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IFTM University, Moradabad, Uttar Pradesh

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IFTM University, Moradabad

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B.Pharm II SEM, Biochemistry (BP209P) Lab Manual

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LABORATORY MANUAL

B.PHARM - II SEM

(Biochemistry Practical)

(**Subject code**: BP 209P)

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IFTM UNIVERSITY, MORADABAD

COURSE: BIOCHEMISTRY- PRACTICAL

COURSE CODE: (BP209P)

B.Pharm II- SEM

S.No.	LIST OF EXPERIMENTS
1.	To perform Qualitative Analysis of Carbohydrates.
2.	To perform Qualitative Chemical Test for Protein.

3.	**Identification test for Protein (albumin and casein)**
4.	**To perform Qualitative Chemical Test for abnormal constituents of urine**
5.	**To prepare phosphate buffer of pH 6.8 and measure its pH using pH meter.**
6.	**To prepare potassium phosphate buffer of pH 6.5 and check its pH using pH meter.**
7.	**To determine the serum total cholesterol.**
8.	**To determine the blood sugar.**
9.	**To study the effect of temperature on salivary amylase activity.**
10.	**To determine creatine in urine.**
11.	**To perform qualitative chemical test for Lipid.**
12.	**To estimate the concentration of glucose in the given sample of urine by Benedict quantitative method.**

Experiment No. 1

AIM: To Perform The Qualitative Tests Of Carbohydrates.

References:

1. S.P. Singh, Practical Manual of Biochemistry, CBS Publishers and Distributors, New Delhi, Fifth Edition, 19-37.

2. R. Chawla, Practical Clinical Biochemistry: Methods and Interpretations, Jaypee brother's medical publishers (P) LTD. New Delhi, Third Edition, 32-38.

Requirements

Glasswares: Test tubes, Test tubes holder, Beaker, Water bath and Glass rod.

Chemicals: Sample solution**,** Molisch reagent, Conc. Sulphuric acid, Iodine solution, Benedict's reagent, Fehling reagent, Barfoed's reagent, Seliwanoff's reagent, Sodium carbonate solution (10%), Phenyl hydrazine hydrochloride, Sodium acetate, Glacial acetic acid.

Introduction

Carbohydrates are polyhydroxy aldehydes and ketones or substances that hydrolyze to yield polyhydroxy aldehydes and ketones. They serve as the primary source of energy. Carbohydrates are mainly divided into monosaccharides, disaccharides and polysaccharides. The commonly occurring monosaccharide includes glucose, fructose, galactose, ribose etc. The two monosaccharides combined together to form disaccharides which include sucrose, lactose and maltose. Starch and cellulose fall into the category of polysaccharides which consists of many monosaccharide residues.

Experimental:

1. Molisch test

Procedure: To take 2 mL of test solution in a test tube and add two drops of Molisch's reagent and mix well. Incline the test tube and add about 1mL of concentrated sulphuric acid along the sides of the test tube. Observe the colour at the junction of the two liquids.

Observation: A red-cum-violet ring appears at the junction of the two liquids (Figure 1).

Inference: Carbohydrate present.

Figure 1.

2. Charring test:

Procedure: Carbohydrates on heating in a test tube or in presence of Conc. H_2SO_4.

Observation: Produces charring with smell like burning sugar.

Inference: Carbohydrate present.

3. Iodine Test

Procedure: To take1 mL of the test solution in a test tube and add a few drops of iodine solution.

Observation: Absence of blue colour.

Inference: This indicates the absence of starch (polysaccharides) in the solution.

4. Benedict test

Procedure: To take 5 drops of the test solution in a test tube and add 2 mL of Benedict's reagent. Boil for five minutes in a water bath. Cool the solution.

Observation: Formation of red, yellow or green colour/precipitate.

Inference: This indicates the presence of reducing sugars in the test solution (Glucose may be present).

5. Fehling test

Procedure: To take 1 mL of Fehling's solution 'A' add 1 mL of Fehling's solution 'B' and a few drops of the test solution in a test tube. Boil for a few minutes.

Observation: Formation of brownish-red yellow or precipitate.

Inference: This indicates the presence of reducing sugars in the test solution (Glucose may be present).

6. Barfoed test

Procedure: To take 1 mL of the test solution in a test tube and add about 2 mL of Barfoed's reagent. Boil it for one minute and allow to stand for a few minutes.

Observation: Formation of brick-red precipitate.

Inference: Only monosaccharides answer this test (Glucose may be present).

