AmbujaNeotia

THE NEOTIA UNIVERSITY

ज्ञानम् आत्म प्रदीपाय

THE NEOTIA UNIVERSITY

BML 192: GENERAL BIOCHEMISTRY LABORATORY MANNUAL

COMPILED BY

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BMLT UNIT

SCHOOL OF HEALTH SCIENCES

1. Methods of Cleaning of the Laboratory Glassware.

To learn about the various types of glassware and plasticware used in the laboratory
To learn detail uses of the glassware and plasticware used in the laboratory
To learn different methods of Cleaning of the Laboratory Glassware

2.Bicarbonate estimation titration method.

Method: Indicators change colour within well defined pH ranges: phenolphthalein changes from colourless to red in the range 8.0 - 9.6; methyl orange changes from red to yellow in the range 3.1 - 4.4. The pH just after the neutralization point depends upon the strength of the acid / base, ie., their pK_a / pK_b.

Hydrochloric acid is standardized against previously standardized sodium hydroxide using phenolphthalein as indicator: $HCl + NaOH = NaCl + H_2O$.

The decomposition of carbonate by acid takes place in two steps: (i) $CO_3^{2-} + H^+ = HCO_3^-$. (ii) $HCO_3^- + H^+ = H_2O + CO_2$. At the end of step (i), the pH will be near about 8 or 9. After the complete neutralization, the next drop of HCl will bring down the pH to near about 3 or 4.

The Na_2CO_3 content of the given sample is determined by titration against standard hydrochloric acid using methyl orange as indicator: $Na_2CO_3 + 2HCl = 2NaCl + H_2O + CO_2$. The equivalent weight of Na_2CO_3 is half its molecular weight (M). This means $M/2$ g of Na_2CO_3 is equivalent to 1000 ml of 1 N HCl. It also means that the molarity of a solution of Na_2CO_3 is half its normality.

The total amount of carbonate and bicarbonate in the mixture is determined by titration against standard hydrochloric acid using methyl orange as indicator: $CO_3^{2-} + HCO_3^- + 3H^+ = 2H_2O + 2CO_2$. Another titration using phenolphthalein as indicator will give the concentration of carbonate. $CO_3^{2-} + H^+ = HCO_3^-$. If the above titre values are V_m and V_p respectively, then carbonate is equivalent to $2V_p$ and bicarbonate is equivalent to $V_m - 2V_p$. *Note that in the second titration the pH change is relatively slow near the neutralization point, therefore the colour change is not sharp and the titration has to be done slowly.*

Procedure:

(i) *Standardization of hydrochloric acid*: Transfer 25 ml of sodium hydroxide solution to a 250 ml conical flask using a pipette. Dilute with a little water (about 10 ml) and add 1-2 drops of phenolphthalein. Titrate against hydrochloric acid taken in a burette. Repeat till concordant titre values are obtained. Calculate the normality of the acid using the given normality of sodium hydroxide.

(ii) *Estimation of Na_2CO_3 content of the given sample*:

A : Weigh accurately about 0.6 g of the sample (w g) into a 100ml standard flask. Dissolve in distilled water and make upto the mark with distilled water. Titrate 25 ml of this solution against standard hydrochloric acid (normality, N_a) using 1-2 drops of methyl orange as indicator. When the colour becomes very faint yellow, continue the titration dropwise until the colour becomes orange or a faint pink. Repeat the titrations till concordant values (V_a) are obtained. Calculate the normality ($N_b = V_a N_a/25$) of the sodium carbonate solution and the percentage purity of the sample. [Amount of Na_2CO_3 in the weighed sample, m = (100/1000) x (N_b/2) x M ; percentage purity = $100m/w$].

B : Repeat the above experiment by accurately weighing about 0.15 g of the solid sample to a 250 ml titration flask (ie., conical flask), dissolving it in water and diluting to about 40 ml and titrating as before. Calculate the percentage purity. The amount of Na_2CO_3 in the weighed sample is, $m = (N_a \times V_a /1000) \times (M /2)$.

Estimation of Na_2CO_3 and $NaHCO_3$ in a mixture: Accurately weigh about 2.0 g of the mixture and prepare a solution in distilled water in a 250 ml standard flask. Slowly titrate 25 ml of this solution against standard hydrochloric acid using phenolphthalein as indicator. Repeat to concordance (V_p ml). Now titrate the same volume using methyl orange as indicator. Repeat to concordance (V_m ml). Calculate the number of equivalents, in the titrated volume(ie., 25.0 ml), of Na_2CO_3 ($E_{carb} = N_a \times 2 \times V_p/1000$) and $NaHCO_3$ [$E_{bicarb} = N_a \times (V_m - 2V_p)/1000$]. Hence calculate the respective amounts and percentages in the given sample.

