TECHNOCRATS

Lab Work Book of

Biochemistry

(BP - 209P)

Department of Pharmacy

Lab Manual of

Biochemistry

(BP - 209P)

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TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

Biochemistry

(BP-209P)

(Strictly According to RGPV Syllabus)

Name : .....

Enrollment No. : .....

Institute : .....

Academic Session : .....

Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

Mission of the Institute

M 1: To inculcate ethical, moral, cultural and professional values in students

M 2: To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

M 3: State of art learning of professionalism by the faculty and students

M 4: To produce well learned, devoted and proficient pharmacists

M 5: To make the students competent to meet the professional challenges of future

M 6: To develop entrepreneurship qualities and abilities in the students

PROGRAM OUTCOMES (POs)

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of thelimitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivationissues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well- being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

PEOs

PEO 1: To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

PEO 2: To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

PEO 3: To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

PEO 4: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

PEO 5: To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

Course Outcomes (COs):

Student will be able to:

- CO1: Identify the Carbohydrates by performing their individual identification tests.
- CO2: Evaluate the Glucose content which is present in Urine.
- CO3: Calculate the percentage of creatinine present in Blood.
- CO4: Identify the proteins by performing their individual identification tests.
- CO5: Identify the Amino acids by performing their individual identification tests.

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Experiment No. -1

OBJECTIVE:

To perform qualitative chemical examination of Urine

THEORY:

Urine is the chief excretory fluid eliminated through kidney. Most of the waste product are eliminated through urine.Urine have mainly two type of ingredient a) Organic & b) Inorganic. The urine constituents have its particular lever. The deviation of limit can produce different disease. The constituents can be evaluated by simply chemical reaction. Organic constituents are Urea (normal limit 25 to 30 gram/24hr), Uric Acid (normal limit 0.7 gram/24hr) and creatinine (normal limit 1.2 to 1.7gram/24hr). Inorganic constituents are Chlorides (normal limit 4 to 6 gram/hr), Phosphate (normal limit 0.8 to 1.3 gram/24hr) and sulphates (normal limit 7 to 1 gram/24hr)

Physical limit of urines :a) Volume: 600 to 2500ml/day, b)Specific gravity: 1.01 to 1.05, c) colour: pale yellow or amber, d) order: Faintly aromatic, Urinod, e) pH : 4.5 to 8.2

In vitamin B6 deficiency more kynurenine is converted to xanthurenic acid due to inactive kynureninase and excreted in urine. So measurement of xanthurenic acid after a test dose of tryptophan is used to detect vitamin B6 deficiency.

APPARATUS REQUIRED:

Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, Water bath, funnel, etc

REFERENCE:

Plumer, D.T., An Introduction to Practical Biochemistry,Tata McGraw Hill, New Delhi.

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

PROCEDURE:

A) INORGANIC CONSTITUENT

Test	Observation	Inference
1.Test for bi-carbonates: 3ml urine + dilute HCl or dilute H_2SO4	Effervescence of CO_2 gas	bi-carbonate present
2.Test for chloride: 5ml urine +1 ml.conc. HNO_3 to prevent precipitation of other ions like phosphate +1ml AgNO3 solution	White curdy ppt. of AgCl solution in NH_4 OH solution	Chlorides present
3.Test for phosphate: 3ml urine +3ml conc. HNO_3 +3ml of ammonium molybdate solution. heat to boil.	Cannary yellow ppt.	Phosphate present
4.Test for sulphates: 5ml urine + 1ml conc. HCl (to prevent phosphate ppt) +2ml $BaCl_2$ solution.	An opaque milkiness or a thick white ppt of $BaSO_4$ insoluble in conc. HCl	Sulphate present
5.Test for ammonia: i).5ml urine +2ml of 40% NaOH, boil ,hold a red litmus paper in vapour.	The red litmus paper turns blue.	Ammonia present
ii).deep the glass rod in phenolphthalein indicator and hold on fumes.		
6.Test for calcium. 5ml urine + few drops of NaOH + 1% acetic acid +2 to3 ml of ammonium oxalate solution.	The colour becomes pink	Ammonia present
	White ppt of calcium oxalate	Calcium present

B) ORGANIC CONSTTUENTS

Test	Observation	Inference
1.Test for urea:	Effervesence of nitrogen	Urea present
a)0.3ml urine + few drops of alkaline sodium hyprobromate solution (NaOBr)	Solution become pink	
b)0.5ml urine +4 drops of phenolphthalein + pinchfull of ureas powder (jack bean/ soybean meal) and mix.Allow it stand for five min.	(If solution is already pink before adding urease add 10% acetic acid to decolourise it)	Urea present
2.Test for Uric acid	Black or yellow brown stain formed	
a)Moisten a strip of filter paper with $AgNO_3$Solution add a drop of urine.	Adeep blue colour develop	Uric Acid present
b)0.5ml Urine + 5 drops benedict uric acid reasent + 3 gms of anhydrous Na_2HCO_3 mix by shaking		Uric Acid present
3.Test forCreatinine		
a)0.5ml Urine + 5 drops of Sodium nitropruside +2 ml of 10% NaOH.	Rubby red colour is formed & soon changed to yellow	Creatinine present
b)0.5ml Urine +1ml saturated solution of picric acid +3 gm of anhydrous Na_2CO_3 mix by shaking	A deep orange colour is formed	Creatinine present

DISCUSSION:

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VIVA QUESTIONS

Q.1 All of the following statements are true for lipids, except

(*a*) Lipids are soluble in organic solvents.

(*b*) They are present in humans, animals and plants.

(*c*) In man they serves as energy source.

(*d*) They are absent in cooking oil and milk.

Q.2 An example for mixed triglyceride is

(*a*) 1,3–distearopalmitin

(*b*) Tripalmitin

(*c*) Triolein

(*d*) 1,3–diacylglycerol

Q.3 Partial acylglycerols are formed

(*a*) During digestion of triglycerides

(*b*) From fats

(*c*) From saponification of fats

(*d*) From none of these

Q.4 Hydrolysis of sphingomyelin yield

(*a*) Sphingosine, 2 fatty acids, phosphate

(*b*) Sphingosine, fatty acid, choline, phosphate

(*c*) Sphingosine, fatty acid, glucose

(*d*) Cerebrosides, sugars, fatty acids

Q.5 An example for ω-3 fatty acid is

(*a*) Palmitoleic acid

(*b*) Arachidonic acid

(*c*) Linolenic acid

(*d*) Linoleic acid

Q.6 Which of the following are susceptible to essential fatty acid deficiency

(*a*) Adults consuming formula diet

(*b*) Pregnant women

(*c*) Infants consuming formula diet

(*d*) Growing children

Q.7 An eicosanoid acting as chemotactic agent is

(*a*) Prostacyclin

(*b*) Leukotriene

(*c*) Lipoxin

(*d*) Thromboxane

Q.8 Which of the following statement is correct regarding protein degradation.

(*a*) Protein degradation is more in well-fed state.

(*b*) It is more in starvation.

(*c*) It is more in diabetes.

(*d*) It is more in starvation and diabetes.

Q.9 All of the following statements are true for amino acids. Except

(*a*) They are required for synthesis of hormones.

(*b*) They are required for the synthesis of purines.

(*c*) They are required for the synthesis of glutathione.

(*d*) They are stored when excess.

