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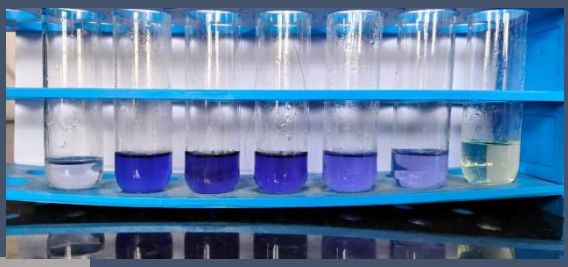
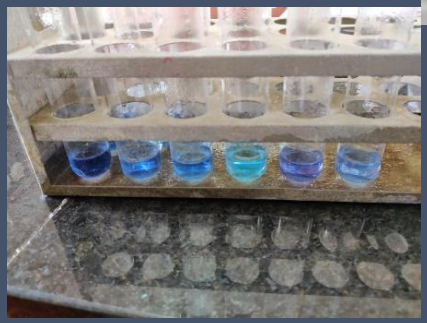
सत्यमेव जयते
DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology
Government of India

BIOCHEMISTRY AND BIOPHYSICS LABORATORY MANUAL

BSc BIOTECHNOLOGY
SEMESTER III


Dr. RADHA DAYANIDHI

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DEPARTMENT OF BIOTECHNOLOGY

Certificate

Reg. No.

This is to certify that _____ _____

has satisfactorily completed the course of experiments in_____

_____prescribed by the MSRCASC-Autonomous

for B.Sc./M.Sc. _____

program in the Biotechnology laboratory during the year 20____- 20___.

Date: _____

Signature of the faculty in charge	Examiners	Head of the department
1.	1.	
2.	2.	

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DEPARTMENT OF BIOTECHNOLOGY AND GENETICS

COURSE: BSc BIOTECHNOLOGY

COURSE CODE: 24BITP301

BIOCHEMISTRY AND BIOPHYSICS

LABORATORY MANUAL

ACADEMIC YEAR 2024-25 ONWARDS

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Program: BSc
Semester: III
Course: Biotechnology Practical

Course Code- 24BITP301: Biochemistry and Biophysics		
Sl. No.	**Name of the Experiment**	**Page No.**
1.	Preparation of Buffers- Citrate and Phosphate	
2.	Estimation of reducing sugars- Glucose/Maltose/ lactose by DNS/ Somogyi's method	
3.	Estimation of protein by Biuret method/ Lowry's method	
4.	Extraction of protein from soaked/ Sprouted green gram by salting out method	
5.	Assay of enzymes activity – Alpha Amylase	
6.	Separation of plant pigments by Paper Chromatography	
7.	Separation of Sugars by TLC	
8.	Estimation of Amino acids by Ninhydrin method	
9.	Estimation of inorganic phosphate by Fiske- Subbarow's method	
10.	a) Determination of saponification and iodine number of fats & lipids b) Determination of iodine number of fats & lipids	
	References	

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Internal Evaluation Report

Sl. No.	Name of the Experiment	Date	Internal Evaluation			Sign
			Record/ Performance	Viva	Total	
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10.	c) Determination of saponification and iodine number of fats & lipids d) Determination of iodine number of fats & lipids					

Faculty in-charge **HOD**

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Experiment no. 1: Preparation of buffers- Citrate and Phosphate

AIM: To prepare phosphate and citrate buffer

INTRODUCTION: A buffer functions to resist change in hydrogen ion concentration as a result of internal and environmental factors. It consists of weak acid and its' conjugates base or weak base and its' conjugate acid. Weak acids and bases do not completely dissociate in water instead exist in solution as an equilibrium of dissociated and undissociated species.

The choice of a buffer is based on

1. The buffering capacity in the deserved pH range with the ability to maintain constant pH range with the ability to maintain constant pH during the fixation.
2. The side effects which vary with the tissue type:

a) Suitably osmolarity so that the cells and the organelles neither swells nor shrink during fixation.

b) Suitable ionic concentration so that the material is neither extracted nor precipitated during fixation

c) The toxicity of the buffer.

CRITERIA OF A GOOD BUFFER

1. The value of the pKa should be usually between 6 and 8 for biological specimen.
2. The maximum solubility should in water, minimum solubility in all the other solvents.
3. It should have reduced an ionic effect.
4. The dissociation of buffer should be least influenced by buffer concentration, temperature and ionic composition.
5. They should be resistant to oxidation.
6. They should be inexpensive and easy to prepare.

PREPARATION OF PHOSPHATE BUFFER

1. Solution A: Prepare 200ml of 0.2M disodium hydrogen phosphate (MW=141.96g)
2. Solution B: Prepare 200ml of 0.2M sodium dihydrogen phosphate (MW=156.01g)

ADVANTAGES OF PHOSPHATE BUFFER

1. Most physiological reactions make use of this commonly used buffer called phosphate buffer as it mimics certain components of extracellular fluid.

2. Non-toxic to cells

3. pH changes slightly with temperature

4. They are stable for several weeks at 4°C

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PREPARATION OF CITRATE BUFFER

1. Solution A: prepare 200ml of 0.1M citric acid(MW=210.14)
2. Solution B: prepare 200ml of 0m1M trisodium citrate

ADVANTAGES OF THE CITRATE BUFFER

1. Citrate buffer are used for RNA isolation for its ability to prevent base hydrolysis.
2. Its' usage is also found in antigen detection by breaking cross-links between antigen and any substances in its fixation medium.

COMMENTS

A buffer solution is a solution that resist the change in pH either in diluted or when limited amount of acids or base are added to it. Such a solution can be prepared by combining a weak acid and its salt With strong base (conjugate base) or analogously, weak base and its salt with strong acid (conjugated acid)

$$\mathbf{pH = pKa + log * C_s/C_{ac}}$$

pKa = negative log of the dissociation constant for the weak acid

$\mathbf{C_s}$ = substance concentration of the salt (conjugate base)

$\mathbf{C_{ac}}$ = substance concentration of weak acid (conjugate acid)

In phosphate buffer, NaH_2PO_4 is weak acid and Na_2HPO_4 is being its' conjugate base. Phosphate buffers are extensively used as it mimics the components of extracellular fluid and has buffering capacity.

