish between Pentose and Hexose monosaccharide

Principle:

Bial's test uses concentrated HCl as a dehydrating acid and Orcinol + traces of Ferric Chloride as condensation reagent. The test reagent dehydrates Pentoses to form furfural. Furfural further reacts with Orcinol and the Iron ion present in the test reagent to produce a greenish blue product, while Hexoses yield muddy-brown to grey condensation product.



Pentose Furfural (Orcinol) Greenish blue dye

Materials

- Ribose, Fructose
- Bial's Reagent (a solution of Orcinol, HCl and Ferric Chloride) 1.5gm of Orcinol in 500 ml of Conc. HCl + 30 drops of 10% Ferric Chloride
- Water bath.

Method:

1. Put 2 ml of a sample solution in a test tube.
2. Add 2 ml of Bial's reagent to each tube.
3. Heat the tubes in boiling water bath for 3 minutes.

A positive test indicated by the formation of a greenish blue product. All other colors indicate a negative result for Pentoses. Note that hexoses generally react to form red, or brown products.

Results:

Tubes	Observation	Result
1		
2		
3		
4		

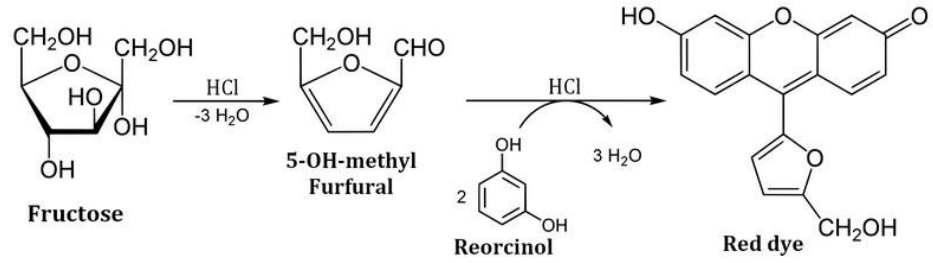
3.1.2.5 Seliwanoff's test:

Objective:

This test is used to distinguish between aldoses (like glucose) and ketoses (like fructose).

Principle:

Seliwanoff's Test uses 6M HCl as dehydrating agent and Resoncinol as condensation reagent. The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further condenses with Resorcinol present in the test reagent to produce a cherry red product within two minutes. Aldohexoses react to form the same product, but do so more slowly giving yellow to faint pink color.



Materials:

- Seliwanoff's reagent (a solution of Resorcinol and HCl) 5.5gm Resorcinol in 269ml of 6M HCl then add dis.H2O up to 1000 ml
- Glucose, Fructose.
- Boiling water bath

Method:

1. Put 1ml of a sample solution in a test tube.
2. Add 2ml of Seliwanoff's reagent (a solution of Resorcinol and HCl).
3. The solution then heated in a boiling water bath for 3 minutes.

Results:

Tubes	Observation	Result
1		
2		

3.2 Carbohydrate (2) (complex carbohydrates):

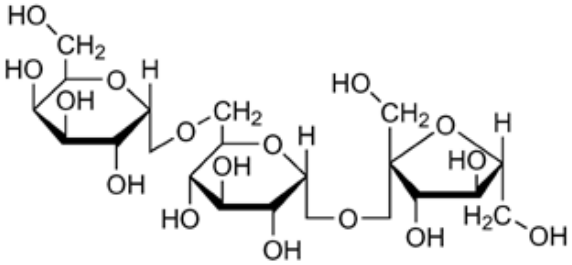
3.2.1 Introduction

The most abundant carbohydrate molecules found in nature are actually large complex structures consisting of mixtures of **Monosaccharide** derivatives. Carbohydrates consisting of several monosaccharides are called **Oligosaccharides**, a designation that could also include disaccharides, whereas, carbohydrates with ~10 or more monosaccharide units are called **Polysaccharides**. Polysaccharides can either be Homopolymeric (same repeating monosaccharide unit) or Heteropolymeric (mixture of monosaccharides).

Disaccharides can be broken into two monosaccharide units by hydrolysis and lost one molecule of water, examples of disaccharides Sucrose, Lactose and Maltose.