Q.10 Creatine formation requires

(*a*) Glycine, Arginine

(*b*) Arginine, Methionine

(*c*) Glycine, arginine and methionine

(*d*) Methionine, glycine

Experiment No:-2

OBJECTIVE:

To perform the Quantitative chemical examination of Urine (Estimation of urine creatinine)

THEORY:

Creatinine is estimated by Folin modified method using colorimeter. In this method urine sample containing creatinine is treated with picric acid in alkaline medium to obtain red coloured creatinine picrate. Optical density of this red coloured solution is compared with that standard solution similarly converted by picric aid to creatinine picrate. By using colorimetry principle concentration of creatinine in given sample of urine can be calculated. Since creatinine is neither secreted nor reabsorbed creatinine is used as endogenous marker of renal GFR. Production of creatinine is also not influenced by diet, age etc. Creatinine clearance test is used to assess renal GFR world wide. In this test first patient is given 500 ml water. So that his body is hydrated properly. After an hour his bladder is emptied and urine is discarded. Then the urine passed for a 4 hour period is collected and volume is measured. Blood samples are also collected during collection of urine. Creatinine concentration in blood and urine samples is determined and by substituting the values in the above mentioned formula creatinine clearance is obtained.

Normal creatinine clearance values are 90-110 ml/min/1.73 square meter body surface area. Low GFR indicates renal dysfunction. It occurs in various kidney diseases and several pre renal conditions.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, Water bath, funnel
2. Instrument- Colorimeter

REFRENCE:

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office.

Jayaraman, J., Laboratory manual in Biochemistry, Wiley eastern Ltd., New Delhi.

PROCEDURE:

Step 1. Label two flasks of 50 ml capacity as “S” standard and “U” unknown.

Step 2. Preparation of standard

In standard flask (S) add following.

Standard creatinine solution – 0.5 ml

Sodium hydroxide solution(10%) – 1.0 ml

Picric acid(1%) – 10.0 ml

Dilute by adding distill water to 50 ml.

Mix and keep for 15 minutes. Estimate optical density at 530 nm wave length.

Step 3. Preparation of unknown:

In unknown flask (U) add followings …

Given urine sample- 0.5 ml.

Sodium hydroxide solution(10%) – 1.0 ml.

Picric acid (1%) - 10.0 ml.

Dilute by adding distill water to make final volume 50 ml.

Mix and keep for 15 minutes.

Step 4. Record the colours (i.e. optical density at 530 nm wave length) obtained of that of standard and unknown by using photoelectric calorimeter.

OBSERVATION:

	Standard (S)	Sample (U)
Optical density		

CALCULATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Creatine formation requires
(*a*) Glycine, Arginine
(*b*) Arginine, Methionine
(*c*) Glycine, arginine and methionine
(*d*) Methionine, glycine

Q.2 Which of the following statement is correct regarding protein degradation.
(*a*) Protein degradation is more in well-fed state.
(*b*) It is more in starvation.
(*c*) It is more in diabetes.
(*d*) It is more in starvation and diabetes.

Q.3 All of the following statements are true for amino acids. Except
(*a*) They are required for synthesis of hormones.
(*b*) They are required for the synthesis of purines.
(*c*) They are required for the synthesis of glutathione.
(*d*) They are stored when excess.

Q.4 Ubiquitin is a protein required for
(*a*) Protein degradation.
(*b*) Amino acid degradation.
(*c*) Glycoprotein degradation.
(*d*) Protein synthesis.

Q.5 In plasma
(*a*) Concentration of glutamine is low.
(*b*) Concentration of aspartate is high.
(*c*) Concentration of glutamine is high where as concentration of aspartate is low.
(*d*) Concentration of glutamine and aspartate is equal.

Q.6 Schizophrenia is associated with altered
(*a*) Dopamine metabolism.
(*b*) Phenylalanine metabolism.
(*c*) Tyrosine metabolism.
(*d*) Epinephrine metabolism.

Q.7 Polypeptide chain formation occurs.

(*a*) From amino terminus to carboxy terminus.

(*b*) From amino terminus.

(*c*) From carboxy terminus.

(*d*) During starvation.

Q.8 Aminoacyl-tRNA synthesis involves

(*a*) Formation of ester bond.

(*b*) Consumption of two high energy bonds.

(*c*) Formation of ester bond and utilization of two high energy bonds.

(*d*) None of the above.

Q.9 Diphtheria toxin inhibits protein synthesis

(*a*) By inactivating initiation factor.

(*b*) By inactivating elongation factor.

(*c*) By preventing peptide bond formation.

(*d*) By combining with ribosomes.

Q.4 Mutations in genes

(*a*) Results in nonfunctional protein production.

(*b*) May cause cancer.

(*c*) Produces non-functional proteins and cancer.

(*d*) May be due to DNA damage.

Q.5 Which of the following statement is correct regarding Hemoglobin Wayne ?

(*a*) It is an example for single point mutation.

(*b*) It contains 146 aminoacids in α-chain.

(*c*) Its α-chain contains 146 aminoacids instead of normal 141 residues.

(*d*) Its β-chain contains only 140 aminoacids.

Experiment No:-3

OBJECTIVE:

To perform the Quantitative chemical examination of Blood (Estimation of blood creatinine)

THEORY:

Creatinine in blood is estimated by Folin modified method using photoelectric calorimeter. In this method protein free blood filtrate is used. The creatinine containing blood filtrate is treated with picric acid in alkaline medium to obtain red coloured creatinine picrate. Optical density of this red coloured solution is compared with that standard solution similarly converted by picric aid to creatinine picrate. By using calorimetry principle concentration of creatinine in given sample of urine can be calculated. Since creatinine is neither secreted nor reabsorbed creatinine is used as endogenous marker of renal GFR. Production of creatinine is also not influenced by diet, age etc. Creatinine clearance test is used to assess renal GFR world wide. In this test first patient is given 500 ml water. So that his body is hydrated properly. After an hour his bladder is emptied and urine is discarded. Then the urine passed for a 4 hour period is collected and volume is measured. Blood samples are also collected during collection of urine. Creatinine concentration in blood and urine samples is determined and by substituting the values in the above mentioned formula creatinine clearance is obtained. Normal creatinine clearance values are 90-110 ml/min/1.73 square meter body surface area. Low GFR indicates renal dysfunction. It occurs in various kidney diseases and several pre renal conditions.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, funnel
2. Instrument- Colorimeter

3.

REFRENCE:

Plumer, D.T., An Introduction to Practical Biochemistry, Tata McGraw Hill, New Delhi.

Jayaraman, J., Laboratory manual in Biochemistry, Wiley eastern Ltd., New Delhi.

PROCEDURE:

Step 1. Label two flasks of 50 ml capacity as “S” standard and “U” unknown.

Step 2. Preparation of standard

In standard flask (S) add following.

- Standard creatinine solution – 5 ml
- Sodium hydroxide solution (10%) –0.5ml

- Picric acid(1%) – 2.0 ml
- Allow to keep for 15 min and obtain optical density at 530nm Mix and keep for 15 minutes.

Step 3. Preparation of unknown:

In unknown flask (U) add followings …

- Folin WU filtrate(protein free filtrate) –5ml.
- Sodium hydro oxide solution (10%) –0.5ml
- Picric acid(1%) – 2.0 ml
- Allow to keep for 15 min and obtain optical density at 530nm Mix and keep for 15 minutes

Step 4. Preparation of Blank:

In unknown flask (B) add followings …

- Distilled water –5ml.
- Sodium hydro oxide solution (10%) –0.5ml
- Picric acid(1%) – 2.0 ml
- Allow to keep for 15 min and obtain optical density at 530nm Mix and keep for 15 minutes

OBSERVATION:

	Standard (S)	Unknown sample (U)	Blank (B)
Optical density			

CACULATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Inhibitors of replication are used as

(*a*) Anti-cancer agents.

(*b*) Anti-viral agents.

(*c*) Anti-bacterial agents.

(*d*) Anti-cancer, anti-viral and anti-bacterial agents.

Q.2 DNA polymerases

(*a*) Are involved in nucleic acid synthesis.