3. Principle, Working & Maintenance of pH Meter.

pH METER

The pH meter is used to measure the pH of a solution.

The pH meter is of two types:

1. Digital pH meter
2. Manual pH meter.

pH meter consists of power pack and two electrodes.

The power pack contains an on/off switch, an indicating meter, a temperature compensation knob, a calibrate knob, and a wire with a plug pin. The on/off switch is used to supply or cut off electric current. The indicating meter shows pH reading. The temperature compensation knob is used to adjust the temperature. The calibrate knob is used to set pH. The plug pin is connected to the main electric line.

The pH meter contains two electrodes— glass electrode and a calomel electrode. In modern pH meters the two electrodes are combined into single unit.

The glass electrode has a hard glass tube. At the base, it has a thin bulb. The bulb contains HCl (0.1 mol/lit.). The bulb is covered by a special membrane of soda glass. It is sensitive to H^+ and it allows H^+ to pass through it. A platinum wire is connected to the HCl through silver – silver chloride electrode. The wire coming out from the glass electrode is connected to the power pack of the pH meter.

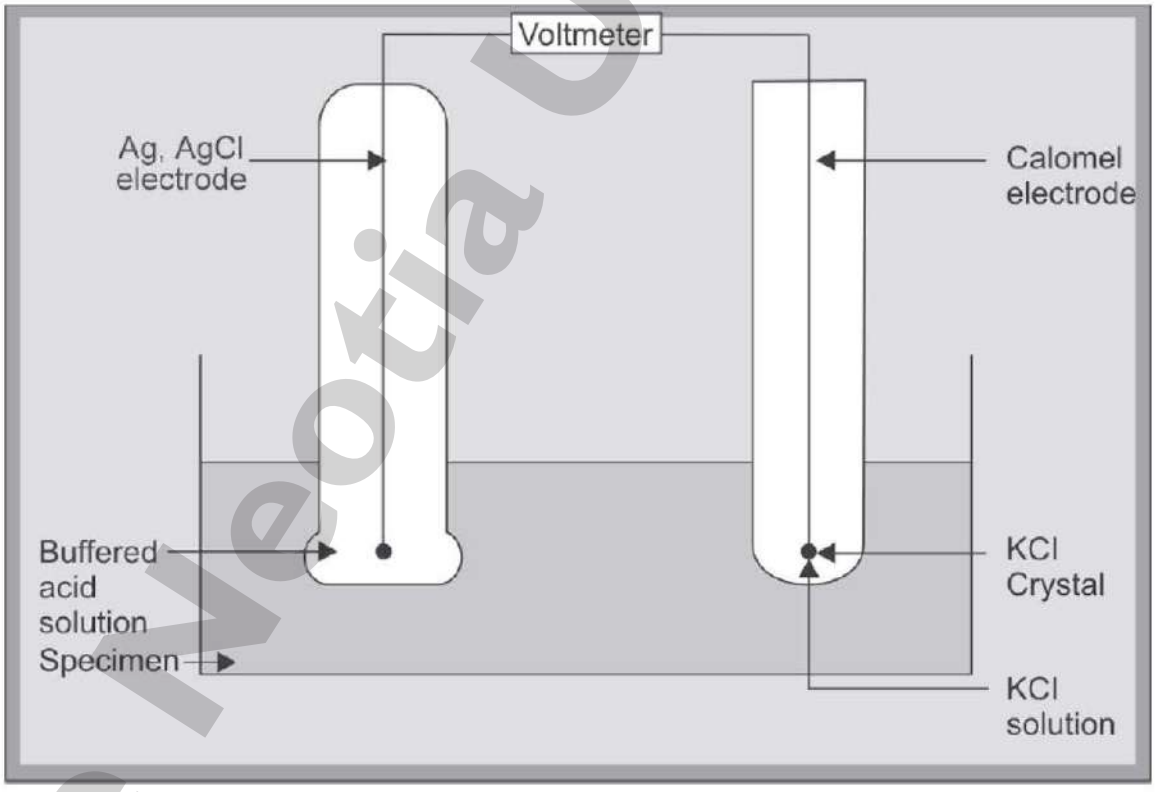
The calomel electrode is a reference electrode. It is not sensitive to H^+. It contains a calomel paste. The calomel paste is connected with a platinum wire through mercury. The free end of the calomel electrode has a porous plug. The base of electrode is deposited with KCl crystal. The remaining portion is filled with KCl solution.