7. Seliwanoff test

Procedure: To take 2 drops of test solution in a test tube and add 2 mL of Seliwanoff's reagent. Heat the mixture to just boiling.

Observation: Appearance of deep red colour.

Inference: Ketoses sugar may be present (Glucose may be absent).

Remark: Seliwanoff's test distinguishes between aldose (glucose) and ketose sugars. A ketohexose like fructose, sucrose will form a deep red color with Seliwanoff's reagent while an aldohexose will show a light pink color and takes a longer time to develop the color.

8. Osazone test

Procedure:

To take 0.5 g of phenyl hydrazine hydrochloride, 0.1 g of sodium acetate and 10 drops of glacial acetic acid in a test tube. To this mixture add 5 mL of test solution and heat on a boiling water bath for about half an hour. Allow the test tube to cool slowly and examine the crystals under a microscope.

Observation: Formation of crystals.

Inference: Glucose produces needle-shaped yellow osazone crystals.

Conclusion: The given sample is glucose.

Experiment No. 2

AIM: To perform Qualitative Chemical Test for Protein.

References:

1. S.P. Singh, Practical Manual of Biochemistry, CBS Publishers and Distributors, New Delhi, Fifth Edition, 31-50.

2. R. Chawla, Practical Clinical Biochemistry: Methods and Interpretations, Jaypee brother's medical publishers (P) LTD. New Delhi, Third Edition, 39-44.

Requirements

Glasswares: Test tubes, Test tubes holder, Beaker, Water bath and Glass rod.

Chemicals: Sample solution, Biuret reagent, Ninhydrin solution, acetic acid (2%), concentrated nitric acid, Picric acid solution, Ethanol, Silver nitrate, Sodium hydroxide, and Millon's reagent.

Introduction

Proteins are complex nitrogenous substance of high molecular weight which on decomposition by acids, alkali, or enzymes yields amino acids. Proteins play a vital role in our body. It is present in the form of skin, hair, callus, cartilage, muscles, tendons and ligaments; it protects and provides structure to the body of a multicellular organism. The enzymes, hormones and antibodies are made up of proteins, which catalyze and regulate the physiological activities of plant and human being.

Experimental:

1. Biuret test

Procedure: To take 3 mL protein solution in a test tube and add an equal volume of 10% NaOH. Mix thoroughly and add a few drops of 0.5% copper sulphate solution.

If the protein tested is *insoluble in water*, then apply the procedure given below:

To take 3 mL acetone and 1.5 mL water into a test tube. Add 1 drop of dilute NaOH and a little piece of protein to be tested. Boil continuously over a small flame for 2 min and cool. Add 0.5 mL 40% NaOH and 2 drops of a 1/10 diluted Fehling's solution A.

Observation: Formation of deep violet color.

Inference: Presence of Protein.

2. Ninhydrin Test:

This test is given by only amino acids and proteins which contain free $-NH_2$ groups in their structure (all amino acids give blue colour but proline and hydroxyproline gives yellow colour).

Procedure: To Take 1 mL amino acid solution in a test tube and add 2 drops of 0.1% ninhydrin solution. Boil over a water bath for 2 min. Allow to cool.

Observation: Formation of blue color

Inference: Presence of Protein.

3. Heat Coagulation

Procedure: To take few mL amino acid solutions in a test tube and heats the upper position of the solution.

Observation: An opalescence was appeared become deep on addition of few of 2% acetic acid

Inference: Protein present

4. Precipitation reactions

Proteins are precipitated by strong acids (e.g. Conc. HNO_3), acidic agents e.g. (picric acid) salt of heavy metals (e.g. $AgNO_3$, copper sulphate and lead acetate etc.) and by organic solvents (e.g. ethanol etc.)

Precipitation by Acid

Procedure: Take few mL amino acid solutions in a test tube and add concentrated HNO_3.

Observation: A white ring appears at the junction of two liquids.

Inference: Protein present.

Precipitation by Acidic agent

Procedure: Take few mL amino acid solutions in a test tube and add picric acid solution

Observation: Formation of precipitate.

Inference: Protein present.

Precipitation by organic solvent

Procedure: Take few mL amino acid solutions in a test tube and add ethanol

Observation: Formation of precipitate.

Inference: Protein present.

Precipitation by heavy metals

Procedure: Take few mL amino acid solutions in a test tube and add $AgNO_3$

Observation: Formation of precipitate.

Inference: Protein present.

