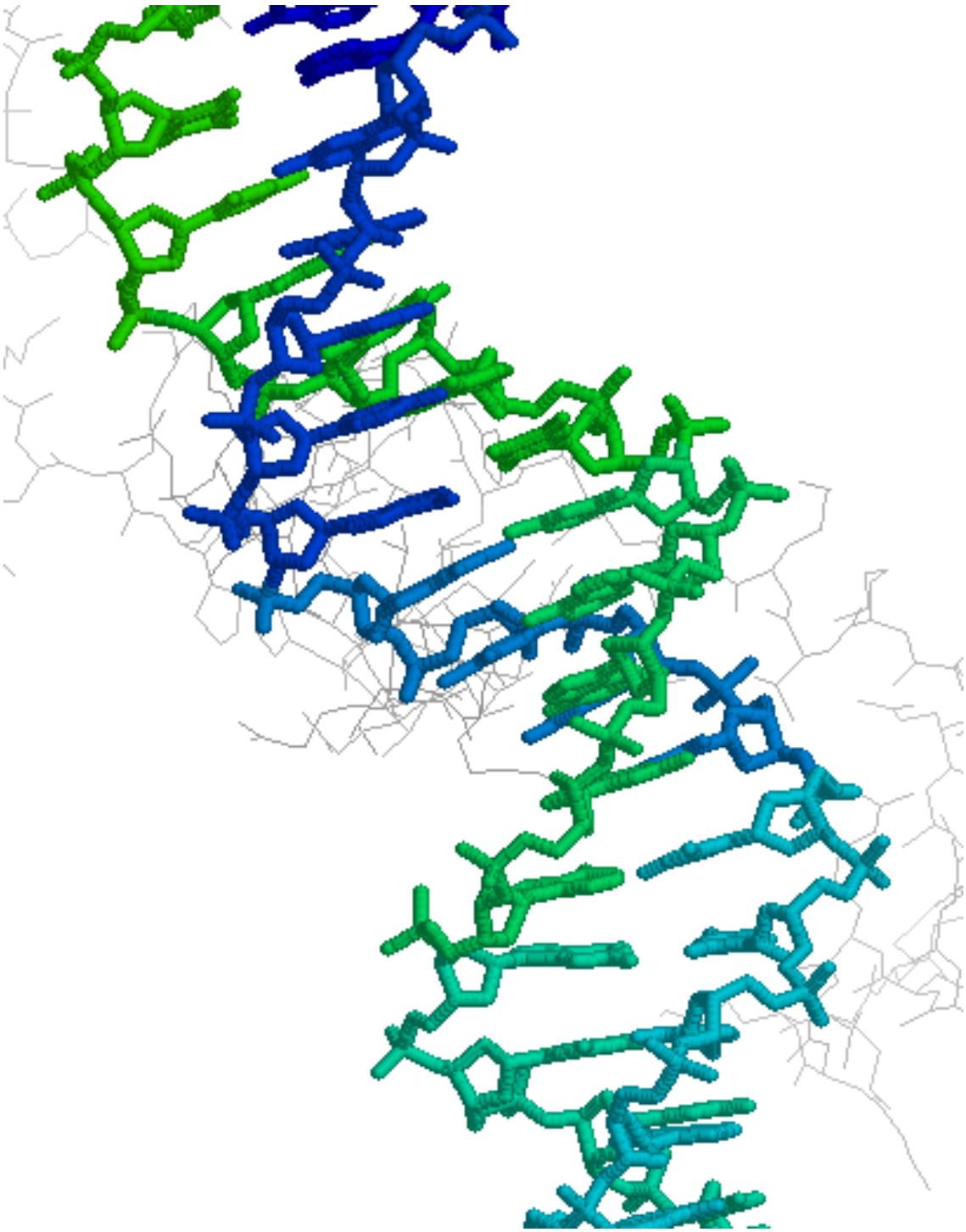
Department of Biochemistry
Government medical college Surat
Student Journal for Practical Biochemistry



Certificate

This is to certify that (name) _____ student of 1st MBBS,Roll No _____ ,Year of Admission _____ ,has completed training in practical Biochemistry at Department of Biochemist Government Medical College Surat.

Tutor
Department of Biochemisty
Government Medical College
Surat

Professor and Head
Department of Biochemistry
Government Medical College
Surat

I N D E X

1. Introduction to Practical Biochemistry

Practical Biochemistry serves several purposes to medical students.
Practical Biochemistry augments concepts learnt in classroom. e.g.
Study of properties of basic biomolecules. e.g Carbohydrate, Proteins
Study of biochemical investigative tools e.g colorimetry, chromatography, electrophoresis
Study of patient case histories in light of its laboratory investigations is fundamental to understanding medical aspects of biochemistry.
It prepares the student for possible use of the practical techniques in clinical practice. e.g.
Many bedside biochemistry diagnostic technologies are used by physicians themselves. Such technologies are called point-of-care-technologies **(POCT).** They are based on many simple concepts studied in practical biochemistry.
Many biochemistry diagnostic technologies are used by patients themselves. Such home monitoring by patients require support from their physicians. Practical biochemistry help medical students for supporting their patient's for such support.

Hazards in Clinical Biochemistry laboratory

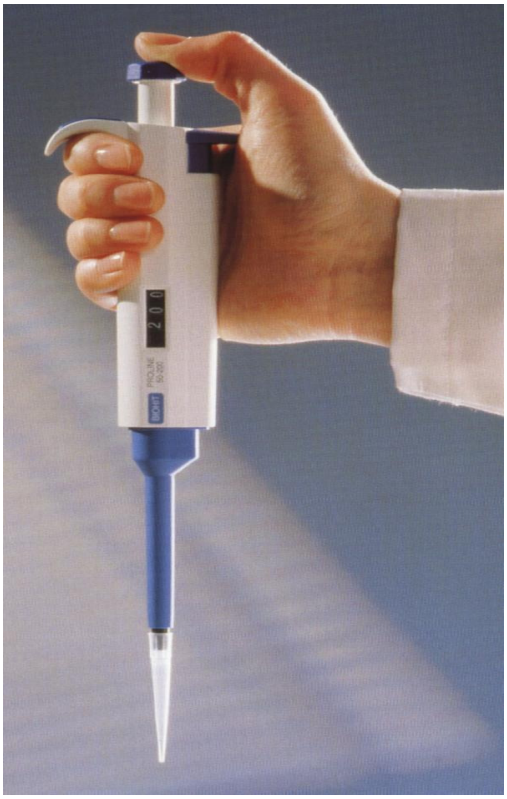
Hazards arises from three main basic sources

1. From dangerous chemicals
2. From infected specimen sent for analysis
3. From faulty apparatus & instruments

These are further increased by carelessness, untidiness, faulty hygiene, conduct of staff, unsatisfactory working condition.
Wide variety of articles are used like conical flasks, ,volumetric flasks, tube, measuring cylinders, pipettes, reagent bottles.

Pipettes

They are available from 0.1 ml to 25 ml delivery volume size.



While working in the laboratory, **personal safety** is of prime importance. Safety of each and every chemical and instrument needs to be understood. Follow specific instructions given to you during each practical.

Chemicals and patient's samples**:**

Never pipette any liquid by mouth.

Do not inhale/smell any thing unless specifically instructed.

Do not allow any chemical to touch your body, especially eyes. Repeated exposures to some chemicals can causes cancers.

While heating a liquid in a test-tube, ensure that it do not burst into yourself or your classmate.

You may use body fluids like serum or urine. Certain bacterial and viral diseases can spread via serum or urine. Never bring any part of your body in their contact. When in doubt about how to handle them, meet your tutor for guidance.

Instruments and equipments**:**

Be away from burner while igniting it. Note location of fire fighters in and around the laboratory. Do use fire fighters in emergency without waiting for any permission from your tutors.

Don't use cracked or broken glassware. Return them to the laboratory technologist.

Disposal of laboratory Waste

There are guidelines to dispose waste.It is recommended that waste should be segregated at the point of generation & disposed in bags with correct colour coding.

YELLOW BAGS	RED BAGS	BLUE BAGS	BLACK CARBOY
Infectious waste, bandages, gauze, cotton or any other objects in contact with body fluids, human body parts, placenta etc.	Plastic waste such as catheters,injection syringes, tubings, iv bottles	All types of glass bottles and broken glass articles, outdated & discarded medicines	Needles without syringes, blades, sharps and all metal articles.

Questions:

Describe any laboratory accident you or your schoolmate has suffered in your school days. How will/was it be first-aid? How will you prevent it?

Give list of Biochemistry POCT and home-monitoring technologies. Explain each of them.

Mention five more points to be noted for laboratory safety.

2.Chemistry of Carbohydrates

Test solution

Glucose solution(400mg/dl) : Dissolve 4 gm of glucose powder in 1000 ml water

Starch solution(1%): Dissolve 10 gm of starch powder in 100 ml of water by slightly Heating & make upto 1000 ml with water

Sucrose solution((400mg/dl) : Dissolve 4 gm of Sucrose powder in 1000 ml water

Fructose solution((400mg/dl) : Dissolve 4 gm of Fructose in 1000 ml water

Maltose solution((400mg/dl) : Dissolve 4 gm of Maltose powder in 1000 ml water

Molisch's test:

Reagent

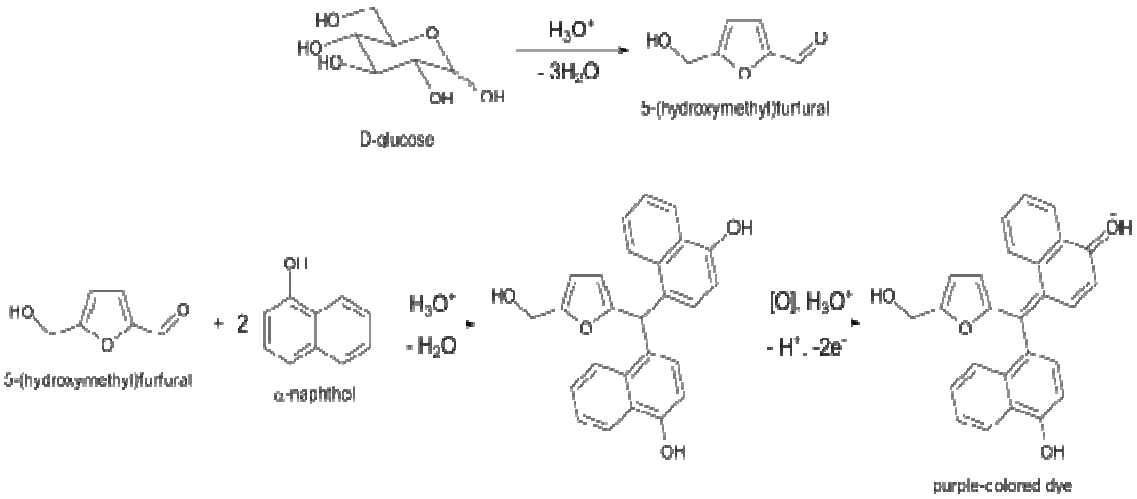
1 % α-Naphthol: Dissove 1 gm α-Naphthol powder in 100 ml methanol

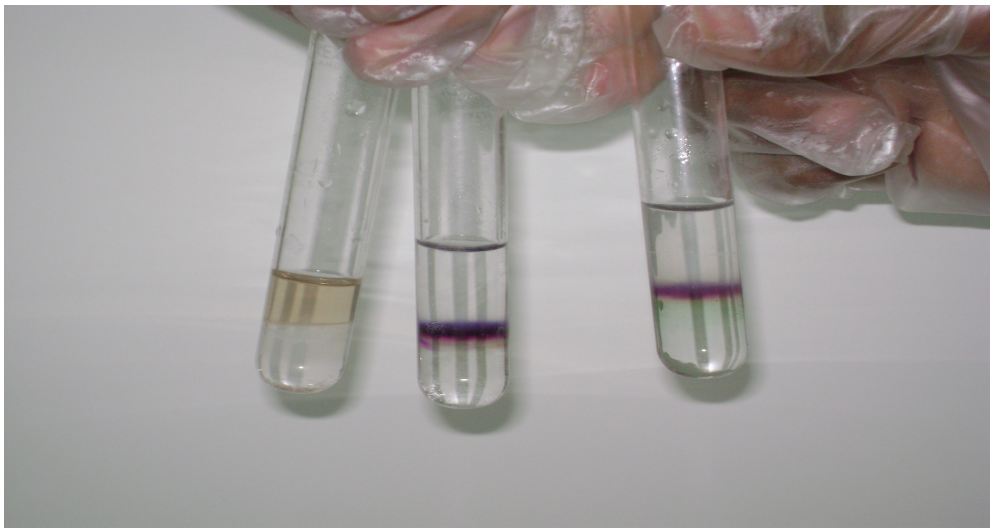
Conc.H_2SO_4

Principle

All carbohydrates when treated with conc. sulphuric acid undergo dehydration to give fufural compounds. These compounds condense with Alpha-napthol to form colored compounds.