The glass electrode in the test solution constitutes a half-cell and the calomel electrode constitutes the other half-cell. The two electrodes complete the circuit.

Principle

If the conductor is immersed into an appropriate electrolyte solution there will be a tendency for its atoms to leave the surface and enter the solution as ions. This is called a half-cell and the conductor is called an electrode. The potential difference between the two half-cell is called the electromotive force. (emf). The emf will change if the flow of electrons is changed.

This is what happens in the measurement of pH. The indicator electrode is dipped in a solution whose hydrogen ion concentration to be measured. H^+ ions are drawn towards the outer surface of the glass membrane. The number of H^+ ions accumulating on the membrane depends on their concentration in the external solution.



The change in the hydrogen ion activity outside the glass bulb changes the potential between the indicator electrode and the calomel electrode. The potential is measured by sensitive voltmeter (Fig. 4.1).

The instrument must be calibrated against buffers of known pH before measuring the pH of unknown specimen.

4. (a) To Prepare 0.1N NaOH Solution (b) To Prepare 0.2N HCl Solution. (c) To Prepare 0.2N H_2SO_4 and 0.2M Na_2CO_3 Solution

To learn concepts of Normality, Molarity and Molality
To prepare the mentioned concentration of solutions using Law of Equivalence

$$V_1S_1 = V_2S_2$$

5. Principle, Working & Maintenance Analytical Weighing Balance.

BALANCES

Balance is used for determination of mass or weight.

There are many types of balance, for example, platform balance, two-pan analytical balance and single-pan analytical balance. Platform balances are capable of measuring mass to within + 0.05 g. while some analytical balances can measure within 10-4 g.

The precaution in using balance is to minimize vibration. To prevent corrosion of the pans, chemicals should never be placed directly on the metal surface. Always use weighing paper. Care and cleanliness are important for balances. Brush up any spilled chemicals and close the balance-case door when done.

Preparing the Balance for Use

Before weighing anything on the analytical balance you must make sure that it is leveled and zeroed.

To check the leveling on the balance, look at the leveling bubble on the floor of the weighing chamber. If it is not centered, center it by turning the leveling screws on the bottom toward the back of the balance.

Once the balance is leveled, close all the chamber doors and press the control bar on the front of the balance. After a few seconds, a row of zeros will appear. This indicates that the balance is zeroed and ready for use.

These substances must always be weighed using an appropriate weighing container.

1. Place the weighing container on the balance pan and close the doors.

2. Tare the container by briefly pressing the control bar. The readout will read zero with the container sitting on the pan. This allows the mass of the sample to be read directly.
3. Add the substance to be weighed. Be careful not to spill chemicals on the balance.
4. With the sample and its container sitting on the pan, close the chamber doors and read the display to find the mass of sample.

Weighing a Solid Object Directly on the Balance

If the object need to weigh is a solid object, one can weigh it directly on the pan. Be sure the balance is zeroed. Open the chamber doors, carefully place the object on the balance pan, close the doors, and read the mass of object.

Cleaning Up and Shutting Down the Balance

When you are done with the balance, make sure you have properly cleaned up any chemicals that may have spilled on the balance. At the end of the day, the balance can be turned off by lifting up gently on the control bar.

6. Qualitative estimation of carbohydrates
a) Benedict's test b) Molisch's c) Phenol Sulfuric Acid

Benedict's Test (Reduction under alkaline condition)

Principle: In mild alkaline medium reducing sugars undergo tautomerization to form enediols which reduce cupric ions to cuprous ions. Cuprous hydroxide is formed. During the process of heating cuprous hydroxide is converted to cuprous oxide which gives different shades of colored precipitate depending upon the concentration of the sugar.

Benedict's Qualitative Reagent contains

- *Copper sulfate:* provides cupric ions.
- *Sodium carbonate:* makes the medium alkaline.
- *Sodium citrate:* chelates cupric ions and releases it slowly for reduction. Thus prevents the precipitation of cupric ions as cupric hydroxide by forming cupric sodium citrate complex. It acts as a stabilizing agent. Improves the shelf-life of the reagent by preventing an interaction between sodium carbonate and copper sulfate, which may otherwise get precipitated as cupric carbonate.