(*b*) Are template directed enzymes.

(*c*) Act in 3 ’→ 5 ’direction.

(*d*) Initiates chain formation.

Q.3 All of the following statements are correct regarding xeroderma pigmentosum. Except

(*a*) Thorny growth of skin is a symptom of this disease.

(*b*) Skin of affected person is in sensitive to UV light.

(*c*) Corneal ulceration is seen in affected people.

(*d*) Patients of this disease die at young age.

Q.4 Rifampicin blocks transcription

(*a*) By inhibiting first phophodiester bond formation.

(*b*) By binding with σ factor.

(*c*) By preventing unwinding of DNA.

(*d*) By forming loops in DNA-RNA hybrid.

Q.5 RFLP is used for

(*a*) Analysis of chromosome structure.

(*b*) DNA estimation.

(*c*) Production of antibodies.

(*d*) Synthesis of nucleic acids.

Q.6 Each polynucleotide chain

(*a*) Has direction.

(*b*) Has 5*'* and 3*'* end.

(*c*) Has direction and two ends.

(*d*) Has phosphodiester linkages.

Q.7 ATTATA is sequence of a DNA segment. Each letter stands for

(*a*) Bases.

(*b*) Nucleosides.

(*c*) Nucleotides.

(*d*) Purine and pyrimidine bases.

Q.8 Shine-Dalgarno sequence is present in

(*a*) Eukaryotic mRNA.

(*b*) Prokaryotic mRNA.

(*c*) At 5*'* end of prokaryotic mRNA.

(*d*) At 3*'* end of eukaryotic mRNA.

Q.9 Ribosomes are

(*a*) Nucleic acids.

(*b*) Proteins.

(*c*) Ribonucleo proteins.

(*d*) Nucleosomes.

Q.10 Loops in RNA molecules are

(*a*) Due to intra strand base pairing.

(*b*) Due to inter strand base pairing.

(*c*) Due to intra strand base pairing between complementary bases.

(*d*) Involved in transfer of genetic information

Experiment No:-4

OBJECTIVE:

To perform the Quantitative chemical examination of Urine (Estimation of urine calcium)

THEORY:

Calcium present in urine is treated with ammonium oxalate to produce a precipitate of calcium oxalate. The precipitate of calcium oxalate is dissolve in sulphuric acid so that it is converted to corresponding equivalent quantity of oxalic acid. The oxalic acid obtain is titrated against 0.01(N) KMnO4.

1ml of 0.1(N) KMnO4=0.2mg of Calcium

Calcitriol, It increases intestinal calcium absorption by promoting synthesis of calcium binding protein. High protein diet increases calcium absorption. Calcium, phosphorous ratio in the diet. Excess phosphate lowers calcium absorption. Phytic acid present in cereals and oxalates present in certain foods inhibit calcium absorption by forming insoluble calcium salts. Faulty digestion and absorption of fats decreases calcium absorption. Neutral and acidic PH favours calcium absorption where as alkaline PH decreases calcium absorption. Dietary fibre if present in excess interferes with calcium absorption.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, Water, funnel, centrifuge tube.
2. Instrument- Centrifuger

REFERENCE:

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office.

Jayaraman, J., Laboratory manual in Biochemistry, Wiley eastern Ltd., New Delhi.

PROCEDURE:

Method Used: Baron & Bell

[A] Unknown titration

1. Collect 24 hour urine sample with 10 ml Con HCl
2. In a 15 ml conical centrifuge tube labeled as "U" pipette 2 ml of urine.
3. Add to the above "U" tube distill water 2 ml.
4. Add to the above "U" tube 2 ml of 4% ammonium oxalate. Mix by rotating in between palms.
5. Keep the above mixture for overnight in ice bath or refrigerator
6. Centrifuge tube for 5 minutes at about 1000 RPM
7. Pour of supernatant fluid. Drain off fluid completely
8. Wash the precipitate twice with 5 ml of washing solution centrifuge again as before. Pour of supernatant fluid. Finally wash the precipitate with 5 ml water and pour off supernatant fluid.
9. To the precipitate in above centrifuge tube add 2ml of 1(N) sulphuric acid.
10. Transfer the content to conical flask for titration .
11. Heat in water bath at 70°C to 80° C.
12. By 1 ml graduated pipette add 0.01N $KMnO_4$ drop by drop till faint pink colour which persists at least 1 minute is obtained.

[B] Blank titration:

1. Lable conical centrifuge tube as "B" and take 2 ml water.
2. Add 2 ml of 4% ammonium oxalate and mix by rotating in between palms. And add few drops of phenil red indicator to this add 2 ml of 2% ammonium solution till red colour change to yellow color.
3. Keep the above mixture for overnight in ice bath or refrigerator
4. Centrifuge tube for 5 minutes at about 1000 RPM
5. Pour of supernatant fluid. Drain off fluid completely
6. Wash the precipitate twice with 5 ml of washing solution centrifuge again as before. Pour of supernatant fluid. Finally wash the precipitate with 5 ml water and pour off supernatant fluid.
7. To the precipitate in above centrifuge tube add 2ml of 1(N) sulphuric acid.
8. Transfer the content to conical flask for titration.
9. Heat in water bath at 70° C to 80°C.
10. By 1 ml graduated pipette add 0.01N $KMNO_4$ drop by drop till faint pink colour which persists at least 1 minute is obtained.
11. Titrate against 0.01N $KMNO_4$ as before till a faint pink colour develops Indicating end point. Record the reading as blank…Y.

OBSERVATION:

	Tube U (unknown sample)	Tube B (blank sample)	Tube U-Tube B
Reading of burette			

CACULATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Which of the following statement is correct regarding trace minerals

(*a*) They are required in large amounts.

(*b*) They are required in less than 100 mg/day.

(*c*) They account for 80% inorganic matter present in body.

(*d*) They are required for nerve impulse transmission.

Q.2 Chloride channel of neurons.

(*a*) Open during propagation of nerve impulse.

(*b*) Are influenced by hormones.

(*c*) Are influenced by fluorine.

(*d*) Are decreased in alcoholism.

Q.3 Calcium is referred as second messenger.

(*a*) Because many hormones mediate their action through calcium.

(*b*) Because it carries message from outside to inside of cell.

(*c*) Because it is related to mRNA.

(*d*) Because it is involved in nerve impulse transmission.

Q.4 Which of the following is correct regarding serum phosphate level.

(*a*) In adults its level is 9-11 mg%.

(*b*) In children its level is 4-6 mg%.

(*c*) It is increased in Fanconi syndrome.

(*d*) It is decreased in hypoparathyroidism.

Q.5 Excess cadmimum causes

(*a*) Hypertension in man.

(*b*) Hypertension in rabbits.

(*c*) Dermatitis in man.

(*d*) Hypopigmentation of skin in rats.

Q.6 Which of the following is correct regarding respiratory quotient.

(*a*) It provides energy values of common food stuffs.

(*b*) It decreases in diabetes.

(*c*) It decreases in starvation.

(*d*) It decreases in diabetes and starvation.

Q.7 Normal woman energy requirement per day

(*a*) Is higher than normal man energy requirement.

(*b*) Is equal to normal man daily energy requirement.

(*c*) It lower than normal man daily energy requirement.

(*d*) Increases with age.

Q.8 All of the following statements are correct for nitrogen balance. Except

(*a*) It is influenced by nitrogen intake.

(*b*) It is influenced by nitrogen output.

(*c*) It is influenced by dietary protein.

(*d*) It is influenced by dietary carbohydrate.

Q.9 Which of the following has high biological value

(*a*) Vegetable proteins.

(*b*) Animal proteins.

