V.V.VANNIAPERUMAL COLLEGE FOR WOMEN

(Belonging to Virudhunagar Hindu Nadars)
An Autonomous Institution Affiliated to Madurai Kamaraj University
Re-accredited with 'A' Grade (3rd cycle) by NAAC

VIRUDHUNAGAR

सत्यमेव जयते
Department of BioTechnology, Government of India

BIOCHEMICAL ANALYSIS OF METABOLITES LABORATORY MANUAL

PREPARED UNDER

DBT STAR COLLEGE SCHEME
DEPARTMENT OF BIOTECHNOLOGY, NEW DELHI

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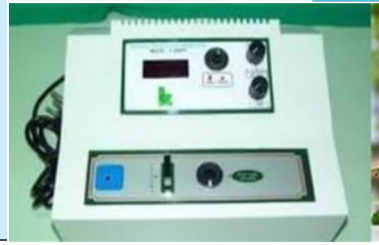

V.V.Vanniaperumal College for Women, Virudhunagar Tamilnadu.
No HRD-11011/163/2020-HRD-DBT-Biochemistry/Lab Manual – Biochemical Analysis of Metabolites

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VIRUDHUNAGAR – 626 001 (TAMIL NADU)

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FOREWORD

This Lab Manual on "**BIOCHEMICAL ANALYSIS OF METABOLITES**" is prepared in accordance with the updated syllabus under DBT Star College Scheme sponsored by the Department of Biotechnology, Ministry of Science and Technology, MHRD, New Delhi to fulfil the needs of students. Students are enabled to analyse the biochemical metabolites like sugars, protein, vitamins and minerals in various food sources and interpret the results. Step by step procedure is given in the manual will help the students to have clear understanding and carry out the experiments easily by themselves.

We thank the **Department of Biotechnology, Ministry of Science and Technology, MHRD, New Delhi** for providing an excellent opportunity under Star College Scheme (No.HRD11011/163/2020-HRD-DBT Dt.24.8.2020).

We hope this manual will definitely improve the student's practical skill and motivate them towards research.

MEMBERSECRETARY/COORDINATOR **CHAIRMAN/PRINCIPAL**

V.V.Vanniaperumal College for Women, Virudhunagar Tamilnadu.
No HRD-11011/163/2020-HRD-DBT-Biochemistry/Lab Manual – Biochemical Analysis of Metabolites

BIOCHEMICAL ANALYSIS OF METABOLITES

LIST OF PRACTICALS

1.ESTIMATION OF GLUCOSE USING BENEDICTS METHOD

TITRATION – I

STANDARDIZATION OF BQR USING STANDARD GLUOSE

S.No	Volume of Benedict's reagent(ml)	Burette reading(ml)		Concordant Value (ml)	End point
		Initial	Final		
1.					
2.					

TITRATION – II

ESTIMATION OF GLUCOSE IN UNKNOWN SAMPLE USING STANDARDIZED BQR

S.No	Volume of Benedicts reagent (ml)	Burette reading(ml)		Concordant Value (ml)	End point
		Initial	Final		
1.					
2.					

CALCULATION

FROM THE STANDARD TITRATION

1 ml of standard glucose solution contains 1mg of glucose

5ml of BQR is reduced by --- mg of glucose.

Volume of unknown glucose solution required to reduce 5 ml of BQR is X ml.

- X ml of unknown glucose contain Y mg of glucose
- 100 ml of unknown glucose solution contain = $\frac{Y}{X}$ x 100

= Z mg

1. ESTIMATION OF GLUCOSE USING BENEDICTS METHOD

AIM:

To estimate the amount of glucose present in the given sample using Benedict's Quantitative reagent.

PRINCIPLE:

Benedict's quantitative reagent contains potassium thiocyanate, copper sulphate and potassium ferrocyanide. When Benedict's reagent is boiled with reducing sugar it leads to the formation of cuprous oxides. Potassium ferrocyanide prevents the precipitation of copper oxide and forming the precipitate of cuprous thiocyanate. A blue coloured solution become colourless, when all the copper sulphate was converted into cuprous thiocyanate.

REQUIREMENTS:

Equipments: Weighing balance

Glasswares : Burette, pipette, conical flask

Chemicals : Benedict's Quantitative Reagent (BQR), anhydrous sodium carbonate, Standard glucose solution

PROCEDURE:

SAMPLE EXTRACTION:

100 mg of sample was weighed into a boiling tube and it was hydrolyzed with 5 ml of 2.5 N hydrochloric acid by heating in a boiling water bath for 3 hours. Then it was cooled at room temperature and neutralized with sodium carbonate until the effervescence ceased. The solution was made up to 100 ml with distilled water. This could be considered as an unknown sample.

TITRATION – I

STANDARDIZATION OF BQR USING STANDARD GLUCOSE:

Pipette out 5 ml of Benedict's quantitative reagent in a clean conical flask and add 2g of anhydrous sodium carbonate to maintain the saturated medium of sodium carbonate. Add a piece of porcelain bit to prevent bumping. The burette should be rinsed with distilled water and filled with standard glucose. Titrate the mixture in the conical flask against the standard glucose solution in the burette. Boil the solution throughout the titration and stir continuously with the glass rod. The formation of chalky white precipitate with the disappearance of last tinge of the blue colour is the end point. The titration is repeated for concordant value. The glucose solution consumed by 5 ml of Benedict's quantitative reagent could be calculated.