Experiment	*Observation*	*Inference*
Take 5 ml of Benedict's reagent in a test tube. Add 8 drops of the given solution. Mix and boil for 2 min. over a small flame. Allow to cool spontaneously.	Brick-red precipitate is formed 	Given solution is a reducing sugar.

Points to Remember:

- This test is also a semi-quantitative test which can be reported as under:

Observation	*Inference*	*Sign*	*Approx. sugar*
No change of blue color: - no precipitate.	Absence of reducing sugar	-	Nil
Green precipitate.	Presence of reducing sugar	+	up to 0.5 %
Yellow precipitate.	Presence of reducing sugar	++	0.5 to 1 %
Orange precipitate.	Presence of reducing sugar	+++	1 to 1.5 %
Red precipitate	Presence of reducing sugar	++++	2 %
Brick red precipitate.	Presence of reducing sugar	++++	> 2 %

- Color of the precipitate depends on the concentration of sugar present.

- Frequently used as screening test for Diabetes mellitus.
- It gives positive result in the presence of other reducing substances like ascorbic acid, glutathione, salicylates, uric acid, glucuronides and homogentisic acid.
- Benedict's quantitative reagent contains potassium thiocyanate and potassium ferrocyanide in addition to copper sulfate, sodium carbonate and sodium citrate present in Benedict's qualitative reagent.

Benedict's tests (In case of sucrose)

Since sucrose is a non-reducing sugar, it does not give a positive Benedict's test. Hence, the below given procedure has to be followed.

Phenol Sulfuric Acid test

The **phenol-Sulphuric Acid method** for determination of carbohydrates and related substances was first proposed in 1956, by Dubois and colleagues. With the exception of certain deoxy-sugars the method is very general, and can be applied to reducing and non-reducing sugars and too many classes of carbohydrates including oligosaccharides. In this assay the carbohydrate react with phenol and Sulphuric acid to form a colored complex with extinction at 490 nm. The amount of sugar present is then determined by comparison with a calibration curve using a spectrophotometer.

METHODOLOGY:

Standards: Sugar of 1 mg/ml stock solution prepared and submitted a carbohydrate standard curve from the following dilution series, used 400,800, 1600, and 2000µl of the stock and made up each sample with H_2O to a final volume of 2000µl to make 0.2µg/µl, 0.4µg/µl, 0.6µg/µl 0.8µg/µl, 1µg/µl concentrations, respectively. The blank of (0µg/µl) was prepared as well by just pipetting 2000µl dH_2O.

To each standard, blank and the sample, added 50 µl of 80% (w/v) Phenol solution, then vortexed thereby added 2.0 ml concentrated Sulphuric Acid in a stream then stood for 10 minutes at room temperature. Red absorbance at 490nm therefore determined the sugar content of the unknown samples provided using the standard curve.

RESULTS

Table1, Shows the absorbencies of the standard glucose measured at 490nm

Absorbance (A^o)	0	0.105	0.072	0.106	0.578	0.847
Concentration (mg/ml)	0	0.2	0.4	0.6	0.8	1

