East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo Nagar Post
Bengaluru – 560049, Karnataka

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka
Bengaluru – 560 041
India

LAB MANUAL

MEDICINAL BIOCHEMISTRY
PHARM D
1st Year

EAST POINT COLLEGE OF PHARMACY
East Point Campus, Jnana Prabha, Virgo Nagar Post,
Bengaluru – 560049, Karnataka

PROGRAM OUTCOMES
DOCTOR OF PHARMACY
PO1: Comprehensive Pharmaceutical Knowledge
Acquire and demonstrate a deep understanding of fundamental concepts in pharmacotherapeutics, clinical pharmacy, pharmacoepidemiology, clinical pharmacokinetics, therapeutic drug monitoring, as well as behavioral, social, and administrative pharmacy. Showcase advanced proficiency by designing and executing effective medication therapy management plans.
PO2: Strategic Planning Proficiency
Cultivate the capability to formulate and execute plans, proficiently organizing tasks to meet deadlines. Demonstrate adeptness in time management, resource allocation, delegation, and organizational skills. Exhibit competence in clinical decision-making by seamlessly integrating pharmaceutical knowledge with patient-specific factors to enhance healthcare outcomes.
PO3: Problem Solving Proficiency
Apply the principles of scientific inquiry, engaging in analytical, clear, and critical thinking to address challenges and make informed decisions in routine clinical practice. Graduates will demonstrate adept communication skills, counseling patients on medication usage, potential side effects, and lifestyle adjustments, fostering patient comprehension and adherence.
PO4: Leadership and Entrepreneurship
Demonstrate leadership skills and entrepreneurial spirit, contributing to the growth and development of the pharmaceutical profession and industry.
PO5: Professional Identity
Exhibit a strong professional identity including a commitment to ethical practice, effective communication, and leadership in advocating for optimal patient care, continuous professional development, and active engagement with the broader healthcare professionals, promoters and stakeholders.
PO6: Adherence to Ethical Standards
Uphold the highest ethical standards in pharmaceutical practice, adhering to the Pharmacy Council of India's code of ethics and promoting patient welfare.
PO7: Communication
Demonstrate effective communication skills, sustaining clear and empathetic interactions with patients, healthcare professionals, and diverse stakeholders. They will proficiently convey pharmaceutical information, contributing to collaborative and patient-centered care. This emphasis on communication ensures graduates are well-equipped to navigate complex healthcare scenarios and advocate for optimal therapeutic outcomes.
PO8: Community Engagement
Participate in community engagement activities, applying pharmaceutical knowledge to address healthcare needs and improve the overall well-being of the community.
PO9: Environment and Sustainability
Demonstrate a profound understanding of environmental issues in the pharmaceutical domain, applying sustainable practices in research, development, and clinical settings. They will champion eco-friendly approaches, fostering a commitment to minimizing ecological impact and promoting responsible stewardship of natural resources.
PO10: Clinical Research Skills
Proficient in conducting clinical research, applying ethical principles, and contributing to advancements in pharmaceutical sciences and healthcare.
PO11: Continuous Professional Development
Embrace a commitment to lifelong learning, staying abreast of advancements in pharmaceutical sciences, healthcare policies, and technological innovations.

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PROGRAM SPECIFIC OUTCOMES	
DOCTOR OF PHARMACY	
PSO1	Acquire a thorough foundational knowledge in pharmaceutical sciences, including pharmacology, pharmaceutics, pharmaceutical chemistry, pharmacognosy and pharmaceutical analysis to excel in further academic pursuits
PSO2	Acquire and apply the pharmacotherapeutic concepts for better patient care enhancing employability across various sectors including clinical research organizations, academic and hospitals
PSO3	Equip with entrepreneurial skills and knowledge of pharmacoepidemiological studies and regulatory aspects to initiate and run successful ventures in the healthcare sector

Course:	**Code: 1.3P** **Medicinal Biochemistry**
CO1	Able to understand principles and reaction involved in the determination of biomolecules in the body fluids
CO2	Able to analyze, determine and estimate normal and abnormal constituents of urine and blood samples sample
CO3	Able to do qualitative and quantitative determination of biomolecules in the body fluids
CO4	Study the enzymatic hydrolysis and factor affecting enzyme activity and viva voce

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QUALITATIVE ANALYSIS OF URINE

EAST POINT COLLEGE OF PHARMACY
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Experiment No: 1

URINE TEST ANALYSIS

INTRODUCTION:

The major function of the urinary system is to remove waste products from our bodies and help regulate the volume and composition of blood by the production and excretion of urine. The urinary system consists of two kidneys, two ureters, one bladder, and one urethra. The composition of urine can vary greatly and constantly fluctuates with dietary intake (food and water) and metabolic activity. Urine consists mostly of water with various organic and inorganic substances such as urea, uric acid, creatine, sodium chloride, ammonia, sulfates, and phosphates as its principal ingredients. A clinical examination of urine can provide a convenient, cost effective and non-invasive means of assessing kidney function and providing an overall assessment of our body's health.

Typically, a complete urinalysis involves an examination of the physical characteristics of urine, a chemical analysis and a microscopic examination of urine sediment. Urine should be collected in a clean container, stored in a cool place, and tested as soon as possible.

Routine Urinalysis (Routine-UA):

- It consists of a group of tests performed as part of physical examination. It involves macroscopic and microscopic analysis.

Type of analysis:

- macroscopic analysis: **physical** characteristics and **chemical** analysis.
- microscopic examination: urine sediment is examined under microscope to identify the components of the urinary sediments.

A. PHYSICAL CHARACTERISTICS OF URINE - The physical characteristics of urine include observations and measurements of colour, turbidity, odour, specific gravity, pH and volume. Visual observation of a urine sample can give important clues as to evidence of pathology.

1. COLOUR

The colour of normal urine is usually light yellow to amber. Generally the greater the solute volume the deeper the colour. The yellow colour of urine is due to the presence of a yellow pigment, **urochrome**. Deviations from normal colour can be caused by certain drugs and various vegetables such as carrots, beets, and rhubarb.

2. ODOUR

Slightly aromatic, characteristic of freshly voided urine. Urine becomes more ammonia-like upon standing due to bacterial activity.

3. TURBIDITY

Normal urine is transparent or clear; becomes cloudy upon standing. Cloudy urine may be evidence of phosphates, urates, mucus, bacteria, epithelial cells, or leukocytes.

4. pH

Ranges from 4.5 - 8.0. Average is 6.0, slightly acidic. High protein diets increase acidity. Vegetarian diets increase alkalinity. Bacterial infections also increase alkalinity.

5. SPECIFIC GRAVITY

The specific gravity of urine is a measurement of the density of urine - the relative proportions of dissolved solids in relationship to the total volume of the specimen. It reflects how concentrated or dilute a sample may be. Water has a specific gravity of 1.000. Urine will always have a value greater than 1.000 depending upon the amount of dissolved substances (salts, minerals, etc.) that may be present. Very dilute urine has a low specific gravity value and very concentrated urine has a high value. Specific gravity measures the ability of the kidneys to concentrate or dilute urine depending on fluctuating conditions. Normal range 1.005 - 1.035, average range 1.010 - 1.025.

Low specific gravity is associated with conditions like *Diabetes insipidus*, excessive water intake, diuretic use or chronic renal failure.

High specific gravity levels are associated with diabetes mellitus, adrenal abnormalities or excessive water loss due to vomiting, diarrhoea or kidney inflammation. A specific gravity that never varies is indicative of severe renal failure.

Specific gravity can be determined by either of two methods using a refractometer or a urinometer.

a. **Refractometer -** measures the refractive index of urine which parallels the specific gravity.

Procedure:

1. Collect mid-stream sample of urine in collection cup.
2. Pipette 1-2 drops of urine into the plastic chamber located on the top of the refractometer. Be sure that the plastic is pressed firmly down in place on the refractometer.
3. Determine the specific gravity of the urine by looking through the refractometer and determining the value on the scale on the left hand side. The specific gravity value is where the light and dark intersect on the scale.
4. Clean the refractometer with kim wipes.

b. **Urinometer -** Is a weighted, bulb shaped device that has a specific gravity scale on the stem end.