Oligosaccharides can be broken into **3 - 6** monosaccharide units by hydrolysis and lost one or more molecules of water, See below example of oligosaccharides.

Raffinose:



Galactose Glucose Fructose

Polysaccharides can be broken into 7 or more monosaccharide units by hydrolysis and lost one or more molecules of water examples Starch.

Plants and animals store glucose in the form of very large polysaccharide glucose homopolymers that contain both α-1,4 and α-1,6 **Glycosidic Bonds**. The glucose homopolymer produced in plants is called **Starch**, while the glucose homopolymer produced in animal cells is called **Glycogen**. Plants synthesize two forms of starch, **Amylose**, a linear polysaccharide containing about ~100 glucose units linked by α-1,4 glycosidic bonds, and **Amylopectin**, a branched polysaccharide containing ~100,000 glucose units connected by α-1,4 and α-1,6 glycosidic bonds.

3.2.2 Practical Experiments

3.2.2.1 Sucrose Hydrolysis test:

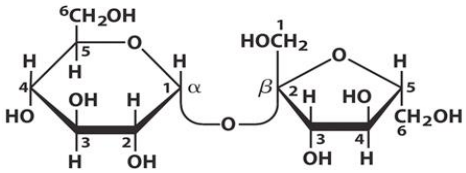
This test is used to convert sucrose (non-reducing disaccharide) to glucose and fructose (reducing mono saccharides).

Objective:

To identify the products of hydrolysis of disaccharides.

Principle:

Sucrose is the only non-reducing disaccharide so it does not reduce the Cu^{++} solution (Bendict's and Fehling's test) because the glycosidic bond is formed between the two hemiacetal bonds. So there is no free aldehydic or ketonic group to give positive reducing properties. This bond can be hydrolyzed and the individual components of sucrose (glucose + fructose) are then able to give positive reducing test.



Materials:

- Sucrose
- Concentrated hydrochloric acid (HCl) 1M
- Concentrated NaOH 1M
- Benedict's reagent

Method:

1. Set up 2 tubes add to each one 2ml of a sucrose solution. Label the tubes (Sucrose with HCl, Sucrose without HCl)
2. Add 5 drops of concentrated hydrochloric acid (HCl) to the 1st tube.
3. Heat the 1st tube in boiling water bath for 10 minutes.
4. Add 1ml NaOH to each tube (why?)
5. In the 1st tube (Sucrose with HCl) add 2 ml of Benedict's reagent mix well and heated for 3 mint then record result.
6. From the tube which contain only sucrose (without HCl) add 2ml of Benedict's reagent mix well and heated for 3 mint then record result.

Results:

Tube	Sucrose with HCl	Sucrose without HCl
Test	Benedict's test	Benedict's test
Result		

Questions:

How you can convert non-reducing sugar to reducing?

.....

.....

.....

3.2.2.2 The Iodine/Potassium Iodide test:

This test used to distinguish between polysaccharides and mono or oligo saccharides.

Objective: to detect the presence of starch in a sample

Principle:
Starch forms deeply blue color complex with iodine. Starch contains □-amylase, a helical saccharide polymer and amylopectin, a branched form of starch. Iodine forms a large complex with □-amylose helix. This complex absorbs light and reflects the blue light only. Simple oligosaccharides and mono saccharides do not form this complex.
Note that other polysaccharides like glycogen may give other colors (red or brown).

Materials:
- Iodine/potassium iodide solution
- Starch- Glycogen- Dextrin, glucose

Method:
1. Take 2 ml of a sample solution in a test tube.
2. Add 3 drops of iodine/potassium iodide solution.
3. A positive test indicated by the formation of a colored complex between Iodine and polysaccharide.
4. Heat the tubes in a water bath and write your observations.

Result:

Tubes	Observation	Discussion
1		
2		
3		
4		

Questions:
Why glucose does not give positive result with iodine test but not starch?
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……

Explain why the blue color disappears upon heating?
……
……

3.2.2.3 Hydrolysis of Starch:

This experiment illustrates the conversion of starch (non-reducing sugar) to a reducing sugar by the action of hydrochloric acid (HCl) at boiling point. The longer the starch is exposed to the acid the further hydrolysis proceeds.