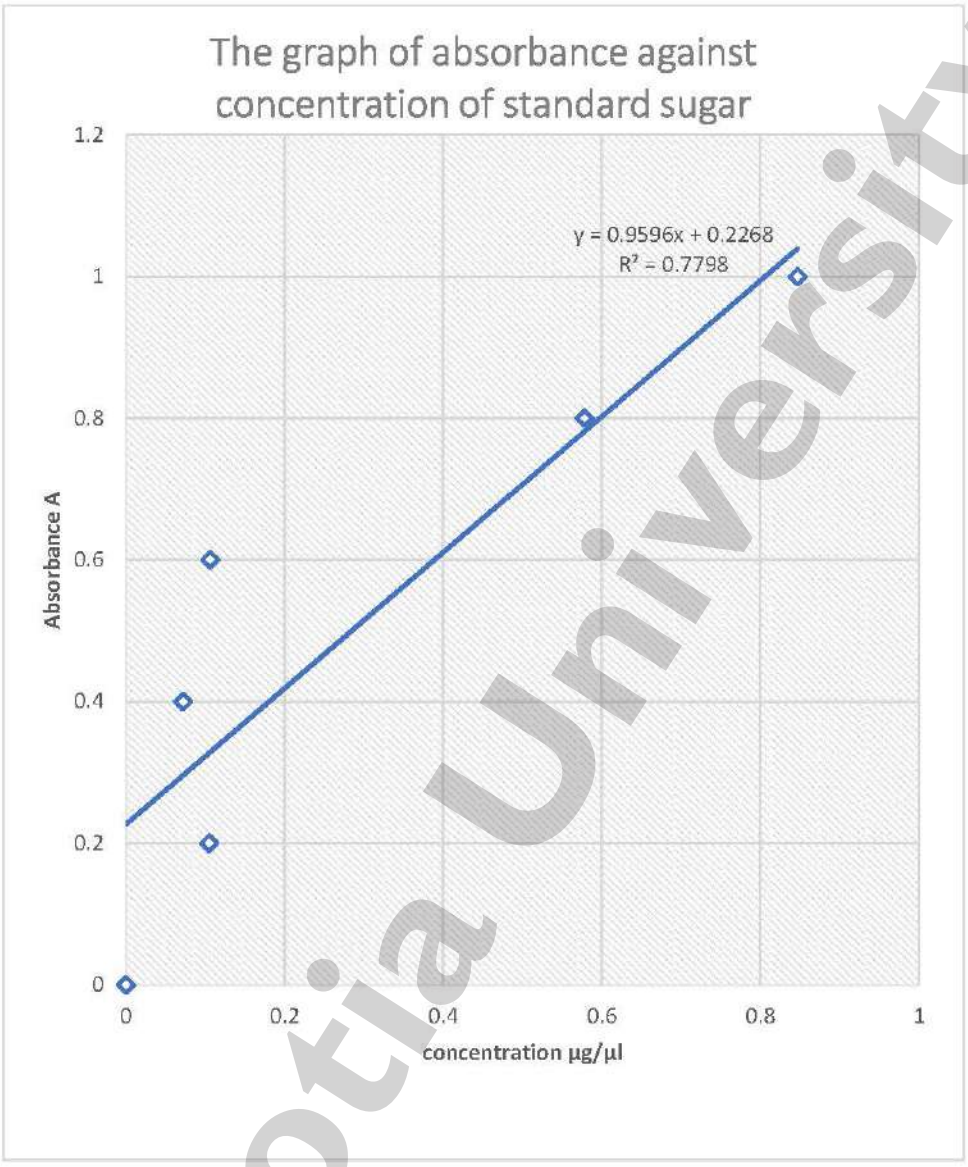


Table 2, Shows the absorbencies of the Unknown sample measured at 490nm

Sample used	A	B	C
Absorbance (A°)	1	1.348	0.992

Table 3, Shows the concentration of the Unknown sample obtained from standard graph.

Sample of unknown	A	B	C
Concentration mg/ml	0.8	1.2	0.8

DISCUSSION

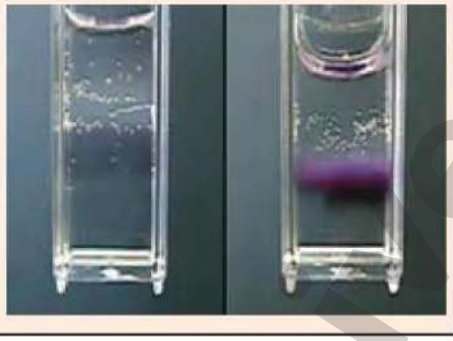
From above result presented, the graph drawn of absorbance against concentration, it shows that as absorbance were increasing; also sugar concentration were increasing. And the nature of the graph was linear; the graph obeyed the Beer's law by including the blank solution. According to Beer's law, the absorbance of a solution should be zero (100%) if there is none of the absorbing species present. A blank solution was not containing any food materials hence has no concentration that is why has 0 A° absorbance. The unknown food sample B has high concentration (1.2mg/ml) compared to other foods, also unknown sample A has contained same concentration as the food C, their concentration is direct proportion to absorbances.

During the practical the sample were not heated enough and gave improper results. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural, hence heat was very important to form a yellow-brown colored product with phenol, and has absorption maximum at 490 nm. This is one of the best methods to estimate total carbohydrate. Under proper conditions, the phenol-sulfuric method is accurate to ± 2%. This seems to be true as shown in tables 2 and 3 in the Absorbance columns. However, the accuracy of the results is completely dependent on the accuracy of the standard curve above.