PROCEDURE:

1. Fill the cylinder with enough urine so that the urinometer will float in the urine and not touch the bottom.
2. Be careful not to drop the urinometer in the cylinder! Gently release it in order not to break or burst the cylinder. It should NOT touch the sides or bottom of cylinder.
3. The specific gravity can be read on the scale on the stern of the urinometer at the meniscus.
4. The specific gravity of water is 1.000 with respect to temperature. The urinometer can be checked periodically against this standard to ensure quality control at that temperature.

B. CHEMICAL ANALYSIS:- Includes various tests to identify normal organic/inorganic ad abnormal constituents which are present in urine.

ABNORMAL CONSTITUENT	ASSOCIATED CAUSES
Protein (albumin)	Albumin is normally too large to pass through glomerulus. Indicates abnormal increased permeability of the glomerulus membrane. *Non-pathological causes are: pregnancy, physical exertion, increased protein consumption. Pathological causes are: glomerulonephritis bacterial toxins, chemical poisons.*
Glucose	Glycosuria is the condition of glucose in urine. Normally the filtered glucose is reabsorbed by the renal tubules and returned to the blood by carrier molecules. If blood glucose levels exceed renal threshold levels, the untransported glucose will spill over into the urine. *Main cause: diabetes mellitus*
Ketones	Ketone bodies such as acetoacetic acid, beta-hydroxybutyric acid, and acetone can appear in urine in small amounts. These intermediate by-products are associated with the breakdown of fat. *Causes: diabetes mellitus, starvation, diarrhea*
Bilirubin	Bilirubin comes from the breakdown of hemoglobin in red blood cells. The globin portion of hemoglobin is split off and the heme groups of hemoglobin is converted into the pigment bilirubin. Bilirubin is secreted in blood and carried to the liver where it is conjugated with glucuronic acid. Some is secreted in blood and some is excreted in the bile as bile pigments into the small intestines. *Causes: liver disorders, cirrhosis, hepatitis, obstruction of bile duct*

Urobilinogen	Bile pigment derived from breakdown of hemoglobin. The majority of this substance is excreted in the stool, but small amounts are reabsorbed into the blood from the intestines and then excreted into the urine. *Causes: hemolytic anemias, liver diseases*
Hemoglobin	Hemoglobinuria is the presence of hemoglobin in the urine. *Causes: hemolytic anemia, blood transfusion reactions, massive bums, renal disease*
Red blood cells	Hematuria is the presence of intact erythrocytes. Almost always pathological. *Causes: kidney stones, tumors, glomerulonephritis, physical trauma*
White blood cells	The presence of leukocytes in urine is referred to as pyuria (pus in the urine). Causes: urinary tract infection
Nitrite	Presence of bacteria. *Causes: urinary tract infection*

C. MICROSCOPIC EXAMINATION

Examination of urine sediment may reveal the presence of different types of cells such as epithelial cells, leukocytes, erythrocytes, or renal cells. Different types of crystals, yeast, bacteria, or casts may also be present. Casts are cylindrical structures created by protein precipitation in the renal tubules.

Procedure:

1. Transfer urine sample to a conical centrifuge tube.
2. Centrifuge your sample at a **moderate speed for 5 minutes**.
3. Discard the supernatant (fluid off the top) by quickly pouring off fluid.
4. Tap tube with index finger to mix sediment with remaining fluid.
5. Make a wet mount of sample by transferring 1 drop of material to a slide and covering with a coverslip.
6. Examine the sample under the microscope under low and high power.
7. Identify what you see by comparing to charts. Draw a few of your observations.

INORGANIC CONSTITUENT	NORMAL VALUE	CLINICAL SIGNIFICANCE
CHLORIDES (expressed as NaCl)	9.0 gm/lit	Reduced in fever, nephritis, diarrhoea, vomiting etc.
Phosphates(expressed as P_2O_4)	2.5 gm/lit	Increased in osteomalacia, rickets, periosteosis, and hyperthyroidism. Decreased in pregnancy and hypothyroidism.
SULPHATES(expressed as SO_4)	1.5 gm/lit	Increased fever and diseases associated with increased metabolism. Decreased in diseases associated with loss of appetite and diminished metabolism.
AMMONIA	0.7 gm/lit	Increased in abnormal protein metabolism, diabetes ,ketosis and cystitis. Decreased in nephritis.
BICARBONATES	In traces...	----------

CLINICAL SIGNIFICANCE: INORGANIC CONSTITUENTS

QUALITATIVE ANALYSIS OF NORMAL INORGANIC CONSTITUENTS OF URINE

TEST	OBSERVATION	INFERENCE
Test for bicarbonate Take 3ml sample add dil.HCl or dilH_2So_4.	Effervescence of Co_2 gas is produced.	Presence of bicarbonates
Test for chloride Take 3 ml urine; add 1ml of HNO_3 (to prevent ppt.of urates by $AgNO_3$ and 1 ml of $AgNo_3$.	White curdy precipitate of silver chloride soluble in ammonium hydroxide solution.	Presence of chlorides
Test for phosphate Take 3 ml of urine, add 2ml of conc. HNO_3 and pinch of Ammonium molybdate. Warm on water bath if green colour is observed add few drops of HNO_3 and heat.	Canary yellow coloured ppt of ammonium phosphomolybdate is formed.	Presence of phosphate
Test for sulphate 5 ml. of urine + 1 ml. Of conc. HCl (to prevent ppt. Of phosphates) + 2 ml. of Barium chloride($BaCl_2$).	An opaque milkiness or a thick white ppt. of $BaSO_4$, insoluble in conc. Hcl.	Presence of sulphates
Test for ammonia i) 10 ml. of urine + 4 drops of phenolphthalein + drop by drop NaOH till a faint pink colour is obtained. Boil the urine, and hold a red litmus paper in vapour. ii) Expose the vapours to a glass rod dipped in con.Hcl	The red litmus turns to blue. Dense white fumes are observed.	Presence of ammonia
Test for calcium 10 ml. of urine + few drops of NaOH + 1 % acetic acid + 2-3 ml of ammonium oxalate solution.	White ppt. of calcium oxalate.	Presence of calcium

Experiment No: 2

QUALITATIVE ANALYSIS OF NORMAL INORGANIC CONSTITUENTS OF UNKNOWN URINE SAMPLE

AIM: To identify normal inorganic constituents of urine in the given sample.

TEST	OBSERVATION	INFERENCE
Test for bicarbonate		
Test for chloride		
Test for phosphate		
Test for sulphate		
Test for ammonia		
Test for calcium		

TEST	OBSERVATION	INFERENCE
Test for urea **a) Effervescence test:** 3ml of urine+5 drops of sodium hypobromite. **b) Specific urease test:** 3ml urine + 2 drops of phenolphthalein + pinch of urease powder. Mix & allow to stand for 5mins.	Brisk effervescence is seen	Urea is present.
Test for uric acid a) **Phosphotungstinic reduction test:** 3ml urine + 0.5ml of Phosphotungstinic acid + few drops of sodium carbonate.	Blue colour develops	Uric acid is present.
Test for creatinine **a) Wayne's test:** 3ml urine+ 3ml sod.nitroprusside + 10% NaOH. **b) Jaffe's test** 3ml urine + 1ml saturated picric acid + 1ml 10% NaOH.	Ruby red colour which changes to yellow Orange red colour is formed. Deep orange colour is seen	Creatinine is present.
Test for Ethereal sulphate: 3ml urine sample+ 1ml of conc. Hcl + 2ml $BaCl_2$. Mix & filter. Divide the filtrate into 2 parts. Boil one test tube & compare control.	Turbidity is not seen	Ethereal sulphate is absent.
Test for Urobilinogen: 3ml urine + 1ml of elhrich reagent. Mix & allow to stand for 5mins.	No reaction	Urobilinogen is absent.