TITRATION – II

DETERMINATION OF GLUCOSE IN UNKNOWN SAMPLE USING STANDARDIZED BQR

Pipette out 5 ml of Benedict's quantitative reagent in a clean conical flask and add 2g of anhydrous sodium carbonate to maintain the saturated medium of Sodium carbonate. Add a piece of porcelain bit to prevent bumping. The burette should be rinsed with distilled water and filled with unknown glucose. Titrate the mixture in the conical flask against the unknown solution in the burette. Boil the solution throughout the titration and stir continuously with the glass rod. The formation of chalky white precipitate with the disappearance of last tinge of blue colour is the end point. The titration is repeated for getting concordant value. The unknown sample consumed by 5 ml of BQR is noted and the concentration of glucose in unknown sample could be determined.

RESULT:

The amount of glucose present in 100 ml of the given unknown sample = Z mg

Outcome:

After the completion of this experiment, the students will be able to estimate the glucose content of unknown samples by volumetric method using Benedict's quantitative reagent.

2. ESTIIMATION OF ASCORBIC ACID USING VOLUMETRIC METHOD

CALCULATIONS

Ascorbic acid in V_1ml of the sample extract = dye factor x V_2 = mg

Therefore, ascorbic acid in 100 ml of the extract = $\frac{\text{Dye factor x } V_2 \text{ x } 100}{V_1} = \text{mg}$

Since Wg sample is made upto 100 ml ascorbic acid content of the sample (mg/ 100 g)

$$= \frac{\text{Dye factor x } V_2 \text{ x } 100 \text{ x } 100}{V_1 x W}$$

$$= \frac{\text{Dye factor x } V_2 \text{ x } 10{,}000}{V_1 x W}$$

2. ESTIIMATION OF ASCORBIC ACID USING VOLUMETRIC METHOD

AIM: To estimate the amount of ascorbic acid present in the given food sample by volumetric method.

PRINCIPLE :

2,6-dichlorophenol indophenol dye, which is blue in alkaline solution and red in acid solution, is reduced by ascorbic acid to a colourless form. The reaction is quantitative and can be performed by titration. This reaction is practically specific for ascorbic acid in fresh fruits and vegetables,Sulphur dioxide present in products like squashes can deduce the dye and thus interferes in the estimation. Condensing SO_2, with formaldehyde can eliminate this interference.

REQUIREMENTS:

Equipments: Weighing balance

Glasswares: Pipette, Standard Measuring Flask, burette, conical flask

Chemicals: Metaphosphoric acid (HPO_3), ascorbic acid, indophenols, sodium bicarbonate, hydrochloric acid, formaldehyde

PROCEDURE

PREPARATION OF REAGENTS:

3% (w/v) Metaphosphoric acid (HPO_3); Prepare by dissolving the sticks or pelletsof HPO_3 in distilled water.

Ascorbic acid standard: Weigh accurately 100 mg of L-ascorbic acid and make upto I00 ml with 3% HPO_3 solution. Dilute 5 ml to 50 ml with 3% HPO_3solution (1 ml= 0. 1 mg of ascorbic acid).

Dye solution: Dissolve 50 mg of the sodium salt of 2,6-dichlorophenol indophenol in approximately 150 ml of hot distilled water containing 42 mg of sodium bicarbonate.Cool, filter and dilute with distilled water to 200 ml. Store in a refrigerator.

PREPARATION OF SAMPLE

Juices and liquid products: Take 10-20 g sample and make up to I00 ml in a volumetric flask with 3% HPO_3 solution. Filter through a Whatman No. 1 filter paper.

Solid or semi-solid products: Blend 10-20 g sample with 3% HPO_3solution and make upto 100 ml in avolumetricflask with 3% HPO_3solution. Filter through a Whatman No. 1filter paper.

TITRATION – I

STANDARDIZATION OF DYE USING STANDARD ASCORBIC ACID

S.No	Volume of Ascorbic acid (ml)	Burette reading(ml)		Volume of dye (ml)	Concordant Value (ml)	End point
		Initial	Final			
1.						
2.						

TITRATION – II

UNKNOWN SAMPLE VS DYE

S.No	Volume ofunknown Ascorbic acid (ml)	Burette reading(ml)		Volume of dye (ml)	Concordant Value (ml)	End point
		Initial	Final			
1.						
2.						

STANDARDIZATION OF DYE

STANDARD ASCORBIC ACID VS DYE- TITRATION I

Pipette out 5 ml ofthe standard ascorbic acid solution into a 100 ml conical flask and add5 ml of the 3% HPO_3solution. Fill the burette with the dye solution. Titrate theascorbic acid solution with the dye solution to a pink colour, which should persist for 15sec. Note the titre value. Calculate the dye factor.

Volume of ascorbic acid solution taken for titration = 5 ml

Volume of dye solution required (titre) = vml

Dye factor = mg of ascorbic acid per ml of the dye

Since 5ml ofthe standard ascorbic acid solution contains 0.5 mg ascorbic acid

Dye factor =0.5/Titre= 0.5/V= mg ascorbic acid per ml dye

UNKNOWN SAMPLE VS DYE TITRATION -II

Pipette out 2-10 ml of the sample extract into a 100 ml conical flask and titrate against the dye solution as that of the standard. The volume of the sample should be such that the titre value is in the range of 3-5 ml. If the sample contains sulphur dioxide, add 1 ml of the formaldehyde solution and 0.1 ml HCl to the pipette out sample extract and keep for 10 min and perform the titration.