In case citrate buffer, citric acid is the weak acid and sodium citrate is its conjugate base. Citrate buffer is mostly used in RNA isolation for its ability to prevent base hydrolysis.

Phosphate buffer

pH	Vol of Na_2HPO_4 (ml) Solution A	Vol of NaH_2PO_4 (ml) Solution B
6.0	6.15	43.85
7.0	30.15	19.50
8.0	47.35	2.65

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Citrate buffer

pH	Vol. of Citric acid (ml) Solution A	Vol. of Trisodium citrate (ml) Solution B
3.0	82.0	18.0
4.0	59	41.0
5.0	35.0	65.0

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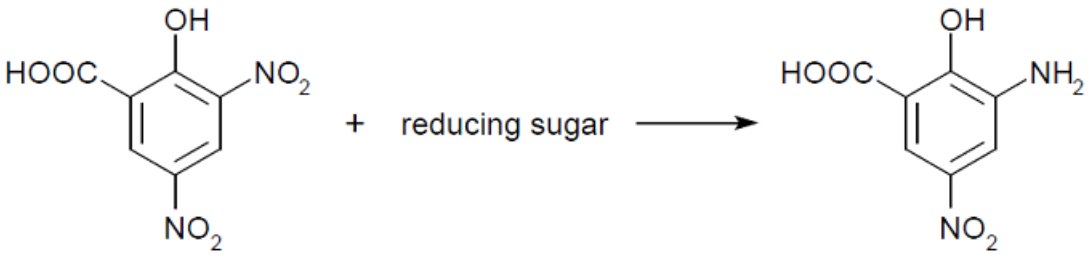
Experiment no. 2: Estimation of reducing sugars (Glucose, Maltose and Lactose) by Dinitrosalicylic (DNS) acid method

AIM: To estimate the maltose by DNS method.

INTRODUCTION:

Carbohydrates are the most abundant class of organic compounds found in living organisms. They are the major source of metabolic energy, for both plants and animals. They also serve as structural material (cellulose) a component of the energy transport compound ATP, recognition sites on cell surfaces, and one of the three essential components of DNA and RNA. To understand the concentration of carbohydrate present, it is necessary to prepare a standard graph by following dinitrosalicylic acid (DNS/DNSA) method which was invented by G. Miller (1959).

PRINCIPLE:



3,5-dinitrosalicylic acid(DNS) 3-amino-5-nitro salicylic acid

3, 5- Dinitrosalicylic acid (DNS/DNSA) is used extensively in Biochemistry for the estimation of reducing sugar. It detects the presence of free carbonyl group (C=O) of reducing sugars which involves the oxidation of the aldehyde functional group (glucose), the ketone functional group (fructose). During this reaction 3, 5 DNS is reduced to 3- amino-5-nitrosalicylic acid (ANSA) which under alkaline condition gives a reddish brown colored complex that has an absorbance maximum at 540 nm.

REAGENTS REQUIRED:

2N NaOH- Dissolve 8g NaOH in 100ml of distilled water. (Prepare 20 ml of 2N NaOH)

Solution A: Weigh 1g DNS and dissolve in 20 ml NaOH (2N).

Solution B: Weigh 30g Sodium potassium tartrate (Rochelle's salt) and dissolve in 50 ml distilled water.

Solution C: DNS reagent - Add solution B to solution A slowly and make up to 100ml with distilled water. Mix properly, decant the contents into an amber coloured bottle and filter it if necessary.

Glucose/ lactose/Maltose Standard Stock solution- Weigh 0.5g maltose and dissolve in 100ml distilled water.

Working Standard maltose stock solution: Take 10ml maltose stock standard solution and make up to 100ml with distilled water.

PROCEDURE:

1. Take 7 test tubes, label them as blank, unknown and 1 to 5 (0.1ml to 1.0ml) with varied volumes of maltose working standard stock solution.
2. Blank preparation- Take 2.0ml of the distilled water; Unknown preparation- take 1.0ml unknown sample.
3. Make up the volume to 2.0ml with distilled water
4. Add 2.0ml DNS reagent to all the test tubes
5. Keep all the test tubes in boiling water bath for 15 min
6. Cool the test tubes to room temperature
7. Record the absorbance maximum at 540nm against the blank

OBSERVATIONS AND CALCULATIONS:

Sl no.	**Vol. of reducing sugar (ml)**	**Vol. of distilled water (ml)**	**Amount of reducing sugar (mg)**	**Vol. of DNS reagent (ml)**		**A_{540nm}**
1	0	2.0	-(Blank)-	↑	Keep all the test tubes in boiling water for 15min and cool at room temp for 5 min	
2	0.2	1.8	0.5			
3	0.4	1.6	1.1			
4	0.6	1.4	1.5	2.0		
5	0.8	1.2	1.5			
6	1.0	1.0	2.5	↓		
7	1.0 (Unknown)	1.0	-			

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RESULT:

The standard graph of maltose is plotted by taking amount of maltose along the X-axis against the absorbance maximum at 540nm along the Y axis. A linear graph is obtained from which amount of maltose in unknown sample is -------- µg.

Paste the graph and photo of the test tubes here

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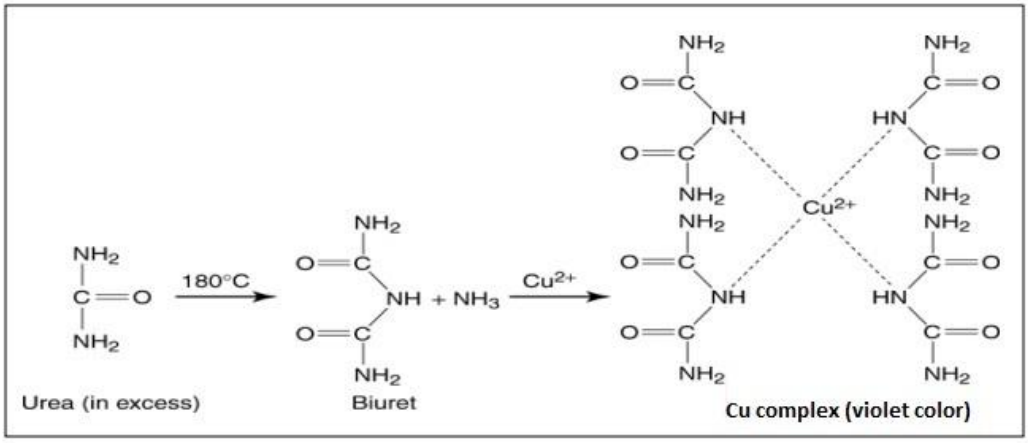
Experiment no. 3: Estimation of protein by Biuret's method

AIM: To estimate the protein by Biuret method.