QUALITATIVE ANALYSIS OF NORMAL ORGANIC CONSTITUENTS OF URINE

CONSTITUENTS	NORMAL	SIGNIFICANCE
Urea	25-30g/lit	Major product of protein metabolism. Increased in fever, diabetes and excessive adrenal cortex activity.
Uric acid	0.7g/lit	Increased in leukaemia, severe liver disease, and certain stages of gout.
Creatine and creatinine	1.2-1.7 g/lit	Increased in dystrophy and starvation.
oxalates	10-30 mg/lit	Increased in diabetes and liver disorder.
Hippuric acid	0.7 g/lit	Increased after vegetable diet.
Urinary pigments	In traces	Increased in lead poisoning

REPORT :

ABNORMAL CONSTITUENTS OF URINE

Proteinuria (protein in urine)

Small molecular weight proteins (such as Peptide Hormones, Insulin, Glucagon, Growth Hormone) can appear in the GF, these filtered proteins are largely reabsorbed by the proximal tubules via pinocytosis. Thus normally only trace amount of proteins are present in the urine that are not readily detected.

Albumin is the major protein present in **PROTEINURIA.**

Causes of Proteinuria:

a) Physiological proteinuria (0.5% of Protein Present)

Examples of such cases include severe exercise, standing for a long time (due to temporary impairment of renal circulation),Pregnancy.

b) Pathological Proteinuria

1. Pre-Renal : when thePrimary causes are factors operating before the kidney, such as a heart problem causing an impaired renal circulation, multiple myeloma.
2. Renal : when the lesion is in the kidney as in glomerulonephritis.
3. Post-Renal : as inflammation of the lower urinary tract.

GLYCOSURIA : It is a term used to refer to the presence of an unusual amount of reducing sugar in urine,once the specific sugar is identified it is given a more specific description glucosuria, fructosuria.

This term is used to indicate an abnormal detection of glucose in urine. Normally not more than 10-20mg/dl,unusual amounts of glucose can be found in urine after anesthesia or emotional stress.
It can be due to hyperthyroidism.
Renal glucosuria which is caused by a disorder of the renal tubular function.

KETONURIA(KETONE BODIES) : It refers to the presence of unusual amount of ketone bodies in urine.Normally only 3-15 mg are excreted per day.
Ketonuria can occur in cases such as ketosis and in starvation,excess fat metabolism.
Bilirubin : it is not present normally in significant amount since it is mainly excreted through bile, unusual amounts of bilirubin is detected in urine in cases of obstructive jaundice and unusual amounts of uribilinogen seen in cases of hemolytic jaundice.

QUALITATIVE ANALYSIS OF ABNORMAL CONSTITUENTS OF URINE SAMPLE

TEST	OBSERVATION	INFERENCE
• ***Test for proteins*** (a) **Haller's nitric acid ring test:** (Principle: Nitric acid causes precipitation of protein) To 3 ml of nitric acid in a tube add 3 ml of urine by the wall of the tube in such a way that the two liquids do not mix.	White ring appears at the junction of the two fluids	Indicates the presence of albumin
b) **Sulphosalicylic acid test:** (Principle: Albumin, the protein, is denatured by sulphosalicylic acid a coagulation.) Add a few drops of Sulphosalicylic acid to 2 ml of urine. Let it stand for 5mins & observe turbidity.	Turbidity appears	Indicates the presence of albumin
c) **Heat coagulation test**: (Principle: The albumin is coagulated after being heated.) Fill 3/4th of the test tube by urine. Heat the upper 1/3rd of the test tube by a small flame.	Turbidity appears on the heated portion of the tube.	Indicates the presence of albumin
• ***Tests for glucose*** a) **Bendict's Test:** (Principle: Copper sulphate of Benedict's qualitative solution is reduced by reducing substances on boiling to form the coloured precipitate of cuprous oxide.)	Blue colour appears Light green precipitate appears Green precipitate appears Yellow precipitate appears Brick red precipitate appears	Sugar Absent 0.1-0.5 % of reducing sugar present 0.5 to 1.0 % of reducing sugar present 1-2 % reducing sugar present Above 2 % reducing sugar present

b) **Fehling's test:** 2ml of Urine and add 2.5ml of Fehling's A & B each. Heat & cool.	Red ppt. is seen.	Glucose is present.
Test for ketone bodies • **Rothera's test:** (Principle: Acetoacetic acid forms a complex with nitroprusside in alkaline solution developing a permanganate colour) Saturate 5 ml of urine with ammonium sulphate by shaking vigorously. Then add 2 drops of freshly prepared 5% solution of sodium nitroprusside and 1 ml of ammonium hydroxide/strong ammonia along the sides of test tube. Allow it to stand in a rack for a while without disturbing. • **Gerhard's test:** To 3ml of urine add 0.5ml of ferric chloridde drop by drop.	A permanganate colour develops just above the layer of un dissolved ammonium crystals. Violet colour is formed.	Indicates the presence of Ketone bodies like acetoacetic acid. (Acetone is absent) Indicates the presence of Ketone bodies like acetoacetic acid.
Tests for Bile salts **Hay's test:** To 5ml of urine in beaker, sprinkle little sulphur powder & observe without shaking.	Sulphur sinks to bottom	Bile salts are present.
Tests for Bile Pigments To 5ml of urine add 2ml of 10% Bacl2 and a pinch of Mag.sulphate. Mix well & allow to stand for 5mins. Filter the solution, unfold the filter paper & add few drops of fouchets reagent on ppt.	Blue or light green colour is observed.	Presence of bile pigments.

Tests for Blood **Benzidine test:** To 3ml of urine add mixture of 2 drops of benzidine & 2 drops of Hydrogen peroxide.	Blue/green which is stable is absent.	Absence of blood.

Experiment No: 3

QUALITATIVE ANALYSIS OF ABNORMAL CONSTITUENTS PRESENT IN UNKNOWN SAMPLE

AIM: To identify any presence of abnormal constituents which can be found in unknown urine sample.

TEST	OBSERVATION	INFERENCE
• ***Test for proteins*** (a) **Haller's nitric acid ring test:** (Principle: Nitric acid causes precipitation of protein) To 3 ml of nitric acid in a tube add 3 ml of urine by the wall of the tube in such a way that the two liquids do not mix. **b) Sulphosalicylic acid test:** (Principle: Albumin, the protein, is denatured by sulphosalicylic acid a coagulation.) Add a few drops of Sulphosalicylic acid to 2 ml of urine. Let it stand for 5mins & observe turbidity. c) **Heat coagulation test**: (Principle: The albumin is coagulated after being heated.) Fill 3/4th of the test tube by urine. Heat the upper 1/3rd of the test tube by a small flame.		
• ***Tests for glucose*** a) **Bendict's Test:** (Principle: Copper sulphate of Benedict's qualitative solution is reduced by reducing substances on boiling to form the coloured precipitate of cuprous oxide.)		

b) **Fehling's test:** 2ml of Urine and add 2.5ml of Fehling's A & B each. Heat & cool.		
Test for ketone bodies • **Rothera's test:** (Principle: Acetoacetic acid forms a complex with nitroprusside in alkaline solution developing a permanganate colour) Saturate 5 ml of urine with ammonium sulphate by shaking vigorously. Then add 2 drops of freshly prepared 5% solution of sodium nitroprusside and 1 ml of ammonium hydroxide/strong ammonia along the sides of test tube. Allow it to stand in a rack for a while without disturbing. • **Gerhard's test:** To 3ml of urine add 0.5ml of ferric chloridde drop by drop.		
Tests for Bile salts **Hay's test:** To 5ml of urine in beaker, sprinkle little sulphur powder & observe without shaking.		
Tests for Bile Pigments To 5ml of urine add 2ml of 10% Bacl2 and a pinch of Mag.sulphate. Mix well & allow to stand for 5mins. Filter the solution, unfold the filter paper & add few drops of fouchets reagent on ppt.		
Tests for Blood **Benzidine test:** To 3ml of urine add mixture of 2 drops of benzidine & 2 drops of Hydrogen peroxide.		

REPORT:

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QUANTITATIVE ANALYSIS OF URINE

Experiment No: 4

ESTIMATION OF REDUCING SUGAR USING BENEDICT'S METHOD

AIM: To estimate the amount of glucose present in the given unknown solution using Benedict's quantitative reagent.