5. Xanthoprotic test:

It is used for detection of aromatic ring containing amino acid in proteins.

Procedure: To take 2 mL the aqueous solution of amino acid (protein) in a test tube and add 2 ml of Conc. HNO_3. A white precipitation is formed. Heat the solution for 1 minute and cool under tape water. Note the yellow colour obtained. Make it alkaline with excess of 40% NaOH.

Observation: Yellow colour deepens into orange.

Inference: Presence of aromatic ring containing amino acids (Albumin may be present)

6. Millons test: Any proteins containing a phenolic hydroxyl group gives Millon's test.

Procedure: Take 1 ml of protein solution in a test tube and add few drops of Millons reagent. Warm the tube in a boiling water bath for 10 min.

Observation: A brick red color is obtained.

Inference: Presence of proteins containing a phenolic hydroxyl group gives (Albumin may be present)

Conclusion: The given sample is protein and albumin may be present.

Experiment No. 3

AIM: To perform identification test for Protein (albumin and casein)

References:

1. S.P. Singh, Practical Manual of Biochemistry, CBS Publishers and Distributors, New Delhi, Fifth Edition, 31-50.

2. R. Chawla, Practical Clinical Biochemistry: Methods and Interpretations, Jaypee brother's medical publishers (P) LTD. New Delhi, Third Edition, 39-44.

Requirements

Glasswares: Test tubes, Test tubes holder, Beaker, Water bath and Glass rod.

Chemicals: Sample solution, Biuret reagent, Ninhydrin solution, acetic acid (2%), concentrated nitric acid, Picric acid solution, Ethanol, Silver nitrate, Sodium hydroxide, and Millon's reagent.

TESTS ON PROTEINS

Albumin

Prepare a solution of egg-albumin as follows: Shake up vigorously one volume of egg-white with ten volumes of water and filter off the precipitate of ovoglobulin -\which forms. (Since the filtration requires a considerable amount of time, it is convenient to prepare the filtrate in bulk for a whole class.) Carry out the following tests with the filtrate ;

Half saturate 10 c.c. of the solution with ammonium sulphate. Note that no precipitation takes place. Add more crystals, little by little, until saturation is complete. Albumin IS precipitated as a flocculent precipitate, easily distinguished from excess of crystals of the Sulphate. Filter off a little, suspend the precipitate in water, shake and observe that it redissolves. Albumins are soluble in water and in half-saturated solutions of ammonium sulphate, but are precipitated by saturation with ammonium sulphate. Saturate 10 c.c. of the solution with sodium chloride crystals. Note that no precipitation of protein occurs. Add 2 or 3 drops of dilute acid and observe the result.

Coagulation Test: Boil 5 c.c. of the albumin solution ina test-tube. The protein coagulates and will no longer redissolve.

Cool and test the suspended coagulum with Millon's reagent. Notice that on warming it reddens.

Precipitation Tests: Add mercuric chloride solution, drop by drop, to 2 c.c. of the egg-albumin

solution in a test-tube.

NITRIC ACID TEST (Heller's test): Transfer 5 C.c. of concentrated nitric acid to a test-tube

and pour on to the surface very carefully, so that mixing does not occur, 2 or 3 c.c. of the egg-albumin solution. A white layer of coagulated albumin appears at the interface between the two liquids. Precipitation by Alcohol: Add to 3 C.c. of the solution 95 per cent. ethyl alcohol, 2 or 3 drops at a time, and note what occurs.

Casein

Transfer to a 500 c.c. flask 40 gm. of commercial casein aqd 200 c.c. of 1 per cent. sodium carbonate solution. Add 50 c.c. of a glycerol extract of pancreas and 20 c.c. of a glycerol

extract of intestinal mucosa. Add a few cubic centimetres of chloroform I and also of toluene (to prevent bacterial action), shake up, and loosely cork the flask. Place it in an incubator at 37° for preferably, eight days. Add more toluene from time to time. At the end of the stated period pour off the supernatant liquid into a smaller flask, raise it to the boiling point and then add glacial acetic acid drop by drop with shaking until the reaction is acid to litmus paper. Cool and filter. The undecomposed protein is filtered off.

Proteins are complex nitrogenous substance of high molecular weight which on decomposition by acids, alkali, or enzymes yields amino acids. Proteins play a vital role in our body. It is present in the form of skin, hair, callus, cartilage, muscles, tendons and ligaments; it protects and provides structure to the body of a multicellular organism. The enzymes, hormones and antibodies are made up of proteins, which catalyze and regulate the physiological activities of plant and human being.

