Ministry of Higher Education and Scientific Research

Al-Mustaqbal University
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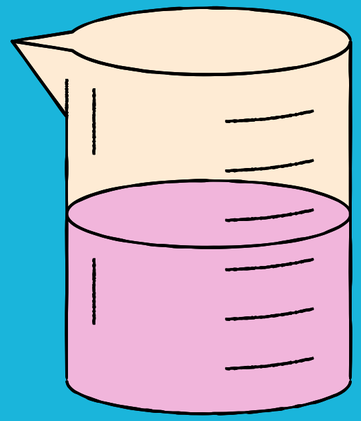
Department of Biology

Biochemistry / Laboratory



Solubility Tests

By:- M.S.c Saja Jawad Abaid

INTRODUCTION

Solubility:

Solubility is a physicochemical property that refers to the maximum amount of a solute that can be dissolved in a given quantity of solvent to form a homogeneous solution.

The Solubility Test is fundamental in biochemistry and is often used to classify and identify biomolecules such as carbohydrates and proteins, based on their interactions with various solvents and reagents.

SOLUBILITY TESTS FOR CARBOHYDRATES (SUGARS)

These tests are based on the ability of carbohydrates to react with specific reagents to produce a characteristic color or precipitate, indicating the presence of a particular functional group or type of sugar.

I. MOLISCH'S TEST – GENERAL TEST FOR CARBOHYDRATES

Molisch's test is a general qualitative test for the detection of all types of carbohydrates — monosaccharides, disaccharides, and polysaccharides.

It is based on the dehydration of carbohydrates by concentrated sulfuric acid (H_2SO_4) to form furfural or hydroxymethylfurfural derivatives, which then react with α-naphthol (Molisch reagent) to produce a violet or purple ring at the interface of the two layers

(MOLISCH TEST)

Principle

Dehydration of carbohydrates using concentrated sulfuric acid to produce furfural or its derivatives (such as hydroxymethylfurfural), which then condense with the Molisch reagent (alpha-naphthol) to form a violet ring.”.

Practical Steps

1. Place 2 mL of the carbohydrate solution into a clean test tube.
2. Add 2–3 drops of Molisch’s reagent (α-naphthol in ethanol).
3. Carefully and slowly add 2 mL of concentrated sulfuric acid (H_2SO_4) along the side of the inclined test tube to form a lower acid layer.
4. (Note: Do not shake or mix the solution.)

✅ Positive result

A distinct violet ring appears at the interface between the two layers.

BENEDICT'S TEST

Benedict's Test – for the detection of .reducing sugars

It is used to detect sugars that contain a free carbonyl group (aldehyde or ketone), such as glucose and fructose (monosaccharides), and maltose and lactose (reducing .disaccharides)

BENEDICT'S TEST

Principle

Reducing sugars reduce the cupric ions {Cu+2}
present in Benedict's reagent (which are blue) to cuprous ions{Cu+}which then precipitate as a brick-red solid of cuprous oxide{Cu2O} upon heating."

Practical Steps

1. Place 1 mL of Benedict's reagent (blue) in a test tube.
2. Add 0.5–1 mL of the sugar solution and shake the tube.
3. Place the tube in a boiling water bath for 3–5 minutes."

✅ Positive result

Appearance of a brick-red precipitate (or green/yellow/orange depending on the sugar concentration)."

Bial’s Test

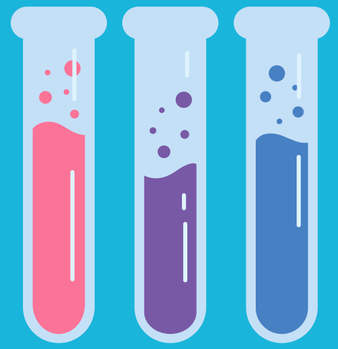
“Bial’s Test – for distinguishing between pentose and hexose sugars.
It is used to differentiate between pentose sugars (such as ribose) and ”.hexose sugars (such as glucose)

Seliwanoff’s Test

”.for distinguishing between ketoses and aldoses

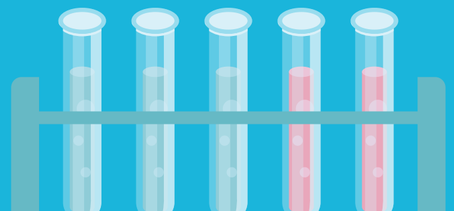
Principle

Concentrated hydrochloric acid dehydrates the sugars. Ketose sugars dehydrate much more rapidly to produce furfural derivatives, which react with resorcinol (a component of Seliwanoff’s reagent) to form a cherry-red dye.”



✅ Positive result

A cherry-red color appears within 1–2 minutes of boiling (for ketoses). Aldoses take much longer or produce a light pink color.”