(*c*) Derived proteins

(*d)* Denatured vegetable proteins.

Q.10 Parenteral feed contains nutrients like

(*a*) Glucose.

(*b*) Denatured proteins.

(*c*) Amylose.

(*d*) Starch.

Experiment No:-5

OBJECTIVE:

To perform the Quantitative chemical examination of Blood (Estimation of blood calcium)

THEORY:

Calcium present in blood serum is treated with ammonium oxalate to produce a precipitate of calcium oxalate. This calcium oxalate is washed for several times with washing solution. The precipitate of calcium oxalate is dissolve in sulphuric acid so that it is converted to corresponding equivalent quantity of oxalic acid. The oxalic acid obtain is titrated against 0.01(N) $KMNO_4$.

1ml of 0.1(N)$KMNO_4$=0.2mg of Calcium

Glyoxalate is oxidized to oxalate, which is excreted in urine. Excess oxalate combines with calcium to form calcium oxalate crystals in urine, which can deposit in kidney and urinary tract. So, the symptoms are bilateral urolithiasis (stones in both ureters), nephrocalcinosis (stones in kidney) and recurrent urinary tract infections. Death occurs in childhood or early adult life due to renal failure or hypertension.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, centrifuge tube, funnel, centrifuge tube.
2. Instrument- Centrifuger

REFERENCE:

Martin, D.W., Mays, P.A. and Redwell, V.M., Harper's Review of Biochemistry, Lange medical Publication.

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office.

PROCEDURE:

Baron & Bell method on Folin WU filtrate

[A] Unknown titration

1. In a15 ml conical centrifuge tube labeled as "U" pipette 2 ml of clear serum.
2. Add to the above "U" tube distill water 2 ml.
3. Add to the above "U" tube 2 ml of 4% ammonium oxalate. Mix by rotating in between palms.
4. Keep the above mixture for overnight in ice bath or refrigerator.
5. Centrifuge tube for 5 minutes at about 1000 RPM
6. Pour of supernatant fluid. Drain off fluid completely
7. Wash the precipitate twice with 5 ml of washing solution centrifuge again as before. Pour of supernatant fluid. Finally wash the precipitate with 5 ml water and pour off supernatant fluid.
8. To the precipitate in above centrifuge tube add 2ml of 3%sulphuric acid.
9. Transfer the content to conical flask for titration.
10. Heat in water bath at 70˚C to 80˚C.
11. By 1 ml graduated pipette add 0.01N $KMNO_4$ drop by drop till faint pink colour which persists at least 1 minute is obtained.

[B] Blank titration:

1. Lable conical centrifuge tube as "B" and take 2 ml water.
2. Add 2 ml of 4% ammonium oxalate and mix by rotating in between palms. And add few drops of phenil red indicator to this add 2 ml of 2% ammonium solution till red colour change to yellow color.
3. Keep the above mixture for overnight in ice bath or refrigerator
4. Centrifuge tube for 5 minutes at about 1000 RPM
5. Pour of supernatant fluid. Drain off fluid completely
6. Wash the precipitate twice with 5 ml of washing solution centrifuge again as before. Pour of supernatant fluid. Finally wash the precipitate with 5 ml water and pour off supernatant fluid.
7. To the precipitate in above centrifuge tube add 2ml of 3% sulphuric acid.
8. Transfer the content to conical flask for titration.
9. Heat in water bath at 70^0C to 80^0C.
10. By 1 ml graduated pipette add 0.01N $KMNO_4$ drop by drop till faint pink colour which persists at least 1 minute is obtained.
11. Titrate against 0.01N $KMNO_4$ as before till a faint pink colour develops Indicating end point. Record the reading as blank…Y.

OBSERVATION:

	Tube U (unknown sample)	Tube B (blank sample)	Tube U-Tube B
Reading of burette			

CALCULATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Most of the amino acids found in human body are

(*a*) L-isomers

(*b*) D-isomers

(*c*) D and L-isomers

(*d*) Optical isomers

Q.2 Which of the following amino acids has more pK values.

(*a*) Glycine

(*b*) Alanine

(*c*) Glutamate

(*d*) Glutamine

Q.3 The isoelectric pH of lysine is equal to

(*a*) Arithamatic mean of amino groups pK values.

(*b*) Half of sum of amino group and carboxyl group pK values.

(*c*) Arithamatic mean of amino groups and carboxyl groups pK values.

(*d*) None of the above.

Q.4 An example for unusual amino acid is

(*a*) Aspargine

(*b*) Taurine

(*c*) Cystine

(*d*) Anserine

Q.5 All of the following statements are correct regarding peptide except

(*a*) It contains amino terminus

(*b*) It contains carboxy terminus

(*c*) It contains peptide bonds

(*d*) It contains only basic amino acids

Q.6 Glucose absorption in intestine requires

(*a*) A carrier and Na+

(*b*) Carrier molecule

(*c*) Na+ only

(*d*) Carrier and K+

Q.7 Aged people are prone to

(*a*) Lactose intolerance

(*b*) Lactase deficiency

(*c*) Primary low lactase deficiency

(*d*) Sucrase deficiency

Q.8 All of the following statements are correct regarding congenital abeta lipoproteinemia. Except

(*a*) It is a genetic disease

(*b*) Triglycerides accumulates in intestine

(*c*) Cholesterol accumulates in liver

(*d*) It is due to lack of apo B-48

Q.9 Peptide bonds of dietary proteins in which amino group is contributed by acidic amino acids are hydrolyzed by :-

(*a*) Renin

(*b*) Pepsin

(*c*) Aminopeptidase

(*d*) Exopeptidase

Q.10 Carriers of amino acid absorption in the intestine are

(*a*) Na+ dependent

(*b*) Na+ independent

(*c*) Na+ dependent as well as Na+ independent

(*d*) None of the above.

Experiment No:-6

OBJECTIVE:

To perform Food Analysis – Analysis of Milk (Estimation of reducing sugar in Milk)

THEORY:

Lactose, the reducing sugar of milk can be obtained by precipitating the protein of milk by copper sulphate & sodium hydroxide solution. It may be estimated by using Benedict's quantitative solution just like reducing sugar in Urine. Benedict's Reagent consists of copper sulphate, potassium thiocyanate and other chemical in alkaline medium. copper sulphate is reduced to cuprous oxide by glucose. Potassium thiocyanate reacts with cuprous oxide and form a white ppt of cuprous thiocyanate instead of usual red ppt of cuprous oxide. The disappearance of blue colour from solution indicate complete reduction of copper sulphate.

10ml of Benedict's Reagent reduce 0.027gm of reducing sugar.

If a sugar rotates plane polarized light to right then it is called as *dextrorotatory* and if a sugar rotates the plane polarized light to the left then it is called as levorotatory. Usually '+' sign or 'd' indicates dextrorotation and '–' sign or 1 indicates levorotation of a sugar. For example, D-glucose which is dextrorotatory is designated as D(+) glucose and D-fructose, which is levorotatory is designated as D(–) fructose. The letter 'D' does not indicate whether a given sugar is dextro or levorotatory.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, funnel, centrifuge tube.
2. Instrument-Photoelectric calorimeter

REFERENCE:

Martin, D.W., Mays, P.A. and Redwell, V.M., Harper's Review of Biochemistry, Lange medical Publication.

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

K.K Pillai, J.S. Quadry. Biochemistry Clinical Pathology, CBS Publishers New Delhi.