Molish test is given by sugars with **at least five carbons** because it involves furfurl derivatives,which are five carbon compounds.





Benedict's Test:

All Reducing sugars give positive benedict's test.Reducing sugars have a free aldehyde or keto group.

Reagent

Benedict's Reagent:One liter of Benedict's solution contains ,
173 grams ----------> sodium citrate,
100 grams ----------> sodium carbonate
17.3 grams ---------->cupric sulphate pentahydrate.

With the help of heat,dissolve 173 gm of sodium citrate & 100 gm of sodium carbonate in 800 ml of water.Dissolve 17.3 gm cupric sulphate pentahydrate in 100 ml of water in different container.
Pour cupric sulfate solution in carbonate- citrate solution with constant stirring& make upto 1000ml.

Role of ingradient of benedict's solution:
1.Sodium citrate:Holding of cupric oxide in alkaline solution
2.Sodium carbonate:provide alkaline pH
3.cupric sulphate pentahydrate:Reducing Agent

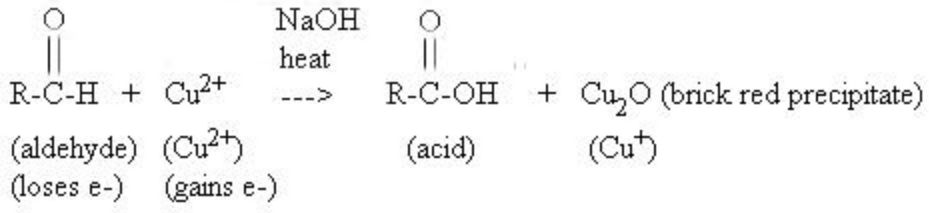
Principle

Glucose (R-CHO) + $2Cu^{2+}$ + $2H_2O$

$\xrightarrow[\text{alkali}]{\text{(Boil)}}$ Gluconic acid (R-COOH) + Cu_2O + $4H^+$

The principle of Benedict's test is that when reducing sugars are heated in the presence of an alkali(pH 10.6), they get converted to powerful reducing compounds known as enediols. Enediols reduce the cupric ions ($Cu2+$) present in the Benedict's reagent to cuprous ions ($Cu+$) which get precipitated as insoluble red copper(I) oxide.

The color of the obtained precipitate gives an idea about the quantity of sugar present in the solution, hence the test is semi-quantitative.



Carbohydrates giving positive Benedict's test:
Glucose, Fructose, Galactose,
Ribose, Glucuronic acid,
Lactose, Maltose
Note: Sucrose with no free reducing group give negative test.

Non-Carbohydrates giving positive Benedict's test:
High concentration of Uric acid , Creatinine and Ketones
Homogentisic acid (solution turns black due to black colored oxidized homogentisic acid)
Vitamin C (even without Boiling)
Certain drugs like aspirin, cephalosporins
Starches
Starches do not react or react very poorly with Benedict's reagent, due to the relatively small number of reducing sugar moieties, which occur only at the ends of carbohydrate chains.
Different concentration of glucose gives different color of solution with Benedict's test, depending on amount of precipitate and residual cupric sulphate.

Grade	Color of Reaction Mixture	Approximate Glucose concentration
+	Green	0.5-1 gm%
++	Yellow	1-1.5 gm%
+++	Orange	1.5-2 gm%
++++	Red	>2 gm%

Benedict's test is frequently used to detect glucose in urine. Although glucose is most frequent reducing substance present in urine, in some patient positive Benedict's test may be due to non-glucose reducing substances listed above. This phenomenon may be called ***false positive*** result.
Following test based on glucose oxidase is positive only with glucose in urine.

Glucose oxidase test:

Reagent:

Glucose strip or liquid reagent based on GOD-POD method

Principle

Glucose + O_2 —(Glucose Oxidase)→ Gluconolactone + H_2O_2

Gluconolactone + H2O —(Spontaneous)→ Gluconate

H2O2 + (reduced colorless dye) —(Peroxidase)→ Oxidized colored dye

Some of the dyes used are O-tolidine, tetramethylbenzidine, and potassium iodide, 4-aminophenazome + phenol .

Reagents for this test are present on a strip of paper in solid form. When the paper is wet with urine, the reagents dissolve in urine on paper and react with glucose in urine. The darkness of color can be correlated with amount of glucose present in urine.

Because **Glucose oxidase enzyme can act only on beta-D-Glucose,** other reducing substances do not give this test positive. (Exception: Galactose can react with glucose oxidase, but very slowly)

Following reaction occur when urine contain compounds reacting with H2O2.

Glucose + O_2 —(Glucose Oxidase)→ Gluconolactone + H_2O_2

H_2O_2 + Vitamin C —→ Oxidized Vitamin C + H_2O

Thus, compounds like Vitamin C, Aspirin utilize H_2O_2 produced in the reaction. Due to lack of H_2O_2, peroxidase can not oxidize dye. Thus, glucose may not be detected even if present, if urine contain Vitamin C or Aspirin in large amount. This phenomenon is called ***false negative*** result.

In neonate, **positive Benedict's test in urine, in presence of negative Glucose oxidase test,** indicate possible presence of Fructose or Galactose in urine. (But note the exception mentioned above). Fructose and galactose are found in some inborn deficiency of enzymes of their metabolic pathways.

Barfoed's Test:

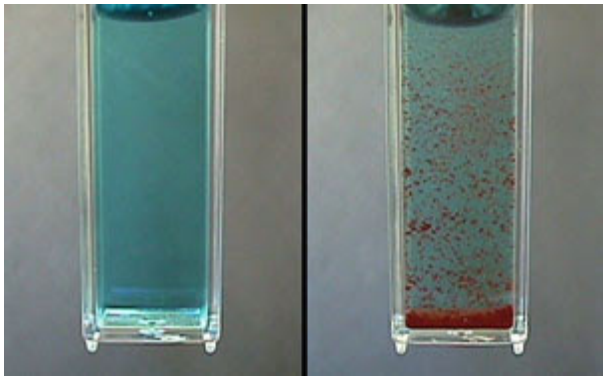
This test is based on the same principle as Benedict's test. But, the test medium is acidic. In acidic medium(pH 4.6) **monosaccharides** react faster than disaccharide. Monosaccharides react fast within 1-2 minutes but disaccharides take longer i.e. 7-12 minutes.

Reagent:

Barfoed's reagent: Dissolve 70 gm of cupric acetate monohydrate in 800 ml of water. Add 9 ml glacial acetic acid & make to 1000 ml with water.

Principle

$$RCHO + 2Cu^{2+} + 2H_2O \xrightarrow[\text{Acidic pH(4.6),Heat}]{} RCOOH + Cu_2O\downarrow + 4H^+$$



Seliwanoff's Test

Seliwanoff's test is a chemical test which distinguishes between aldose and ketose sugars. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses.

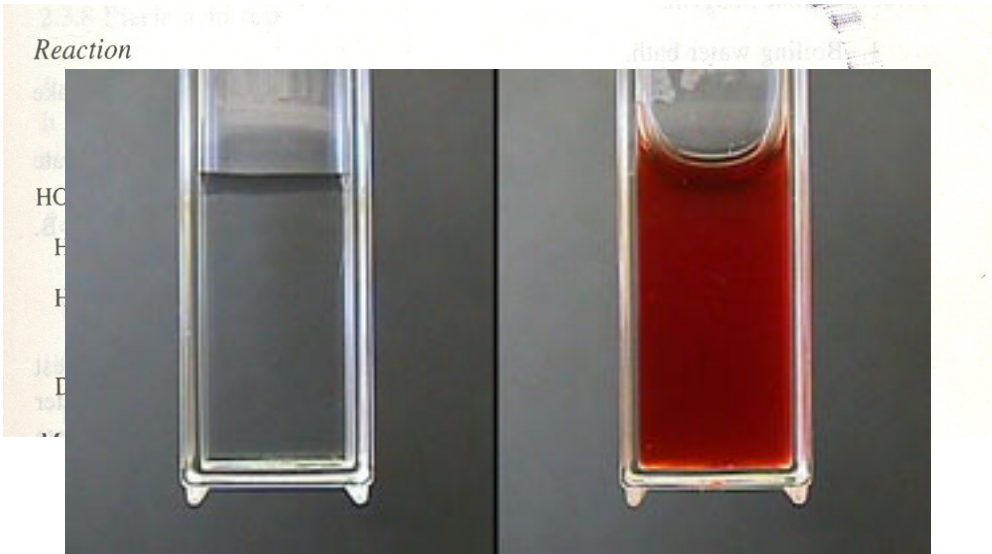
Reagent

Seliwanoff's reagent:Dissolve 0.05 gm of Resorcinol in 100 ml. of dilute (1:2) Concentrated hydrochloric acid(approximate 4 M HCL).

Principle

Ketohexoses like fructose on treatment with HCl form 5-hydroxymethylfurfural, which on condensation with resorcinol gives a cherry red complex.

Sucrose is hydrolyzed into glucose and fructose when boiled in acidic medium of Seliwanoff's reagent. Fructose, present in hydrolysate gives positive Seliwanoff's test.



Inversion Test:

Reagent

Conc.HCL

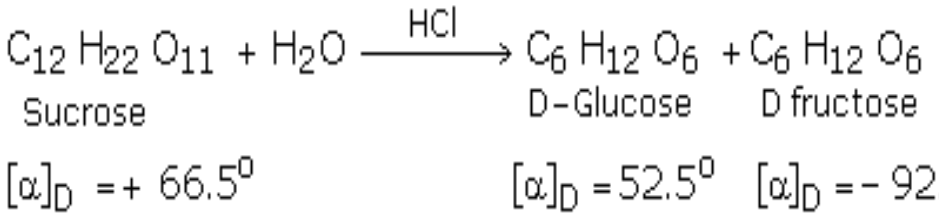
40% NaOH : dissolve 40 gm of NaOH pellet in 100ml Water

Benedict's reagent

Seliwanoff's reagent

Principle

When sucrose is boiled with conc. HCl, It is hydrolyzed into its constituent monosaccharides i.e. fructose and glucose. The hydrolyzed glucose and fructose give Benedict's test. Fructose gives seliwanoff's test.



Sucrose is dextrorotatory.

The optical rotation changes from dextrorotatory to leavorotatory on hydrolysis, since fructose causes a much greater leavorotation than the dextrorotation caused by glucose. This is known as inversion. The resultant hydrolysate is called invert sugar, which is sweeter than sucrose because fructose is sweeter than sucrose.