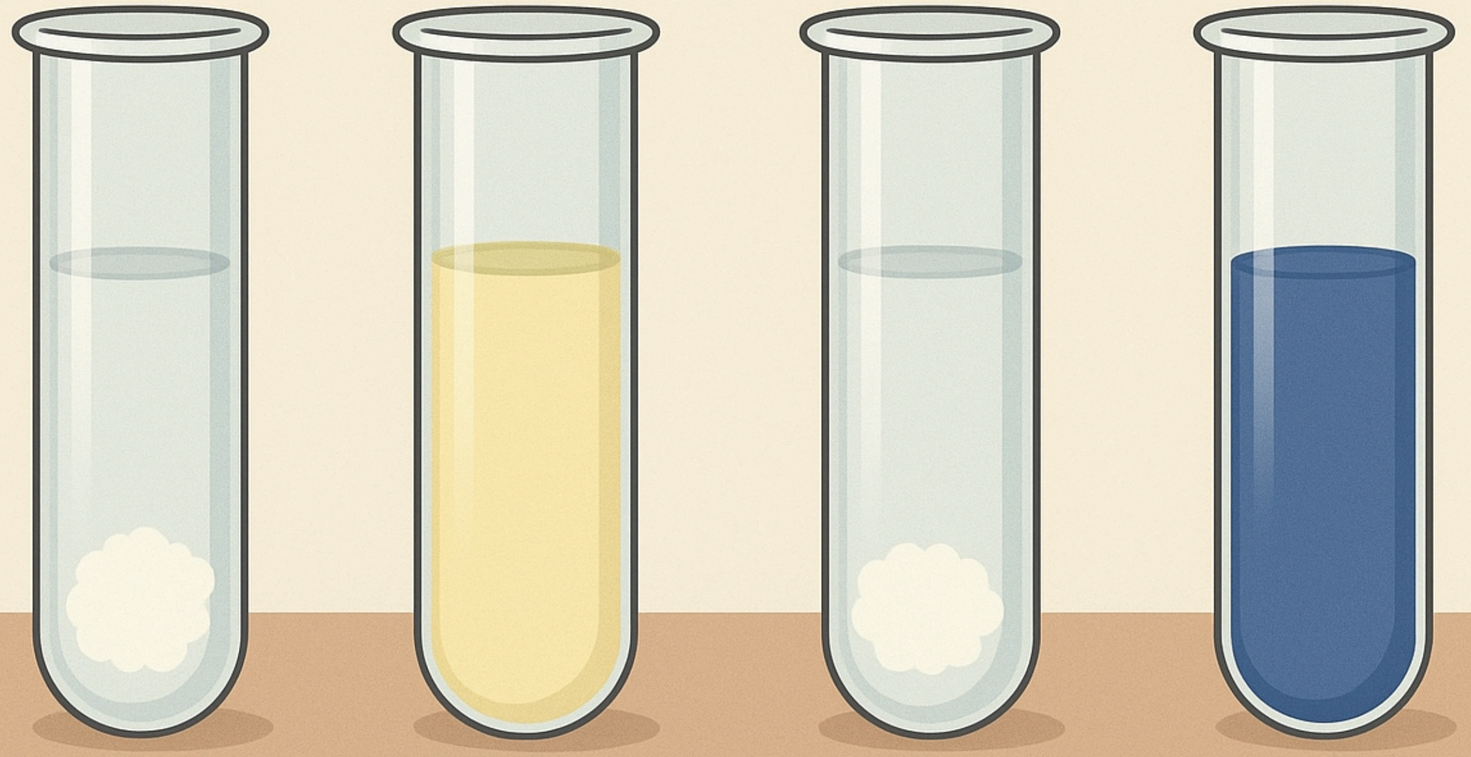
Differential Solubility Tests for Proteins (Precipitation)

The solubility of proteins depends largely on factors such as pH, the ionic strength of the solution, and temperature. Changes in solubility (precipitation) can be used as a method to identify proteins.”

IMPORTANT PRACTICAL NOTES:

1. SAFETY FIRST: WHEN USING CONCENTRATED SULFURIC ACID OR CONCENTRATED HYDROCHLORIC ACID, WEAR SAFETY GOGGLES, GLOVES, AND A LAB COAT, AND WORK UNDER A LABORATORY FUME HOOD.
2. HEATING: HEAT IN A BOILING WATER BATH, NOT OVER A DIRECT FLAME, TO ENSURE EVEN AND SAFE HEATING.
3. MIXING IN MOLISCH TEST: IN THE MOLISCH TEST, ADD SULFURIC ACID SLOWLY AND CAREFULLY ALONG THE SIDE OF THE TEST TUBE WITHOUT SHAKING, TO AVOID MIXING THE LAYERS AND BURNING THE SAMPLE, ENSURING A CLEAR VIOLET RING FORMS AT THE INTERFACE."

CARBOHYDRATE SOLUBILITY TEST



STARCH – INSOLUBLE IN COLD WATER

SUCROSE – PARTIALLY SOLUBLE

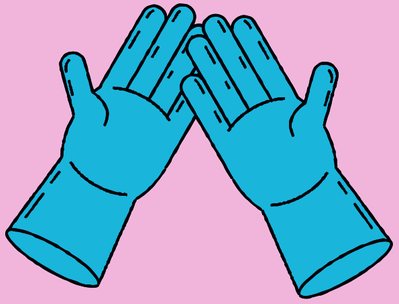
GLUCOSE COMPLETELY SOLUBLE

STARCH + IODINE – BLUE INDICATING STARCH

THANK YOU!

- أنت تعيش الحياة مرة واحدة فقط ، فما أسوأ أن تصل إلى نهايتها وتُدرك بأنك لم تعشها جيدًا.

- إدوارد ألبي 📚.



ELEMENTS