OBSERVATIONS

Weight of sample taken for extraction with HPO_3 =W g

Volume of the sample made up with HPO_3solution = 100 ml

Volume of sample extract taken for dye titration = V_1ml

Volume of dye required (titre) =V_2 ml

RESULT

Ascorbic acid content of the sample = ...mg per 100 g.

OUTCOME:

After the completion of this experiment, the students will be able to determine the ascorbic acid content of food samples volumetrically using 2,6 dichlorophenol indophenol dye.

3. ESTIMATION OF LACTOSE USING BENEDICT'S METHOD

TITRATION – I

TITRATION OF BENEDICTS REAGENT VS STANDARD LACTOSE SOLUTION

S.No	Volume of Benedict's reagent(ml)	Burette reading(ml)		Volume of lactose standard solution(ml)	Concordant Value (ml)	End point
		Initial	Final			
1.						
2.						

TITRATION – II

TITRATION OF BENEDICTS REAGENT VS UNKNOWN SAMPLE

S.No	Volume of Benedict's reagent(ml)	Burette reading(ml)		Volume of unknown sample (ml)	Concordant Value (ml)	End point
		Initial	Final			
1.						
2.						

3. ESTIMATION OF LACTOSE USING BENEDICT'S METHOD

AIM:

To estimate the amount of lactose present in the given milk sample using Benedict's reagent.

PRINCIPLE:

Benedict's quantitative reagent contains potassium thiocyanate, copper sulphate and potassium ferrocyanide. When Benedict's reagent is boiled with reducing sugar, it leads to the formation of cuprous oxides. Potassium ferrocyanide prevents the precipitation of copper oxide and forming the precipitate of cuprous thiocyanate. A blue coloured solution become colourless, when all the copper sulphate was converted into cuprous thirocyanate.

REQUIREMENTS:

Equipments: Weighing balance

Glasswares: Pipette,burette, conical flask, Standard Measuring Flask

Chemicals: Benedict's quantitative reagent, anhydrous sodium carbonate,lactose

PROCEDURE:

SAMPLE PREPARATION:

Take 20 ml of milk sample in a beaker and make up the volume to 100 ml with distilled water. Heat the diluted milk sample with 2 ml of 10 % acetic acid in order to precipitate the protein. The precipitation is removed by filtration. Use the filtrate as the unknown lactose sample.

TITRATION OF BENEDICTS REAGENT WITHSTANDARD LACTOSE SOLUTION

Fill the burette with standard lactose solution. Pipette out 5 ml of Benedict's quantitative reagent into a clean conical flask and add about 2 g of sodium carbonate. A small piece is added to prevent bumping. This mixture is titrated against standard lactose filled in the burette. This mixture is boiled throughout the titration and stir well continuously with the glass rod. The formation of chalky white precipitate with the disappearance of last tinge of blue colour is the end point. The titration is repeated to get concordant value. Similarly, the experiment is repeated using the filtrate

(milk sample) as burette solution. The amount of lactose in milk sample is calculated using these values.

RESULT:

The amount of lactose present in 100 ml of unknown sample is ----- mg.

OUTCOME:

After the completion of this experiment, the students will be able analyse the Lactose content of milk samples volumetrically using Benedict's quantitative reagent.

4. ESTIMATION OF GLUCOSE USING ANTHRONE METHOD

S.No	Value of standard glucose /unknown sample (ml)	Concentration of standard glucose solution (mg)	Volume of water (ml)	Volume of Anthrone reagent (ml)	Incubation time (min)	%Transmission	Optical Density
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							

4. ESTIMATION OF GLUCOSE USING ANTHRONE METHOD

AIM:

To estimate the amount of glucose present in the given sample using Anthrone reagent.

PRINCIPLE:

If carbohydrate is present in the form of free carbohydrate as poly- or monosaccharide or bound in a glycoprotein or a glycolipid, the concentrated acid in the Anthrone reagent hydrolyses it into component monosaccharide. Similarly, the concentrated acid then catalyzes the dehydration of the monosaccharides to form furfural (from pentoses) or hydroxyl furfural (from hexoses). The furfural or hydroxyl furfural formed condenses with two molecules of naphthol from the Anthrone reagent to form a blue-green complex. The complex can then be quantified by measuring the absorbance of 620 nm wavelength in a spectrophotometer or in a red filter colorimeter.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask

Chemicals: Anthrone reagent, Glucose stock solution, sulphuric acid, hydrochloric acid, sodium carbonate

PROCEDURE:

PREPARATION OF REAGENTS:

Anthrone reagent: 2g of Anthrone is dissolved in 1 litre of concentrated H_2SO_4. The freshly prepared reagent should be used for the assay

Glucose stock solution: 200µg glucose per mL distilled water stock solution of glucose is to be prepared from the stocked solution. Note: Other carbohydrates of the same concentration can be used as samples if desired.

EXTRACTION OF THE SAMPLE:

100 mg of sample is weighed into a boiling tube and hydrolyse it by keeping it in boiling tube water bath for 3 hours with 5 ml of 2.5N hydrochloric acid. Cool at room temperature then it has to be neutralized with sodium carbonate until the effervescence ceases out and make up the volume to 100 ml with distilled water. This will be considered as an unknownglucose sample.