Experimental:

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If the protein tested is *insoluble in water*, then apply the procedure given below:

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Observation: Formation of deep violet color.

Inference: Presence of Protein.

2. Ninhydrin Test:

This test is given by only amino acids and proteins which contain free $-NH_2$ groups in their structure (all amino acids give blue colour but proline and hydroxyproline gives yellow colour).

Procedure: To Take 1 mL amino acid solution in a test tube and add 2 drops of 0.1% ninhydrin solution. Boil over a water bath for 2 min. Allow to cool.

Observation: Formation of blue color

Inference: Presence of Protein.

3. Heat Coagulation

Procedure: To take few mL amino acid solutions in a test tube and heats the upper position of the solution.

Observation: An opalescence was appeared become deep on addition of few of 2% acetic acid

Inference: Protein present

4. Precipitation reactions

Proteins are precipitated by strong acids (e.g. Conc. HNO_3), acidic agents e.g. (picric acid) salt of heavy metals (e.g. $AgNO_3$, copper sulphate and lead acetate etc.) and by organic solvents (e.g. ethanol etc.)

Precipitation by Acid

Procedure: Take few mL amino acid solutions in a test tube and add concentrated HNO_3.

Observation: A white ring appears at the junction of two liquids.

Inference: Protein present.

Precipitation by Acidic agent

Procedure: Take few mL amino acid solutions in a test tube and add picric acid solution

Observation: Formation of precipitate.

Inference: Protein present.

Precipitation by organic solvent

Procedure: Take few mL amino acid solutions in a test tube and add ethanol

Observation: Formation of precipitate.

Inference: Protein present.

Precipitation by heavy metals

Procedure: Take few mL amino acid solutions in a test tube and add $AgNO_3$

Observation: Formation of precipitate.

Inference: Protein present.

5. Xanthoprotic test:

It is used for detection of aromatic ring containing amino acid in proteins.

Procedure: To take 2 mL the aqueous solution of amino acid (protein) in a test tube and add 2 ml of Conc. HNO_3. A white precipitation is formed. Heat the solution for 1 minute and cool under tape water. Note the yellow colour obtained. Make it alkaline with excess of 40% NaOH.

Observation: Yellow colour deepens into orange.

Inference: Presence of aromatic ring containing amino acids (Albumin may be present)

6. Millons test: Any proteins containing a phenolic hydroxyl group gives Millon's test.

Procedure: Take 1 ml of protein solution in a test tube and add few drops of Millons reagent. Warm the tube in a boiling water bath for 10 min.

Observation: A brick red color is obtained.

Inference: Presence of proteins containing a phenolic hydroxyl group gives (Albumin may be present)

Conclusion: The given sample is protein and albumin may be present.

Experiment No. 4

AIM: To Perform The Qualitative Tests For Abnormal Constituents Of Urine

References:

1. S.P. Singh, Practical Manual of Biochemistry, CBS Publishers and Distributors, New Delhi, Fifth Edition, 65-119.

2. R. Chawla, Practical Clinical Biochemistry: Methods and Interpretations, Jaypee brother's medical publishers (P) LTD. New Delhi, Third Edition, 51-64.

Requirement: Urine sample, pH paper, Silver nitrate, Conc. HNO_3, Barium chloride, conc. HCl, 1% acetic acid, Potassium oxalate, Ammonium molybedate, 2% sodium carbonate, red litmus paper, Sodium hypobromite, Phosphotungstic acid, Sodium nitroprusside, Picric acid, Sodium hydroxide.

Introduction

Urine is an excretory product of the body, formed in the kidneys. Normal urine contains of approximately 95% water, the rest contains both inorganic constituents viz: chlorides, sodium, potassium, calcium, phosphate, sulphate, ammonia and organic constituents viz: urea, uric acid creatinine and hippuric acid.

Experimental

For qualitative analysis, fresh urine 20-30 ml is collected in a clean dry container and use for the following investigations

1. Color: normal freshly excreted urine is colorless to straw color

2. pH: pH of urine varies between 4.5- 8.0 with a mean of 6.0 in 24 hr.

3. Odour: Normally aromatic or ammonical/foul smell/ bad or food like odor.

4. Test for chloride:

Procedure: Take 2 mL urine in a test tube and add 0.5 mL of concentrated HNO_3 and 1 mL of $AgNO_3$.

Observation: Formation of white precipitate.