Iodine test for starch

Reagent:

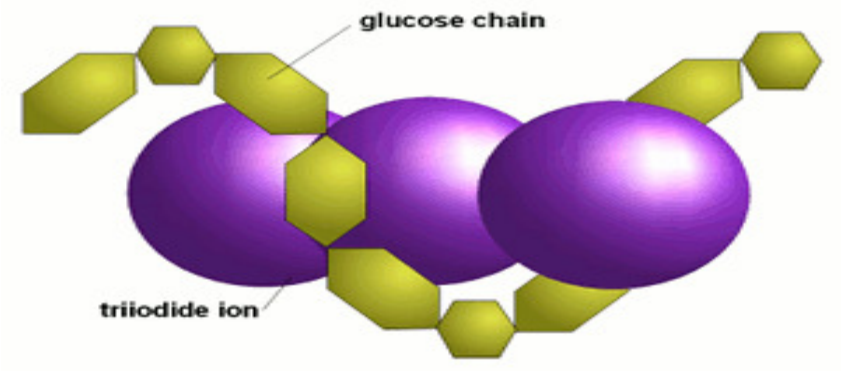
Iodine solution : Dissolve 1.27 gm Iodine and 3 gm potassium iodide crystals in 100 ml water.Dilute 1:10 in water before use.

Iodine by itself is very poorly soluble in water. One way to dissolve iodine in water is to add potassium or sodium iodine. Those salts dissolve into potassium or sodium ions and iodine ions. The iodine ion (I^-) reacts with the free iodine (I_2) to form a triiodide ion (I_3^-) which is soluble in water and can react with glucose chains.

Principle

Iodine binds starch to give blue colored complex.

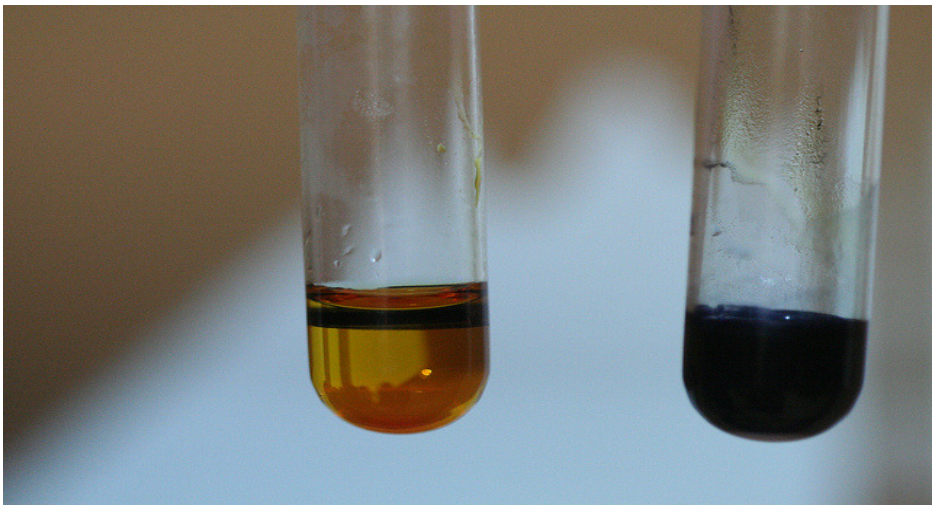
When glucose chains are sufficiently long they coil up like springs. This coil is supported by weak links between the glucose molecules. These links break down at high temperatures and the glucose chains uncoil. When the chains are longer than about 9 glucose molecules a triiodide ion (I_3^-) fits inside the coil (Figure).The longer the glucose chains are the more iodine molecules fit into the coils and the more intense the color reaction will be.



The resulting color depends on the length of the glucose chains. Shorter chains (starting at about 9 glucose molecules in unbranched chains and up to 60 glucose molecules in branches chains) give a red color .

Amylose, which consists of very long glucose chains between occasional branch points and very large **dextrines** give a dark blue color .

while **amylopectin**, which has much more branch points and shorter glucose chains between these branch points, gives a more reddish color in the presence of iodine.



Hydrolysis Test for starch

When starch/dextrin is boiled with HCl, It is hydrolyzed into its constituent monosaccharides i.e. glucose. Glucose, thus formed, gives Benedict's test.

TEST	METHOD	OBSERVATION	INFERENCE
Molisch's Test	1ml OS + 2 drops of α-napthol solution mix. add 2 ml. of conc. Sulphuric acid carefully through the side of the test tube without shaking.	Purple ring is formed at the junction of acid and solution.	Carbohydrate present.
Benedict's Test	5ml of Benedict's reagent + 8 drops of OS, mix Boil and cool.	Green / Yellow / Orange / Red / Brick Red precipitates seen	Reducing Group present.
Barfoed's Test	1 ml OS + 1 ml Barfoed's reagent Boil for 30 sec , Cool Excess boiling or may give false positive results.	Red colored precipitates. At the bottom of the tube.	Disaccharides absent. Monosaccharide present
Seliwanoff's Test	1 ml O.S. + 1 ml Seliwanoff's reagent. Boil	Red colored formed.	Keto sugars present e.g. Fructose
Iodine Test	1 ml OS + 2 drops of iodine solution, Mix	Blue color develops. Violet colour develops.	Starch present. Dextrine present.
Inversion Test	5 ml OS + 2 drops of conc. HCl. Boil for 2 mins. Cool. Make it alkaline with 5 drops of 40% NaOH. From this solution perform Benedict's test and Saliwanoff's test.	Benedict's and Saliwanoff's test are positive	Sucrose is present if OS give negative Benedict's test.
Hydrolysis test for starch/dextrin	**Step-1:** Perform Benedict's Test with OS. **Step-2:** 5 ml OS + 2 drops of conc. HCl . Boil for 2 mins. Cool. Make alkaline with 5 drops of 40% NaOH. From this solution perform Benedict's test	Benedict's test is negative/ weakly positive Benedict's test is positive	Starch present (weak Benedict's test with OS is due to free reducing groups at end of starch molecules.)
Glucose oxidase test (on strip or with liquid reagents)	Method for the test will be provided in the laboratory	Observation will be explained in the laboratory	Glucose present in the solution

What you will do:

Perform tests mentioned in above table with various carbohydrates given to you. Note down your observation and inference in tables as shown below.

TEST	OBSERVATION	INFERENCE
Molisch's Test		
Benedict's Test		
Barfoed's Test		
Seliwanoff's Test		
Iodine Test		
Inversion Test		
Hydrolysis test for starch/dextrin		
Glucose oxidase test (on strip or with liquid reagents)		

Questions:

Explain biochemical reason why Sucrose gives negative Benedict's test.

Why the hydrolysis of sucrose is called 'Inversion test'?

Does alpha-D-Glucose in the solution react with Glucose Oxidase? Explain.

3. Chemistry of Proteins and Amino acid

Proteins are made up of amino acids. Amino acids differ from each other in their side chain (-R group). The differing –R groups in different amino acids are responsible for many reactions mentioned below.

Preparation of Protein solutions:

Egg albumin solution (1:21): Mix 50 ml of egg(both white and yellow) in 1 liter of tap water. Use only for 24 hours

Gelatine solution(0.5%): Dissolve 5 gm of Gelatin powder in 50 ml of water by slight Heating & make upto 1 liter

Peptone solution(0.5%) :Dissolve 5 gm of Peptone powder in 50 ml of water by slight Heating & make upto 1 liter

Casein solution(0.5%) : Dissolve 5 gm of Casein powder in 20 ml of 40% NaOH & make upto 1 Liter with water

Biuret Test

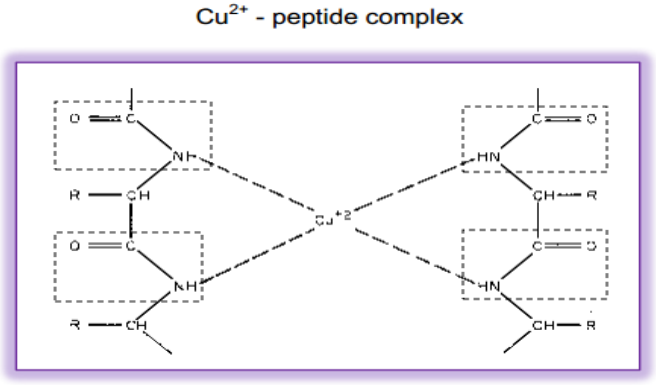
This test is given by all peptides having at least two peptide bonds. So, it is given by all proteins.

Reagents:

10% NaOH: Take 10gm NaOH pellets and make it up to 100ml with DI water.

1% CuSO4: 1 gm of CuSO4 in 100 ml DI water.

Principle:



Cupric ions of copper sulphate solutions in alkaline medium form coordinate complex with at least two nitrogens of the peptide bonds to form purple colored complex. Thus color intensity is proportionate to the presence of number of peptide linkages.

Minimum of 2 peptide bonds (3 amino acids) are required for binding of Cu2 + with peptide. single amino acids and dipeptides do not give positive test.

The name of reaction is derived from organic compound **biuret** which is formed by condensation of 2 urea molecules at high temperature.

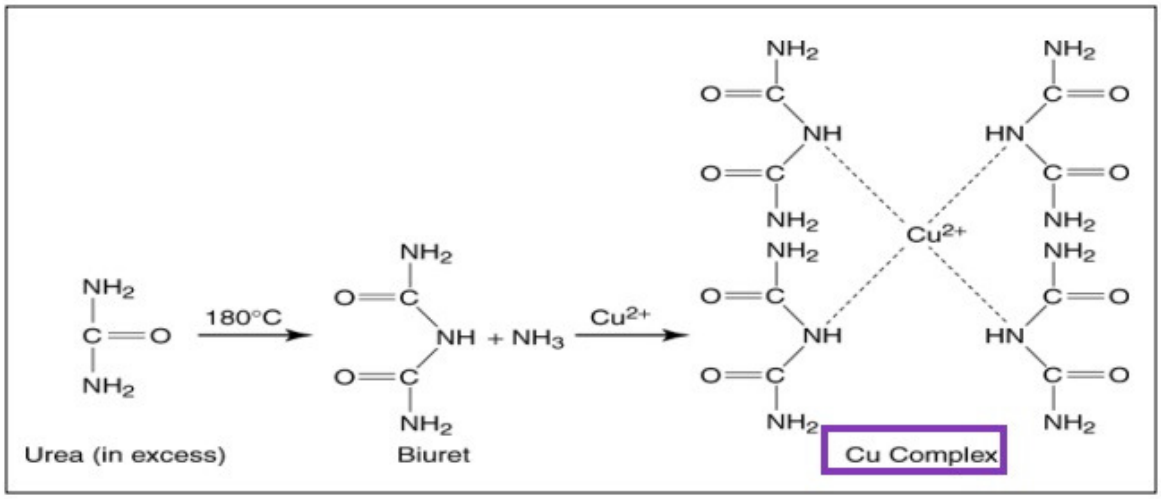


Figure of Biuret

Biurat is formed when solid urea powder is heated in a tube. The resultant Biurat is solid at room temperature and soluble in water.

The test produces color proportionate to number of peptide bonds which can be correlated with amount of protein. Similar reagent is used for estimation of serum proteins quantitatively.

Ninhydrin Test

This test is given by all compounds having free α-Amino groups. ex: peptides, proteins, free α- Amino acid. Different

Proline and hydroxyproline give yellow color in this test.