ESTIMATION OF THE AMOUNT OF GLUCOSE:

- Pipette out different volumes (50 µl, 100 µl, and so on) of glucose solution from the supplied stock solution (200µg /ml) into a series of test tubes and make up the volume to 1 ml with distilled water.
- Take a tube labelled as one as blank containing 1ml of just distilled water and the rest of the tubes labelled 2 to 9 for plotting standard curve. Tubes 10 and 11 are for the unknown samples.
- Add 5 ml of the anthrone reagent to each tube and mix well.
- Cool the tubes.
- Cover the tubes with caps on top and incubate at 90°C for 17 minutes or boiling water bath for 10 minutes.The presence of a blue-green complex indicates the presence of carbohydrates in the given solution.
- Cool the tubes to room temperature and measure the optical density of the solutions at 620 nm against a blank.
- Prepare a standard curve of absorbance (OD) against glucose concentration (in milligram/ microgram)
- Determine the amount of glucose in the unknown sample using the standard curve.

RESULT:

The amount of glucose present in 100 ml of the given unknown sample is ----- mg.

OUTCOME:

After the completion of this experiment, the students will be able to determine the Glucose content of unknown samples colorimetrically using Anthrone reagent. They will also learn to handle colorimeter to find out the concentration of unknown compounds.

5. ESTIMATION OF FRUCTOSE USING SELIWANOFF'S METHOD

S.No	Volume of standard/ unknown fructose Solution (mg)	Concentration of fructose solution (mg)	Volume of water (ml)	Volume of resorcinol (ml)	Volume of dil.HCl (ml)	Incubation time (min)	% transmission	O.D at 520 nm
1.								
2.								
3.								
4.								
5.								
6.								
7.								

5.ESTIMATION OF FRUCTOSE USING SELIWANOFF'S METHOD

AIM:

To estimate the amount of fructose present in the given unknown sample by Seliwanoff's method.

PRINCIPLE

Seliwanoff's reagent consists of resorcinol and concentrated HCl. The acid hydrolysis of polysaccharides and oligosaccharides yields simpler sugars. Ketoses are more rapidly dehydrated than aldoses. Ketoses undergo dehydration in the presence of concentrated acid to yield 5-hydroxymethyl furfural. The dehydrated ketose reacts with two equivalents of resorcinol in a series of condensation reactions to produce a complex (not a precipitate), termed xanthenoid, with deep cherry red color. Aldoses react slightly to produce a faint pink to cherry red color, if the test is prolonged. Fructose in the presence of strong acid, dehydrated to give furfural derivatives which complexes with resorcinol to give a red-coloured complex which is measured calorimetrically at 520 nm. The product and reaction time of the oxidation reaction helps to distinguish between carbohydrates.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask

Chemicals: Resorcinol, fructose, Hydrochloric acid(HCl)

PROCEDURE:

PREPARATION OF REAGENTS:

SELIWANOFF'S REAGENT: Add 0.05% resorcinol (m-hydroxybenzene) in 3 N HCl. Dissolve 50 mg resorcinol in 33 ml concentrated HCl and make it 100 ml with water.

STOCK STANDARD FRUCTOSE SOLUTION:

100 mg of fructose is dissolved in 100 ml of distilled water.Concentration of fructose in standard solution = 1 mg / 1 ml

30% HCl

30 g of HCl is dissolved in 100ml of water

SAMPLE PREPARATION:

1 ml of honey is diluted to 100 ml with distilled water.

ESTIMATION OF FRUCTOSE

- Pipette out different volumes (1ml, 2 ml, and so on) of standard fructose solution (1mg /ml) into a series of test tubes and make up the volume to 5 ml with distilled water.
- Take 5 ml of distilled water alone as a blank. Pipette out 1 ml of unknown sample in a separate test tube.
- Add 1 ml of resorcinol and 2 ml of 30% hydrochloric acid to all test tubes and boil them in a water bath at 80^0C.
- The contents of the tubes have to be cooled under tap water.
- The colour developed is read in a colorimeter at 520 nm.
- Plot the standard graph using concentration on X axis and OD on Y axis.
- The concentration of fructose present in the unknown sample is determined using the standard graph.

RESULT:

The amount of fructose present in 100 ml of the given unknown sample ----- mg.

OUTCOME:

After the completion of this experiment, the students will be able to determine the fructose content of fruits and honey colorimetrically using Selivanoff's reagent..

6. ESTIMATION OF PENTOSE USING BIAL'S METHOD

S.No	Volume of pentose solution (ml)	Concentration of pentose (mg)	Volume of water (ml)	Volume of Bial'sreagent (ml)	Incubation time (min)	%Transmission	Optical Density at 660 nm
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							

6.ESTIMATION OF PENTOSE USING BIAL'S METHOD

AIM:

To estimate the amount of ribose present in the given solution using Bial's reagent.