Inference: Chloride present

5. Test for sulphate:

About 85-95% of sulphur is excreted as inorganic sulphate (Na_2SO_3) and the rest as ethereal sulphate (cystine etc.)

Procedure: Take 2 mL urine in a test tube and add 2 mL of 10 barium chloride.

Observation: Formation of white precipitate.

Inference: inorganic sulphate present

Filter the precipitate, and in the filtrate, add 1 mL of conc. HCl and about 3 mL of 10% barium chloride. Boil the mixture for 1-2 minutes. Note the appearance of turbidity. This is test for ethereal sulphate.

6. Test for calcium:

Procedure: Take 2 mL urine in a test tube and add 5 drops 1% acetic acid and 5 mL of potassium oxalate.

Observation: Formation of white precipitate.

Inference: Calcium present

7. Test for inorganic phosphorus:

Procedure: Take 5 mL urine in a test tube and add few drops of concentrated HNO_3 and a pinch of ammonium molybedate. Warm it.

Observation: Formation of yellow precipitate/ solution.

Inference: Phosphorus present

8. Test for ammonia:

Procedure: Take 5 mL urine in a test tube and add 2% sodium carbonate till the solution is alkaline to litmus. Boil the solution, and place a piece of moistened red litmus paper at the mouth of test tube.

Observation: Litmus paper turns to blue due to evaluation of ammonia.

Inference: Ammonia present

9. Test for urea

Procedure: Take 2 mL urine in a test tube and add 4-5 drops of sodium hypobromite.

Observation: Note the effervescence of nitrogen gas.

Inference: Urea present

10. Test for uric acid:

Procedure: Take 2 mL urine in a test tube and add few drops of phosphotungstic acid followed by a few drops of 20% sodium carbonate.

Observation: Blue colour produced.

Inference: Uric acid present

Procedure: Take a wet piece of filter paper with a few drops ammonical silver nitrate solution. Add 2-3 drops of urine on wet paper.

Observation: Black colour produced due to precipitation of silver.

Inference: Uric acid present

11. Test for creatinine

Nitroprusside test

Procedure: Take 5 ml of urine in a test tube, add few drops of sodium nitroprusside and then make the solution alkaline with NaOH.

Observation: A ruby red color is first formed which turns yellow

Inference: Creatinine present

Picric acid test

Procedure: Take 5 ml of urine in a test tube, add aqueous solution of picric acid and then make the solution alkaline with NaOH.

Observation: A red color is formed which turns to yellow by acidification.

Inference: Creatinine present

Conclusion: The given urine sample contains above constituents.

Experiment No. 5

AIM: To prepare phosphate buffer of pH 6.8 and measure its pH using pH meter.

Reference: Indian Pharmacopoeia 1996, vol. 2

Theory: A buffer solution is one which resists changes in pH when small quantities of an acid or an alkali are added to it. An acidic buffer solution is simply one which has a pH less than 7 Acidic buffer solutions are commonly made from a weak acid and one of its salts- oftes sodium salt. An alkaline buffer solution has a pH greater than 7. Alkaline buffer solutions are commonly made from a weak base. one of its salts.

The pH scale is alternatively sometimes called the pH-acid-base scale and sometimes just the acid-base scale.

Procedure: Place 50 ml of 0.2 M potassium dihydrogen phosphate in a 200 ml volumetric flask. Add the sufficient volume of 0.2 m sodium hydroxide and then make up the volume upto 100 ml with water.

Result: The phosphate buffer was prepared and pH was found to be.....

Experiment No. 6

AIM: To prepare potassium phosphate buffer of pH 6.5 and check its pH using pH meter.

Reference: Indian Pharmacopoeia 1996, vol. 2

Theory: A buffer solution is one which resists changes in pH when small quantities of an acid or an alkali are added to it. An acidic buffer solution is simply one which has a pH less than 7 Acidic buffer solutions are commonly made from a weak acid and one of its salts- oftes sodium salt. An alkaline buffer solution has a pH greater than 7. Alkaline buffer solutions are commonly made from a weak base. one of its salts.

The pH scale is alternatively sometimes called the pH-acid-base scale and sometimes just the acid-base scale.

Procedure: Place 50 ml of 0.2 M potassium dihydrogen phosphate in a 200 ml volumetric flask. Add the sufficient volume of 0.2 m sodium hydroxide and then make up the volume upto 100 ml with water.

Result: The phosphate buffer was prepared and pH was found to be.....

Experiment No. 7

AIM: To determine the serum total cholesterol.