PRINCIPLE: Benedict's quantitative reagent is a modification of qualitative aspects. It contains copper sulphate-sodium acetate and sodium carbonate. It also contains potassium thiocyanate and small amount of potassium ferricynate. The inclusion of acetate prevents the precipitation of copper car boning by chelating Cu3+ ions. The thiocynate causes with the precipitation of white cuprous thiocynate rather than red cupric oxide. On the reduction of Cu3+ ions, which inhibits the end point of the titration digest the transition from blue to white to be readily observed. Methylene blue will be used as an additional indicator. The small amount of potassium ferricyanaide prevents the pre oxidation of copper .The non-stiochemetric reaction is on which not follow a defined pathway and cannot be described by an equation either quantitatively or qualitatively. The reduction of Cu3+ ions by sugar is a nonstiochemetric equation and is only constant over a small range of sugar concentration. To obtain accurate results the volume of sugar added must be within 6-12ml for 10ml of Benedict's regent.

REAGENTS REQUIRED:

(i) Urine sample to be filled in burette.

(ii) **Benedict's quantitative reagent:** 100mg of sodium citrate and 62.5gm potassium thiocynate were dissolved in 300ml of distilled water by warming gently and filtered.18% of copper sulphate is dissolved in 50ml of water, added with continuous stirring. 2.5ml of 5% potassium ferricyanide is added and volume is made up to 500ml with water.

(iii) Anhydrous sodium carbonate.

PROCEDURE:

- 10ml of Benedict's reagent was pipetted out into a clean conical flask.
- Add two spatulas of anhydrous sodium carbonate was added to provide the required alkalinity with a few porcelain bits and heated to boiling over a moderate flame directly on the wire gauze & titrate against urine sample which is in burette. When the Benedict's solution boils continuously, add urine sample drop by drop (1 drop/sec) till last trace of blue colour disappears which indicates end point. The volume of urine rundown is noted and the titrations are repeated to get concordant values.

NOTE: Titration should be done in BOILING CONDITIONS ONLY until disappearance of blue colour to white ppt.

CALCULATIONS:

TITRATION TABLE:-

S.NO	CONTENTS IN FLASK	BURETTE READING		VOLUME OF TITRANT CONSUMED	ENDPOINT
		INITIAL	FINAL		
1					
2					
3					

50ml Benedicts quantitative reagent oxidises 100mg of glucose
10ml of BQR oxidises 100*10/50 = 20mg
Therefore, Amount of glucose present in urine = 20mg of glucose/burette reading *100ml

=_____50mg/dl

REPORT:

Experiment No: 5

ESTIMATION OF CHLORIDE IN URINE BY VOLHARD'S METHOD

AIM: To estimate chloride in given urine sample.

Apparatus: Conical flask, burette, pipette.

Chemicals and reagents: Standard $AgNO_3$, standard NH_4 CNS solution, 10% ferric alum, conc. HNO_3

Clinical significance:

On an average diet 8-15 gm (or 170-250 m Eq) of chloride as NaCl are excreted per day. Low serum chloride levels results in Vomiting and diarrhea, and diminished excretion in urine. When the serum levels are much below 103 m Eq of chloride / liter, the urinary excretion of chloride becomes very small.

In Cushing's syndrome and steroid therapy, decreased excretion of chloride is observed. When there is retention of chloride in the body fluids as in some cases of chronic nephritis and inflammation leading to formation of large exudates, chloride excretion diminishes.

Principle:

Urine chloride is precipitated quantitatively as silver chloride when treated with an excess of silver nitrate and concentrated HNO_3

$$AgNO_3 + NaCl \longrightarrow AgCl + NaNO_3$$

The precipitate is filtered off. Unreacted silver nitrate in the filtrate is treated with ammonium thiocyanate using ferric alum as indicator. Silver thiocyanate is formed during the titration and is precipitated.

$$AgNO_3 + NH_4CNS \longrightarrow AgCNS + NH_4NO_3$$

When all the silver nitrate has been converted to silver thiocyanate, further addition of ammonium thiocyanate results in a dark red brown colour due to the formation of ferric thiocyanate. This is the end point of titration.

$$6\ NH_4CNS + Fe_2(SO_4) \longrightarrow 2\ Fe(CNS)_3 + 3\ (NH4)\ SO_4$$

PROCEDURE:

Pipette 10ml urine in to a 5o ml standard flask, Add 10 drops of concentrated HNO_3 and 2 ml ferric alum. Slowly add 10ml $AgNO_3$ solution with constant rotation of the flask. After 10 min, make up to 50ml mark with distilled water and mix. Filter through a dry filter paper into a clean dry conical flask. Pipette 20 ml of the filtrate into another conical flask and Titrate against ammonium thiocyanate solution taken in a 2ml a graduated pipette.

Note: The titer value when a permanent tinge of reddish brown colour appears. Repeat the titration with blank. (Take 10 ml of water instead of sample).

OBSERVATIONS:

Sample:

Sl.No	Burette reading		Volume Consumed V_1
	Initial	Final	
1.			
2.			

Blank:

Sl.No	Burette reading		Volume Consumed V_2
	Initial	Final	
1.			
2.			

Calculations:

1 ml AgNO3 solution = 10mg Nacl

The volume of free $AgNO_3$ in the 20ml of the filtrate = $\mathbf{V_1}$

The volume of $AgNO_3$ added in the sample = $\mathbf{V_2}$

Volume of $AgNo_3$ that has reacted with chloride in the 20 ml of urine sample = $\mathbf{V_2 - V_1}$

Amount of chloride present in 20 ml of urine = $\mathbf{(V_2 - V_1) \times 10}$

% of chloride present in the urine sample = $(V_2 - V_1) \times 10 \times 5$

=_____mg

REPORT:

Note:

- When the tubular re absorption of chloride is impaired as in the case of Addison's disease, urinary excretion of chloride is appreciable even when the serum level is as low as low as 85mEq/litre

Experiment No : 6

ESTIMATION OF CREATININE IN URINE BY JAFFE'S METHOD

AIM: To determine the amount of creatinine in given urine sample.

APPARATUS REQUIRED: Pipette, volumetric flask, beaker, cuvette etc.,

CHEMICALS REQUIRED:1% Picric acid,10% NaOH,stnd.creatinine sol.,urine sample,water etc.,

PRINCIPLE:

Creatinine is a breakdown product of creatine, which is an important part of muscle. Creatinine is removed from the body entirely by kidneys. Creatinine can also be measured by a blood test. Creatinine in urine is estimated by modified Folin method using photoelectric method. Urine is allowed to react the picric acid in an alkaline medium to form an orange coloured complex i.,e creatine picrate. The O.D of this complex is measured at a wavelength of 520nm. The O.D of sample sol. is compared to that of Stnd. solution having known amount of creatinine.

PROCEDURE:

	TEST	STANDARD	BLANK
DISTILLED WATER	-	-	2.5ml
URINE SAMPLE	2.5ml	-	-
STND.CREATININE	-	2.5ml	-
10% NaOH	1ml	1ml	1ml
1% PICRIC ACID	1ml	1ml	1ml

Mix well and allow to stand for 5mins and makeup the solution to 50ml with distilled water. After 15mins, measure the O.D of each solution @ 520nm.

CALCULATIONS:

O.D OF BLANK =

O.D OF STND =

O.D OF TEST =

Concentration of stnd. creatinine =_____mg/ml

2.5ml of sample creatinine sol. contains = O.D SAMPLE/O.D STND * CONC.OF SOLID CREATININE SOL*VOLUME OF STND CREATININE SOLUTION

=X mg/ml

If 2.5ml of sample creatine contains Xmg/ml,

Therefore, 50ml of sample creatinine solution contains = X/2.5*50

=_____mg/ml.

REPORT:

The total amount of creatinine present in 50ml urine sample was found to be_____.

Experiment No : 7

ESTIMATION OF CALCIUM IN URINE BY PRECIPITATION

AIM: To determine the amount of calcium in the given urine sample.