INTRODUCTION:

Proteins are highly diversified class of biomolecules differences in their chemical properties such as charges, shapes, size and solubility enable them to perform many biological functions. These functions include enzyme catalyst, metabolic regulation, building and transport of small molecules, gene regulation, immunological defence and cell structure. The cellular activities and functions involve one or more proteins. Their central place in the cell is reflected in the fact that genetic information is ultimately expressed as proteins.

PRINCIPLE:



Biuret test is a general test based on the fact that the components such as proteins having a peptide bond (CO-NH) form a purple complex with copper ions in an alkaline medium. Biuret is a compound formed by heating urea to 180^0C when biuret is treated with dilute copper sulphate in alkaline condition a purple coloured compound is formed due to the formation of a copper co-ordinated complex or a chelate complex cupric ions or Cu^{2+} ions form a violet coloured chelate complex with unshared electron pairs of peptide nitrogen and oxygen of water. This is the bases of Biuret's test widely used for identification of proteins and amino acids. Some substances like urea and Biuret interfere because they possess the CO-NH group; the interfering materials are reducing sugars like glucose which interacts with cupric ions in the reagent.

REAGENTS REQUIRED:

1. *Biuret reagent*: Dissolve 3g of sulphate pentahydrate ($CuSO_4.5(H_2O)$) and 9g of sodium potassium tartrate in 500ml of 0.2 N NaOH Add 5g of potassium iodide and make up to 1L with 0.2 N NaOH.

2. *Protein standard stock solution*: Dissolve 1g BSA in 100ml distilled water.
3. *Working stock solution*: 50ml standard stock is diluted to 100ml with distilled water to get 5mg/ml concentration.

PROCEDURE:

i. Pipette out 0.0, 0.1, 0.3, 0.5, 0.7, 0.9 ml of working standard into the series of labelled test tubes.

ii. Pipette out 1ml of unknown sample into another test tube.

iii. Make up the volume to 2ml in all the test tubes with distilled water.

iv. Add 3ml of Biuret reagent to all the test tubes.

v. Mix the contents of the tubes by vortexing or shaking the tubes and warm it at 37^0C for 10min.

vi. Cool the contents to room temperature and record the absorbance at 540nm against blank.

vii. Plot the standard curve by taking amount of protein along x-axis and absorbance at 540nm along y-axis.

OBSERVATIONS AND CALCULATIONS:

Sl No.	**Protein working Std (ml)**	**Vol. Of DW (ml)**	**Amount of protein (mg)**	**Vol. of Biuret's reagent (ml)**		**A_{540nm}**
1	0	2.0		3.0	Keep all tubes at 37^0C for 10mins and cool	
2	0.1	1.9				
3	0.3	1.7				
4	0.5	1.5				
5	0.7	1.3				
6	0.9	1.1				
7	1.0 (Unknown)	1.0				

RESULT:

The amount of protein present in unknown sample from the linear graph is _____mg.

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Paste the graph and photo of the assay here

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Experiment no. 4: Extraction of Proteins from Soaked/ Sprouted Green Grams by Salting out method

AIM: To extract and estimate the amount of protein from soaked or sprouted green grams by salting out method.

INTRODUCTION

Salt precipitation can be a very powerful tool to purify proteins. Ammonium Sulfate is usually the salt of choice since it is cheap, very soluble in water and is able to become much more hydrated (interacts with more water molecules) than any ionic solvent. In practice, Ammonium Sulfate is either added directly as a solid or added as a saturated solution to precipitate the desired proteins. At low salt concentration (<0.19M), addition of more salt in general tends to increase the solubility of proteins, as ions shield the proteins molecules from the changes of other molecules which is termed as "Salting In". At some point, the ionic strength becomes too high and starts to have a negative effect on the solubility of proteins, termed as "Salting Out". This happens because the dissolved salts compete with the proteins for scarce water molecules, increasing the surface tension of water and therefore, causing the protein to fold tighter. The reduction in protein surface area means less protein-water interaction which allows for more hydrophobic interactions between protein molecules causing aggregation and subsequently precipitation.

PRINCIPLE

There are various binds of proteins present in all the cells and tissues which vary in their solubility in different solvents. In some solvents, proteins get extracted in low salt concentration, which is commonly used to produce purified proteins. The peptide bond present in the protein reacts with $CuSO_4$ in an alkaline medium to give purple-coloured complex, which is measured at 540nm.

REAGENTS REQUIRED

1) *Biuret Reagent:* Dissolve 3g $CuSO_4$ and 9g Sodium Potassium Tartarate in 500ml NaOH (0.2N). Add 5g Potassium Iodide and make upto 1 litre with 0.2N NaOH.

2) *Protein Standard Stock Solution:* Dissolve 1g BSA (Bovine Serum Albumin) in 100ml distilled water.

PROCEDURE

i) Preparation of Plant Extract- Weigh 3g of green gram and grind with 5ml of cold distilled water using mortar and pestle.

ii) The homogenate is taken in the centrifuge tube. Centrifuge the tube at 1000rpm for 5mins.Take the supernatant and add 1.6g of solid NH_4SO_4 to procedure 45% saturation. Keep the above tubes on ice for 45mins. Centrifuge the tubes at 3000rpm for 10mins.Collect the precipitate and dissolve in 5ml distilled water.

iii) Pipette out BSA standard stock solution into two test tubes (t1- 1.3 mL & t2- 1.7mL) and label the test tubes as t1 and t2

iv) Pipette out 0.5mL and 1 ml of green gram sample into two test tubes labelled as S1 & S2 respectively (unknown).

v) Take 2ml distilled water in a test tube which serves as a blank.

vi) Make up volume to 2ml in all test tubes with distilled water. Add 3ml of biuret reagent to all the test tubes. Mix the contents of the test tubes by shaking and incubate at 37℃ for 10mins and read the absorbance at 540nm.

vii) Plot the standard graph by taking observations on the Y-axis and the protein concentration in X-axis.