Objective: to establish the effect of concentrated HCl on a glycosidic bond in starch**.**

Principle:
Although starch has free hemiacetal in the terminal glucose residue, it has no reducing properties, because the percentage between the free residues is very low in comparison to the whole molecule. Heating starch solution in acid medium hydrolyses the glycosidic bonds giving many free glucose residues. These glucose molecules give reducing properties to the hydrolysis product.

Materials:

- Starch
- Benedict's reagent
- Iodine reagent (10 ml of concentrated iodine in 100 ml of dis.H2O)
- Water bath
- concentrated NaOH

Method:

1. Take 2 ml of starch in large tube
2. Add 5 drops of HCl, heated in boiling water bath for 10 mints. then cool the solution.
3. Add 2ml of NaOH to convert the medium to basic.
4. Divide the whole amount in 2 tubes (A and B)
5. In tube (A) add 3 drops of iodine solution and note the result.
6. In tube (B) add 1 ml of Benedict reagent, mix well and heated for 3 mint then record result.

Result:

Tube	**Starch with HCl**	
Test	(A) Iodine test	(B) Benedict's test
Result		

Questions:

Although starch has free hemiacetal bonds it is non reducing sugar, explain?

……

……

4. Quantitative Estimation of Glucose by Enzymatic Methods

4.1 Introduction:

There are three main methods of estimation the reducing sugar content in solution:

a. **Reduction of cupric to cuprous salts:**
   Reducing sugars contains an **aldehyde** or **keto** groups reduced alkaline copper to cuprous oxide. Cuprous oxide allowed reacting with phosphomolybdate solution, which reduced and forms blue color. The intensity of color measured on colorimeter against standard.
b. **Reduction of ferricyanide to ferrocyanide:**
   Reduction of ferricyanide to ferrocyanide by reducing sugars in alkaline solution. In presence of zinc ions, the ferrocyanide formed precipitated as a zinc complex.
c. **Enzymatic methods**

Of the above three methods, method "**C**" is the most commonly used in clinical laboratories for glucose estimation.

4.2 Enzymatic methods

Glucose commonly measured using an enzyme to covert the glucose to a product that can be easily detected, common enzymes used are glucose oxidase, glucose dehydrogenase and hexokinase.

4.2.1. Glucose Oxidase:

Converts glucose, in the presence of oxygen, to gluconolactone and hydrogen peroxide:

$$\text{Glucose} + O_2 \xrightarrow{Glucose\ oxidase} \text{Gluconolactone} + H_2O_2$$

Hydrogen peroxide can convert to water and oxygen by the action of the enzyme peroxidase. A chromogenic oxygen acceptor (the reduced form) captures the released oxygen (now the oxidized form), and this allows quantitation of the glucose. The oxygen acceptor is usually a non-colored substance that becomes colored when oxidized.

$$\underset{\text{(colorless)}}{H_2O_2 + \text{o-Dianisidine}} \xrightarrow{Peroxidase} \underset{\text{(colored)}}{\text{Oxidized o-dianisidine} + H_2O}$$

4.2.2 Glucose Dehydrogenase

$$\text{Glucose} + \text{NAD}^{+} \xrightarrow{Glucose\ dehydrogenase} \text{Glucono-}^{\delta}\text{-lactone} + \text{NADH} + \text{H}^{+}$$

The amount of reduced nicotinamide adenine dinucleotide (NADH) produced is proportional to the concentration of glucose in the sample. The production of NADH is monitored by an increasing absorbance at 340 nm.

4.2.3 Methods using Hexokinase

produce glucose-6-phosphate, which is used as a substrate with NAD^+ in a second enzyme reaction using glucose-6-phosphate dehydrogenase. NADH is produced in proportion to the glucose concentration in the sample.

$$\text{Glucose} + \text{ATP} \xrightarrow{hexokinase} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NAD}^{+} \xrightarrow{G-6-P\ dehydrogenase} \text{6-Phosphogluconate} + \text{NADH} + \text{H}^{+}$$

Objective:

Measurement of glucose concentration by enzymatic method.