PRINCIPLE:

On hydrolysis pentosans are hydrolyzed into pentoses. Further, pentoses are dehydrated to yield furfural, which in turn condense with orcinol to form a blue-green precipitate. In the presence of hexoses, hydroxyfurfural is formed instead of furfural which upon condensation with orcinol forms a muddy brown colored precipitate. The intensity of the precipitation is directly proportional to the concentration of the pentose(Ribose) in the sample. The intensity of the color developed depends on the concentration of HCl, ferric chloride, orcinol, and the duration of boiling. The concentration of the Ribose is determined by measuring the absorbance of 620 nm wavelength in a colorimeter.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask, test tubes

Chemicals: Orcinol, Ribose, Ferric chloride($FeCl_3.6H_2O$)

PROCEDURE

PREPARATION OF REAGENTS

- **Bial's Reagent:** 300 mg of orcinol is dissolved in 5 ml ethanol. Add 3.5 ml of this mixture to 100ml of 0.1% solution of $FeCl_3.6H_2O$. The reagent thus formed is to be stored in a dark bottle and used within a couple of hours.
- **Ribose stock solution:** 200mg/100 ml of distilled water (2mg/1ml)
- **Working standard** solution of ribose is to be prepared from the stock solution (1 in 10 dilution – 10ml of stock solution has to be made up to 100ml with distilled water).

ESTIMATION OF PENTOSE BY BIAL'S METHOD

- Pipette out different volumes (0.2- 1ml) of ribose solution from the working standard solution into a series of test tubes and make up the volume to 1 ml with distilled water.
- Take a tube labeled as one as blank containing 1ml of just distilled water and the rest of the tubes labeled 2 to 6 for construction of a standard curve. Tubes 7 and 8 are for the unknown samples.

- Add 5 ml of the Bial's reagent to each tube and mix well.
- Cool the tubes.
- Cover the tubes with caps on top and incubate at 90°C for 17 minutes or boiling water bath for 10 minutes.
- Cool the tubes to room temperature and measure the optical density of the solutions at 620 nm against a blank.
- Prepare a standard curve of absorbance against ribose concentration.
- Determine the amount of ribose in the unknown sample by plotting a standard curve of A620 on the Y-axis and concentration of Ribose on the X-axis.

RESULT: The amount of pentose present in the 100 ml of given unknown solution ----- mg.

OUTCOME: After the completion of this experiment, the students will be able determine the pentose content of unknown samples using colorimetric method.

7. ESTIMATION OF PROTEIN USING BIURET METHOD

S.No	Volume of Protein Solution (ml)	Concentration of Protein (mg)	Volume of Water (ml)	Volume Biuret reagent (ml)	Incubation time (min)	% transmission	O D at 520 nm
1.							
2.							
3.							
4.							
5.							
6.							
7.							

7. ESTIMATION OF PROTEIN USING BIURET METHOD

AIM: To estimate the protein using Biuret method.

PRINCIPLE:

The –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a purple colour which can be measured at 540 nm.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask, test tubes

Chemicals : Copper sulphate($CuSO_4.5H_2O$), protein, sodium potassium tartarate, sodium hydroxide , potassium iodide

PROCEDURE:

PREPARATION OF REAGENTS:

Biuret Reagent: Dissolve 3 g of copper sulphate ($CuSO_4.5H_2O$) and 9 g of sodium Potassium tartarate in 500 ml of 0.2 mol/liter sodium hydroxide; add 5 g of potassium iodide and make up to 1 liter with 0.2 mol/liter sodium hydroxide.

Protein Standard: 5 mg BSA/ml.

PROCESSING OF SAMPLE:

100 mg of sample is extracted with 5 ml of phosphate buffer centrifuge, and the supernatant ismade upto 100 ml with distilled water and used as unknown sample.

ESTIMATION OF PROTEIN BY BIURET METHOD

- Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
- Pipette out 1 ml of the given sample in another test tube.
- Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
- Now add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
- Mix the contents of the tubes by shaking the tubes and keep all test tubes at 37 ºC for 10 min.

- Record the absorbance at 540 nm against blank.
- Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis.
- Then from this standard curve, calculate the concentration of protein in the given sample.

RESULT: The given unknown sample contains ----mg protein/100 ml.

OUTCOME:

After the completion of this experiment, the students will be able to quantify the protein content of food samples colorimetrically using Biuret reagent..

8.ESTIMATION OF PROTEIN USING LOWRY'S METHOD

S.No	Volume of Working standard Protein (ml)	Concentration of Protein (mg)	Volume of Water (ml)	Volume of Lowry's reagent (ml)	Incubation of time (min)	Volume of folin's reagent	Incubation of time (min)	Percentage transmission	O.D 650 nm
1.									
2.									
3.									
4.									
5.									
6.									
7.									

8.ESTIMATION OF PROTEIN USING LOWRY'S METHOD

AIM:

To estimate the amount of protein present in the given food sample using Lowry's method.

PRINCIPLE:

Under alkaline conditions, the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that is reduced to molybdenum/tungsten blue.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask, test tubes

Chemicals: Bovine Serum albumin, sodium carbonate, sodium hydroxide, potassium tartarate, Folin's Phenol Reagent

PROCEDURE:

PREPARATION OF REAGENTS:

1.**Stockstandard protein solution:**100mg of Bovine Serum albumin is weighed accurately and dissolved in100ml of distilled water in a standard flask (concentration 1 µg /ml).

2. **Working Standard protein solution:** The stock solution of 10 ml is diluted to 100ml with distilled water in astandard flask (concentration 100 mg/ml)

3.**Alkaline copper reagent:**

Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide.

Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate.

Mix Solution A& B in 50:1 ratio.

4..**Folin's Phenol Reagent:**

Folin's Phenol Reagent is mixed with distilled water in the ratio 1:2.