Reference: K.K pillai and J.S. Qadry biochemistry and clinical pathology. CBS Publishers & Astributors Pvt. LTD. First edition, PP 136-137.

Requirements: Alcohol, Acetone, Chloroform, Sulphuric acid, Std. cholesterol, Test tube

Principle: Blood or serum is excreted with an alcohol-acetone mixture which removes cholesterol and other lipids and participates protein. The organic solvent is removed by evaporation on a boiling water bath. The residue is dissolved in chloroform and colour developed by the Liebermann-Burchard reaction and compared with the standard.

Procedure: Place 10 ml of the alcohol-acetone solvent in centrifuge tube and add 0.2 ml of serum or blood. Cork the tube and immerse the tube in a boiling water bath with shaking until the solvent begins to ball. Remove the tube and continue shaking the mixture for further 5 minutes. Cool to room temperature and centrifuge it at 1500 r.p.m. for minutes. Decant the supernatant fluid in a smallbeaker, and evaporate to dryness on a boiling water bath or a hot plate. Cool and dissolve the residue in 6 ml of chloroform and transfer it to a dry test tube. At the same time,label two other test tubes, standard and blank. Add 6 ml of cholesterol working standard into the standard tube. Take 6 ml of pure chloroform in the blank tube. Add 2 ml of acetic anhydride and 0.1 ml of conc. Sulphuric acid to all tubes and thoroughly mix. Leave the tubes in the dark at room temperature for 15 minutes and read the extinction at 680 nm. Set the photometer to zero optical density with blank.

Serum cholesterol (mg/100ml)=O.D of Test/O.D of Std X 3000

Result: After performing the above experiment serum cholesterol in blood was found to be.....

Experiment No. 8

AIM: To determine the blood sugar.

Reference: K.K Pillai and J.S. Qadry Biochemistry and clinical pathology. CBS Publishers & distributors Pvt. LTD. First edition, P.P 127-128. Requirements: Sodium tungstate, Sodium Sulphate, Copper Sulphate, Sodium carbonate, Test tube

Principle: Protein free filtrate is heated with alkaline copper sulphate, in a test tube. Alkaline copper sulphate is reduced by glucose to form cuprous oxide. It is treated with phosphomolybdic acid to develop a blue colour. The blue colour is developed due to the reduction of the phosphomolybdic acid. It is compared with a colour developed by a standard glucose solution.

Procedure: Add 0.1 ml of blood into 3.8 ml of reagent A in a test tube and mix it. Then add 0.1 ml of 10% sodium tungstate. Mix it well and centrifuge. To 1 ml of the supernatant liquid add 1 ml of reagent B. Plug the test tube with cotton wool and heat it on a boiling water bath for 10 minutes. Cool and add 3 ml of phosphomolybdic acid reagent and 3 ml of distilled water. Mix well and keep it for 5 minutes. Read the optical density at 680 mu using a red filter.

Preparation of Standard: Take 1 ml of saturated glucose 25 µg/ml (Prepared in Reagent A) in a test tube. Add 1 ml of reagent B. Plug the test tube with cotton wool and heat it on a boiling water bath for 10 minutes. Cool and add 3 ml of phosphomolybdic acid reagent and 3 ml of distilled water. Mix well and keep it for 5 minutes. Read the optical density at 680 mu.

Preparation of blank: Take 1 ml of reagent A in a test tube and proceed in the same way as that

for the standard. Mg of glucose in 100 ml of blood Optical density of test Optical density of test 100

Result: After performing the above experiment sugar level in blood was found to be.....

Experiment No. 9

AIM: To study the effect of temperature on salivary amylase activity.

REFERENCE: Enzyme Kinetics (2012).

Determination of Salivary amylase activity

The study of saliva affords a convenient method of introduction, not only to the nature of a typical digestive juice, but also to the nature of enzyme action. I

Preparation of Saliva.

Chew a small piece of paraffinwax; the act of chewing stimulates a flow of saliva. Collect 20 or 30 c.c. in a beaker. Filter a portion. Since the((filtration frequently takes a considerable amount of time, carry out the following tests with filtered or unfiltered saliva as directed :

Experiment 1. Transfer sufficient unfiltered saliva to a small clean urinometer tube to nearly fill it, and measure the phenolphthalein paper, and congo red paper. (If these paper strips are not available add I drop of litmus paper, specific gravity with the urinometer. (It should lie between

1'002 and 1'008.) Test the saliva with the corresponding indicator solution to 1 C.c. of saliva în a test-tube.) The saliva will react alkaline to congo red. very different acid to phenolphthalein, and neutral or very faintly acid to litmus, owing to the fact that these indicators change colour at degrees of hydrogen-ion concentration. Use up the saliva in tests calling for unfiltered saliva.