APPARATUS REQUIRED: Burette, pipette, vf, beaker & centrifuge tube.

CHEMICALS REQUIRED: 4% ammonium oxalate, 0.01N KmnO4, conc. H2SO4, ammonia sol.

PRINCIPLE:

Calcium present in urine sample is treated with ammonium oxalate to produce a precipitate of calcium oxalate. The precipitate of calcium oxalate is dissolved in sulphuric acid so that it converts into corresponding equivalent oxalic acid. The oxalic acid so obtained is titrated against 0.01N potassium permanganate.

- The amount of oxalate = The amount of calcium present in the urine.
- The amount of pot permanganate is required = The amount of oxalic acid.
- The amount calcium oxalate = The amount calcium present in the urine.

$Ca^{++} + (NH_4)\,C_2O_4 \longrightarrow CaC_2O_4 + 2NH_4^+$

$CaC_2O_4 + H2SO4 \longrightarrow H_2C_2O_4 + CaSO_4$

$2Kmno_4 + 3H_2SO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 3H_2O + 5[0]$

$H_2C_2O_4 + [0] \longrightarrow H_2O + 2CO2\ x$

$2Kmno_4 + 3H_2SO_4 + 5H_2C_2O_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 10\ CO2$

PROCEDURE:

1. In a centrifuge tube labelled as sample and add 2ml of urine, 2ml of distilled water, 2ml of 4% ammonium oxalate solution & 3-4 drops of methyl red indicator.
2. To the above solution add 2% ammonia solution slowly until the red colour solution turns yellow.
3. Allow to stand for 20-30mins & centrifuge in 1500rpm for 15mins. Discard the supernatant liquid.
4. Add 3ml of ammonia solution to wash all the oxalate from the sides of test tube.
5. Dissolve the precipitate in 1N H2SO4 taken in conical flask and heat on water bath until the precipitate completely dissolved.
6. The resultant solution is titrated with KMnO4 till pale pink colour is obtained. A blank could be carried out by omitting urine sample.

CALCULATION:
TITRAION TABLE:

S.NO	CONTENTS IN FLASK	BURETTE READING		VOLUME OF TITRANT CONSUMED	ENDPOINT
		INITIAL	FINAL		

1ml of 0.01N KMnO4 = 0.2mg Ca++

CA++ IN SAMPLE = (TITRE VALUE-BLANK VALUE) * 0.2*100/2
REPORT:

Experiment No : 8

QUANTITATIVE ESTIMATION OF SERUM CHOLESTEROL BY LIBERMANN BURCHARD'S METHOD

AIM: To estimate the amount of serum cholesterol present in the given sample by Libermann Burchard's method.

Apparatus required: Pipette, centrifuge tube, beaker, cuvette, colourimeter.

Reagents required:

a) Alcohol & ether mixture - 90% ethyl alcohol and ether mixed in ratio of 3 : 1
b) Chloroform
c) Acetic anhydride & sulphuric acid mixture - mixed in the ratio of 20 : 1 before use
d) Stock standard solution - 200 mg of chemically pure cholesterol is dissolved in chloroform and made upto 1000ml with the same.
e) Working standard cholesterol solution - 1 ml of stock standard cholesterol solution is diluted to 25 ml using chloroform. 5 ml of resultant solution consists of 0.4 mg of cholesterol.

Principle:

Cholesterol and cholesteryl esters from serum is extracted into alcohol and ether mixture. The contents in centrifuge tube and protein free extract is evaporated to dryness. The cholesterol residue is then dissolved in chloroform and measured calorimetrically by Libermann Burchard's method. In this method, cholesterol reacts with acetic anhydride and H_2SO_4 mixture to give green colour, whose colour intensity can be measured using calorimeter at a wavelength of 680 nm.

PROCEDURE:

Pipette 12 ml of alcohol ether mixture in dry centrifuge tube. Add 0.1 ml of serum and shake vigorously for 1 min. Keep tube in vertical position for 30 minutes, then centrifuge at 1800 rpm for 5 minutes. Pour the supernatant fluid into a beaker and evaporate it on steam bath. Extract the residue twice with 2 ml portion of chloroform into a dry test tube. Make then volume to 5ml using chloroform and label it as test. Label the other two test tubes as standard and blank. Pipette 5 ml of standard cholesterol solution and 5 ml of chloroform in to blank respectively. Add 2ml of acetic anhydride - H_2SO_4 mixture into all the 3 test tubes and place it in dark for 15 minutes. Measure the absorbance at 680 nm.

Data Collection:

Optical density of blank =
Optical density of standard =
Optical density of sample =

Calculation:

Cholesterol stock = 20 mg in 100 ml of $CHCl_3$ = 0.2 mg

Cholesterol working standard = 1 ml to 25 ml $CHCl_3$ (0.2 mg in 25 ml)

$$5\text{ml} = \frac{0.2 \times 5}{25} = 0.04\ mg\ (C_s)$$

$$\text{Cholesterol in serum} = \left(\frac{0.D\ of\ test - 0.D\ of\ blank}{0.D\ of\ standard - 0.D\ of\ blank}\right) \times \frac{conc.of\ standard \times 5}{vol.of\ serum\ (0.1 \times 5)} \times 100$$

Clinical Significance:

The normal total serum cholesterol in adults ranges from 130- 200 mg/dl. Hypercholestremia ia associated with conditions like hypothyroidism, uncontrolled diabetes, cholestasis, nephrotic syndrome, hypolipoproteinemia etc. Cholesterol level increases with age and also in third trimester of pregnancy. It also increases in those individuals who consume food rich with saturated fatty acids. Increased levels of cholesterol predisposing factor for atherosclerosis, coronary artery disease, myocardial infarction and other disorders of the heart. Hypercholestremia is seen in hyperthyroidism..

REPORT:

The amount of cholesterol present in given serum sample was found to be_____mg/dl.

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QUANTITATIVE ANALYSIS OF BLOOD

Experiment No : 9

PREPARATION OF FOLIN-WU FILTRATE FROM BLOOD

AIM : To prepare follin - wu filtrate.

Apparatus required : Pipette, volumetric flask, beaker and funnel.

Chemicals required : 0.65N H_2SO_4, 10% sodium tungstate solution.

Principle:
Since the blood is coloured , it cannot be used directly for calorimetric reactions. A follin - wu filtrate is prepared by precipitating the protein in the blood by the use of sodium tungstenic acid. In order to complete the precipitation , H_2SO_4 is added.

Experimental procedure:
In a centrifuge tube, add 7ml of distilled water and 1ml of blood. The contents are mixed well, 1 ml of sodium tungstate solution is added along with 1 ml of 0.66N H_2SO_4 and is added to above mixture in portions by using a graduated pipette with constant shaking. The colour of precipitate gradually turns from red to brown. The solution is kept for 10 minutes and filtered. The filtrate is cleaned and colourless. The obtained filtrate is known as follin - wu filtrate.

Conclusion:
Follin - wu filtrate was prepared and submitted.

Expirement No : 10

ESTIMATION OF CREATININE IN SERUM

AIM : To determine the amount of creatinine in given serum sample

Apparatus required: Pipette, volumetric flask, beaker and cuvette,

Chemicals required: 1% picric acid, 10% NaOH, standard creatinine solution, urine sample, distilled water.

Principle:

Creatinine is the breakdown product of creatine, which is an important part of muscles. Creatinine is removed by the body entirely by the kidneys. Creatinine can also be measured by blood test. Creatinine in urine is estimated by modified follin - wu method using photoelectric method. Urine is allowed to react with picric acid in alkaline medium to form a orange coloured complex, creatinine picrate (Jaffe's reactions). The optical density or absorbance of this complex is measured at a wavelength of 520nm. The O.D of sample is compared to that of standard solution having known amount of creatinine.

Procedure:

	TEST	STANDARD	BLANK
Distilled water	-	-	2.5 ml
Urine sample	2.5 ml	-	-
Standard creatinine		2.5 ml	-
10% NaOH	1 ml	1 ml	1 ml
1% picric acid	1 ml	1 ml	1 ml

Mix well and allow to stand for 5 minutes. Measure the optical density of each solution at 520 nm.