PROCESSING OF SAMPLE:

100 mg of sample is extracted with 5 ml of phosphate buffer centrifuge, and the supernatant is made upto 100 ml with distilled water and used as unknown sample.

PROCEDURE:

- Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes(S1-S5)
- Pipette out 0.5 ml and 1 ml of the given sample in another test tube (T1 and T2)
- Make up the volume to 1 ml in all the test tubes.
- A test tube with 1 ml of distilled water serves as the blank.
- Now add 4.5 ml of Alkaline copper reagent to all the test tubes including the test tubes labeled as 'blank' and ‘unknown'.
- Mix the contents of the tubes by shaking the tubes and keep all the tubes at room temperature for 10 min.
- Add 0.5 ml of Folin phenol reagent to all test tubes.
- Mix the contents well. A blue colour is developed after 15 minutes.
- Record the absorbance at 640 nm against blank.
- Then plot the standard curve by taking concentration of protein along X-axis and absorbance along Y-axis.
- Then from the standard curve, calculate the concentration of protein in the given sample.

RESULT : The given unknown sample contains ----mg protein/100 ml.

OUTCOME: After the completion of this experiment, the students will be able to quantify the protein content of food samples colorimetrically using Lowry’s method.

9.DETERMINATION OF NIACIN USING COLORIMETRIC METHOD

S.No	Volume of Niacin Solution (ml)	Concentration of Niacin (mg)	Volume of Water (ml)	Volume of cyanogen bromide (ml)	Volume of aniline (ml)	Percentage transmission	O.D
1.							
2.							
3.							
4.							
5.							
6.							
7.							

9. DETERMINATION OF NIACIN USING COLORIMETRIC METHOD

AIM:

To estimate the amount ofniacin present in the given sample by colorimetric method

PRINCIPLE:

Acidification with hydrochloric acid stabilizes the maximum color developed by the reaction of nicotinic acid with cyanogen bromide and aniline. Color development is immediate and the intensity is greater than that obtained with many other amines.The intensity of the colour produced is propositional to the amount of Niacin present and it is read at 420 nm.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask, test tubes

Chemicals:bromine,sodium cyanide, niacin, aniline

PROCEDURE

PREPARATION OF REAGENTS:

CYANOGEN BROMIDE:

Bromine is kept in the ice bath for few minutes and then pipette out 25 ml of bromine into a beaker containing 500 ml of distilled water. Take a 10 % solution of sodium cyanide in a burette and delivered slowly to the bromine water with constant shaking unit the solution become colourless.

STOCK STANDARD SOLUTION

100 mg of niacin is dissolved in 100 ml of distilled water concentration = 1 mg / ml.

WORKING STANDARD SOLUTION:

100 ml of stock standard solution is made upto 100 ml with distilled water.

Concentration = 100 mg / ml

4 % ANILINE:

4 g of aniline in 100ml of absolute alcohol.

DETERMINATION OF NIACIN

- Pipette out the aliquots of working standard niacin ranging from 0.1 – 0.5 ml in a series of test tubes.
- All the test tubes are made upto 6 ml with distilled water.
- Take 6 ml of distilled water alone as blank
- Pipette out 1 ml of unknown sample in a separate test tube.
- Add 5 ml of cyanogen bromide to all the test tubes and mix well
- After 5 minutes, add 1 ml of 4 % aniline to all the test tubes. yellowcolour is developed.
- Measure the absorbance (Optical density)at 420 nm
- Draw the standard graph using the concentration of niacin on x-axis and optical density on y-axis.From the standard graph, the amount of Niacin present in the given sample can be calculated.

RESULT:

The amount of niacin present in 100 ml of the given sample ------ mg.

OUTCOME:

After the completion of this experiment, the students will be able to determine the niacin content of food samples colorimetrically using cyanogen Bromide reagent.

10.ESTIMATION OF PHOSPHOROUS USING COLORIMETRIC METHOD

S.No	Volume of phosphorous Solution (ml)	Concentration of standard phosphorous solution(mg)	Volume of Water (ml)	Volume of molybdnum reagent (ml)	Volume of ANSA (ml)	Percentage transmission	O.D at 680 nm
1.							
2.							
3.							
4.							
5.							
6.							
7.							

10. ESTIMATION OF PHOSPHOROUS USING COLORIMETRIC METHOD

AIM: To estimate the amount of phosphorous in the given sample by colorimetric method.

PRINCIPLE:

When ammonium molybdate solution is added to a solution of phosphate containing conc. H_2SO_4, it produces a yellow crystalline precipitation of ammonium phospho-molybdate. Phospho-molybdate reacts with amino-naphthol-sulphonic acid and produces a molybdenum complex which forms a blue- coloured solution.The appearance of colour is due to the formation of a coloured complex in the reaction mixture. The intensity of the colour against known concentration is plotted on a graph paper to prepare a standard curve. By comparing the intensity of the colour of unknown samples with the standard curve, the concentration of phosphate in the unknown sample can be estimated.

REQUIREMENTS:

Equipments: Weighing balance, Colorimeter

Glasswares: Pipette, Standard Measuring Flask, test tubes

Chemicals: Ammonium molybdate,sulphuric acid,potassium dihydrogen phosphate

PROCEDURE:

PREPARATION OF REAGENTS

MOLYBDATE REAGENT:

Dissolve 5 g of ammonium molybdate in about 200 ml of double distilled water and add 250 ml of 10 N sulphuricacid. Make up the solution to 500 ml with double distilled water and mixwell.