Experiment 2. To 2 c.c. of filtered saliva in a test-tube add 1 drop of dilute acetic acid. A white amorphous precipitate of the glucoprotem Mucin separates. This protein aids, by virtue of its viscous nature, in the mastication and balling together of solid food prior to swallowing. It contains carbohydrate radicals.

Experiment 3. For this and the following two experiments use filtered saliva, one volume diluted with four volumes of water. To 2 C.c. of saliva add an equal volume of 40 per cent. sodium hydroxide and 1 or 2 drops of very dilute copper sulphate. A purplish-red colour is formed, due to the protein Mucin. (This is an example of a positive biuret test; see Exercise V.)

Principle: The study of enzymes is an integral part of the biochemistry curriculum in universities. Therefore, it is necessary to familiarize the students with the knowledge of how to develop an enzyme assay and how to observe the effects of various factors on the activity of an enzyme. In this laboratory, we will examine the kinetics of a-amylase as found in saliva. Enzyme reacts differently in different environments. The properties of the environment may influence the rate of an enzyme to react. For example, pH, temperature or substrate concentration. In this lab, we will determine the effect of such to amylase.

Procedure:

A) Preparation of standard reference

1. Prepare starch solutions from the stock solution (1.0 mg/ml) into dilutions of 0.01, 0.025, 0.05.

0.1.0.3, 0.5, 0.7, and 1.0 mg/ml from the starch stock solution.

2. Iodine solution is prepared by adding 5 g potassium iodide to 100 ml water. The dissolved potassium iodide is added with 1 g of iodine and is allowed to dissolve.

3. Prepare a standard curve of Absorbance (@590 nm) vs. Concentration of a starch/iodinemixture.

The effect of temperature

Prepare as the following for the experiment of different temperature.

Test Tube	8ml starch of x mg/ml	Water (ml)	Amylase (ml)	Incubate each sample at 20, 28, 35, 40 °C for 10 mins	Iodine	Place all test tubes in an ice bath. Measure the absorbance at 590nm.
1	0	8	1			
2	0.01	0	1			
3	0.025	0	1			
4	0.05	0	1			
5	0.1	0	1			
6	0.3	0	1			
7	0.5	0	1			
8	0.7	0	1			
9	1.0	0	1			

Data analysis

Plot the Lineweaver-Burke line for the result of 20, 28, 35 and 40°C.

Compare all three plots.

What are the values of Vmax and Km for all plots?

Experiment No. 10

AIM: To determine creatinine in urine

Reference: K.K Pillai and J.S. Qadry Biochemistry and clinical pathology. CBS Publishers & distributors Pvt. LTD. First edition, PP 136-137.

Requirements: Sodium tungstate, Sulphuric acid, Picric acid, Test tube, Principle: Creatinine gives an amber colour on reaction with picric acid in the presence of strong alkali. The amber colour obtained due to the formation of cretinine picrate.

Test for creatinine

Nitroprusside test

Procedure: Take 5 ml of urine in a test tube, add few drops of sodium nitroprusside and then make the solution alkaline with NaOH.

Observation: A ruby red color is first formed which turns yellow

Inference: Creatinine present

Picric acid test

Procedure: Take 5 ml of urine in a test tube, add aqueous solution of picric acid and then make the solution alkaline with NaOH.

Observation: A red color is formed which turns to yellow by acidification.

Inference: Creatinine present

Procedure: Take 7 ml of water in a test tube. Add 1 ml of urine and 1 ml of 10% sodium tungstate. Mix., Add 1 ml of 2/3 N sulphuric acid with constant shaking. Allow it to stand for a few minutes, centrifuge and filter. Take 5 ml of the filtrate into a tube labeled as T. Take 0.2 ml of saturated creatinine in a test tube labeled as S. and make up the volume upto 5 ml with distilled water. Take 5 ml of water in a tube labeled as B. Add 2 ml of 1% picric acid followed by 0.15 ml of 10% sodium hydroxide solution. Mix gently and allow standing for 15 minutes. Transfer a portion of the blank and adjust the colorimeter to zero 0.Dat 520 nm. Then take the reading for stand and unknown sample.

$$\text{creatinine (mg/100ml)} = \frac{\text{O.D of Test}}{\text{O.D of Std.}} \times 2$$

Result: After performing the above experiment creatinine in urine was found to be.....