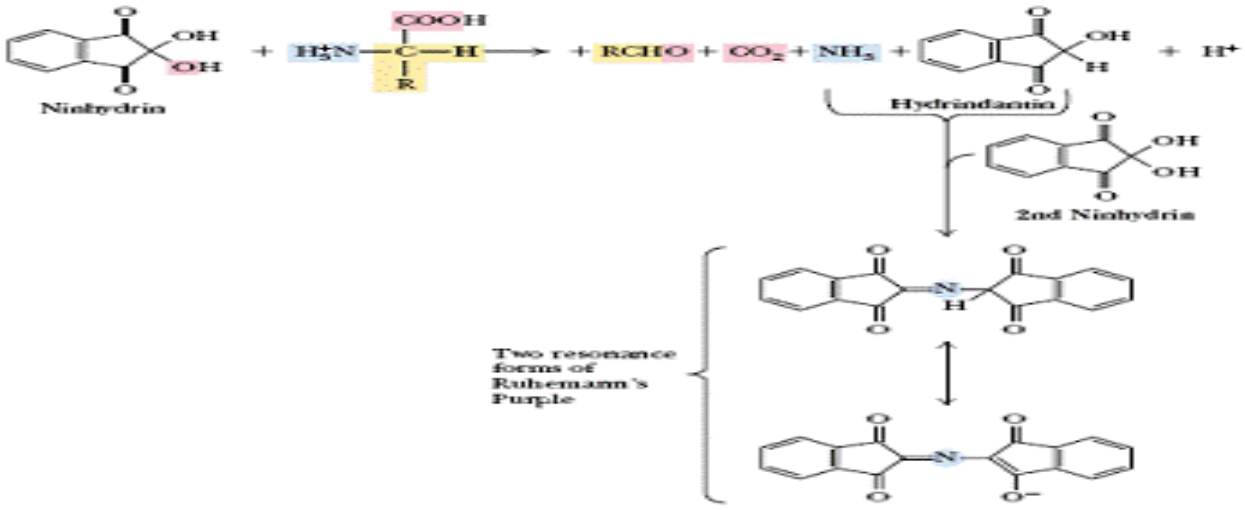
Prepare reagent:

1 % Ninhydrine solution : 1 gm of Ninhydrine powder disolved in 100 ml DI water.

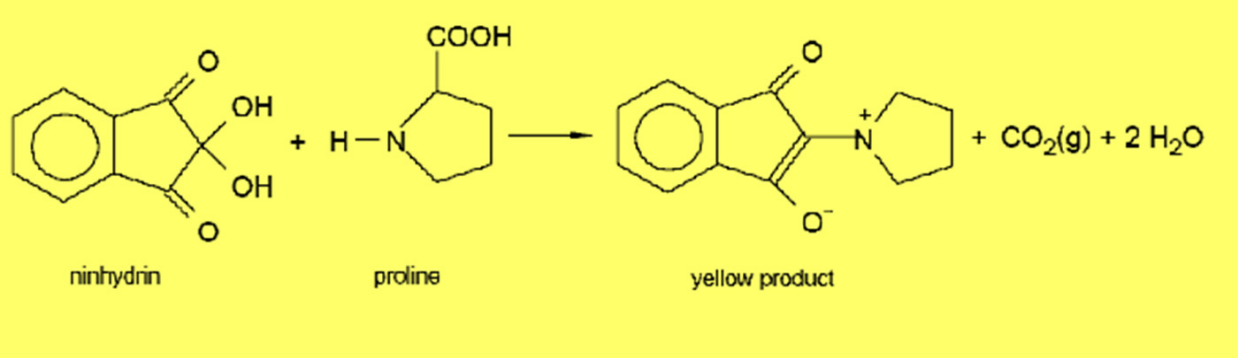
Principle:

Ninhydrine +α- Amino acid → hydrindantin + aldehyde + CO2 + NH3

Hydrindantin + NH3 + Ninhydrine → blue colored complex



Ninhydrin oxidises an α-amino acid to an aldehyde liberating NH3 and CO2 and is itself reduced to hydrindantin. Hydrindantin then react with NH3 and another molecule of ninhydrine to form a purple colored complex.



All amino acids that have a free amino group will give positive result (purple color) .
While not free amino group-proline and **hydroxy-proline** (amino acids) will give a (yellow color).

Note: Many substances other than amino acids, such as amines will yield a blue color with ninhydrin, particularly if reaction is carried out on filter paper.

Xanthoproteic Test:

This test is answered by aromatic amino acids. (Tyrosine, Tryptophane)

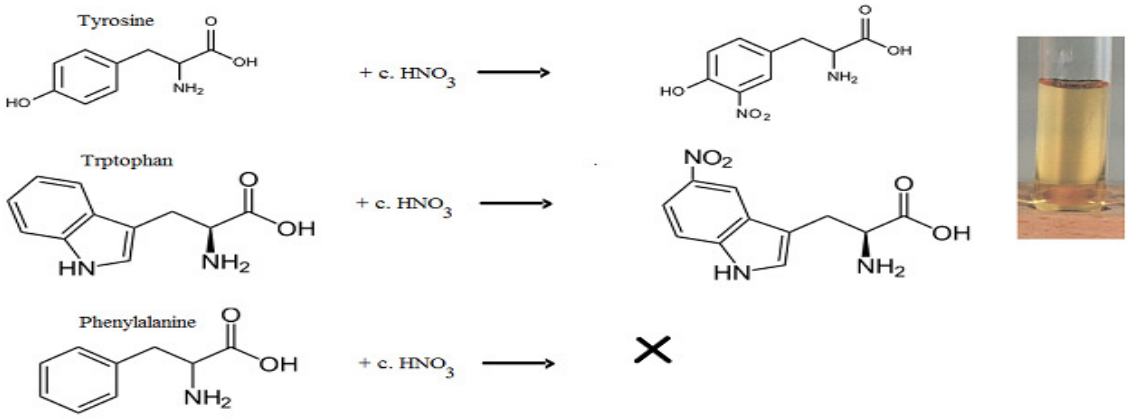
Reagent:

Concentrated HNO3

40 % NAOH : 40 gm NAOH in 100 ml DI water.

Principle

Concentrated nitric acid causes nitration of activated benzene ring of tyrosine and tryptophan. The nitrated activated benzene is yellow in color. It turns tro orange in alkaline medium.. Phenylalanine also contains benzene ring, but ring is not activated, so it does not undergo nitration. The reaction can be hastened by heating. The heat may be produced by dilution of concentrated HNO3 with OS or may require heating.



Aldehyde Test

Reagents

1:500 Formaldehyde Reagent:

Take 1 ml of Formaldehyde solution (37-41 % W/V) and make upto 500 ml with

DI water.Use only for 1 week. Old Formaldehyde may not give test.

1 % Sodium Nitrite solution :

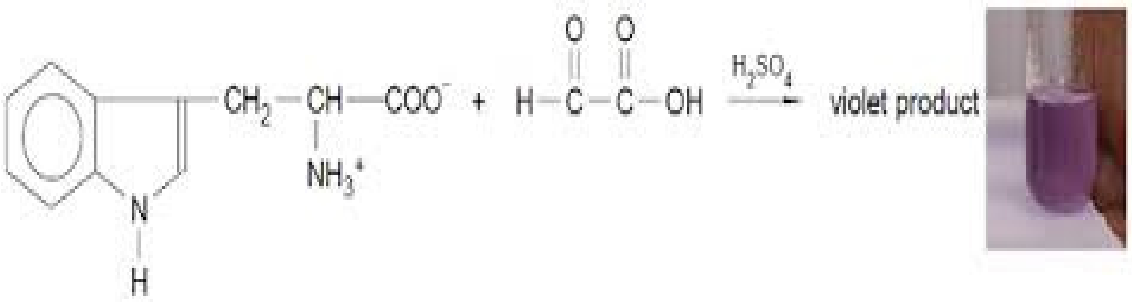
Take 1 gm sodium nitrite powder and make upto 100 ml with DI water. Use only for 1 week. Old Sodium nitrite may not give test.

Sulphuric acid AR :

Use sulphuric acid Bottle directly for use as reagent. Use for 1 week.Old Sulphuric acid may not give test

Principle

Indole ring is present in tryptophan. Formaldehyde react with indole ring to give violet colored complexes in presence of H2SO4. Addition of Sodium nitrite intensify and stabilize colour.



Millon's reagent

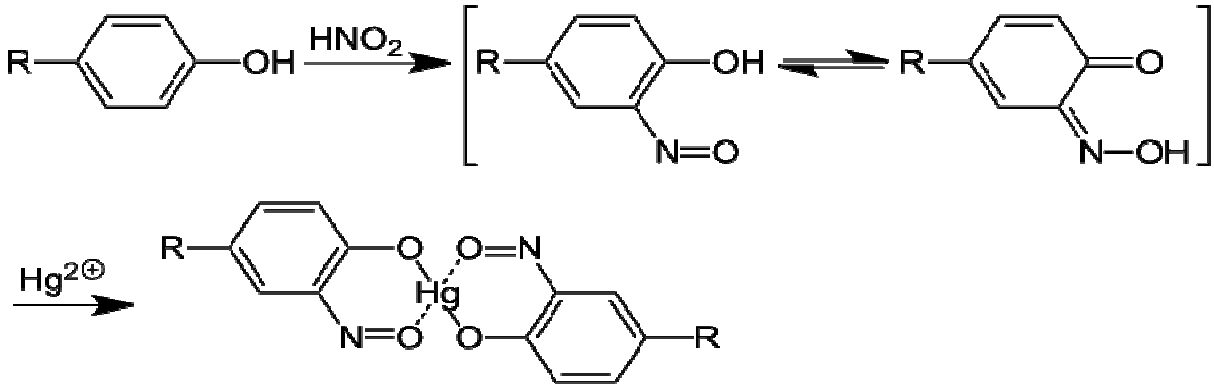
Reagent:

Millon's reagent**:**

Dissolve 10 gm of mercuric sulphate(HgSO4) +100ml DI water + 7 ml Conc.H2SO4

1% sodium nitrite:1 gm in 100 ml DI water

Principle



Tyrosine has hydroxyphenyl(Phenol) group. The hydrophobic group is in the core of protein. The protein is denatured by mercuric sulphate in boiling water exposing hydroxyphenyl group. Sodium nitrite reacts with sulfuric acid to form nitrous acid. The exposed hydroxyphenyl groups react with nitrous acid & give red colour precipitates.

Sakaguchi's Test

This test is for Guanido group Which is the R-group of arginine.

Reagent:

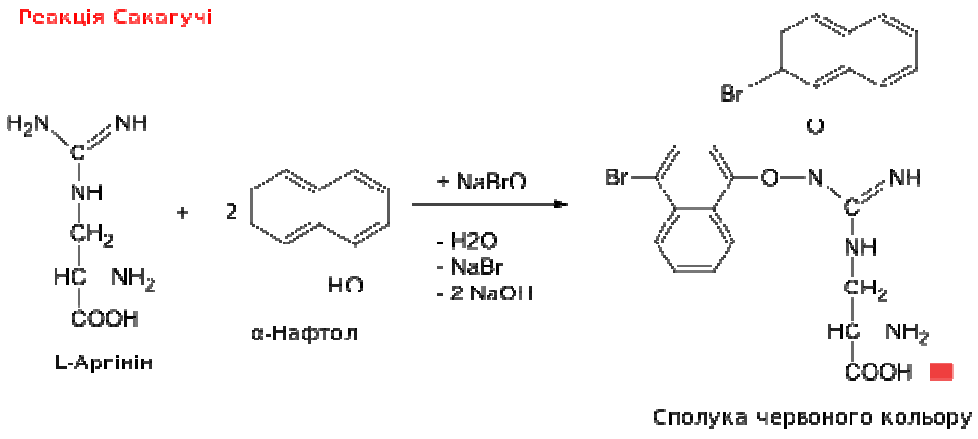
1%w/v α-Napthol: Dissolve 1 gm α-Napthol in 100 ml of methanol

10%w/v NaOH: Dissolve 10gm of NaOH & make it upto 100ml with DI water.

Alkaline hypochloride : Make 100 ml 10 % NaOH & add 8 ml 5-6 % Analytical grade Sodium hypochloride.