Materials and method:

As shown in the provided kit.

Results

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Discussion

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5. Lipids

5.1 Introduction

Lipids found naturally in all living organisms. It has a structural function in the cell, since it presents in cell membranes, and also it is an essential source of energy in the body. It gives more energy than carbohydrate and proteins.

It can be defined as nonpolar organic compound insoluble in polar water, but soluble in organic solvents such as benzene, ether, chloroform and boiling alcohol.

Fatty acids:

The building blocks of lipid. Fatty acid has a long hydrocarbon chain containing a carboxyl group at the end. They are divided into: **saturated** fatty acids and **unsaturated** fatty acids (unsaturated contain double bonds). The general formula for fatty acids:

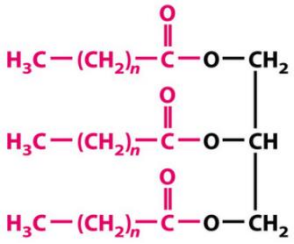
$$CH_3(CH_2)_n\,COOH$$

Fats can be divided according to their chemical composition to:

A- Simple lipids:

Triglycerides are esters of fatty acids with glycerol. It is found in fats and oils. The triacyglycerol is the simplest and most common fat. It is the form in which lipids are stored in the cell.

The general formula of fats and oils



Triacylglycerol

B - Compound (conjugated) lipids:

Lipids are linking with other compounds, such as phospholipids and glycolipids.

C - Derived lipids:

They are substances that are soluble in lipid or derived from the above groups of lipids by hydrolysis; for examples, cholesterol and fat-soluble vitamins.

5.2 Qualitative tests of lipids:

5.2.1 Solubility test:

Objective: to test the solubility of oils in different solvent.

Principle:

Fats are not dissolved in water due to their nature, non-polar (hydrophobic), but it is soluble in organic solvents such as chloroform, benzene, and boiling alcohol. Different lipids have ability to dissolve in different organic solvent. This property enables us to separate a mixture of fat from each other for example, undissolved phosphatide lipid in acetone; undissolved cerebroside, as well as sphingomyelin in the ether.

Materials:

- Olive oil (or cotton seed oil) - butter - corn oil.
- Solvents: diluted acid - dilute alkaline - ethanol - ether - chloroform - acetone
- Test tubes
- Water bath

Method:

1. Place 0.5ml of oil in 6 test tubes clean, dry containing 4ml of different solvents (acetone, chloroform and ether and ethanol, cold ethanol and hot water),
2. Shake the tubes thoroughly, then leave the solution for about one minute,
3. Note if it separated into two layers, the oil is not dissolve; but if one layer homogeneous transparent formed, oil be dissolved in the solvent.

Results:

Tube	Solvent	Degree of solubility

Questions:

Which solvent is the best for lipid?

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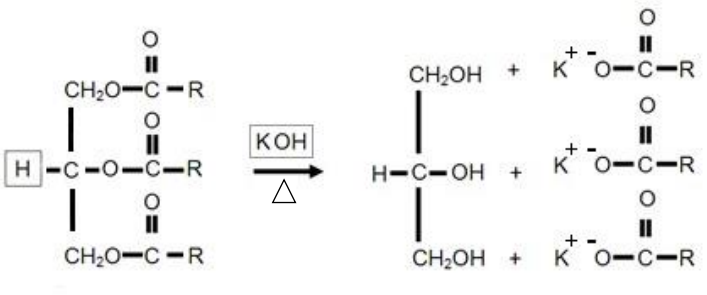
5.2.2 Saponification test:

Triacylglycerol can be hydrolyzed into their component of fatty acids and alcohols. This reaction can also be carried out in the laboratory by a process called **saponification** – where the hydrolysis is carried out in the presence of a strong base (such as NaOH or KOH).

Objective: to form the soap.