PROCEDURE:

1. Pipette 10 ml of milk into 100 ml volumetric flask.
2. Add 2 ml of 10% $ZnSO_4$ solution and 1 ml of 1(N) NaOH.
3. Mix well, make up the volume upto 100 ml with distilled water.
4. Allow to stand for 1 to 2 minutes and then put in boiling water bath for a few minutes.
5. Filter the solution and pour the filtrate into burette
6. Titrate with Benedict's quantitative reagent
7. Take three reading and take the mean for calculation

OBSERVATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Most of the carbohydrates found in human body are

(*a*) D-isomers

(*b*) L-isomers

(*c*) D- and L-isomers

(*d*) None of these

Q.2 The linkage between aldehyde group of glucose and its hydroxyl group of 5th carbon atom is

(*a*) Hemiacetal linkage

(*b*) Hemiketal linkage

(*c*) Glycosidic linkage

(*d*) Ester linkage

Q.3 Glucose and fructose are the examples for

(*a*) Functional isomers

(*b*) Optical isomers

(*c*) Geometric isomers

(*d*) Non-reducing sugars

Q.4 Polyol is formed from

(*a*) Oxidation of sugars

(*b*) Reduction of sugars

(*c*) Polysaccharides

(*d*) Monosaccharides

Q.5 O-glycosidic bond is formed

(*a*) When a sugar reacts with acid.

(*b*) When sugar reacts with alkali.

(*c*) When an anomeric carbon of sugar reacts with an alcohol.

(*d*) When an anomeric carbon of sugar reacts with an acid.

Q.6 Trehalose is a disaccharide present in

(*a*) Milk

(*b*) Blood

(*c*) Hemolymph

(*d*) Tubers

Q.7 An example for NADP+ dependent dehydrogenase is

(*a*) Phosphogluconate dehydrogenase.

(*b*) Succinate dehydrogenase.

(*c*) Acyl-CoA dehydrogenase.

(*d*) None of these.

Q.8 All of the following statements are correct for oxidases. Except

(*a*) They catalyze removal of hydrogen from substrates.

(*b*) They use oxygen as hydrogen acceptor.

(*c*) They produce H_2O_2.

(*d*) They produce H_2O.

Q.9 In iron-sulfur proteins

(*a*) Iron is complexed with organic sulfur.

(*b*) Iron is complexed with inorganic sulfur.

(*c*) Iron is complexed with organic and inorganic sulfur.

(*d*) Iron is complexed with proteins.

Q.10 Which of the following is correct for endergonic reaction.

(*a*) It occurs with release of energy.

(*b*) Its ΔG is negative.

(*c*) It occurs when energy supplied.

(*d*) It occurs with decrease in free energy.

Experiment No:-7

OBJECTIVE:

To perform Food Analysis – Analysis of Milk (Estimation of fat in Milk)

THEORY:

Concentrated sulphuric acid when added to the milk charrs organic matter breaks emulsion of fat. The centrifugation of the acid treated milk in a special tube (Babcock tube) brings the fat in the narrow calibrated part of the tube from which fat content is directly read. Reagent required are concentrated sulphuric acid (Sp gr. 1.83 to 1.84) and Acid alcohol mixture (Equal volume of HCl Acid and alcohol)

Foods like meat, animal fat, butter, milk, cheese, egg yolk and cooking oils and ghee contain

lipids. The lipids present in them are mainly triglycerides, phospholipids, glycolipids, cholesterol and its esters, fatty acids, sterols and carotenes. An adult may consume 50-150 gms of lipid per day. However, triglycerides accounts for 90% of dietary lipids. Rennin is present in the infant stomach. It causes coagulation of milk. It converts casein of milk to para casein in presence of calcium ions on which pepsin acts and converts into proteoses and peptones.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, funnel, babcock tube, purmic stone.

REFERENCE :

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

K.K Pillai, J.S. Quadry. Biochemistry Clinical Pathology, CBS Publishers New Delhi

PROCEDURE:

1. Introduce milk into the Babcock tube upto 5 ml mark.
2. Add Conc. sulphuric acid to fill the body of tube, 1 ml at a time. Mix well by rotation after each addition, cool than further acid to fill the body once again.
3. Fill the neck of the tube to the zero mark with an acid-alcohol mixture.
4. Centrifuge the tube for 2 to 3 minutes and read the percentage of fat from the calibrated part of neck
5. In case fat content is over 5 percent, the milk should be diluted with equal volume of water

OBSERVATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 All of the following statements are true for lipids, except

(*a*) Lipids are soluble in organic solvents.

(*b*) They are present in humans, animals and plants.

(*c*) In man they serves as energy source.

(*d*) They are absent in cooking oil and milk.

Q.2 An example for mixed triglyceride is

(*a*) 1,3–distearopalmitin

(*b*) Tripalmitin

(*c*) Triolein

(*d*) 1,3–diacylglycerol

Q.3 Partial acylglycerols are formed

(*a*) During digestion of triglycerides

(*b*) From fats

(*c*) From saponification of fats

(*d*) From none of these

Q.4 Hydrolysis of sphingomyelin yield

(*a*) Sphingosine, 2 fatty acids, phosphate

(*b*) Sphingosine, fatty acid, choline, phosphate

(*c*) Sphingosine, fatty acid, glucose

(*d*) Cerebrosides, sugars, fatty acids

Q.5 An example for ω-3 fatty acid is

(*a*) Palmitoleic acid

(*b*) Arachidonic acid

(*c*) Linolenic acid

(*d*) Linoleic acid

Q.6 Which of the following are susceptible to essential fatty acid deficiency

(*a*) Adults consuming formula diet

(*b*) Pregnant women

(*c*) Infants consuming formula diet

(*d*) Growing children

Q.7 An eicosanoid acting as chemotactic agent is

(*a*) Prostacyclin

(*b*) Leukotriene

(*c*) Lipoxin

(*d*) Thromboxane

Q.8 The isoelectric pH of lysine is equal to

(*a*) Arithamatic mean of amino groups pK values.

(*b*) Half of sum of amino group and carboxyl group pK values.

(*c*) Arithamatic mean of amino groups and carboxyl groups pK values.

(*d*) None of the above.

Q.9 An example for unusual amino acid is

(*a*) Aspargine

(*b*) Taurine

(*c*) Cystine

(*d*) Anserine

Q.10 All of the following statements are correct regarding peptide except

(*a*) It contains amino terminus

(*b*) It contains carboxy terminus

(*c*) It contains peptide bonds

(*d*) It contains only basic amino acids

Experiment No:-8

OBJECTIVE:

To perform Quantitative estimation of Amino Acid (Estimation of SGPT)

THEORY:

Transaminase are the enzyme which promote the process of removal of α- amino grops of most of L-Amino acid to an α- keto acid. As a result number of α-amino acid and α-keto acid are formed. One of this are serum alanine transaminase. This catalyses the reaction as follow. L- α-oxoglutarate+L-alanine= L-glutamate+L-pyruvate. This pyruvate produced by "glutamate-pyruvate-transaminase" reacts with di-nitrophenyl hydralazin (DNPH solution) in an alkaline medium which is measured at 510nm filter. Aspartate aminoa transferase (AST) and alanine amino transferase (ALT) are two transaminases most frequently measured. Normal levels are 3-20 U/L for AST and 4-20 U/L for ALT (Units-U). The former enzyme is also referred as GOT (Glutamate oxalo acetate transaminase) and latter is referred as GPT (Glutamate Pyruvate Transminase). These two enzymes differ in distribution. Heart is rich in AST where as liver contains both of them in equal amounts. Hence, AST estimation is most commonly done in diseases that affect heart. AST level increases in plasma following heart attack or myocardial infarction. Since liver contains more of ALT, its elevation in plasma is specific indicator of liver damage. Plasma ALT level is more in liver diseases like alcoholic cirrhosis, biliary obstruction, cancer and toxic hepatitis. Both the enzymes are elevated in acute infective hepatitis because liver contains both of them in significant amount. After the onset of viral hepatitis, the levels of these enzymes reaches peak rapidly and come back to normal reference level within a week. Since the skeletal muscle contains appreciate amounts of ALT, its level is increased in muscle damage as in severe trauma and in muscular dystrophy. Serum transaminases are also elevated in lung disease.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, funnel
2. Instrument-Photoelectric calorimeter

REFERENCE:

Martin, D.W., Mays, P.A. and Redwell, V.M., Harper's Review of Biochemistry, Lange medical Publication.