Principle

In an alkaline medium, alpha-Napthol combines with guanidino group of arginine to form a complex, which is oxidized by bromine/chlorine.



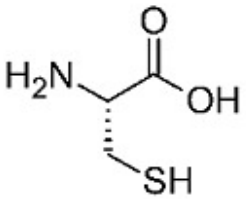
Sulphur Test (Lead acetate test):

Reagent:

2% Lead acetate in 10% NaOH**:** add 20 gm lead acetate, 100 gm NaOH in 1 liter of water. There is no need to make exactly up to 1 liter. Above solution will be more than 1 liter in volume.

Principle:

When protein containing cysteine & cystine is boiled with strong alkali, organic sulphur(R-SH) is converted to sulphide (Na_2S]. Addition of lead acetate to this solution causes precipitation of insoluble lead sulphide (PbS), which is black-gray in colour. Methionine does not give this test due to the presence of thioether linkage (H3C-S-CH2-R) which does not allow the release of sulphur in this reaction.



Cysteine

$NaS \rightarrow Na^+ + S^{2-}$

$Pb(CH_3COO^-)_2 + S^{2-} \rightarrow PbS\downarrow + 2CH_3COO^-$

Heat coagulation test:

Reagent:

1% acetic acid: 1 ml acetic acid up to 100 ml with DI water.

Principle

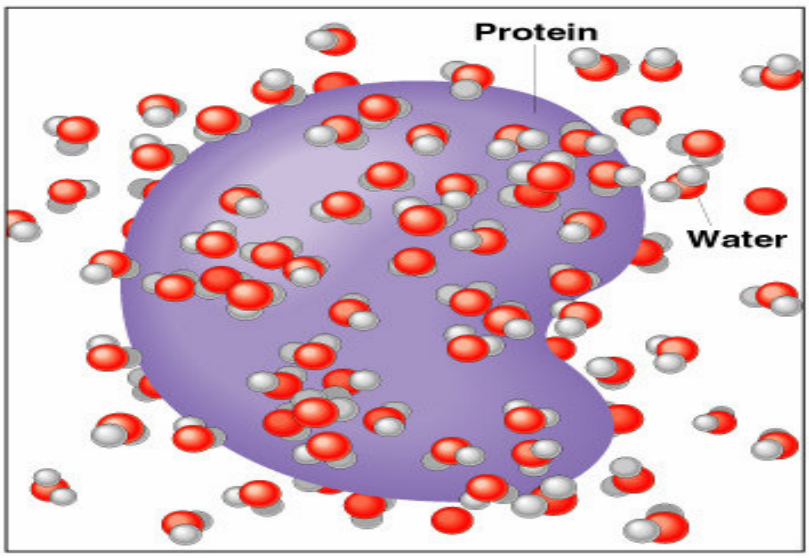
Proteins have net zero charge at their iso-electric pH (pI). So, at pI, protein molecules have minimum repelling force. Thus proteins are easily precipitated at pI. When proteins are heated, weak bonds like hydrogen-bonds, salt bonds and van-der-wal forces are broken. Proteins are said to be denatured.

Core hydrophobic regions of denatured Albumin can form intermolecular associations and cause precipitation.Thus, in order to precipitate proteins like albumin, two conditions are required. 1) Bring albumin to its pI(5.4) by adding few drops of 1% acetic acid. 2)Heat the solution

Half & Full Saturation Test:

Reagent:
Saturated ammonium sulphate [(NH4)2SO4]: Add ammonium sulphate in 500 ml DI water till it stops dissolving.
Ammonium sulphate [(NH4)2SO4] power

Principle
When ammonium sulphate is added to protein solution, water concentration decreases. This removes shell of water from outer surface of protein molecules, favoring formation of hydrogen bonds among protein molecules and causing their precipitation. While proteins like globulin, gelatin and casein are precipitated in half-saturated ammonium sulphate solutions, albumin is precipitated in full-saturated ammonium sulphate solution.



Protein molecules contain both hydrophilic & hydrophobic aminoacids.
In aqueous medium, hydrophobic amino acids form protected areas while hydrophilic amino acids form hydrogen bonds with surrounding water molecules (solvation layer).When proteins are present in salt solutions (e.g.ammonium sulfate), some of the water molecules in the solvation layer are attracted by salt ions. When salt concentration gradually increases, the number of water molecules in the solvation layer gradually decreases until protein molecules coagulate forming
a precipitate; this is known as “salting out”.

For example,albumin requires higher salt concentration for precipitation than casein or gelatin.Albumin particals are smaller in size & so have larger surface area,so they hold more water molecules around them.so a higher concentration of Ammonium sulphate is required.The salt concentration used is described as 'half saturation'(for

casein,gelatin,globulin) or ' full saturation' (for albumin).

PROCEDURES

TEST	METHOD	OBSERVATION	INFERENCE
BIURET	➢ 10% NaOH (2 ml) + 1% CuSO4 (2 ml) ➢ divide above mixture in two parts of 2 ml ➢ part 1: add 2 ml OS part 2: add 2 ml H2O	Pink or Violet Colour develops in part 1. No such color develop in part 2	Two or more peptide linkages present. Protein present
XANTHO-PROTEIC TEST	➢ OS (0.5 ml) + $HNO3_{con}$ (1 ml) Mix it. (Solution turns yellow) + 40%NaOH (1 ml) in above mixture. Solution turns orange Note: **Use Fresh(tightly packed) conc.HNO otherwise test come negative.**	Yellow-Orange colour develops.	Aromatic Amino Acids Tyrosine and Tryptophan present in protein.
NINHYDRIN TEST	➢ OS (1 ml) + 1% Ninhydrine (2 drops) ➢ Mix, Boil (1 min). ➢ Cool.	Blue or Purple colour develops.	Alpha Amino groups of proteins at N-terminal are responsible for positive test with proteins.

Aldehyde Test	➢ 1 ml Protein Solution + 1 drop of 1:500 formalin. Mix. ➢ Slant the test tube and slowly add 1 ml of conc. H_2SO_4 . Mix. ➢ Add 1 drop of 1% sodium nitrite solution in Test tube. Mix. ➢ **Use Fresh(tightly packed) conc.H2SO4 &1:500formaline otherwise test come negative.**	Violet color is formed.	Indole group present in protein. Tryptophan present in the protein.
\ MILLION'S TEST	➢ 0.5 ml protein sol. +50 ul sodium nitrate sol.^{n}+100 ul Millon's reagent. mix well & Heat	Red coloured precipitate 0bserved.	Hydroxyphenyl group present in protein. Tyrosine present in protein.
S AKAGUCHI'S TEST	1 ml Protein sol.^{n} + 2 drops of alpha Napthol + 1 ml Alkaline sodium hypoochloride	Carmine Red colour observed.	Guanidino group present in protein. Arginine present in protein.
MOLISCH'S TEST	➢ 1ml OS + 2 drops of α-napthol solution, mix ➢ Add 2 ml. of conc. Sulphuric acid carefully through the side of the test tube without shaking.	Purple ring is formed at the junction of acid and solution.	Proteins contain Carbohydrates
SULPHER TEST (Lead acetate test)	➢ 0.5 ml OS + 0.5 ml Lead acetate reagent ➢ Boil for 1 minute	Black- Grey colour seen.	Sulfhydryl group (-SH) present in protein. Cysteine & Cystine present in protein
HEAT COAGULATION TEST	Heat upper part of 5 ml Protein solution. After heating ,add 2-4 drops of 1% acetic acid.	White precipitates seen in upper part of solution, as compared to clear lower part of solution	Albumin is precipitated when denatured at its pI~5.4
HALF SATURATION TEST	2 ml of the protein sol.^{n} + 2 ml of saturated sol.^{n} of $(NH_4)_2 SO_4$ (Thus, saturated $(NH_4)_2 SO_4$ is half diluted)	White precipitate formed.	Casein, Gelatin and Globulin are precipitated at half saturation with $(NH_4)_2 SO_4$

FULL SATURATION TEST	5 ml. Of protein sol.^{n} + a pinch of Ammonium Sulphate powder, Shake Repeat above steps till some undissolved $(NH_4)_2\ SO_4$ remains at the bottom of the test tube.	White precipitate formed	Albumin precipitates at full saturation with $(NH_4)_2\ SO_4$

What you will do:

- Perform tests mentioned in above table with various Protein Solutions given to you. Note down your observation and inference in tables as shown below.

TEST	OBSERVATION	INFERENCE
Biuret Test		
XANTHO-PROTEIC TEST		
NINHYDRIN TEST		
Aldehyde Test		
MILLION'S TEST		
SAKAGUCHI'S TEST		
MOLISCH'S TEST		
SULPHER TEST		
HEAT COAGULATION TEST		
HALF SATURATION TEST		
FULL SATURATION TEST		

- Fill up the table given below.

Use: 'P' for positive test 'N' for negative test 'W' for weakly positive test

Test	Amino acids responsible for the test	Albumin	Casein	Gelatin	Peptone
Xanthoproteic test					
Ninhydrin test					
Hopkin's and Cole test					
Million's test					
Sakaguchi's test					
Lead acetate test					

- Mention food sources of Albumin, Casein and Gelatin.

- Which of the Albumin, Casein and Gelatin is nutritionally best? Explain.

- If by mistake Ninhydrin touches your skin while doing the ninhydrin test, skin gets bluish stain. Explain.

4. Chemistry of lipids

Lipids are heterogeneous group of compounds soluble in non-polar solvents like chloroform but not soluble in polar solvents like water.

While body is water medium, lipids of body require specialized methods for digestion, absorption and transport.

Bile salts cause emulsification of oil due to their amphipathic nature and ability to reduce surface tension. Thus making bile salts essential for digestion and absorption of lipids of food.

Lipids of blood are transported as lipoproteins. Without lipoproteins, lipids would be insoluble is plasma (93% water).

Reagent

Any oil : Ground nut oil, coconut oil
Non polar Solvent : Acetone/ Methanol
Bile salt solution : Dissolve 0.6 gm sodium deoxycholate in 100 ml DI water. Donot take tape water for making bile salt solution,Precipitation occur due to interference by calcium.