10 N SULPHURICACID:

55.5 ml of concentrated sulphuric acid is diluted with 144.5 ml of double distilled water.

AMINONAPHTHOLSULPHONIC ACID:

Add 0.5 g of ANSA to 195 ml of 15 % sodium bisulphate and add 5 ml of 20 % sodium sulphate. Shake the contents well.

STOCK STANDARD PHOSPHATE SOLUTION:

35.2 mg of potassium dihydrogen phosphate is weighed accurately and it is dissolved in double distilled water. 1 ml of 10 N sulphuric acid is added and make up the solution to 100 ml with double distilled water in a standard measuring jar.

WORKING STANDARD SOLUTION:

10 ml of standard solution is diluted to 100 ml with double distilled water in a standard flask.

ESTIMATION OF PHOSPHOROUS

- Take the aliquots of working standard phosphate solution from 0.1 – 0.5 ml into a series of test tubes.
- Pipette out 0.5 ml and 1 ml of unknown sample in separate test tubes
- Make up the volume of all test tubes to 8.6 ml with double distilled water.
- Take 8.6 ml of water alone as Blank.
- To all the tubes, add1 ml of molybdate reagent and 0.4 ml of aminonaphtholsulphonic acid.
- Mix gently after each addition.
- Keep all the test tubes for 5 minutes at room temperature.
- Measure the absorbance at 680 nm,when the blue color is developed.
- Record the percentage of transmission values.
- Plot the standard graph and calculate the amount of phosphorous present in unknown sample.

RESULT:

The amount of phosphorous present in 100 ml of the given sample–--- mg.

OUTCOME: After the completion of this experiment, the students will be able to determine the phosphorous content of food samples colorimetrically using Fiske subbarow method.

11.DETERMINATION OF SODIUM AND POTASSIUM CONTENT USING FLAME PHOTOMETER

Solution No.	Sodium stock solution (ml)	Potassium stock solution (ml)	Na^+ Conc.(mEq/L)	K^+ conc(mEq/L)
1	1.4	2	0.28	0.02
2	1.5	3	0.30	0.03
3	1.6	4	0.32	0.04
4	1.7	5	0.34	0.05
5	1.8	6	0.36	0.06
6	1.9	7	0.38	0.07
7	2.0	8	0.40	0.08
8	2.1	9	0.42	0.09

11. DETERMINATION OF SODIUM AND POTASSIUM CONTENT USING FLAME PHOTOMETER

AIM

To determine the sodium and potassium content in unknown samples using Flame Photometry

PRINCIPLE

Quantitative estimation of Na^+ and K^+ in various types of samples such as water, soil, plant material, body fluids etc. is done by flame photometric method. It is a rapid, convenient,reliable and sensitive method for determination of these two minerals. Na^+ and K^+ present in the sample emit light of characteristic wavelengths, on introducing the sample as afine mist into a non-luminous flame. The intensity of lightemitted at their characteristic wavelengths is proportional to the amount of that mineral.

REQUIREMENTS

Equipments: Weighing balance, Flame photometer

Glasswares: Pipette, Standard Measuring Flask, test tubes

Chemicals:
(i) Sodium stock solution (100mEq/L):Dissolve 5.85 g ofNaCl in 1 L of deionized DDW.

(ii) Potassium stock solution (5mEq/L): Dissolve 3.73 g ofKCI in 1L of deionizedDDW.

(iii) Combined working standard solution of Na and K:Pipette out the stock solutions of Na' and K into 500ml volumetric flasks, according to the table givenbelow, and make the final volume with deionized DDW.These working standards are quite stable and can bestored in polythene bottles.

Preparation of sample:
Plant tissue samples:
1 Dilute the mineral extracts prepared by dry ashing or wet digestion with DDW before estimation.

PROCEDURE

1. Take three test tubes for standard solutions. Prepare
   a) one standard with highest sodium and potassium concentration,

CALCULATION

The method for calculating Na and K content in the sample is illustrated below with the help of an example.

Reading of the sample for Na = 80 (RT)

Reading of the standard solution with lower value = 72.5 (RS_1)

Reading of the standard solution with higher value = 85.0 (RS_2)

Suppose, sodium equivalent in the standard solution giving reading lower than unknown sample

= 0.28 (CS_1)

Sodium equivalent in standard solution, with reading slightly higher then the unknown sample

= 0.32 (CS_2)

$$\text{Sodium conc. (mEq/L) in unknown sample} = \frac{CS_1 + (RT - RS_1)(CS_2 - CS_1)}{RS_2 - RS_1}$$

$$= \frac{0.28 + 7.5 \times 0.04}{12.5}$$

$$= 0.304$$

Potassium conc. in the test sample is also calculated in a similar manner as described for Sodium. Use double distilled water (DDW) or deionised water.

b) one with a slightly higher concentration than that of the sample.

c) one with a slightly lower concentration than that of the sample according to the table.

2. Connect the instrument to an air compressor and a cooking gas cylinder.

3 Switch ON the air compressor and adjust air pressure passing through the instrument to 10 psi.

4. Open outlet of the gas cylinder and ignite the burner and adjust the flame in such a way that cones seen in the flame do not fuse with one another and the flame remains non luminous.