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

PROCEDURE:

[A] PREPARATTION OF UNKNOWN SAMPLE

1. In a tube labeled as 'U' take alanine substrate 0.5ml.

2. Add 0.1 ml of serum sample.

3. Incubate the tube at 37°C for 30 minutes.

4. Remove the tube and add 0.5ml DNPH solution kept 20 minutes at room temperature.

5. Add 5ml of 0.4(N) NaOH in the tube

6. Take the optical density using photoelectric calorimeter with green filter(520nm) Note it as 'Eu'

[B] PREPARATTION OF CONTROL:

1. In a tube labeled as 'C' take alanine substrate 0.5ml.

2. Add 0.1 ml of serum sample.

3. Incubate the tube at 37°C for 30 minutes. Keep for 20 minutes at room temp.

4. Add 5ml of 0.4(N) NaOH in the tube

5. Take the optical density using photoelectric calorimeter with green filter (520nm). Note it as 'Ec'

[C] PREPARATTION OF STANDARD

1. In a tube labeled as 'S' take alanine substrate 0.5ml and 0.5ml of DNPH solution

2. Add 0.1 ml of Standard pyruvate.

3. Incubate the tube at 37°C for 30 minutes.

4. Remove the tube and kept 20 minutes at room temperature.

5. Add 5ml of 0.4(N) NaOH in the tube

6. Take the optical density using photoelectric calorimeter with green filter (520nm) Note it as 'Es'

[D] PREPARATTION OF BLANK SAMPLE

1. In a tube labeled as 'B' take alanine substrate 0.5ml.

2. Add 0.5 ml of DNPH and 0.1ml of water.

3. Incubate the tube at 37°C for 30 minutes.

4. Remove the tube and kept 20 minutes at room temperature.

5. Add 5ml of 0.4(N) NaOH in the tube

6. Take the optical density using photoelectric calorimeter with green filter (520nm) Note it as 'Eb'

OBSERVATION:

	Eu	Ec	Es	Eb
Optical density				

CALCULATION FORMULA

SGPT= [(Eu-Ec)/(Es-Eb)]x67 IU

RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Most of the amino acids found in human body are

(*a*) L-isomers

(*b*) D-isomers

(*c*) D and L-isomers

(*d*) Optical isomers

Q.2 Which of the following amino acids has more pK values.

(*a*) Glycine

(*b*) Alanine

(*c*) Glutamate

(*d*) Glutamine

Q.3 The isoelectric pH of lysine is equal to

(*a*) Arithamatic mean of amino groups pK values.

(*b*) Half of sum of amino group and carboxyl group pK values.

(*c*) Arithamatic mean of amino groups and carboxyl groups pK values.

(*d*) None of the above.

Q.4 An example for unusual amino acid is

(*a*) Aspargine

(*b*) Taurine

(*c*) Cystine

(*d*) Anserine

Q.5 All of the following statements are correct regarding peptide except

(*a*) It contains amino terminus

(*b*) It contains carboxy terminus

(*c*) It contains peptide bonds

(*d*) It contains only basic amino acids

Q.6 All of the following statements are true for amino acids. Except

(*a*) They are required for synthesis of hormones.

(*b*) They are required for the synthesis of purines.

(*c*) They are required for the synthesis of glutathione.

(*d*) They are stored when excess.

Q.7 Ubiquitin is a protein required for

(*a*) Protein degradation.

(*b*) Amino acid degradation.

(*c*) Glycoprotein degradation.

(*d*) Protein synthesis.

Q.8 In plasma

(*a*) Concentration of glutamine is low.

(*b*) Concentration of aspartate is high.

(*c*) Concentration of glutamine is high where as concentration of aspartate is low.

(*d*) Concentration of glutamine and aspartate is equal.

Q.9 Schizophrenia is associated with altered

(*a*) Dopamine metabolism.

(*b*) Phenylalanine metabolism.

(*c*) Tyrosine metabolism.

(*d*) Epinephrine metabolism.

Q.10 Lysosomes contain mainly

(*a*) Hydrolases

(*b*) Proteases

(*c*) Lipases

(*d*) Cathepsins

Experiment No:-9

OBJECTIVE:

To perform Quantitative estimation of proteins.

THEORY:

The protein of urine is completely precipitated with the help of alkaloid Esbach's reagent. The precipitate is centrifuged in tube (Aufrecht's tube) which is graduated to indicate the amount of protein present in 100 ml of urine. The Esbach's reagent prepared by addition of 1% solution of citric acid with saturated picric acid.

Proteins have high molecular weight, *e.g.,* the lactalbumin of milk molecular weight is 17000 and pyruvate dehydrogenase molecular weight is 7 × 106. Proteins are colloidal in nature. Proteins have large particle size. Different kinds of proteins are soluble in different solvents.

Proteins differ in their shape. Some proteins yield amino acids only on hydrolysis where as others produce amino acids plus other types of molecules.

Charge properties: Charge of a protein depends on the surroundings like amino acids. So, by changing the pH of surroundings the charge of protein can be altered. This property is used for separation of proteins.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, funnel, filter paper, centrifuge tube, Aufrech's tube
2. Instrument-Centrifuge, stop watch,

REFERENCE:

Plumer, D.T., An Introduction to Practical Biochemistry, Tata McGraw Hill, New Delhi.

Martin, D.W., Mays, P.A. and Redwell, V.M., Harper's Review of Biochemistry, Lange medical Publication.

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

PROCEDURE:

1. Filter the urine using the dry filter paper until the filtrate is clear.
2. Fill up the Aufrech's tube to the mark 'U' with clear filtered urine.
3. Add Esbach's reagent upto mark 'R'
4. Stopper and invert the tube a few time to mix the contents thoroughly but not shake. Turbidity will appear due to precipitation of protein.
5. Stand for 5 minutes and then centrifuge for 10 minutes. At the end of it take the redings of the horizontal level of the precipitate protein.
6. Centrifuge again for 5 minutes and read
7. If the consecutive two reading are same stop further centrifugation and take the reading

OBSERVATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 An allosteric enzyme

(*a*) Is usually made-up of many subunits.

(*b*) Obeys Michaelis Menten kinetics.

(*c*) Undergo covalent modification.

(*d*) Exist in pro-enzyme form.

Q.2 In fibrous proteins, polypeptide chains are

(*a*) Extended

(*b*) Folded

(*c*) Twisted

(*d*) Coiled

Q.3 Hair pin turn of polypeptide chain is called as

(*a*) β-Turn

(*b*) α-Turn

(*c*) γ-Turn

(*d*) β-pleated turn

Q.4 In the body, one gram of albumin holds

(*a*) 10 ml of fluid

(*b*) 18 ml of fluid

(*c*) 25 ml of fluid

(*d*) 20 fatty acids

Q.5 Tumour marker present in liver cancer patient blood is

(*a*) Haptoglobulin

(*b*) Acid protein

(*c*) α-Feto protein

(*d*) Thyroxine

Q.6 The concentration of Ig E class of immunoglobulin increases in blood in

(*a*) Allergic reactions

(*b*) Cancers

(*c*) Cold conditions

(*d*) Neonatal life

Q.7 A competitive inhibitor

(*a*) Binds at active site

(*b*) Does not bind at active site

(*c*) Alters Vmax only

(*d*) Binds at allosteric site

Q.8 A competitive inhibitor used in hypertension is

(*a*) Malonate

(*b*) Allopurinol

(*c*) Captopril

(*d*) Oxaloacetate

Q.9 A non-competitive inhibitor that is used as nerve gas in World War II is

(*a*) Iodo acetate

(*b*) Cyanide

(*c*) Di-isopropyl fluorophosphate (DFP)

(*d*) Arsenite

Q.10 In metalloenzymes metals are

(*a*) Attached to enzyme through coordinate bonds.