This method has provided the best and accurate values than other methods, as the result above show no unclear result. Chemical methods such as the phenol–sulfuric acid assay can be utilized to provide an approximate value, but because different sugars in solution react differently with the assay reagents, a measurement of the total sugar would be an estimate based on the reactivity of the sugar used to construct the calibration curve (usually glucose). If an approximate or estimated value is all that is required then this method is sufficient. When the exact amount of each sugar present is required, highly specific enzymatic methods are more appropriate

Molisch Test

Principle: Carbohydrates, when treated with concentrated Sulfuric acid undergo dehydration to form furfural/ hydroxymethyl furfural derivatives which on condensation with alphanaphthol form colored products (chromogens).

Experiment	*Observation*	*Inference*
Take 2 ml of given solution in a clean dry test tube; add 1-2 drops of Molisch reagent. Mix. Incline the test tube slightly and overlay 2 ml of conc. sulphuric acid along the sides of the test tube so as to form two layers.	Violet ring at the junction of the two liquids is formed. 	Given solution is a carbohydrate.

Molisch reagent: A 5% solution of alpha naphthol in ethyl alcohol.

Points to Remember:

- This is a general test for all carbohydrates.
- Molisch test is given by sugars with at least five carbons because it involves furfural derivatives which are five carbon compounds.
- Rapid pouring of sulfuric acid down the test tube leads to water- acid interaction which produces heat and can cause charring of carbohydrates, resulting in the formation of black ring. Therefore, acid should be layered very slowly and carefully to minimize this interaction.
- Charring occurs due to the precipitation of carbon as a result of dehydration of the carbohydrate. This occurs as a result of the action of concentrated sulfuric acid on it.
- Impurities in the reagent tend to give a green ring, which is negative test.

- A green ring even in absence of carbohydrates is due to excess of alpha naphthol.
- In case of oligo and polysaccharides, they are first hydrolyzed to monosaccharides by acid, which undergoes dehydration to form furfural or its derivatives.
- Some proteins and lipids can also give positive Molisch test. This occurs if these substances have a bound carbohydrate moiety attached to them, e.g. albumin.

7. Quantitative estimation of proteins. a) Lowry Method b) Bradford test

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

AIM:

To estimate the amount of Protein present in given unknown solution.

PRINCIPLE:

Alkaline CuSo4 catalyses the oxidation of aromatic amino acids with subsequent reduction of sodium potassium molybdate tungstate of Folin's reagent giving a purple colour complex the intensity of the colour is directly proposition to the concentration of the aromatic amino acid in the given sample solution.

REAGENTS REQUIRED:

1. Stock Solution:

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

2. Working Standard:

The Stock Solution of 10 ml is distilled to 100ml with distilled water in a standard flask (concentration 100 mg/ml).

3. Folin's Phenol Reagent:

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

4. 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.

Solution A, B, C is mixed in the proportion of 50:1:0.5.

Unknown Preparation:

The unknown protein is made upto 100 ml with distilled water.

PROCEDURE:

Working standard of 0.2 -1ml is pipette out into clean test tube and labeled as S1-S5. Test solution of 0.2ml is taken into test tube and labeled as T1. The volume is made upto 1ml of distilled water. Distill water of 1ml serve as blank.

To all the test tube 4.5ml of alkaline CUSO4 reagent is added and incubated at room temperature for 10 minutes. All the test tube 0.5ml of folin's phenol reagent is added. The contents are mixed well and the blue colour developed is read at 640 rpm after 15 minutes. From the standard graph the amount of protein in the given unknown solution is calculated.

RESULT:

The amount of protein present in the given unknown solution is mg (µg of protein).

2. ESTIMATION OF PROTEINS BY BRADFORD METHOD

Aim:

To estimate the amount of protein in the given sample by Bradford Assay.

Principle

The protein in solution can be measured quantitatively by different methods. The methods described by Bradford uses a different concept- the protein's capacity to bind to a dye, quantitatively. The assay is based on the ability of proteins to bind to coomassie brilliant blue and form a complex whose extinction coefficient is much greater than that of free dye.