Principle:

Saponification is a process of hydrolysis of oils or fat with alkaline and result in glycerol and salts of fatty acids (soap) and can be used the process of saponification in the separation of saponifiable materials from unsaponified (which are soluble in lipid). The process of saponification as follows:



Triacylglycerol Glycerol Salts of Fatty Acids (SOAP)

Soap can be defined as mineral salts of fatty acids. The soap is soluble in water but insoluble in ether. Soap works on emulsification of oils and fats in the water as it works to reduce the attraction surface of the solution.

Materials:

- Types of oils like corn oil, butter, olive oil.
- KOH solution in alcohol (2O% KOH)
- Water bath (boiling)

Method:

1. Place 1 ml of oil in a large test tube (or flask).
2. Add 5 ml of alcoholic potassium hydroxide (preferably add little small pieces of porcelain to regulate the boiling point).
3. Boil the solution for 10 minutes. After this period, make sure it is perfectly saponification process, by taking a drop of the solution and mix with the water if oil separated indicates that the non-completion of the saponification. In this case, continued to boil until all the alcohol evaporates.
4. Take the remaining material (soap), add about 10 ml of water, and keep it for the following tests.
5. Shake the solution after it cools and noted to be thick foam.

Results:

Tube	Observation	Conclusion

Questions:

What is the chemical composition of soap?

.....

.....

Why potassium hydroxide is used in this test?

.....

.....

If you use cocoa butter, what kind of soap that will get it?

.....

.....

5.2.3 Testing the separation of soap from the solution by salting out:

Objective: to investigate the effect of NaCl on soap solubility.

Principle:

To get the soap out of solution by salting out when added solid sodium chloride to the solution until saturation; separated soap in the form of insoluble and floats above the surface.

Materials:

- Soap (which was prepared in the previous experiment)
- Solid sodium chloride NaCl (or solution)
- A small beaker.

Method:

Place about 3 ml of soap in a 25ml beaker, then add small amounts of sodium chloride in batches, stirring until solution saturated.

Results:

Tube	Observation	Conclusion

Questions:

Why is a separation layer on the surface of soap when you add salt?

.....

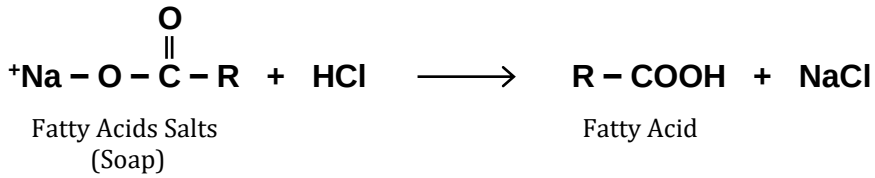
.....

5.2.4 Testing the formation of free fatty acids from soap:

Objective: to form a free fatty acids.

Principle:

Treatment of a soap solution with dilute hydrochloric acid (HCl) produces a mixture of free fatty acids. Fatty acids are long-chain carboxylic acids (C10 to C18) which may be saturated or unsaturated.



Materials:

- Soap (which was prepared in the previous experiment)
- Hydrochloric Acid (HCl) 10%
- Ice path

Method:

1. Add about 3ml of soap in big test tube then put it in the ice path.
2. Add 5 drops of HCl.
3. Notice the floating oily layer (free fatty acids)

Results:

Tube	Observation	Conclusion

5.2.5 Testing the formation of insoluble fatty acids salts (insoluble soaps):

Objective: to investigate the effect of different cations on soap solubility.

Principle:

Working calcium, magnesium, lead or iron ions to the deposition of soap and make it insoluble in water, where solve these ions replace the sodium or potassium ions are present in soap. Due to the hard water to contain significant quantities of Ca^{+2} ,Mg^{+2} ,Pb^{+2} or some Fe^{+3} are difficult to foam.

Potassium soap + calcium sulfate ===> calcium soap + potassium sulfate.

Materials:

- Soap (which was prepared in the previous experiment)
- Calcium chloride ($CaCl_2$) 5%
- Magnesium chloride or sulfate 5%
- Lead-acetate 5%

Method:

4. Add about 2 ml of soap in three test tubes.
5. Add 5 drops to the first tube of Calcium chloride, to second tube Magnesium chloride, and third tube Lead-acetate.

Results:

Tube	Observation	Conclusion

Questions:

Write the equation of reacting calcium chloride with soap?