OBSERVATIONS & CALCULATIONS

Sl. No.	Vol. of sample (mL)	Vol. of DW (mL)	Vol of Biuret's reagent (mL)		A_{540nm}
1.	0.0	2.0	3.0	Incubate all test tubes at 37^0 C for 10 mins	
2.	1.3(t1)	0.7			
3.	1.7 (t2)	0.3			
4.	0.5 (S1)	1.5			
5.	1.0 (S2)	1.0			

RESULT: The amount of protein present in the given a) Green Gram Sample (t1) is _____μg & b) Green Gram Sample (t2) is _____μg.

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Paste the graph and photo of the biochemical assay here

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Experiment no. 5: Assay of enzyme activity- Alpha Amylase

AIM: To determine the activity of α amylase enzyme present in saliva by DNS method.

INTRODUCTION:

Salivary amylase is the enzyme produced by the salivary glands, formerly known as ptyalin which breaks down starch into maltose and isomaltose. Amylase like other enzymes works as a catalyst. All catalysts are enzymes and not all enzymes are catalysts. A catalyst is a substance that hastens a chemical reaction but does not become part of the end product. The activity of enzymes is strongly affected by changes in pH and temperature. Each enzyme works optimally at a certain pH and temperature, its' activity decreases at values above and below that point due to denaturation. (For enzymes, denaturation can be defined as the loss of structure rendering the enzyme inactive)

PRINCIPLE:

Amylase is the hydrolytic enzyme which breaks down polysaccharides such as, starch, amylose and dextrins yielding a disaccharide, maltose.

$$(C_6H_{10}O_5)_n + H_2O \longrightarrow n\,(C_{12}H_{22}O_{11})$$

REAGENTS REQUIRED:

(a) *Substrate solution*: Dissolve 1g starch in 100ml phosphate buffer (0.1M, pH-6.8), boil for 3min and cool to room temperature. Filter it if necessary.

(b) *0.1M phosphate buffer*: Dissolve 2.72g of KH_2PO_4 (0.1M) in 100ml distilled water, to this solution add 0.5M KOH (2.8g in 100ml) drop by drop till the pH is set to 6.8, make up to 200ml with distilled water. Thereby the final concentration is 0.1M of 200ml phosphate buffer.

(c) *Enzyme source*: Saliva is the best source of alpha amylase enzyme. Collect the saliva in a beaker and dilute it to 1:10 or (1:20 dilution) with 0.1 M Phosphate buffer (or distilled water).

(d) *DNS reagent*: Dissolve 1.6gm NaOH in 20ml distilled water. Take 1gm of 3,5 DNS in NaOH solution. In another beaker, weigh 30gm sodium potassium tartrate, dissolve in 50ml distilled water. Mix the above solutions and finally make up the volume to 100ml with distilled water.

e) Maltose standard solution: Dissolve 0.25gm maltose in 100ml distilled water.

PROCEDURE

2) *Blank preparation*: Add 2.0ml Phosphate buffer to a test tube labelled as blank

3) *Standard sample*: take two test tubes, add 0.5ml and 1.0ml maltose std solution, label as s1 & s2, and make up the volume to 2.0ml with phosphate buffer

4) *Test samples:* Take two test tubes, add 1.4ml starch (1%) to each test tube and label it as t1 & t2; add 0.2ml and 0.4ml salivary amylase to t1 & t2 respectively; make the volume to 2.0ml with phosphate buffer. Incubate the test tubes at room temperature for 10min.

4) Stop the reaction by adding 2.0ml DNS reagent, mix well and keep all the test tubes in boiling water for 15min. Cool all the test tubes

5) Read the absorbance of the coloured samples at 540nm.

6) Calculate the enzyme activity using the formula.

OBSERVATIONS AND CALCULATIONS

Sl. No.	**Vol. of starch (1%)**	**Vol. of Salivary amylase (ml)**	**Std maltose (ml)**	**Std maltose (mmoles)**	**Vol. of Phosphate buffer (ml)**	Incubate all test tubes at room temp. for 10 min	**DNS reagent (ml)**	Incubate all test tubes at room temp. for 10 min.	**A_{540nm}**
1.	0.0	0.0	0.0	-	2.0		2.0		
2.	0.4 (t1)	0.2	-	-	0.4				
3.	0.4 (t2)	0.4	-	-	0.2				
4.	0.0	-	0.5		1.5				
5.	0.0	-	1.0		1.0				

$$Enzyme\ activity = \frac{ODtest\ X\ Conc.of\ Std\ X\ dilution\ of\ enzyme}{ODstd\ X\ Incubation\ time\ (min)}$$

= …….μmol/min/ml

RESULT: a) Enzyme activity of salivary amylase which is breaks down starch into maltose in t1/t2 sample is ----------μmoles maltose/min/ml

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Paste the graph and photo of the biochemical assay here

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Experiment no. 6: Separation of plant pigments by Paper Chromatography

AIM: To distinguish and study the various pigments present in plants through the process of paper chromatography

INTRODUCTION

Plants carry out the process of photosynthesis, during which light energy from the sun is converted into chemical energy (food). The capturing of light energy is carried out by molecules known as pigments, which are present within the plant cells. Pigments are chemical compounds, which are able to reflect only a particular range of wavelengths of visible light. Leaves of plants primarily contain different types of pigments within their tissues. The four different types of pigments are listed below in a tabular column along with their colours. In order to view and distinguish the primary four plant pigments, a simple technique known as chromatography can be used. It is a technique that is used to distinguish between different molecules. This differentiation is based on these attributes-shape, size, charge, mass, adsorption and solubility.

MECHANISM OF PAPER CHROMATOGRAPHY

In this technique, the interaction between three components is involved – solid phase, separation of a mixture and a solvent.