List of Reagents and Instruments

A. Equipment

- Test tubes
- Graduated cylinder
- Weight Balance
- UV spectrophotometer

B. Reagents

- Dissolve 100mg of Coomassie-Brilliant blue G250 in 50 ml of 95% Ethanol.
- Add 100 ml of 85% phosphoric acid and make up to 600 ml with distilled water.
- Filter the solution and add 100 ml of glycerol, then make upto 1000ml.
- The solution can be used after 24 hrs.
- BSA

Procedure

- Prepare various concentration of standard protein solutions from the stock solution (say 0.2, 0.4, 0.6, 0.8 and 1.0 ml) into series of test tubes and make up the volume to 1 ml .
- Pipette out 0.2ml of the sample in two other test tubes and make up the volume to 1ml.
- A tube with 1 ml of water serves as blank
- Add 5.0 ml of coomassie brilliant blue to each tube and mix by vortex or inversion.
- Wait for 10-30minutes and read each of the standards and each of the samples at 595nm.
- Plot the absorbance of the standards verses their concentration.
- Plot graph of optical density versus concentration. From graph find amount of protein in unknown sample.

Tabulation

Sl No	Vol. of BSA (ml)	Conc of BSA (mg/ml)	Vol. of Distilled water (ml)	Vol. of Bradford reagent (ml)	Incubation for 10 min	OD at 595 nm

8. Demonstration of Osmosis and Dialysis.

Definition of osmosis.
Definition of Dialysis.
Demonstration of Osmosis and Dialysis
Importance and significance of osmosis and dialysis

8. Quantitative Estimation of a) Glucose concentration b) Urea concentration c) Cholesterol Concentration

Blood Glucose

GLUCOSE OXIDASE METHOD

Principle

Glucose oxidase (GOD) oxidizes glucose to gluconic acid. Hydrogen peroxide is produced in this reaction. In presence of peroxide hydrogen peroxidase (POD) reacts with 4-aminoantipyrine and phenol to form red coloured quinoneimine dye. The intensity of colour is directly proportional to the glucose concentration. This method is highly specific for glucose and does not involve any other sugar.

$$\text{Glucose} + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine dye}$$

Reagents

1. Glucose oxidase reagent
2. Phenol solution
3. Glucose stock standard (200 mg/dl in 0.2% benzoic acid solution)
4. Glucose working solution.

Procedure

1. Take three test tubes and label them as blank standard and test.
2. Add 2 ml of glucose oxidase reagent into the three test tubes.
3. Add 0.5 ml of distilled water; 0.5ml of ten times diluted serum, 0.5ml of working standard in blank, test and serum respectively.
4. Add 2 ml of phenol reagent into the three test tubes.
5. Shake well and allow it to stand for 30 min. at room temperature or 15 min. at 37°C.
6. Read the absorbance at 515 nm.

Reagent	*Blank*	*Standard*	*Test*
Glucose oxidase	2 ml	2 ml	2 ml
Glucose std.	—	0.5 ml	—
Diluted test serum	—	—	0.5 ml
Distilled water	0.5 ml	—	—
Phenol reagent	2 ml	2 ml	2 ml

Table showing glucose oxidase procedure

Clinical Significance

Blood glucose level is mainly determined to diagnose diabetes mellitus. It provides the valuable information about the course, severity and therapeutic control of diabetes mellitus.

With the FPG test, a fasting blood glucose level between 100 and 125 mg/dl signals pre-diabetes. A person with a fasting blood glucose level of 126 mg/dl or higher has diabetes. The early symptoms of untreated diabetes mellitus are related to the elevated blood glucose levels. Excess glucose in the blood ultimately results in high levels of glucose being present in the urine (glucosuria). This increases the urine output, which leads to dehydration and increased thirst. Other symptoms include extreme tiredness, weight loss, blurred vision, itchy skin and repeated minor infections such as thrush and boils.

Beside the diabetes, blood sugar level is also increased in —

With increased circulatory epinephrine, pancreatitis, some CNS lesions, or effects of drugs like alcohol, phenytoin, etc

Decreases in—Extrapancreatic tumors, hepatic disease, endocrine disorders, pediatric abnormalities, enzyme diseases and malnutrition.

Calculation

$$\text{Glucose mg/100 ml} = \frac{\text{O.D. of Test}}{\text{O.D. of Std.}} \times \text{Conc. of Std.}$$

Normal Values

- Fasting 65 — 110 mg/dl
- Post meal 120 —140 mg/dl
- Random 70 — 140 mg/dl

SERUM UREA

DIACETYLMONOXIME (DAM) METHOD

Principle

Urea reacts with diacetylmonoxime in hot medium and in the presence of thiosemicarbazide and ferric ions to form a pink coloured compound. The colour intensity is directly proportional to amount of urea in specimen. It is measured at 520 nm.