Clinical Significance:

Any structure that impairs the functions of kidney is likely to raise the creatinine level in blood. It is important to recognize whether the process leading to kidney dysfunction is longstanding or recent. Recent elevation may be more easily treated and reversed.

The most common cause of longstanding kidney disease in adults are high blood pressure and diabetes mellitus. Certain drugs can sometimes cause abnormally elevated creatinine level. Serum creatinine can also transiently increase after injection of a large amount of dietary meat, thus nutrition can play a role in creatinine measurements.

Data collection:

Optical density of standard =
Optical density of blank =
Optical density of test =

Calculations:

Concentration of standard creatinine = 1 mg/ ml

= 1ml of stock + 99 ml of H_2O

Working stock = $\frac{1mg}{100ml}$

Amount of creatinine = $\frac{0.D\ of\ test - 0.D\ of\ blank}{0.D\ of\ standard - 0.D\ of\ blank} \times \frac{conc.of\ std \times vol.of\ std \times 100}{vol.of\ std \times dilution\ factor\ of\ sample}$

REPORT: The total amount of creatinine is found to be_____mg/dl.

Expirement No : 11

ESTIMATION OF GLUCOSE IN BLOOD (FOLLIN - WU METHOD)

Introduction and purpose of the experiment:

The importance of testing the blood glucose level comes from the fact that the brain cells are very dependent on the extracellular glucose concentration for their energy supply. Hypoglycemia is likely to impair cerebral functions as well as do the hyperglycemia especially of rapid onset, which can cause cerebral dysfunction by affecting extracellular osmolarity.

AIMS AND OBJECTIVES :

To estimate the amount of glucose in blood.

Apparatus : Pipette, volumetric flask, beaker, spectrophotometer, cuvette, water bath and follin- wu.

Chemicals : Alkaline $CuSO_4$, phosphomolybdic acid, 0.66 N H_2SO_4, sodium tungstate.

Principle:

The proteins in the blood are precipitated by addition of tungstenic acid and H_2SO_4. The protein free filtrate containing glucose is then made to react with alkaline $CuSO_4$ to form cuprous oxide. The cuprous oxide formed is treated with phosphomolybdic acid and is reduced to phosphomolybdus acid which gives blue solution. The intensity of blue solution is measured using colourimeter and compared with standard and blank. O.D are used to estimate amount of blood glucose in 100 ml of blood.

PROCEDURE:

In a 50ml beaker, 7 ml of distilled water and 1 ml of blood is added. The contents are mixed well, 1 ml of sodium tungstate solution is also added along with 1 ml of 0.66 N H_2SO_4 and is added to above mixture in portions by using a graduated pipette with constant shaking. The colour of the precipitate gradually turns from red to brown. The solution is kept for 10 minutes and then filtered. The filtrate obtained is clean and colourless. The obtained filtrate is known as follin - wu filtrate.

	TEST	STANDARD	BLANK
Distilled Water	-	-	0.2 ml
Serum (Follin-wu)	0.2 ml	-	-
Std. glucose	-	0.2 ml	-
Alkaline $CuSO_4$	2 ml	2 ml	2 ml
Phosphomolybdic acid	2 ml	2 ml	2 ml

Boil the test tubes for 15 - 20 minutes on a boiling water bath and cool for 2 minutes.
Mix well and allow to stand for 5 minutes. Make up the volume of the solution to 25 ml with distilled water. Measure the optical density of each solution at 530nm.

REPORT:

The amount of glucose present in 100 ml of blood =_____mg.

Experiment No: 12

ESTIMATION OF SGOT IN SERUM

AIM : To determine the amount of SGOT in the given serum sample.

Apparatus required: Test tube, micro pipette, water bath and micro centrifuge.

Principle:

Some of the reasons for higher level of AST are:

a) Liver damage caused by infection such as viral hepatitis or mononucleosis
b) Gall bladder disease
c) Poisons, such as too much alcohol
d) Cancer
e) Muscle damage caused by:
    i. A muscle disease
    ii. An injury, such as after a fall, auto accident or seizure.
f) Kidney, pancreas, heart or liver disease or damage
g) Medicine that affects the test results

PROCEDURE:

- Pipette out 800µL of reagent I to a test tube.
- Pipette out 200µL of reagent II to the same solution.
- Keep it in water bath maintained at 37°C for 1 minute.
- After 1 minute, pipette out 100µL of serum to the reagent mixture.
- Mix the solution and keep it for 1 minute in water bath at 37°C.
- After 1 minute, measure the absorbance at 340nm by UV spectrophotometer, which is set autozero using distilled water.
- Measure the absorbance after 2 minutes and 3 minutes.
- Calculate the rate of change in absorbance to determine the activity of SGOT enzyme.

Clinical significance:

This blood test measures an enzyme called aspartate amino transferase (AST). Enzymes are the chemicals that help the cells of your body work. AST is found in RBC's, liver cells and muscle cells, including heart. It is released into the blood when the C cells are damaged. This enzyme is also called serum glutamic oxaloacetic transaminase or SGOT. SGOT is estimated by UV method using the kit. The absorbance is measured at 340nm. SGOT catalyses the transfer of amino group from aspartate to α- ketoglutarate. The oxaloacetate, so formed is converted to malate by malate dehydrogenase present in reagent R1. The rate of absorbance is measured to determine the activity of SGOT.

REPORT:

The SGOT activity was found to be_____U/L.

Experiment No: 13

ESTIMATION OF SGPT IN SERUM

AIM : To determine the amount of SGPT in the given serum sample.
Apparatus required: Test tube, micro pipette, water bath and micro centrifuge.
Chemicals required : SGPT kit, serum.

Principle:
Some of the reasons for higher level of ALT are:

a) Liver damage from an viral infection as hepatitis
b) Liver damage caused by medicines taken
c) Drinking too much alcohol
d) Mononucleosis
e) Muscle injury
f) Liver or gall bladder diseases such as gall stones, liver cancer, or liver failure.
g) Medicine that affects the test results

PROCEDURE:

- Pipette out 800µL of reagent I to a test tube.
- Pipette out 200µL of reagent II to the same solution.
- Keep it in water bath maintained at 37°C for 1 minute.
- After 1 minute, pipette out 100µL of serum to the reagent mixture.
- Mix the solution and keep it for 1 minute in water bath at 37°C.
- After 1 minute, measure the absorbance at 340nm by UV spectrophotometer, which is set auto zero using distilled water.
- Measure the absorbance after 2 minutes and 3 minutes.
- Calculate the rate of change in absorbance to determine the activity of SGPT enzyme.

Clinical significance:
This blood test measures an enzyme called alanine transferase (ALT). Enzymes are the chemicals that help the cells of your body work. ALT is an enzyme made in liver cells. It is released into the blood when tissues are damaged. This enzyme is also called serum glutamic pyruvate transaminase or SGPT. SGPT catalyses the transfer of amino group from L - alanine and α- ketoglutarate to form pyruvate and L - glutamate. The pyruvate, so formed is converted to lactate by lactate dehydrogenase present in reagent R1. The rate of absorbance is measured to determine the activity of SGOT.

REPORT:

The total amount of SGPT was found to be_____ U/L.

Experiment No: 14

ESTIMATION OF UREA IN SERUM

Introduction and Purpose of experiment :

Normal blood urea level in blood ranges from 13 - 36 mg/ml.

Increased levels : In healthy individuals, urea concentration with dietary proteins, ageing in late pregnancy.

Pathological : Incomplete urea clearance from bogy as in renal failure, acute and chronic glomerular nephritis, polycystic kidney, malignant hypertension, pancreatitis, vomiting, septicaemia.

Decreased levels : In children during growth than in adults, in early normal pregnancy, in individuals on low protein diet and high carbohydrate intake.

Pathological : Acromegaly, acute necrosis of liver, after haemodialysis, toxic hepatitis.

AIM: To estimate the amount of urea in serum sample.

Apparatus required : Test tube, stand, pipette, cuvette.