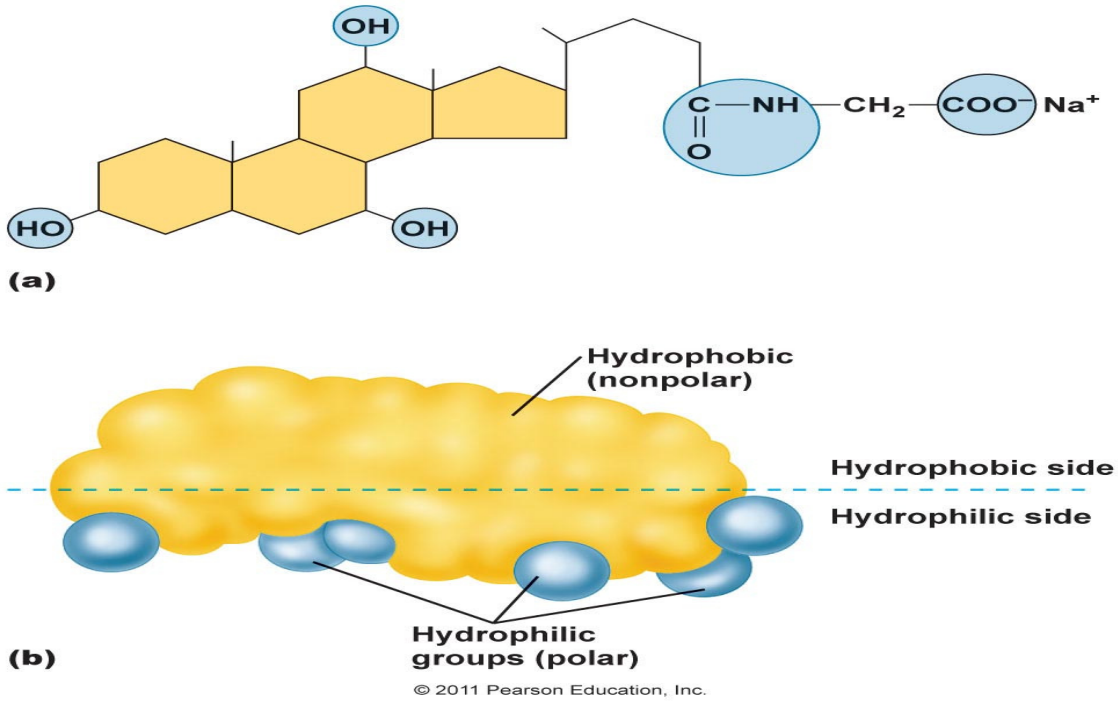
TEST	METHOD	OBSERVATION
Solubility of oil in water	➢ 0.1 ml of oil + 1 ml water, mix, for 15 sec.	Big oil drops are observed
Solubility of oil in non-polar solvent	➢ 0.1 ml of oil + 1 ml Acetone/Methanol, mix, for 15 sec.	oil droplets are not observed
Emulsification of oil in Bile salts.	➢ Take 2 test tubes T1 and T2 ➢ Take 1 ml H2O in T1 test tube. ➢ Take 1 ml Bile salt solution(Sodium deoxycholate solution) in T2 test tube. ➢ Add 0.1 ml of oil in T1and T2. ➢ Mix T1 & T2,all together for 15 sec. against palm of your hand.	Compare size of oil drops and turbidity immediately . **T1**: Big oil drops, Clear water(Compared to T2) **T2**: Small oil drops, Turbid solution (Compared to T1)

What will you do:

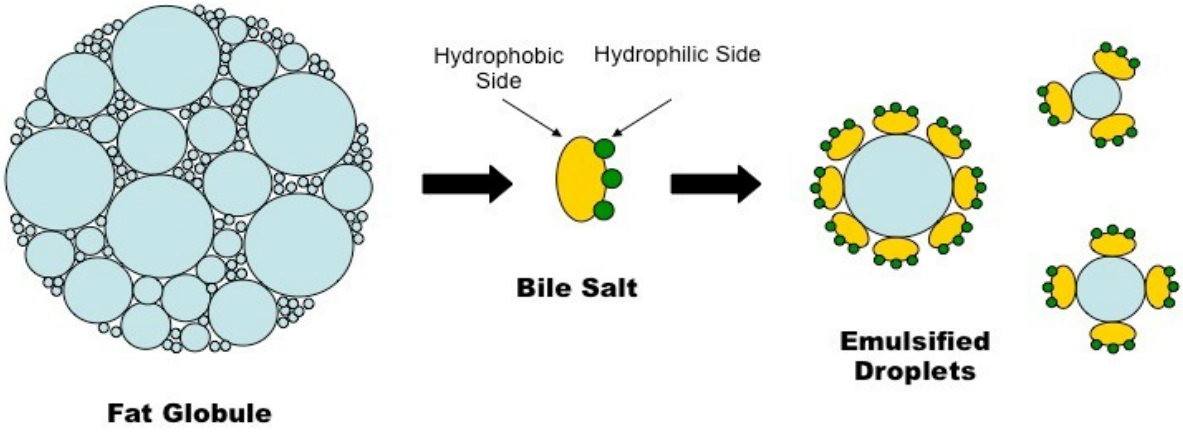
- Perform the test shown above with the oil provided. Draw table showing the tests and your observations.

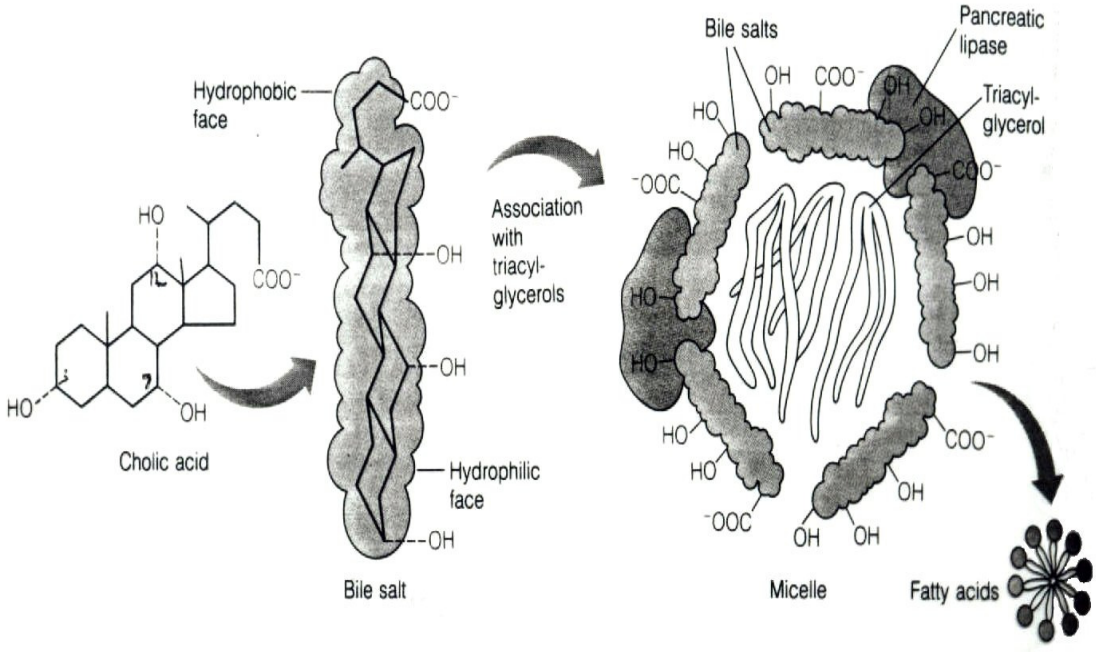
TEST	OBSERVATION	INTERFERENCE
Solubility of oil in water		
Solubility of oil in non-polar solvent		
Emulsification of oil in Bile salts.		

Draw structure of bile salt

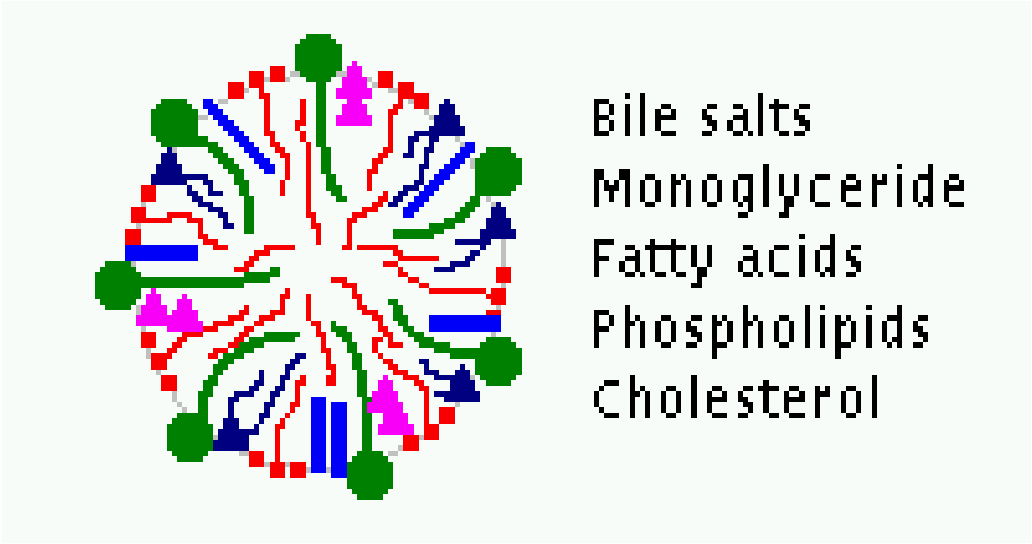


Draw structure of an oil droplet in a bile salt solution.

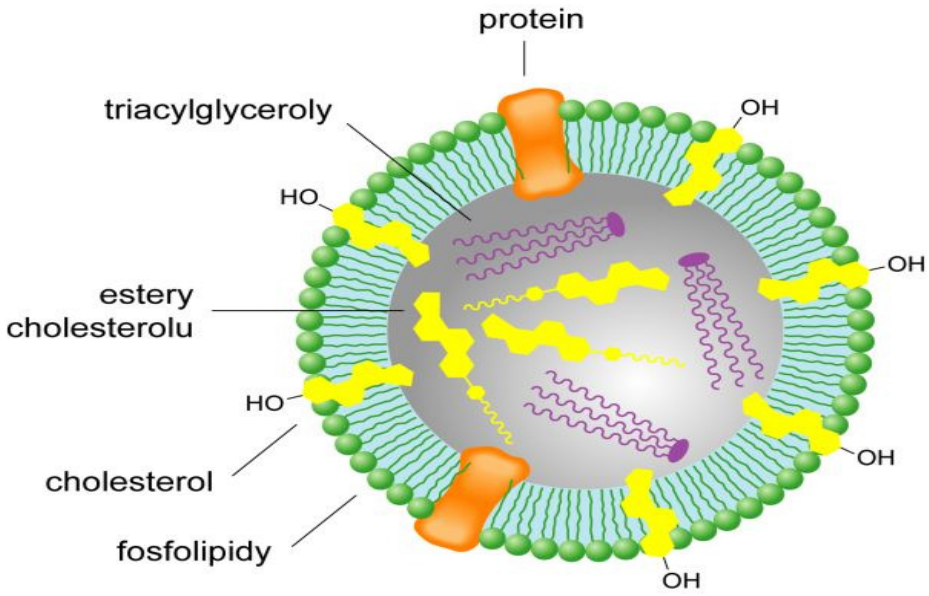




Draw structure of a micelle. Write its function in body.



Draw structure of a lipoprotein particle. Write its function in body.



5. Physiological Urine

Artificial Urine sample:

Ammonium sulfate	2 gm
Sodium phosphate dibasic(monobasic	2 gm
Pottasium dihydrogen phosphate	2 gm
Urea powder	2 gm
creatinine powder	2 gm
Uric acid powder	1 gm
Calcium carbonate/Calcium chloride :	1 gm.
NaCl	4 gm,

And make upto 2 liters

Urine is examined by (1) Physical method (2) chemical method

(1) Physical method

Physical characteristics of urine

Volume: Normal adult excretes 800-2000 ml of urine daily

Factors affecting urine volume:

According to quantity of fluid ingested, environment temperature, physical activity, loss of water in feces, via skin, in vomitus etc.

Collection of urine to measure volume:

Discard the first morning urine. Then collect urine during each micturition in a vessel up to, including the next morning urine.

Some conditions with increased urine volume:

- Diabetes mellitus
- Diabetes insipidus (low specific gravity of urine)
- Diuretics drug therapy

Some conditions with decreased urine volume:

- Dehydration
- Renal failure

Urine output volume is measured

- In patients dependent on IV fluid input (to detect dehydration and overhydration)
- To monitor treatment of dehydration
- To adjust water intake of patients of renal failure.

Appearance: Normal urine is clear and transparent when freshly voided. On standing bacterial urease converts urea into CO2 and Ammonia. Ammonia makes urine alkaline. Phosphates precipitate in alkaline urine making it turbid.

Colour: Fresh urine is amber yellow. This colour is due to urobillin.

Odour: Fresh urine has an aromatic odor due to presence of volatile organic acids produced by body and intestinal bacteria.

Reaction: Fresh urine is normally acidic (pH<7.0). Post-prandial urine is alkaline due to secretion of HCl in stomach, the condition known as “Alkaline Tide”.

Specific gravity: Normal range-1.003 to 1.035 gm/ml of urine. The greater the amount of solutes per unit volume of urine, the greater the specific gravity. It is high in diabetes mellitus, while low in diabetes insipidus.