1. At first, the mixture is spotted onto the paper and is dried.

2. The solvent is made to flow through the capillary attraction.

3. While the solvent moves through the paper, the various components of the mixture differentiate into varied coloured spots.

4. Later the paper is allowed to dry and the position of various compounds is viewed.

5. The substance, which is the most soluble moves further on the paper as compared to the other substances that are less soluble.

Materials Required: Test tubes, Spinach leaves, Mortar and pestle, Scissors, Petroleum ether & acetone solvent (9:1), Capillary tube, Pencil, ruler, Filter paper strips

PROCEDURE

1. In this experiment, spinach leaves are used to separate different pigments.

2. Pick a few fresh and green leaves of spinach and wash it.

3. Cut out small pieces of spinach using scissors. Add them to the mortar.

4. Accurately measure 5ml acetone using a measuring cylinder and add it into the mortar.

5. With the help of mortar and pestle, grind the spinach leaves into a smooth paste.

6. Place a filter paper strip with a tapering notch towards one ending of the strip.

7. Horizontally trace a line with a scale and a pencil that is 2 to 3 cm apart from the notch's tip.

8. Using a capillary tube, add 1 drop of the extract of the pigment in the midsection of the line.

9. Let the drop dry. Repeat the same process of adding a drop and allowing it to dry for 4-5 times.

10. In the test tube, pour the petroleum ether acetone solvent.

11. Make sure to fold and suspend the strip in the chamber.

12. The loading spot remains about 1 cm above the level of the solvent.

13. Let the test tube remain uninterrupted for a while.

14. Observe the solvent passes along the paper scattering various pigments of the blend to different distances.

15. Once the solvent reaches 3/4th of the strip, carefully take the strip off.

16. Allow the strip to dry. Calculate the Rf value using the formula

$$Rf = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by solvent}}$$

OBSERVATION: The dried paper strip displays four different bands. Discrete pigments can be distinguished with the help of colours.

1. The Carotene pigment is observed at the topmost as an orange-yellow band of pigments distinctively.
2. Just below this band, a yellowish band appears which indicates the pigment xanthophyll.
3. The third band appearing dark green indicates chlorophyll-a pigment.
4. The yellowish-green band present at the bottom is the chlorophyll b pigment.

Precautions

- The leaves that are selected should be green and fresh spinach leaves
- From the tip of the notch, the loading spot needs to be 2 to 3 cm apart

- While suspending the filter paper strips in the test tube, one need to ensure that the loading spot needs to be set up above 1 cm from the level of the solvent.

RESULTS:

a) Rf of Chlorophyll b -

b) Rf of Chlorophyll a -

c) Rf of xanthophyll -

d) Rf of Carotene -

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Experiment no. 7: Separation of Sugars by Thin Layer Chromatography (TLC)

AIM: To separate sugar by thin layer chromatography (TLC).

INTRODUCTION:

Great attention has been given recently to some relatively techniques of analysis. Thin layer chromatography is already accepted as a lab tool for routine work. Its low cost, ease and rapidity along with its capacity for separating and identifying small qualities of compound mixtures make the technique a prime tool for research as well.

PRINCIPLE:

The TLC technique allows to carry out qualitative and also sometimes quantitative analysis of chemical components in complex mixtures, substance to be determined are transferred onto the layer of sorbent as a spot using a capillary or microsyringe, sorbent may comprise of a layer (0.05.....0.2mm thick) of fine grain material [silica gel. cellulose, aluminium oxide] on a solid support (glass, plastic, metal). Spots of samples are applied on the plate in a straight line called starting line. The mobile phase starts moving upwards due to capillary forces when the plate edge is inserted into the eluent in chromatographic chamber. While the eluent is moving the components of sample separate from each other because of the interactions between the molecules of eluent, sorbent and substances to be separated. The elution process is stopped, when the eluent has travelled up the plate until 5-10mm from the upper edge of chromatography plate. The higher the interaction of sugar with silica, will be carried by eluent to a small distance whereas the lesser interaction will be moved further.

REAGENTS REQUIRED:

1. *Sugar sample*: Samples like glucose, fructose, and maltose were prepared with different concentrations of 0.1 g to 0.7g/100ml are prepared.
2. *Eluent mixture*: Butane/ethanol/water = 3:2:1.
3. *Chromatography*: TLC plates and TLC chamber was kept ready. The sample were spotted on 0.1mm plate with a capillary tube.
4. *Detection reagent:*

a) *Silver nitrate solution*: $AgNO_3$ solution is prepared by dissolving 3g $AgNO_3$ in 12ml of DW adding 500ml of acetone.

b) *Ethanolic Sodium hydroxide solution*: It is prepared by diluting 50ml NaOH (10N) in 450ml of ethanol.

c) *Sodium thiosulphate* (5%): 5gm Sodium thiosulphate in 100ml DW.

PROCEDURE:

1. On the TLC plates, two lines is drawn from 2cm from one edge of the plate and 1cm from another edge.

2. Aliquots (3µl) were spotted on the TLC plates with a capillary tube and dried. They are allowed to develop by ascending technique.

3. TLC plates are later placed in TLC chamber containing solvent mixture-butane/ethanol/water.

4. The eluent is allowed to dry at room temperature and dipped in $AgNO_3$ solution until dark brown spots appeared in a brown background.

5. If plates were kept for a long time, they are sprayed with 5% $Na_2S_2O_3$ solution once they are dry.

6. The spots are circled with pencil and the distance is measured from the sample loaded spot till sugars are separated as spots.