5. Dip the suction tube in the blank containing only deionised DDW. Ensure that water is sprayed as mist by the atomizer into the flame.

6 Adjust the galvanometer so that background radiation is zero when the sodium filter is at the proper place.

7. Remove the beaker containing water and keep the container having standard solution of the highest concentration of Na' and K and adjust the digital display to 100. Again adjust with double distilled Glass water and recheck with standard solution. The instrument is now ready for the analysis of th samples.

8. Feed the diluted sample into the instrument through the suction tube and note down the reading.

9. Then feed two standard solutions separately, one which gives a lower and other slightly higher reading than the sample.

10. Proceed as above for estimation of potassium in different-samples, but now using potassium filter.

RESULT:

The amount of Sodium/Potassium present in the given sample---- mEq/L

OUTCOME: After the completion of this experiment, the students will be able to determine the Sodium /Potassium content of food samples using Flame photometer.

12. **DETERMINATION OF CALCIUM CONTENT OF MILK SAMPLES USING FLAME PHOTOMETER**

Solution No.	Calcium Stock Solution(ml)	Calcium stock Concentration (ppm)	Volume of milk sample (ml)	Concentration of Calcium in milk(ppm)	Calcium content in %
1					
2					
3					
4					
5					

12.DETERMINATION OF CALCIUM CONTENT OF MILK SAMPLES USING FLAME PHOTOMETER

AIM: To determine the Calcium content in milk samples using Flame Photometry

PRINCIPLE:

Quantitative estimation of Calcium in various types of samples such as milk, soil, plant material, body fluids etc. is done by flame photometric method. It is a rapid, convenient, reliable and sensitive method for the determination of Calcium. Calcium present in the sample emit light of characteristic wavelengths, on introducing the sample as afine mist into a non- luminous flame. The intensity of light emitted at their characteristic wavelengths is proportional to the amount of Calcium.

REQUIREMENTS

Equipments: Weighing balance, Flame photometer, silica crucible, Muffle furnace.

Glasswares: Pipettes, Standard Measuring Flask, filtration funnel, conical flasks,beakers and test tubes

Chemicals: Concentrated Hydrochloric Acid
10% Lanthanum Chloride solution
Deionised Water
Calcium Carbonate

REAGENT PREPARATION;

1. Prepare a dilute hydrochloric acid by adding 1 part concentrated acid to 4 parts deionised water in a beaker (NB take great care and always add the acid to water, **not** water to acid)
2. Label the beaker clearly as diluted Hydrochloric Acid

BLANK PREPARATION;

1. Pipette 2.5ml of diluted hydrochloric acid into a 100ml volumetric flask
2. Pipette 2.0ml of 10% Lanthanum Chloride solution into the same flask
3. Take up to the 100ml mark with Deionised Water
4. Clearly label the flask as Blank Solution

STANDARD PREPARATION:

1. Weigh out 1.249g Calcium Carbonate into a 1000ml volumetric flask
2. Add approximately 50ml Deionised Water
3. Add concentrated Hydrochloric Acid dropwise using the dropper pipette, swirling the flask between additions, until the Calcium Carbonate has dissolved (should take around 10ml)
4. Make up the solution to the 1000ml mark with Deionised Water
5. Label the flask as 500ppm Calcium Stock Solution
6. Prepare standards of lower concentrations in 100ml volumetric flasks, by taking the stated volume of the stock solution and diluting with Deionised Water to the 100ml mark. Remember to label each flask as you go;
    1. 2.5 ppm standard = 0.5ml of 500 ppm stock, remainder Deionised Water
    2. 5.0 ppm standard = 1.0ml of 500ppm stock
    3. 7.5 ppm standard = 1.5ml of 500 ppm stock
    4. 10.0 ppm standard = 2.0ml of 500 ppm stock

SAMPLE PREPARATION:

1. Weigh 4g of milk into the dry silica crucible
2. Ash the sample in a furnace at 525°C
3. When cool, add 5ml of the dilute Hydrochloric Acid solution using the pipette to dissolve it
4. Transfer the dissolved ash solution into a 100ml volumetric flask, and make up to the 100ml mark with Deionised Water, remembering to invert the flask for thorough mixing
5. Fold the filter paper and place into the filtration funnel, over the conical flask. Filter the solution to remove any remaining precipitates.

6. Using the volumetric pipette, transfer 50ml of the filtrate into a 100ml volumetric flask
7. Add 2.0ml of 10% Lanthanum Chloride solution, then make up to the 100ml mark with Deionised Water
8. Label clearly with the sample name or reference. Repeat for each sample.

PROCEDURE

1. Ensure that fuel, air and drain are all connected correctly, and the instrument is set to Calcium. Start
   the photometer as per the user manual instructions.
2. Aspirate the blank solution and set the zero
3. Aspirate the 10ppm calcium standard solution and set the full scale
4. Reset the zero

5. Aspirate the intermediate standards one by one, noting the readings down to prepare your calibration curve
6. Aspirate the samples one by one and note the readings.

CALCULATION

Plot your calibration curve using the standard solution results, then evaluate the sample results using the curve – the result is given in ppm of calcium in milk

To convert to % calcium, multiply the ppm reading by 0.025 to give the % calcium in milk

RESULT:

The percentage of Calcium present in the given milk sample is –---

OUTCOME:

After the completion of this experiment, the students will be able to determine the Calcium content of unknown food samples using Flame photometer.