Determination of specific gravity: Wipe the urinometer by a filter paper and allow it to float in the urine contained in the cylinder. See carefully that the

apparatus do not touch the sides or bottom of the cylinder, when it is at rest take the reading from lower meniscus (true surface) of urine. Note the temperature of urine. If it differs from the standard temperature written on the urinometer, add one unit (0.001) for every 3 degree rise from the standard temperature.

(2) chemical method

Inorganic Chemical constituents

Ammonia:

Reagent:

1% phenolphthalein : Dissolve 0.5 gm of phenolphthalein in 50 ml of methanol. Phenolphthalein is insoluble in water

2% sodium carbonate : Dissolve 10 gm of sodium carbonate in 500 ml of water

Principle:

Urinary ammonia is derived from glutamine in kidney. It is secreted as a buffer against H+ secreted by tubules.

$NH_4^+ + OH^- \rightarrow NH_3 + H_2O$

On heating NH3 evaporate, dissolve in water around a glass road and make it alkaline. At alkaline pH phenolphthalein ions are formed which is pink coloured.

Phenolphthalein is a weak acid, which can lose H^+ ions in solution. The phenolphthalein molecule(HIn) is colorless, and the phenolphthalein ion(In^-) is pink. When a base is added to the phenolphthalein, the molecule ions equilibrium shifts to the right, leading to more ionization as H^+ ions are removed.

$$HIn \text{ (colourless)} \xrightarrow[\text{PH } >8.2]{} H^+ + In^- \text{ (Red colour)}$$

For phenolphthalein: pH 8.2 = colorless; pH 10 = red

Procedure:

- Take 5ml urine in a test tube and add a drop of phenolphthalein. Add drop wise 2% sodium carbonate solution till the solution turns faint pink. Boil and hold a glass rod dipped in phenolphthalein at the mouth of the test tube. Phenolphthalein turns pink due to gaseous ammonia.

Chloride:

Reagent:

Concentrated HNO3

3% AgNO3 : Dissolve 15 gm of AgNO3 in 500 ml of water.

Principle:

$AgNO_3(aq) + NaCl(aq) \rightarrow AgCl(s) + NaNO_3(aq)$

White precipitation

When acidified urine reacts with silver nitrate,a white precipitate of silver chloride is formed.

Procedure:

- [3 ml of urine] + [1.0ml concentrated HNO3] + [1.0 ml 3% AgNO3]

  Curdy white precipitate of AgCl is formed.

(Concentrated HNO3 is added to prevent precipitation of urate and acid phosphates by AgNO3)

Calcium:

Reagent:

Saturated ammonium oxlate solution: Dissolve ammonium oxalate powder in 500 ml of water till it become undissolved.

Principle:

Calcium precipitated as insoluble calcium oxalate with ammonium oxalate

$CaCl_2(aq) + (NH_4)_2C_2O_4(aq) \dashrightarrow CaC_2O_4(s) + 2\ NH_4Cl(aq)$

Procedure:

- Sulkowitch Test: To 5 ml urine and add 3 ml saturated ammonium oxalate solution.

  Calcium precipitated as insoluble calcium oxalate is observed as turbidity.

Phosphorus:

Reagent:

Concentrated HNO3

5% Ammonium Molybdate : Dissolve 5 gm of Ammonium Molybdate in 100 ml of water

Principle: Inorganic phosphorus reacts with ammonium molybdate in an acidic medium to form a phosphomolybdate complex.

$$H_3PO_4 + 12(MoO_3) \xrightarrow[H_2O]{H^+} H_3PMo_{12}O_{40}$$

Molybdophosphate complex

Procedure:

- [2-ml of urine] + [0.5 ml concentrated HNO3] + [3 ml of 5% Ammonium Molybdate], Heat

Canary yellow precipitate of Ammonium phosphomolybdate are formed

Sulphate:

Reagent:

1 % HCL : Take 1 ml of concentrated HCL & make upto 100 ml
10% Barium chloride :Dissolve 50 gm of Barium chloride in 500 ml of water

Principle:

$$SO_4^{-2} + BaCl2 \xrightarrow{HCL} BaSO4 + KCL$$

Procedure:

[5 ml urine] + [1 ml 1 % diluted HCL] + [2 ml of 10% Barium chloride].
White precipitate of BaSO4 are formed

Organic Chemical constituents

Urea:

(Specific Urease Test)

Reagent:

1% phenolphthalein :Dissolve 1 gm of phenolphthalein in 100 ml methanol

Principle:

$$\text{Urea} \xrightarrow[\text{Urease}]{} NH3 + CO2$$

CO2 evaporates.

$$NH3 + H2O \longrightarrow NH4^{+} + OH^{-}$$

In this reaction the liberation of NH3 changes the pH to alkaline side, turning phenolphthalein to pink colour.
On heating NH3 evaporate, dissolve in water around a glass road and make it alkaline. At alkaline pH phenolphthalein ions are formed which is pink coloured.

Phenolphthalein is a weak acid, which can lose H^+ ions in solution. The phenolphthalein molecule(HIn) is colorless, and the phenolphthalein ion(In^-) is pink. When a base is added to the phenolphthalein, the molecule ions equilibrium shifts to the right, leading to more ionization as H^+ ions are removed.

HIn ----------> H^+ + In^-
colourless PH >8.2 Red colour

Procedure:
[2 ml Urine] + [2 drops phenolphthalein]
Add 2% Na2CO3 till faint pink color is seen.
Add acetic acid, one drop at a time, with mixing, till faint pink color just disappears.
Add a spatula of Urease powder(Jack Bean Meal Powder), mix.
Pink color develops after few minutes.

Uric acid:

Phosphotungstic acid reduction test:
Reagent:
10% Sodium carbonate: Dissolve 10gm of sodium carbonate in 100ml of water
Phosphotungstic acid Reagent :
Stock : Dissolve 50 gm of sodium tungstate in 400 ml of water & add 40ml of 85% phosphoric acid .Make final volume to 500ml.
Working : Dilute 50ml of stock to 500ml with water.
Principle:
Uric acid is reducing agent in alkaline medium.It reduced phosphotungstic acid into tungsten blue.

Procedure
To 2.5 ml of urine add 0.5 ml of sodium carbonate and 0.5 ml of Phosphotungstic acid reagent working reagent

Creatinine

Reagent:
Refer SOP in dokuwiki document
Creatinine R1 (NaOH)

1. Weigh 24 gm NaOH.
2. Dissolve in approximately 500 ml DI water.
3. Add 20 ml of 30% brij in above mixture.
4. Weigh 2 gm SDS and pour it into approximately 200 ml water in beaker. Heat the solution until SDS dissolve.
5. Add SDS containing solution in main mixture.
6. Make upto 2 liter with DI water.

Creatinine R2 (Picric acid)

1. Dry picric acid between filter paper pieces
2. Weight 9.16 gm dry picric acid
3. Dissolve in approx. 600 ml water

4. Add 20 ml of 30% Brij in above mixture.
5. Remove froth with a clean object of glass or plastic dipped in capryl alcohol
6. Make 2 liter with water

5% NaOH : Dissolve 10 gm of NaOH in 200 ml of water
Picric acid :

Principle:

Creatinine forms creatinine picrate in alkaline medium which is orange in colour

Procedure

2 ml alkaline picrate solution + 1 drop of urine & mix

What will you do:

Note physical characteristics of urine given to you, Draw table.

physical characteristics of urine	Observation	Interference
Volume		
Appearance		
Colour		
Odour		
Reaction		
Specific gravity		

Perform tests for inorganic constituents of urine. Draw table.

Inorganic constituents of Urine	Observation	Interference
Ammonia		
Chloride		
Calcium		
Phosphorus		
sulphate		

Perform tests for organic constituents of urine. Draw table.

Organic constituents of Urine	Observation	Interference
Urea		
Uric acid		

Creatinine		

6. Pathological Urine-I

Appearance:
Turbid: infection (cells make urine turbid)
Color:
Yellow: Hepatic jaundice & obstructive jaundice (Conjugated bilirubin)
Red: Hematuria, rifampicin therapy
Red on exposure to air: porphyria
Black on exposure to air: alkaptonuria
Odour:
Fruity: diabetic ketoacidosis (acetone)
Mousy smell: Phenylketonuria. (Phenylacetyl glutamine)
Foul smell: Urinary tract infections. (H2S etc.)

I.Protein:

Reagent :

Sample preparation :

10 mg% albumin : Dissolve 100 mg bovin albumin in 1000 ml of water
50 mg% albumin: Dissolve 500 mg bovin albumin in 1000 ml of water
100 mg% albumin: Dissolve 1000 mg bovin albumin in 1000 ml of water
1% Acetic acid: 5 ml of acetic acid in 500 ml of water
30% Sulphosalisylic acid: Dissolve 150 gm of Sulphosalisylic acid in 500 ml of water

Proteinuria and albuminuria

Category	Protein	Albumin
Normal Adult	<150 mg /day	
Proteinuria	>=150 mg /day	
Proteinuria (Nephrotic range)	>3500 mg / day	
Normal Adult		<30 mg /day
Microalbuminuria		30-300 mg /day
Macroalbuminuria		>300 mg /day

Albumin (Filtered but not reabsorbed) and Tamm-Horsfall protein (secreted by renal tubules) are normally present.

Causes of Proteinuria:

Pre-renal: (overload proteinuria)
(Many non-Albumin proteins)
Multiple myeloma (light chains of immunoglobulins)
Severe hemolysis (Hemoglobin)
Severe muscle injury (Myoglobinuria)

Renal:*Glomerular diseases* (Mainly albumin, being small)
After streptococcal infection
Diabetes mellitus
Hypertension
Lipoid Nephrosis (Nephrotic range proteinuria)
Tubular diseases (decreased reabsorption of proteins)
(Small, normally reabsorbed, proteins like Beta2 microglobulin, Retinol Binding protein)
Tubular necrosis due to Drugs and toxins

Post Renal: (various blood and cellular proteins)
Bleeding in urinary tract
Infection in urinary tract
Tumor in urinary tract

Other causes:
Postural: on standing posture.
Exposure to cold, physical activity, fever.
Last weeks of pregnancy

Heat coagulation Test:

Principle :

Proteins have net zero charge at their iso-electric pH (pI). So, at pI, protein molecules have minimum repelling force. Thus proteins are easily precipitated at pI.

When proteins are heated, weak bonds like hydrogen-bonds, salt bonds and van-der-wal forces are broken. Proteins are said to be denatured.

Core hydrophobic regions of denatured Albumin can form intermolecular associations and cause precipitation.

Thus, in order to precipitate proteins like albumin, two conditions are required. 1) Bring albumin to its pI(5.4) by adding few drops of 1% acetic acid

2) Heat the solution

Procedure

Fill 3/4 th of the test tube with urine sample,Heat the upper part on the flame till either turbidity appears or urine starts boiling.Then add few drops of 1% acetic acid if turbidity develops & note change.

In case of multiple myeloma, light chains of immunoglobulin precipitate between 40-60 degrees centigrade. With further heating turbidity disappears. Turbidity appears again on cooling to 40-60 degree centigrade.

Sulphosalisylic Test:

Principle
Test is based on the precipitation of urine protein by a strong acid, sulfosalicylic acid. Precipitation of protein in the sample seen as increasing turbidity)
Unlike the routine urine protein chemistry dipstick pad, the SSA reaction will detect globulin and Bence-Jones proteins, in addition to albumin

Method: 3 ml of urine + 0.3 ml of 30% Sulphosalisylic acid, mix.
Turbidity indicates presence of urinary proteins.
Iodinated contrast agents used for evaluation of renal disorders can give the test positive.
False positives:
X-ray contrast media
High concentration of antibiotics, such as penicillin and cephalosporin derivatives.
False negatives:
Highly buffered alkaline urine. (The urine may require acidification to a pH of 7.0 before performing the SSA test.)
Dilute urine
Turbid urine - may mask a positive reaction. Again, best practice is to always used supernatant from a properly spun urine sample.