Specimen

Serum is preferred, however heparinized plasma or fluoride plasma can be used.

Reagents

1. DAM – TSC Reagent (diacetylmonoxime thiosemicarbazide)
2. Uric Acid Reagent
3. Stock Urea Std. 1g%
4. Working Std. (50 mg%)

Procedure

Take 3 test tubes, mark them as T, S and B, for Test, Std. and Blank respectively. Add the reagents as per following table.

Reagent	*Blank*	*Std.*	*Test*
Distilled water	3.6 ml	3.5 ml	3.5 ml
Blood	—	—	0.1 ml
Working Std. (50 mg %)	—	0.1 ml	—
10 % sodium tungstate	0.2 ml	0.2 ml	0.2 ml
2/3 N sulphuric acid	0.2 ml	0.2 ml	0.2 ml

Mix well and allow to stand for 5 min; centrifuge into 3 test tubes pipette out as follows:

	Blank	Std.	Test
Supernatant	2 ml	2 ml	2 ml
DAM-TSC Reagent	3 ml	3 ml	3 ml
Urea acid Reagent	3 ml	3 ml	3 ml

Clinical Significance

Determination of BUN is used in diagnosis of renal insufficiency. A BUN of 50 –150 mg/dl implies serious impairment of renal function. Markedly increased BUN (150-250 mg/dl) indicates severely impaired glomerular function. BUN is also increased in Hemorrhage to GI tract, stress, shock, congestive heart failure, acute myocardial infarction, vomiting-diarrhea etc. BUN is found to be lowered in over hydration, severe liver damage, increased utilization of proteins for synthesis, malnutrition, low protein diet, poisoning, hepatitis etc.

SERUM TOTAL CHOLESTEROL

Cholesterol is a lipid and is classified as sterol. It is widely distributed in various animal tissues and vegetable oils and consumed with food. It can also be synthesized in the liver. It is a normal constituent of bile, and is principal constituent of most gallstones. It is important in metabolism serving as precursor of various steroid hormones, e.g. sex hormones and adrenal corticoids.

Serum total cholesterol includes esterified cholesterol as well as non-esterified cholesterol.

Principle

Cholesterol reacts with acetic anhydride in the presence of glacial acetic acid and conc. sulfuric acid to form green coloured complex. Intensity of the colour is proportional to the cholesterol concentration. It is measured at 520–580 nm.

Reagent

1. Cholesterol reagent (this should be stored in amber coloured bottle at room temperature)
2. Conc. sulfuric acid
3. Cholesterol std. (200 mg/dl in glacial acetic acid)

Procedure

Cholesterol reagent is highly corrosive so, it should not be pipetted by mouth. Take three test tubes and mark them as T, S, and B. Add 2.5 ml cholesterol reagent in each tube. Add 0.1 ml of serum, 0.1 ml of cholesterol std. and 0.1 ml of distilled water in test, std. and blank respectively. This is an exothermic reaction. So, cool the tubes to room temperature by placing in water bath. Now carefully add 0.5ml of sulfuric acid to each tube mix thoroughly and keep in water bath at room temperature for 10 min. Read the absorbance of test and std. against blank at 575nm.

Reagent	*T*	*S*	*B*
Cholesterol reagent	2.5 ml	2.5 ml	2.5 ml
Serum	0.1 ml	—	—
Cholesterol std.	—	0.1 ml	—
Distilled water	—	—	0.1 ml
Cool to room temperature			
Conc. Sulfuric acid	0.5ml	0.5ml	0.5ml

Calculations

$$\text{Serum cholesterol mg/dl} = \frac{\text{OD of T}}{\text{OD of S}} \times 200$$

Clinical Significance

Total cholesterol estimation is useful in monitoring for increased risk factor for coronary artery disease, screening and monitoring for hyperlipidemias.

It is high in hyperlipoproteinaemias, hypothyroidism, nephrosis, pancreatic disease like diabetes mellitus, chronic pancreatitis, biliary obstruction like stone, carcinoma, biliary cirrhosis, it is also increased in cholesterol ester storage disease and Von Gierke's disease. Usage of some drugs like birth control pills, amiodarone, or vitamins can also show increased cholesterol value.

The serum total cholesterol levels are decreased in severe liver cell damage, hyperthyroidism, chronic anaemia, cortisone and ACTH therapy. The levels of total cholesterol are also low in Tangier disease and in some infections.