(*b*) Covalently attached to enzymes.

(*c*) Non-covalently attached to enzymes.

(*d*) Loosely attached to enzymes.

Q.11 All the following statements are correct regarding protein except:

(*a*) Proteins are involved in transport of gases.

(*b*) Proteins are involved in defence.

(*c*) Proteins act as buffers.

(*d*) Proteins are not found in all cells

Experiment No:-10

OBJECTIVE:

To determine glucose present in Urine

THEORY:

It can be estimated by using Benedict's quantitative solution. Benedict 's reagent consists of copper sulphate, potassium thiocyanate and other chemical in alkaline medium. copper sulphate is reduced to cuprous oxide by glucose. Potassium thiocyanate reacts with cuprous oxide and form a white ppt of cuprous thiocyanate instead of usual red ppt of cuprous oxide. The disappearance of blue colour from solution indicate complete reduction of copper sulphate.

10ml of Benedict's Reagent reduce 0.027gm of reducing sugar.

Glucose in blood or urine can be detected by using immobilized glucose oxidase. In pharmaceutical industry, glucose isomerase is used to produce fructose from glucose. D-glucose which is dextrorotatory is designated as D(+) glucose and D-fructose, which is levorotatory is designated as D(–) fructose. The letter 'D' does not indicate whether a given sugar is dextro or levorotatory.

APPARATUS REQUIRED:

1. Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, funnel, piece of porcelain, Burette
2. Instrument- stop watch,

REFERENCE:

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

Martin, D.W., Mays, P.A. and Redwell, V.M., Harper's Review of Biochemistry, Lange medical Publication.

PROCEDURE:

1. Wash and clean the required apparatus
2. Pipette10 ml of Benedict's quantitative Reagent in 100 ml flask
3. Add 20 ml of water and 5gms of anhydrous sodium bicarbonate and few piece of procelain

4. Heat the flask on flame, the titration mixture must be kept in boiling throughout the titration period.
5. Fill the burette with diluted urine
6. When the contant of flask begin to boiling start titration and add urine ml wise rapidly until white precipitate appears.
7. After this urine is added drop by drop at 10 minutes intervel
8. Continue the titration until the lasts trace of blue colour disappear.

OBSERVATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 Glucose and fructose are the examples for

(*a*) Functional isomers

(*b*) Optical isomers

(*c*) Geometric isomers

(*d*) Non-reducing sugars

Q.2 Polyol is formed from

(*a*) Oxidation of sugars

(*b*) Reduction of sugars

(*c*) Polysaccharides

(*d*) Monosaccharides

Q.3 O-glycosidic bond is formed

(*a*) When a sugar reacts with acid.

(*b*) When sugar reacts with alkali.

(*c*) When an anomeric carbon of sugar reacts with an alcohol.

(*d*) When an anomeric carbon of sugar reacts with an acid.

Q.4 All of the following statements are correct regarding congenital abeta lipoproteinemia. Except

(*a*) It is a genetic disease

(*b*) Triglycerides accumulates in intestine

(*c*) Cholesterol accumulates in liver

(*d*) It is due to lack of apo B-48

Q.5 Peptide bonds of dietary proteins in which amino group is contributed by acidic amino acids are hydrolyzed by

(*a*) Renin

(*b*) Pepsin

(*c*) Aminopeptidase

(*d*) Exopeptidase

Q.6 Which one of the following statement is correct regarding pyruvate dehydrogenase?

(*a*) It is present in cytosol

(*b*) It is a multienzyme complex

(*c*) It is multi enzyme complex present in mitochondria

(*d*) Acetyl-CoA is its substrate

Q.7 Glycogen isolated from liver of Type VI glycogen storage disease patient had normal structure. So, glycogen accumulation is due to deficiency of

(*a*) Muscle phosphorylase

(*b*) Glucose-6-phosphatase

(*c*) Liver phosphorylase

(*d*) Glycogen synthase

Q.8 In man, uronic acid pathway is unable to produce ascorbic acid due to lack of

(*a*) Gulonolactone oxidase

(*b*) Lactonase

(*c*) Xylulose

(*d*) Xylitol

Q.9 All of the following statements are correct for oxidases. Except

(*a*) They catalyze removal of hydrogen from substrates.

(*b*) They use oxygen as hydrogen acceptor.

(*c*) They produce H2O2.

(*d*) They produce H2O.

Q.10 Which of the following statement is correct regarding protein degradation.

(*a*) Protein degradation is more in well-fed state.

(*b*) It is more in starvation.

(*c*) It is more in diabetes.

(*d*) It is more in starvation and diabetes.

Experiment No:-11

OBJECTIVE :

To Seperate Serum protiens by Folin-WU filtrate method

THEORY:

Folin-WU filtrate or protein free blood filtrate is essential to perform the various experiment on blood. Here blood proteins are denatured by proton ion of Sulphuric Acid. The denatured protein precipitated and can be filtered through filter paper. So other constituents come in the filtrate which can be subjected for different test. A 70 kg human adult body contains about 12 kg of protein. Body proteins have life times. They undergo degradation and re-synthesis. About 400gm of body protein is synthesized and degraded per day i.e., about 6 gm of protein is synthesized and broken down per kg body weight per day. Aged proteins, damaged or modified proteins and non-functional proteins of the body undergo degradation. Further degradation is one way of controlling enzyme activity. Hence, continuous re-synthesis and degradation of proteins is a quality control mechanism. Protein degradation may play important role in shaping tissues and organs during pregnancy and development.

APPARATUS REQUIRED:

Test tube, Glass rod, Beaker, Measuring Cylinders, Conical Flask, Pipettes, funnel, filter paper

REFERENCE:

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

Jayaraman, J., Laboratory manual in Biochemistry, Wiley eastern Ltd., New Delhi.

PROCEDURE :

1. Collect 3 ml of oxalated blood in a 500 ml flask.
2. Add 21 ml of distill water.
3. Add 3 ml of sodium tungstate (10%) and mix well
4. Add 3 ml of 2/3 (N) H2SO4 by a graduated pipette drop by drop with constant shaking. Stopper with rubber cock shake well and keep for 5 minutes.
5. The colour of the precipitate gradually changes from red to brown.

Note: If change in colour does not occur, it means that precipitation is incomplete due to much use of oxalate as anti-coagulant. In such an emergency the sample may be saved by adding few drops of H2SO4, one drop each time shake vigorously each time and Continue till dark brown colour may set in.

6. Filter the above mixture in a funnel covered with filter paper. Obtain a clear filtrate as clear as water.
7. This obtained filtrate is known as "Folin-WU" filtrate.

Note:

1. 10 ml of folin-WU filtrate represent 1 ml of blood.
2. Filtrate prepared from 3 ml of blood is sufficient for routine investigations.
3. If the readings are to taken on a photoelectric a calorimeter a "blank" must always be prepared. The optical density of the blank must be subtracted from that of unknown and standard, to get the optical density.

OBSERVATION:

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RESULTS:

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DISCUSSION:

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VIVA QUESTIONS

Q.1 All the following statements are correct regarding protein except:

(*a*) Proteins are involved in transport of gases.

(*b*) Proteins are involved in defence.