7. The distance moved by solvent mixture is also noted down.

8. The retention factor is calculated using the formula.

$$\text{Retention factor} = \frac{Distance\ moved\ by\ substance\ from\ origin}{Distance\ moved\ by\ solvent\ from\ origin}$$

RESULT: RF for the dextrose of concentration 0.5% is =--------

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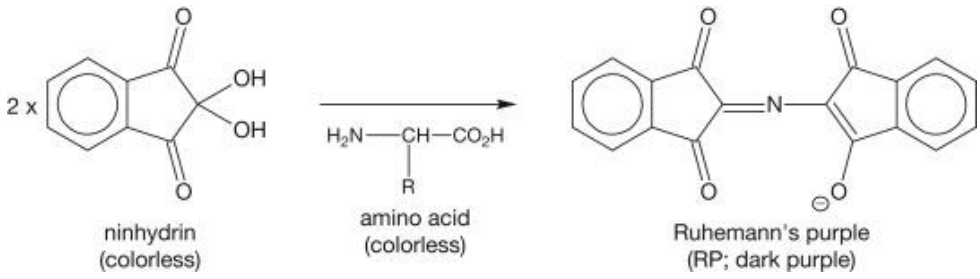
Experiment no. 8: Estimation of amino acids by Ninhydrin method

AIM: To quantify the amount of amino acids by utilizing Ninhydrin reaction.

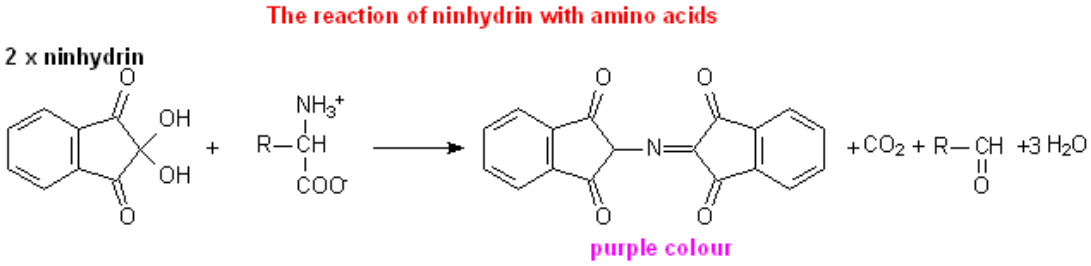
INTRODUCTION:

Amino acids are the building blocks of all proteins. There are twenty different amino acids found in proteins which are comprised of a carboxyl and an amino group attached to the same carbon atom (α-Carbon). They vary in size, structure, electric charge and solubility in water because of variation in their side chains (R-group). Detection, quantification and identification of amino acids in any sample constitute an important step in the study of protein.

PRINCIPLE:



OR



Ninhydrin which is originally yellow, reacts with amino acids and turns deep purple detected in this method. Ninhydrin will react with the free α-amino acid group that is present in all amino acids, proteins or peptides. Whereas the decarboxylation reaction will proceed for a free amino acid, it will not happen for peptides and proteins. Theoretically only amino acids produce colour with Ninhydrin reagent (Ruhemann's Purple). When amino acids with a free alpha amino groups are treated with an excess of Ninhydrin, they yield a purple colored product. Under appropriate conditions, the color intensity produced is proportional to the amino acid concentration.

REAGENTS REQUIRED:

1. *Glycine Standard Stock Solution*- Dissolve 100mg of Glycine in 100ml distilled water.

2. *Working Standard Glycine stock solution:* Take 50 ml of solution and make up to 100ml distilled water.
3. *8% Ninhydrin Reagent*: 8gm of Ninhydrin reagent and dissolve in 100ml Acetone.
4. *70% Ethanol*: Mix in proportion, 70ml Ethanol: 30ml distilled water.

PROCEDURE:

1. Pipette out different volumes (0.1-1.0) ml of working standard amino acids into test tubes from test tube no. 2 to test tube no. 5 with an increment of 0.2ml and test tube no. 6 with 1.0 ml unknown.
2. Make up the volume to 4ml with distilled water in test tubes; while test tube no. 1 with 4.0ml distilled water serves as blank
3. Add 1ml Ninhydrin reagent to all the test tubes including blank.
4. Mix the contents of the test tubes by vortexing.
5. Cover the mouth of the test tubes with aluminium foil and place them in water bath for 5min.
6. Cool the test tubes and add 1ml ethanol to each test tube and mix well.
7. Record the absorbance at 570nm using Colorimeter.
8. Plot the standard graph with the absorbance of amino acid present in the sample along Y-axis against the amount of glycine along X-axis

OBSERVATIONS AND CALCULATIONS

Sl No.	**Vol. of glycine (ml)**	**Amount of glycine (mg)**	**DW (ml)**	**Volume of Ninhydrin reagent (ml)**	**Incubate all the test tubes for 5 min at room temperature**	**Volume of Ethanol (ml)**	**Absorbance at 570 nm**
1	0.0		4.0	↑ 1.0 ↓		↑ 1.0 ↓	
2	0.2		3.8				
3	0.4		3.6				
4	0.6		3.4				
5	0.8		3.2				
6	1.0		3.0				
7	1.0 (Unknown)		—				

RESULT: In the present study, the standard graph of amino acids is plotted against 570nm. A linear graph was obtained from which the amount of amino acid (glycine) present in unknown sample is ___µg/ml.

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Paste the graph and photo of the biochemical assay here

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Experiment no. 9: Estimation of inorganic phosphate by Subbarow method

AIM: Estimation of inorganic phosphate by Fiske-Subbarow method (1925).

INTRODUCTION:

Phosphorus in the body is mainly present in the bones; small portion is present in cells and soft tissues. Eg., some proteins, lipids, nucleic acid & co-enzymes. It plays an important role in acid-base regulation particularly by the kidneys. The serum phosphate may exist as free ions (40%) or in a complex form (50%) with cations, such as calcium, sodium, potassium, etc… About 10% of serum phosphate is bound to proteins. A quantitative method for the estimation of inorganic phosphate was developed by Fiske-Subbarow (1925).

PRINCIPLE:

Phosphate will readily react with molybdic acid forming phosphomolybdic acid further reacts with 1,2,4 -aminonapthol sulphonic acid (ANSA) to form a blue coloured complex, the intensity of which is directly proportional to the concentration of phosphate present in the solution.

Ammonium molybdate + Phosphate ⟶ Ammonium phosphomolybdate +ANSA ⟶ Blue complex

$(NH_4)_2\ MoO_4\ (PO_4)^{3-}$ $(NH_4)_3[PO_4(MoO_3)_{12}]$ + $H_2NC_{10}H_5(OH)SO_3H$

REAGENTS REQUIRED:

1. *Ammonium molybdenum reagent* (2.5 % w/v): Dissolve 2.5g ammonium molybdate in 100ml of 3M sulphuric acid (16.8ml concentration sulphuric acid and dilute to 100ml with distilled water).