Chemicals required:

- Diacetyl monoximine : 2 % in water made upto 100 ml.
- Acid reagent : 75 mg semi carbazide + 100 ml water + 30 ml phosphoric acid + 160 ml of H_2SO_4 acid. Volume made upto 1 litre after cooling.
- Standard urea solution : 40 mg of urea is dissolved in 100 ml of water into which 0.3 ml of 4 N sulphuric acid and 4 mg of phenyl mercuric acetate have been dissolved earlier.

Principle :

Blood sample is treated with diacetyl monoximine under strong acidic condition and heat. Diacetyl released from the reagent condenses with urea present in blood sample to form yellow coloured complex which turns pink on reaction with thiosemicarbazide. The colour intensity for the resulting solution is measured using a green filter at 540 nm and the concentration of urea in mg/100 ml is calculated.

Calculations :

Optical density of blank =
Optical density of standard =
Optical density of test =
Concentration of standard urea solution = 40 mg/ml

0.2 ml of follin - wu filtrate contains= $\frac{\text{O.D of sample}}{\text{O.D of std solution}}$ ×conc. of std urea soln× vol of std urea

=

10 ml of follin - wu filtrate is equivalent to 1 ml of blood.

0.2 ml of follin - wu filtrate = $\frac{1}{10} \times 0.2$

=

If 0.2 ml of Follin - wu filtrate contain 0.118 mg of urea.

0.2 ml of blood contains 0.118 mg urea.

Hence 100 ml of blood contains = $\frac{0.118}{0.02} \times 100$

=_____mg of urea

PROCEDURE:

1. Label 3 test tubes as blank, standard and test.
2. Add 0.2 ml of follin - wu filtrate into test, 0.2 ml of standard urea in standard and 0.2ml of distilled water into blank.
3. Add 1 ml of diacetyl monoximine and 5 ml of cid reagent to all the test tubes.
4. Mix well and heat the test tubes in boiling water bath for 5 minutes. Allow to cool and measure the O.D at 540 nm.

REPORT:

Amount of urea present in 100 m of blood sample was found to be_____mg of urea.

Experiment No: 15

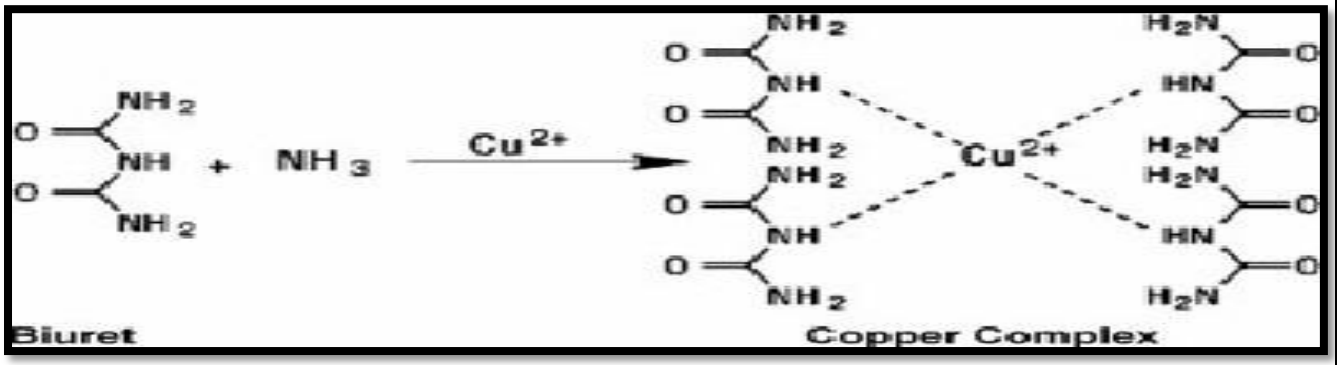
QUANTITATIVE ANALYSIS OF PROTEINS BY BIURET REAGENT METHOD

AIM: To estimate the amount of protein by using Biuret reagent method.

Apparatus and Glass wares required: Test tubes, Pipettes, Colourimeter, etc.,

Chemicals & reagents: Biuret reagent, distilled water, protein standard(Albumin).

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.



Reagents Required:

- PREPARATION OF BIURET REAGENT-
- Dissolve 45gm of sodium pot.tartarate in 400ml of 0.2M NaOH. Add 15gm CuSO4 in powder to this & stir well. After dissolving the salt, add 5gms Potassium Iodide & make up the sol. to 1 litre with 0.2M NaOH, filter if necessary.
- Protein Standard: 5 mg BSA/ml.

Procedure:

1. 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.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and warm at 37 ^{o}C for 10 min.

6. Now cool the contents to room temperature and record the absorbance at 540 nm against blank.
7. Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis.
8. Then from this standard curve calculate the concentration of protein in the given sample.

Volume of Standard BSA (ml)	Volume of Distilled water (ml)	Conc. of protein (in mg)	Vol. of Biuret reagent (ml)		Absorbance @ 540nm
				INCUBATE AT 37°C FOR 10MINS & COOL	

(graph need to be attached)

Notes:

Bovine serum albumin (also known as **BSA** or **"Fraction V"**) is a serum albumin protein derived from cows. It is often used as a protein concentration standard in lab experiments.

The nickname "Fraction V" refers to albumin being the fifth fraction of the original Edwin Cohn purification methodology that made use of differential solubility characteristics of plasma proteins. By manipulating solvent concentrations, pH, salt levels, and temperature, Cohn was able to pull out successive "fractions" of blood plasma. The process was first commercialized with human albumin for medical use and later adopted for production of BSA.

REPORT:

The given unknown sample contains ---- mg protein/ml.

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ENZYMES

Experiment No: 16

Determination of hydrolysis of starch by Amylases

AIM: To estimate the amount of hydrolysis of starch by Amylases.

Apparatus and Glass wares required: Test tubes, Pipettes, Colourimeter, etc.,

Chemicals & reagents: Starch, distilled water.

Principle:

Enzyme activity depends on several factors, including enzyme concentration, substrate concentration, pH, temperature, and presence of heavy metal cations. Your own saliva containing the amylase enzyme will be used for this experiment, although the levels of amylase vary considerably from one person to another. Each experiment must be timed. As you proceed with each experiment, you will check enzyme activity by reacting a few drops of the reaction mixture with iodine. The time at which the blue-black colour of starch does not appear will be noted in each experiment. The time required for the hydrolysis of starch will be correlated to the relative enzyme activity. When enzyme activity is high, the time for the starch to hydrolyze will be very short. When the enzyme is operating poorly or not at all, the activity is low, and more time will be required for the starch to hydrolyze. In some cases, the enzyme will be completely inactivated and the blue-black colour of the starch and iodine will persist throughout the entire experiment. Graphs will be prepared showing the effects of concentration, pH, temperature, and heavy metal cations on the relative enzyme activity.

PROCEDURE:

1. Collect approximately 5 mL of saliva in a medium test tube.
2. Place 2 droppers full of 1 % starch solution in each of 5 medium test tubes. Number the test tubes 1-5.
3. Place the tubes in a 37-40 °C water bath. After 5 minutes, add the following amounts of saliva to the test tubes as quickly as possible, mixing each solution thoroughly, and then returning the tubes to the water bath. Do not overheat the bath or you will inactivate the enzyme.
4. Prepare a spot plate for testing for the presence of starch in the samples by placing one drop of iodine reagent in each of five depressions on the spot plate.
5. Two minutes after the addition of saliva, transfer one drop from each test tube (using a different pipet for each tube) to a separate drop of iodine in the spot plate. Note the colour produced for each. Remember that the complex formed by starch and iodine is an indigo blue. If the colour of the iodine solution remains red or gold after adding the starch solution, the starch has been completely hydrolyzed.

6. As soon as one of your starch solutions has hydrolyzed, use it to begin preparing the solutions for Parts B and C below. Continue to test the remaining starch solutions that have not yet hydrolyzed.
7. Clean the spot plate and then prepare it for the next testing by placing one drop of iodine reagent in each of five depressions.
8. Repeat the testing at 5 minutes after the addition of saliva, and at 5 minute intervals thereafter. Continue testing for 20 minutes, or until the blue-black colour no longer appears for each sample.
9. Record the time required for the hydrolysis of starch in each sample.