……

……

What happens to the soap when washing with water hardness?

……

……

5.2.6 Unsaturation test:

Principle:

All neutral lipid contains glycerides of some unsaturated fatty acids. These unsaturated fatty acids become saturated by taking up iodine. If the fat contains more unsaturated fatty acids, it will take up more iodine.

Materials:

- Hub's iodine reagent (alcoholic solution of iodine containing mercuric chloride)
- Chloroform
- Mustard oil, coconut oil, olive oil, and groundnut oil

Method:

1. Add 10 drops of Hub's iodine reagent to 10 ml of Chloroform. The chloroform shows pink color due to presence of iodine.
2. Divide solution equally into 4 tubes:
3. To one test tube add Mustard oil drop by drop shaking the tube vigorously for about 30 seconds after addition of each until the pink color is discharged and count the number of drops. The pink color is discharged owing to the taking up of iodine by the unsaturated fatty acids of the oil.
4. Repeat above experiment with the remaining three test tubes taking fats (coconut oil, olive oil, and groundnut oil).
5. Compare unsaturation, it should be remembered that more the number of drops required to discharge the pink color, the less is the saturation.

Result:

Tube	Observation	Conclusion

5.2.7 Copper Acetate test:

Objective: to distinguish between oil or neutral fat and fatty acid saturated and unsaturated.

Principle:

The copper acetate solution does not react with the oils (or fats), while saturated and unsaturated fatty acids react with copper acetate to form copper salt. Copper salt formed in the case of unsaturated fatty acids can only be extracted by petroleum ether.

Materials:

Olive oil - oleic acid (polyunsaturated fatty acid) - stearic acid (saturated fatty acid) - petroleum ether - copper acetate solution (5%)

Method:

1. Take three test tubes put 0.5 gm of each sample and then added 3 ml of petroleum ether and an equal volume of a solution of copper acetate.
2. Shake the tube and leave it for some time.
3. In the case of olive oil notice that petroleum ether upper layer containing the dissolved oil and appears colorless, aqueous solution remains blue in the bottom.
4. In the case of oleic acid, the upper layer of petroleum ether becomes green as a result of copper oleate. The lower layer becomes less in blue.
5. In the case of stearic acid notice that the petroleum ether upper layer remains colorless, while consists of pale green precipitate of copper stearate at the bottom.

Results:

Tube	Observation	Conclusion

5.2.8 Qualitative Estimation of Cholesterol by Liebermann - Burchard test:

Objective:

To detect the presence of cholesterol

Principle:

Liebermann - Burchard Test, is a chemical estimation of cholesterol, the cholesterol is reacting as a typical alcohol with a strong, concentrated acids; the product is colored substances.

Acetic anhydride is used as solvent and dehydrating agents, and the sulfuric acid is used as dehydrating and oxidizing agent. A positive result is observed when the solution becomes red, then blue, and finally bluish –green color.

Materials:

- cholesterol
- Acetic anhydride
- Concentrated sulfuric acid
- Chloroform

Method:

1. Dissolve a few crystals of cholesterol in 2 ml of chloroform in a dry test tube
2. Now add 10 drops of acetic anhydride
3. Add 2 to 3 drops of conc. sulfuric acid
4. Record your result

Result:

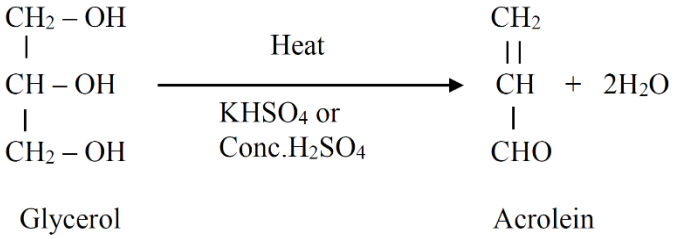
Tube	Observation	Conclusion
Cholesterol		

5.2.9 Acrolein test:

Most lipids found in the form of triglycerides, an ester formed from glycerol and fatty acids. When a fat heated strongly in the presence of a dehydrating agent such as $KHSO_4$, the glycerol portion of the molecule dehydrated to form the unsaturated aldehyde, acrolein

Principle:

When a fat heated strongly in the presence of a dehydrating agent such as $KHSO_4$ or H_2SO_4, the glycerol portion of the molecule is dehydrated to form the unsaturated aldehyde Acrolein, which can be distinguished by its irritating acrid smell and as burnt grease.