Experiment No.11

AIM: To Perform The Qualitative Tests Of Lipid

References:

1. S.P. Singh, Practical Manual of Biochemistry, CBS Publishers and Distributors, New Delhi, Fifth Edition, 38-44.

2. R. Chawla, Practical Clinical Biochemistry: Methods and Interpretations, Jaypee brother's medical publishers (P) LTD. New Delhi, Third Edition, 45-47.

Requirements

Glasswares: Test tubes, Test tubes holder, Beaker, Water bath and Glass rod.

Chemicals: Sample solution, Chloroform, Ether, Benzene, Ethanol, conc. H_2SO_4 and acetic anhydride.

Introduction

Lipids are heterogeneous group of compounds related to fatty acids. (e.g. Fats, Cholesterol, Oils and wax etc.) Lipids are insoluble in water and soluble organic solvents like ether, chloroform, and benzene. Chemically, the lipids are either esters of fatty acids or substances capable of forming such esters. Lipids act as heat insulators and as reserve substances of energy.

Experimental:

1. Solubility test

Procedure: Take few drops of sample in test tubes. Add 4 mL of solvents (water, chloroform, ether and benzene etc.) to each test tube. Mix each of the test tubes vigorously for 15 seconds. Wait 2 minutes.

Observation: Note the miscibility in each test tube; Lipid is soluble in organic solvents but insoluble in water.

Inference: Lipid is present.

2. Salkowski reaction

Procedure: Dissolve few crystals of cholesterol in 5-6 ml chloroform and add and 2-3 ml conc. H_2SO_4 slowly through the side of the test tube and mix.

Observation: A reddish color is produced in upper chloroform layer and the acid lower layer assumes a yellow color with a green fluorescence.

Inference: Cholesterol present

3. Liebermann-Burchard test

Procedure: Dissolve few crystals of cholesterol in 5-6 ml chloroform and add 1 ml of acetic anhydride, 1-2 ml of conc. H_2SO_4.

Observation: Solution turns violet, blue and finally green.

Inference: Cholesterol present

4. Crystal formation test

Procedure: Dissolve few crystals of cholesterol in 2-3 ml alcohol and slightly heat to get a saturated solution. Add a few drops of water and allow to cool.

Observation: A precipitate appears at the bottom which contains rhombic shaped crystal with broken margin.

Inference: Cholesterol present

Conclusion: The given sample is cholesterol.

Experiment No. 12

AIM: To estimate the concentration of glucose in the given sample of urine by Benedict quantitative method.

References:

1. S.P. Singh, Practical Manual of Biochemistry, CBS Publishers and Distributors, New Delhi, Fifth Edition, 65-119.

2. R. Chawla, Practical Clinical Biochemistry: Methods and Interpretations, Jaypee brother's medical publishers (P) LTD. New Delhi, Third Edition, 51-64.

Requirement

Urine sample, Benedict's qualitative reagent and test tubes.

Introduction

Urine is an excretory product of the body, formed in the kidneys. Normal urine contains of approximately 95% water, the rest contains both inorganic constituents viz: chlorides, sodium, potassium, calcium, phosphate, sulphate, ammonia and organic constituents viz: urea, uric acid creatinine and hippuric acid. Many of the pathological constituents are present in small amounts viz: glucose, bile pigments, bile salts, ketone bodies and proteins in normal urine but they escape detection due to low sensitivity of the method employed. Normal urine contains small amounts of reducing sugar, i.e. 1-1.5 gm/24 hrs. Out of this glucose is present in the concentration of 50- 300 mg/24 hrs. The concentrations of these constituents in urine are increased markedly in different pathological conditions like glucosuria.

Experimental

Test for glucose in urine

Procedure: Take 1-2 mL given unknown urine sample and 1-2 ml of fresh normal urine in a test tube, add 5 mL of Benedict's qualitative reagent in both test tube and boil in a water bath for 5 minutes.

. **Observation:** Unknown urine sample shows brick red color and normal urine shows light green color.

Remark: Light green, yellow or brick red color is produced depending on whether urine contains 0.5%, 1% or more than 2% glucose.

Inference: Glucose is present in given unknown urine sample and glucose is absent in normal urine.

Conclusion: The given unknown urine sample contain abnormal amount (increased amount) of

glucose.

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