2. *1-Amino-2-naphthol-4-sulphonic acid (ANSA) reagent:*

0.1% w/v of ANSA reagent in 15% w/v of sodium metabisulphite and 20% w/v sodium sulphite.

a. 15g of sodium metabisulphite is dissolved in 100ml distilled water.

b. Weigh 20g of sodium sulphite dissolved in 100ml of distilled water.

100mg of ANSA (0.1g) is dissolved in 97.5ml of 15% w/v of sodium metabisulphite and 2.5ml of 20% w/v of sodium sulphite, mix well and store at room temperature.

3. (a) *Standard phosphate stock solution*: 20 mg (0.02g) of potassium dihydrogen orthophosphate is dissolved in 100ml distilled water.

(b) *Working standard Phosphate stock solution*: Take 50ml of standard phosphate stock solution and make up the volume to 100ml with distilled water.

PROCEDURE:

1. Pipette out different volumes (0.1ml to 1ml) of standard phosphate solution into five different test tubes (test tube no.2- test tube no. 5)

2. Make up the volume to 1.0ml with distilled water; pipette 1.0ml dw to test tube no.1 serves as blank.
3. Add 1ml of 2.5% ammonium molybdate reagent and 0.5ml of ANSA reagent.
4. Mix reaction mixture well and allow the tubes to stand for 20min at room temperature.
5. Read the colour developed in each test tubes at 620nm against blank.
6. A standard graph is prepared taking different concentration of standard phosphorous against the absorbance at 620nm.

OBSERVATIONS AND CALCULATIONS:

Sl. No.	Phosphate working std. soln. (ml)	Vol. of dw (ml)	Amount of phosphate (μg)	Vol. of Ammonium molybdate (ml)	Vol. of ANSA reagent (ml)		Absorbance at 620 nm
1.	0.0	1.0	0.0	↑	↑	Incubate all test tubes for 5 mins at room temp	
2.	0.2	0.8	20				
3.	0.4	0.6	40				
4.	0.6	0.4	60	1.0	0.5		
5.	0.8	0.2	80				
6.	1.0	0.0	100				
7.	1.0 (Unknown)	0.0	---	↓	↓		

RESULT:

The standard graph of inorganic phosphate plotted against the absorbance at 620nm. A linear graph was obtained from which the amount of inorganic phosphate in unknown sample was found to be ---------μg.

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Paste the graph and photo of the biochemical assay here

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Experiment no. 10a: Determination of saponification value of fats & lipids

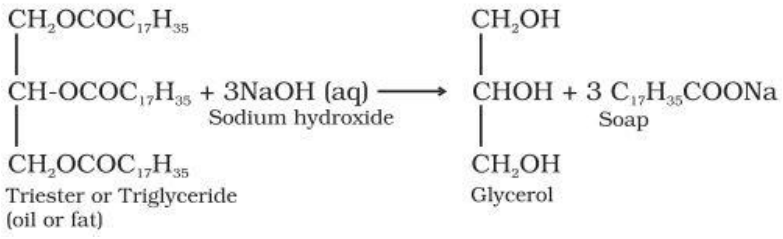
AIM: To determine the saponification value of peanut oil

INTRODUCTION:

Saponification value is defined as number of mg of KOH required to neutralise the fatty acid obtained by complete hydrolysis of 1g of fat/oil. The esters of the fatty acids of low molecular weight require the more alkali for saponification, so that the saponification value is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present. As many oils have somewhat similar values (e.g. those in the olive oil series fall within the range 188-196), the saponification value is not, in general, as useful for identification purposes as the iodine value. The saponification value is of most use for detecting the presence of coconut oil (SV 255), palm-kernel oil (SV 247) and butterfat (SV 225), which contain a high proportion of the lower fatty acids. The presence of paraffin hydrocarbons may be detected as cloudiness when water is added to the ethanolic saponified oil or fat solution.

- Saponification is used by wet chemical fire extinguishers to convert burning fats and oils into non-combustible soap which helps in extinguishing the fire. Further, the reaction is endothermic and lowers the temperature of the flames by absorbing heat from the surroundings.
- In an undesirable scenario, saponification damages oil paintings. In oil paintings, the heavy metals used in pigments react with the oil containing free fatty acids and form soaps. This way, the paintings get damaged gradually.
- Soaps formed are used in everyday life like sodium soaps are used for laundry, potassium soaps are used for cleaning and lithium soaps are used as lubricating greases. There are various other soaps which are used for different purposes.

PRINCIPLE:



When a fat is boiled with an excess of alcoholic potassium hydroxide (KOH), the triglycerides hydrolyse, and glycerol and soap are formed. The alkali consumed by this hydrolysis is a measure of the Saponification Value (S.v.). It is defined as the number of milligrams of potassium hydroxide (KOH) required to saponify completely one gram of the oil or fat.

REAGENTS REQUIRED:

1. *Ethanolic KOH*: 35g in 20ml distilled water, and make up to 1000ml with 95% (v/v) ethanol
2. *0.5 N HCl:* 4.2ml of 37% HCl is made up to 100ml with distilled water
3. *Peanut Oil/ fat sample*
4. *Phenolphthalein indicator*: 1gm Phenolphthalein powder in 100ml Ethanol (v/v-95%)

Procedure:

1. Take 2of coconut oil (with sample) into 250ml conical flask (3 in no.) and take three more conical flasks for blank (without sample) serves as blank
2. Add 25ml alcoholic KOH into all the 6 conical flasks
3. Keep all the three flasks with samples in the water bath at 85^0C for 1 hour with occasional stirring
4. After 1 hour stop heating and cool the conical flasks to 37^0-40^0 C
5. Add few drops of Phenolphthalein indicator
6. Note the colour change (Pink Colour)
7. Take 0.5 N HCl in the burette
8. Titrate excess KOH (unreacted) against 0.5 N HCl, till the solution changes to colourless.
9. Record the volume of HCl run down for at least 3 concordant values.