Lipid can detected by dye Sudan IV (general dye for lipid), which produce red color with lipid.

Materials:

- Different types of vegetable oils (such as corn oil, olive oil, butter)
- Glycerol.
- Solid potassium hydrogen sulfate $KHSO_4$
- Test tube
- Water bath (boiling)

Method:

1. Place an amount of solid potassium hydrogen sulfate $KHSO_4$ to about 0.5ml of glycerol in a test tube, then carefully heat the tube in boiling water bath and observed the emergence of the smell of acrolein (irritating to the membranes).
2. Repeat the previous step using oil from different sources instead of glycerol.

Results:

Tube	Observation	Conclusion
Glycerol		
Palm oil		
Sun Flower oil		

Questions:

Why acrolein test is used as a general test for oils and fats?

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.....

Do you expect to get a positive result if you use free fatty acid like oleic acid or palmitic acid and why?

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.....

Do you expect to get a positive result if you use beeswax and why?

.....

.....

.....

6. Spectral Characterization of DNA

Objective:

1. To establish the effect of temperature on the absorbance of DNA
2. To determine the optimum wave length for DNA
3. To determine the concentration of DNA in the sample.

Principle:

Nucleic acids are characterized by a strong absorption at wave length 260 nm.

Denaturation of a double stranded nucleic acids (DNA), produce an increase in absorption (this is called hyper-chromic effect)

Materials:

- DNA concentrated sample (extracted from yeast)
- 1X saline solution (NaCl with Tri Sodium Citrate)
- Quartz Cuvette
- UV Spectrophotometer

Method:

1. Pipette 0.5 ml of isolated DNA solution and add to it 4.5 ml of 1X saline-citrate as blank.
2. Read the absorbance (A) or Optical density (OD) of the solution at 260 nm using 1X saline - citrate as blank. **Note:** (if the absorbance is greater than 1.0, dilute the solution until you obtain OD260 of 1.0 or slightly less.)
3. When the absorbance of the solution ≈ 1.0 is obtained, read the absorbance of the solution at the following wave lengths: (240, 245, 250, 255, 260, 265, 270, 275, 280), using a blank of 1X saline citrate. **Note:** (for each wave lengths, zero the spectrophotometer against a blank of 1X saline citrate)
4. Using the same isolated DNA solution (solution of OD ≈ 1.0), heat the DNA solution in a boiling water bath for 15 min.
5. Rapidly determine the absorbance of heated sample at different wave lengths: (240, 245, 250, 255, 260, 265, 270, 275, 280), using a blank of 1X saline citrate. **Note:** (for each wave lengths, zero the spectrophotometer against a blank of 1X saline citrate)
6. Plot the absorption spectra of the native DNA solution and the denatured DNA heated 15 min at 100 °C.

Results and calculations:

Part 1: The Effect of Temperature on the Absorbance of DNA

Wave length (nm)	OD of native DNA	OD of heated DNA
240		
245		
250		
255		
260		
265		
270		
275		
280		

Plot the absorption spectra of the native DNA solution and the denatured DNA heated 15 min at 100 °C.

Part 2: Determine the Optimum Wave Length for DNA

For calculation of DNA concentration of samples free of RNA, the following conversion factor is used: (OD_{260}) 1 = 50ug of DNA/ml.

You can calculate the concentration of the DNA in your sample as follows:

$$\text{DNA Concentration } (\mu g/ml) = \frac{(\text{OD260}) \text{ x (dilution factor) x (50 } \mu\text{g of DNA/ml)}}{(\text{OD280})}$$

Part3: Determine the Concentration of DNA in the Sample

Purity of DNA:

OD_{260} / OD_{280} = 1.7 - 1.8

If a value out of this range, it may indicate the DNA sample are contaminated (i.e., protein)

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