(*c*) Proteins act as buffers.

(*d*) Proteins are not found in all cells.

Q.2 In fibrous proteins, polypeptide chains are

(*a*) Extended

(*b*) Folded

(*c*) Twisted

(*d*) Coiled

Q.3 Hair pin turn of polypeptide chain is called as

(*a*) β-Turn

(*b*) α-Turn

(*c*) γ-Turn

(*d*) β-pleated turn

Q.4. In the body, one gram of albumin holds

(*a*) 10 ml of fluid

(*b*) 18 ml of fluid

(*c*) 25 ml of fluid

(*d*) 20 fatty acids

Q.5 Tumour marker present in liver cancer patient blood is

(*a*) Haptoglobulin

(*b*) Acid protein

(*c*) α-Feto protein

(*d*) Thyroxine

Q.6 The concentration of Ig E class of immunoglobulin increases in blood in

(*a*) Allergic reactions

(*b*) Cancers

(*c*) Cold conditions

(*d*) Neonatal life

Q.7 All of the following statements are correct for enzymes. Except

(*a*) Enzymes are proteins

(*b*) Enzymes are catalysts

(*c*) Enzymes speed up chemical reactions by lowering energy of activation.

(*d*) Enzymes alters equilibrium constant of the reaction which they catalyze.

Q.8 The pH optimum of pancreatic proteases is

(*a*) 7.6

(*b*) 8.0

(*c*) 6.0

(*d*) 2.5

Q.9 A competitive inhibitor

(*a*) Binds at active site

(*b*) Does not bind at active site

(*c*) Alters Vmax only

(*d*) Binds at allosteric site

Q.10 A competitive inhibitor used in hypertension is

(*a*) Malonate

(*b*) Allopurinol

(*c*) Captopril

(*d*) Oxaloacetat

Experiment No:-12

OBJECTIVE:

To identify Unknown Carbohydrate

THEORY:

All the tests are chemical reaction which can be perform in different process. The particular chemical reagents are utilized for particular reaction. Glucose is the major fuel for all types of cells in the body. Its oxidation produces energy. Glucose consumption per day varies from one organ to another. Some organs like brain prefers glucose as fuel than fat and protein. Brain consumes about 100 gm of glucose per day. Rate of glucose oxidation is more in cancer cells. Glucose is used for the formation of glycogen, pentoses, lactose and mucopoly saccharides. Since brain is totally dependent on glucose for its energy needs glucose is synthesized from glycogen or other non-carbohydrates during starvation or when food is in short supply. Deficiency or absence of enzymes of glycogen metabolism causes glycogen storage diseases. Dietary galactose and fructose are converted to glucose. Deficiency of enzymes of galactose and fructose metabolism causes galactosemia and fructosemia, respectively.

APPARATUS REQUIRED:

Test tube, Glass rod, Beaker, Measuring Cylinders, pH meter, Conical Flask, Pipettes, Water bath, funnel, Gas burner

REFERENCE:

Martin, D.W., Mays, P.A. and Redwell, V.M., Harper's Review of Biochemistry, Lange medical Publication.

S.R . Kale Practical Biochemistry and Clinical Pathology, Nirali Prakashan, Mumbai office

Jayaraman, J., Laboratory manual in Biochemistry, Wiley eastern Ltd., New Delhi.

PROCEDURE:

TEST	OBSERVATIONS	INFERENCE

1.MOLISH REAGENT: Aq. Or alcoholic solution of bubs. + 10% Alc. solution of A Napthol shake + conc. Sulphuric acid along the side of the tube.	Violet ring at the junction of two liquids.	Carbohydrate present
2.SOLUBILITY: Com. + water	Soluble Insoluble	Mono & disaccharides Polysaccharides.
3.FEHLING'S TEST: 2ml. of fehling's Sol. A + 2ml.of fehlings sol. B + 2ml of sugar solu. Boil	Yellow or brick red ppt	Reducing sugars present
4.BENEDICT'S TEST: 5ml. of Benedict's reagent + 3ml sugar solu. Boil for 2 minutes cool.	Green,yellow or red ppt.	Reducing sugars present
5.TOMMER'S TEST: 2ml. of tommers reagent + 3ml of sugar solution boil for 2 imnutes cool.	Yellow or red ppt	Reducing sugar present
6.BARFOED'S TEST: 2ml. of test soluation + 2ml of barfoed's reagent. Boil on water bath .	Bricks red ppt. at the bottom of the test tube	Monosaccharides present.
7.SELIWANOFF'S TEST: 3ml. of seliwanoff's reagent + 1ml of sugar solution. boil for 2minutes.	Red ppt	Ketoses like fructose,sucrose present.
8.RAPID FURFURAL TEST: 1-2ml.sugar solution +1ml.of A-napthol solution (1% in alcohol)+ 5ml.conc. HCl Boil	Deep purple colour	Ketoses like fructose, sucrose present.
9.OSAZONE TEST: 0.2 gms of sugar + 0.4 gms of phenyl hydrazine hydrochloride + 0.6 gms.of sodium acetate + 4ml water. heat on a water bath for 20 minuts. cool and allow crystalisation. observe crystals under microscope.	(a) Greenish yellow needle shaped crystals arranged in fan-shape (b) This small needle shaped crystals appear like ball of prinkles. (c) Plat like sunflower. crystals appear like	Glucosazone is glucose present Lactosazone i.e. lactose present. Maltosazone i.e. maltose present.

RESULTS

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DISCUSSION

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VIVA QUESTION

Q.1 Phosphofructokinase-1 is

(*a*) An enzyme of glycolysis

(*b*) Inhibited by fructose-6-phosphate

(*c*) An allosteric enzyme of glycolysis

(*d*) Activated by ATP

Q.2 Which one of the following statement is correct regarding pyruvate dehydrogenase?

(*a*) It is present in cytosol

(*b*) It is a multienzyme complex

(*c*) It is multi enzyme complex present in mitochondria

(*d*) Acetyl-CoA is its substrate

Q.3 Glycogen isolated from liver of Type VI glycogen storage disease patient had normal structure. So, glycogen accumulation is due to deficiency of

(*a*) Muscle phosphorylase

(*b*) Glucose-6-phosphatase

(*c*) Liver phosphorylase

(*d*) Glycogen synthase

Q.4 In man, uronic acid pathway is unable to produce ascorbic acid due to lack of

(*a*) Gulonolactone oxidase

(*b*) Lactonase

(*c*) Xylulose

(*d*) Xylitol

Q.5 Synthesis of glucose from pyruvate requires

(*a*) Six high energy bonds

(*b*) Two high energy bonds

(*c*) Reduced NADP

(*d*) NADH

Q.6 Glucose absorption in intestine requires

(*a*) A carrier and Na+

(*b*) Carrier molecule

(*c*) Na+ only

(*d*) Carrier and K+

Q.7 Aged people are prone to

(*a*) Lactose intolerance

(*b*) Lactase deficiency

(*c*) Primary low lactase deficiency

(*d*) Sucrase deficiency

Q.8 All of the following statements are correct regarding congenital abeta lipoproteinemia. Except

(*a*) It is a genetic disease

(*b*) Triglycerides accumulates in intestine

(*c*) Cholesterol accumulates in liver

(*d*) It is due to lack of apo B-48

Q.9 Peptide bonds of dietary proteins in which amino group is contributed by acidic amino acids are hydrolyzed by

(*a*) Renin

(*b*) Pepsin

(*c*) Aminopeptidase

(*d*) Exopeptidase

Q.10 Carriers of amino acid absorption in the intestine are

(*a*) Na+ dependent

(*b*) Na+ independent

(*c*) Na+ dependent as well as Na+ independent

(*d*) None of the above.