OBSERVATIONS AND CALCULATIONS

Sl. No.	**Vol. of KOH solution (ml)**	**Burette reading**		**Volume of 0.5N HCl run down (ml)**
Blank		**Initial reading**	**Final reading**	
1				
2				
3	**25.0**			
Oil Sample				
1				
2				
3				

$$\text{Saponification value} = \frac{28.05^{*} \times \text{Molarity of HCl} \times (V_0 - V_1)}{\text{Sample weight}}$$

V_0 –Volume of HCl run down for titration of blank

V_1 –Volume of HCl run down for titration of sample

Sample weight- gms

RESULT: The saponification value of given sample is ------- mg KOH/ g sample

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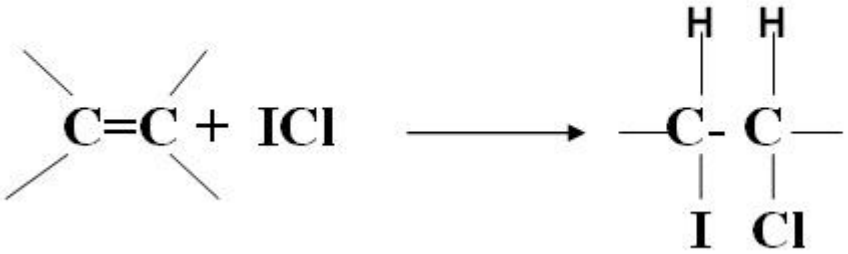
Experiment no. 10(b): Determination of iodine number of lipids

AIM: To determine the iodine number of peanut oil

PRINCIPLE:

The iodine number is an important characteristic of oils as it indicates the proportion of unsaturated fatty acids present. The iodine number equals the number of mg of iodine required to saturate the fatty acids present in 100 mg of the oil or fat. Oils rich in saturated fatty acids have low iodine numbers, while oils rich in unsaturated fatty acids have high iodine numbers. Fatty acids react with a halogen (iodine) resulting in the addition of the halogen at the C=C double bond site. In this reaction, iodine monochloride reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine.



After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product.

$$ICl + KI \text{ ----------> } KCl + I_2$$

This causes the remaining unreacted ICl to form molecular iodine. The liberated I_2 is then titrated with a standard solution of 0.1N sodium thiosulfate.

$$I_2 + 2\ Na_2S_2O_3 \text{ ----------> } 2\ NaI + Na_2S_2O_4$$

Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is an oil. Starch is used as the indicator for this reaction so that the liberated iodine will react with starch to give purple coloured product and thus the endpoint can be observed.

REAGENTS REQUIRED:

1. *Sodium thiosulphate (0.1N)* – Dissolve 24.8g ($Na_2S_2O_3.\ 5H_2O$) in 800ml of freshly boiled and cooled water by shaking for 15mins and make up to volume to 1000ml with distilled water.

2. *Potassium iodide solution*- 30% Potassium iodide solution

3. *Iodine Monochloride Solution, Wijs Reagent*- Dissolve 26.0 g of reagent grade iodine (I_2) in 2000 ml of reagent grade glacial acetic acid, heating gently if necessary to hasten reaction. Cool to room temperature. Pipette 25 mL into a 250 mL Erlenmeyer flask. Add 100 ml purified water and titrate immediately with standard 0.1 N thiosulfate solution until the yellow iodine color almost disappears. Add 1 mL of starch indicator solution and complete the titration dropwise with vigorous swirling to the disappearance of the blue starch iodine complex. This is the original titre.

PROCEDURE:

1. Weigh accurately 0.22-0.25 g of oil sample into a 250 mL Erlenmeyer flask
2. Add 20 mL of carbon tetrachloride.
3. Take 20 mL carbon tetrachloride in three additional flasks which serve as blanks.
4. Pipette 25.0 mL Wijs reagent into all the flasks. Stopper the flasks, mix the contents by swirling and store in a dark place at 25 ± 5 °C.
5. At the end of 30 mins, add 10 mL of 30% potassium iodide solution and 150 mL of purified water to the reaction mixture.
6. Titrate immediately with standard 0.1 N sodium thiosulfate solution until the yellow color almost disappears.
7. Add 1 mL of starch indicator solution and titrate dropwise with vigorous swirling to disappearance of the blue starch-iodine color.
8. Titrate the blanks in the same manner. If the sample titer is not between 50 and 60% of the average of the average blank titer, adjust sample size accordingly and repeat the determination.

OBSERVATIONS & CALCULATIONS:

Blank (Wij's solution Vs Sodium thiosulphate solution)

Sl. No.	**Volume of ICl (Wij's solution) (ml)**	**Burette reading (ml)**		**Vol. of sodium thiosulphate solution run down (ml)**
		Initial reading	**Final reading**	
1				
2				
3				

Sample (Oil sample + Wij's solution Vs Sodium thiosulphate solution)

Sl. No.	**Oil sample + Volume of ICl (Wij's solution) (ml)**	**Burette reading**		**Vol. of sodium thiosulphate solution run down (ml)**
		Initial reading	**Final reading**	
1				
2				
3				

$$Iodine\ number = \frac{\text{Atomic mass of iodine X N X (V0 } - \text{ V1)}}{m\ (g)}$$

Atomic mass= 12.69; N= 0.1; V_0 =Blank (ml); V_1 = Sample (ml); m = Weight of the sample (oil) in g

RESULT: The iodine number of the given sample is ------- g I_2 / 100g oil or fat

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Department of Biotechnology and Genetics in the M S Ramaiah College of Arts, Science and Commerce, was established in the year 2000 offering both UG and PG programs. The main objective of the program is to provide conducive learning environment for the students and to mitigate the shortage of biotechnologists in the field of food, agriculture, medicine and environmental management. Highly qualified and experienced faculty members deliver the lectures and conduct the practical in various subjects of as per the curriculum developed by the Bangalore City University. The department focuses mainly on teaching the basics, applications and hands-on training in a state-of-the-art classroom and laboratory environment. It also facilitates students to broaden their knowledge for multitasking.

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This manual is authored by Dr. Radha Dayanidhi, Assistant Professor, Department of Biotechnology and Genetics, MSRCASC, Bengaluru.

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