REPORT:

Experiment No : 17

EFFECT OF pH ON ENZYME ACTIVITY

AIM : To study the effect of pH on enzyme activity of a sample of salivary amylase.

Apparatus required : Pipette, volumetric flask, beaker, test tube, and funnel.

Chemicals required : Saliva sample, DNS reagent and buffer reagent.

Principle:

Enzyme has an optimum pH and are inactive in higher or lower pH. The optimum pH is the temperature at which the enzyme shows maximum activity. The optimum pH for most of the enzymes are 5-9. The curve of pH against enzyme activity is a bell shaped and indicates the activity falls rapidly on both sides of optimum pH. The optimum pH depends on temperature, substrate and buffer concentration.

PROCEDURE:

1. Setup 12 test tubes into which add 0.5ml starch and 0.5 ml of saliva, 1.5 ml of different buffers having different pH.
2. The test tube is incubated for 15minutes at 37°C and then the reaction was started by adding 1 ml of DNS to each test tube respectively.
3. Boil the test tubes for 10 minutes. Cool and add 5 ml of distilled water into each test tube. Mix and measure the O.D at 540 nm.

REPORT:

Experiment No : 18

EFFECT OF TEMPERATURE ON ENZYME ACTIVITY

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

Apparatus required : Pipette, volumetric flask, beaker, test tube, thermometer and funnel.

Chemicals required : Saliva sample, DNS reagent and buffer reagent.

Principle: The enzyme activity increases with increase in temperature upto a certain point, normally 37-40 C, after the enzyme gets inactivated as a result of denaturation. At elevated temperature, the enzyme protein undergoes thermal decomposition, that decrease the effective concentration of enzyme. Therefore the effect of temp on activity of amylase is studied at pH 6.9 with varying the temp. The amylase is incubated with the starch substrate and varying temp in pH buffer for a specified period of time and the reaction is arrested with addition of DNSA reagent. The amylase activity is determined by measuring the amount of maltose released, for each temp with the help of Standard maltose curve.

The optimum temp of amylase enzyme is noted by plotting the graph of temperature on X-axis and enzyme activity on y-axis.

PROCEDURE:

Preparation of salivary α-amylase: Wash the mouth thoroughly with distilled water. Take 20 ml of distilled water into the mouth and gargle it for 15 minutes, Collect the diluted saliva into the beaker and used for estimation.

1. Setup 8 test tubes into which add 0.5ml starch and 1.5 ml of distilled water.
2. Maintain the temperature for 5 minutes at the following temperatures 0°C, 10°C, 25°C, 37°C, 48°C, 60°C, 75°C, 95°C with occasional shaking.
3. Then add a5 ml of salivary amylase mix and incubate for respective temperature for 5 minutes.
4. Prepare a blank and eliminate the saliva sample.
5. After 15 minutes add 1 ml DNS reagent and 5ml of distilled water into each test tube. Cool and measure the optical density at 540 nm.
6. Plot a graph of optical density *vs* temperature and prepare optimum temperature of salivary amylase.

Clinical Significance:

The velocity of enzyme activity increases with temperature and reaches a maximum the declines. The optimum temperature is the temperature at which the enzyme shows maximum activity. The temperature at which maximum amount of reactant is converted into product in a unit time is called optimum temperature. Temperature coefficient is defined as the increase

in enzyme activity when the temperature is increased by 10°C. Increase in temperature increases the activation energy of reactants and collision increases and more amount of reactant is converted to product. The optimum temperature for most of the enzymes are 40 - 45 °C. Above that the enzyme denaturation occurs in lower temperatures, the enzyme becomes inactive and rate of enzyme reaction is also diminished . However a few enzymes are active in low and higher temperatures. This may be due to the stable confirmation or rigid structure.

REPORT:

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BUFFERS

Experiment No: 19

PREPARATION OF BUFFER AND THEIR pH

Introduction and purpose of experiment :
Buffer solutions are defined as those solutions which changes the pH on addition of small quantity of acid or alkali solution from Henderson - Hasselback equation. The pH of buffer solution depends upon pKa constant and ratio of salt and acid. The ratio is considered to be same as the amount of salt and acid mixed together over the pH range 4 - 10 when the concentration of hydrogen and hydroxyl ions are very low and can be ignored.

AIM AND OBJECTIVES :
To prepare buffer solutions and measure their pH.

Experimental Setup :
Volumetric flask, burette, pipette, beaker.

Principle:
The solutions that are able to resist the change in pH are termed as buffer solutions. A buffer solution consists of a mixture of weak acid and its salt or a weak base and its salt. To such solutions, when a small amount od acid or alkali is added, no significant change in pH takes place.
Types of buffer solutions
These are mainly 2 types of buffer solutions:

- Acid buffer solution - The solution having a mixture of weak acid and its salt.

- Basic buffer solution - The solution having a mixture of weak base and its salt.

Significance of buffer solutions:
Buffer solutions help to maintain acid - base balance in our body. The maintenance of normal pH range within the body fluids becomes essential since the biochemical reactions that takes place in the living systems are very sensitive to even small changes in acidity and alkalinity. Saliva has a pH between 6 and 7.5 which is necessary for functioning of ptylin , an enzyme in saliva which digests the carbohydrates.
Buffer systems of the body-
In our body, there are many buffer systems which prevent rapid changes in pH of a body fluid by converting strong acids and bases into weak acuds and bases. Thus, buffers are able to remove excess H^+ions from body fluids.
Following are the major buffer systems of the body fluids:

1) Carbonic acid - bicarbonate buffer system: It is found in plasma and kidney, an important regulator of blood pH. If there are excess of H^+ ions, the HCO_3^- ions act as a weak base and accepts H^+ to form carbonic acid. The carbonic acid dissociates further to yield CO_2 and H_2O molecules. If there is shortage of H^+, then the carbonic acid ionises to release H^+ ions and maintains pH.

2) Phosphate buffer system : The buffer system is able to maintain physiological pH at 7.4. K^+ ion is high in intracellular fluid. Therefore it is an important regulator of pH in the cytosol. This system occurs in the cells and kidneys. The system consists of monohydrogen or dihydrogen phosphate anion (HPO_4^- or $H_2PO_4^-$). When there is excess of H^+, HPO_4^- ion acts as a weak base by accepting a proton.

3) Protein (haemoglobin) buffer system: The system is most abundant buffer in body cell and plasma. The plasma protein and haemoglobin together constitute protein buffer of the blood. Proteins are composed of amino acids having carboxyl group and NH_2 group. When there occurs an excess of H^+ ions, the amino group acts as a base and accepts proton. The free carboxyl group can liberate proton to neutralize an alkaline condition.

Biological buffers-
Blood is an example of biological buffer where pH is maintained at 7.4 due to plasma and erythrocytes. Plasma contains carbonic groups and sodium salt of phosphoric acid as buffer. Erythrocytes contain hemoglobin or oxyhemoglobin and potassium salts of phosphoric acid as buffers.
Pharmaceutical Buffers-
These are important in ophthalmic solutions and parenteral preparations. Deviation from normal pH causes irritation. The buffering agents used in parenterals are acetate phosphate , citrate and glutamate buffers.

PROCEDURE:

Preparation of acid phosphate buffer:-
Preparation of reagent-

1) Preparation of potassium dihydrogen phosphate :

2) Dissolve 27.218 g of potassium dihydrogen phosphate in little amount of H_2O and make upto 1000 ml.

3) Preparation of 0.2 N NaOH :

Dissolve 8 g NaOH in small amount of water and make upto 1000 ml.

Preparation of alkaline - borate buffer- Dissolve 12.36 g of boric acid and 19.91 g of potassium chloride in little amount of water and make upto 1000 ml.
Preparation of 0.2 N NaOH : Dissolve 8 g in little water and make upto 1000 ml.

REPORT :

Vision and Mission of the Institution

Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

Mission

M1 Create pharmacy graduates through **quality education**

M2 Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**

M3 **Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning

M4 **Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.