Dip-Stick Test:

Principle
Testing for protein is based on the phenomenon called the "**Protein Error of Indicators**" (ability of protein to alter the color of some acid-base indicators without altering the pH).

This principle is based on the fact that proteins alter the colour of some pH indicators even though the pH of the media remains constant. This occurs because proteins (and particularly albumin) acquire hydrogen ions at the expense of the indicator as the protein's amino groups are highly efficient acceptors of H+ ions.

Indicator-H+(Yellow) + Protein → Indicator(Blue-green) + Protein-H+

At pH 3 and in the absence of proteins both indicators are yellow, as protein concentration increases the colour changes through various shades of green until it becomes a dark blue.

According to the manufacturer, the strip's protein pad contains tetrabromophenol blue or 3',3,5',5-tetrachlorophenol-3,4,5,5-tetrabromosulphonphthalein, as well as an acid buffer substance to maintain pH at a constant level.

The main problem with the protein tests found on urine test strips is that very alkali urine can neutralise the acid buffer and produce a false positive reading

that is unrelated to the presence of proteins. Another similar error occurs if the strip is left submerged in the urine sample for too long.

This method is more sensitive to albumin than to globulin, Bence Jones protein and mucoprotein are examples of globulin components that are sometimes present in urine, but are not distinguishable by the dipstick method for protein

Method: Dip the strip for Albumin in urine. Drain excess urine from strip. Read the color chart. (Read instruction manual provided with the strips for time of reading after dip.).

Because the dipstick test detect albumin, it can not identify many pre-renal proteinuria caused by Hb, Mb and light chains of Igs.

All the three tests mentioned above are qualitative and used for screening proteinuria and albuminuria. Once proteinuria is found quantitative estimation of proteinuria and albuminuria is required for clinical decision making.

What Will You Do:
Perform all three tests with urine. Draw table of your observations.

Sr. no.	Concentration	Heat coagulation test	Dipstick test	Sulphosalisylic acid	Interference
1	10 mg %				
2	50 mg %				
3	100 mg %				
4	Urine sample				

Which of the three tests is most sensitive?

Write biochemical explanation of proteinuria in diabetes mellitus and hypertension.

II.Acetone & acetoacetic acid (Ketone Bodies):

Reagent

Ammonium sulphate powder
Small crystals of sodium nitroprusside
liquor Ammonia
Rothera's powdered reagent : Sodium Nitroprusside 1 gm
Sodium carbonate 20 gm
Ammonium sulphate 20 gm
Mix & grind all in fine particales & stored in air-Tight container.

Sample Preparation

0.1 ml/L Acetone : Take 0.1 ml Acetone in 1000 ml DI water
1 ml/L Acetone : Take 1 ml of Acetone in 1000 ml DI water
10 ml/L Acetone :Take 10 ml of Acetone in 1000 ml DI water

Principle

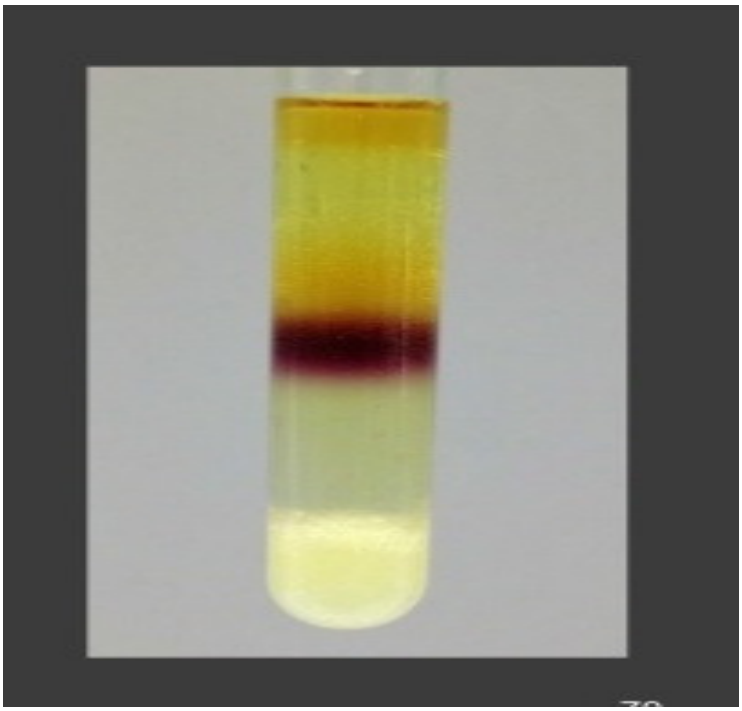
Acetoacetic acid and acetone form a violet coloured complex with sodium nitroprusside in alkaline medium. Acetoacetic acid reacts more sensitively than acetone. Values of 10 mg/dl of acetoacetic acid or 50 mg/dl acetone are indicated. Phenylketones in higher concentrations interfere with the test, and will produce deviating colours. ß-hydroxybutyric acid (not a ketone) is not detected.

Sodium Nitroprusside : acetone form a violet coloured complex with sodium nitroprusside in alkaline medium

Sodium carbonate: Provide Alkaline medium
Ammonium sulphate : Precipitate other protein which give purple colour with sodium nitroprusside & make solution Heavier than liquire Ammonia, so Ammonia may be remain on top of solution ,so purple ring is formed.

Rothera's test, liquid reagent

Saturate 2ml urine with ammonium sulphate powder.
Add a small crystal of sodium nitroprusside. Mix.
Add 0.5 ml liquor ammonia by side of the tube to form a ring.
Permanganate/Purple color ring is formed



Rothera's test, powdered reagent

Take a pinch of Rothera's powdered reagent
Add 1-2 drops of urine on powder.
Permanganate/purple color is formed

What Will You Do:(Draw table of your observations where required)
Perform both tests with given sample of urine.
Perform both tests with 0.1ml/L ,1/ml/L , 10 ml/L acetone

Sr. no.	Concentration	Rothera's test,powdered reagent	Rothera's test,Liquid reagent	Interference
1	0.1 ml/L			
2	1 ml/L			
3	10 ml/L			
4	Urine sample			

Perform both tests with acetone and ethyl acetoacetate.

Which other tests in blood and urine are usually done when tests for ketone bodies are positive?

III.Bile Salts:

REAGENT

Bile salt sample : Dissolve 2 gm of Bile salt powder into 1000 ml of water.
Sulfur powder

Principle

Sulphur powder is non-polar. It floats on water surface due to surface tension of water. Bile salt reduces surface tension of water and thereby sulphur powder sinks.

Procedure

Hay's sulfur flower Test:
Sprinkle a pinch of sulphur
powder over 2 ml urine in a test tube & Sprinkle a pinch of sulphur powder over 2 ml Water in a test tube. Observed & compare immediately without shaking of test tubes.
Sulphur powder sink to the bottom of the test tube if bile salts are present.

What Will You Do:

Perform the Hay's sulfur flower test with given sample

Sample	Observation	Interference
Bile salt solution		
Water		
Urine sample		

7. Pathological Urine-II

Glucose:

Perform both the tests with urine. Draw table of your observation.
Perform both tests with 100 mg%, 500 mg%, 1 gm% glucose. Note color of the test.
Draw table showing the results as follows.

Glucose %	Benedict's Test color	GOD Strip test color	Interferance
100 mg%			
500 mg%			
1000 mg%			
Urine sample			

Perform Benedict's test and Glucose oxidase strip test with following compounds and fill up the table given.

Compound	Benedict's test	Glucose oxidase strip test	Interference
Fructose			
Vitamin C			
Glucose with Vitamin C			
A cephalosporin drug			

8. Estimation of acid output by stomach.

Parietal cells of gastric mucosa secrete H+ using H^+-K^+-ATPase.
Gastrin, acetylcholine (from vagus) and histamine stimulate H+ secretion.
Thus, abnormality of *parietal cells, G cells and Vegas* are important in disturbances of gastric acid secretion.

Hypochlorhydria: (decreased acid output, pH>4)
Pernicious anemia
Autoimmunity to parietal cells destroys them.
Antibodies to Na+-K+-ATPase are found
Chronic *Helicobacter Pylori* infection of gastric mucosa.
Treatment with Proton pump inhibitors, H2-Blocker
Vagotomy

Hyperchlorhydria: (increased acid output)
Zollinger-Ellision Syndrome
G cells tumors in GIT

Reagent

0.1 mol/L NaOH : Dissolve 20 gm of NaOH in 5000 ml of water
1 % phenolphthalein : Dissolve 1 gm of phenolphthalein in 100 ml methanol

Sample preparation

Gastric juice Sample : 0.1mol/L HCL solution
How 0.1 mol/L HcL will be prepared?
1000ml of HCL solution contain=11.5 mol H^+
??????? =0.08 mol H^+
=1000x0.1/11.5
=8.6 ml
So add 17 ml of concentrated HCL & make upto 2 liter with water.

Examples

Example-1:

If you want your **result** will be Gastric Acid Output (mmol/hr) = 5 mmol/hr and You give Fasting Gastric juice output in 1 hour =100 ml/hr then prepare gastric juice sample as follow,

Fasting Gastric juice output =100 ml/hr
BAO = 5 mmol/L

100 ml of fasting gastric juice contain = 5 mmol/L HCL
1000 ml of fasting gastric juice contain = ???

$$= \frac{1000 \times 5}{100}$$

= 50 mmol/L HCL
= 0.05 mol/L HCL

Now We use fixed 10 ml of Gastric juice sample & titrate with fixed 0.1 mol/L NaOH

10 ml of 0.05 mol/L HCL =-------ml of 0.1 mol/L NaOH
V1=10 ml of Gastric juice V2=???? ml of NaOH

NI=0.05 mol/L HCL N2=0.1 mol/L NaOH

V2=10 x 0.05/0.1
=5 ml of 0.1 mol/L NaoH
Thus 5 ml of 0.1 mol/L NaOH is required to titrate 10 ml of 0.05 mol/L HCL.
Now ,Check your sample of gastric juice is made proper or not by following formula,

$$\text{Gastric Acid Output} = \frac{[\text{Average Reading R}] * [\text{Gastric Juice Output in one hour}]}{100}$$

We require 5 ml of NaOH & give 100 ml/hr Gastric output,so our result is

Gastric Acid Output = 5 x 100/100

=5 mmol/hr ,that is our BAO.

Example-2:
If you want your **result** will be Gastric Acid Output (mmol/hr) = 8 mmol/hr and You give Fasting Gastric juice output in 1 hour =80 ml/hr then prepare gastric juice sample as follow,

Fasting Gastric juice output =80 ml/hr
BAO = 8 mmol/L

80 ml of fasting gastric juice contain = 8 mmol/L HCL
1000 ml of fasting gastric juice contain = ???
= 1000 x 8/80
= 100mmol/L HCL
= 0.1 mol/L HCL

Thus Take 8.6 ml of Concentrated HCL solution and make upto 1000ml with water is made to 8.6 mol/L HCL solution.
Now We use fixed 10 ml of Gastric juice sample & titrate with fixed 0.1 mol/L NaOH

10 ml of 0.1 mol/L HCL = ------- ml of 0.1 mol/L NaOH
V1=10 ml of Gastric juice V2=???? ml of NaOH
NI=0.08 mol/L HCL N2=0.1 mol/L NaOH

V2=10 x 0.1/0.1
=10 ml of 0.1 mol/L NaoH
Thus 10 ml of 0.1 mol/L NaOH is required to titrate 10 ml of 0.1 mol/L HCL.

Now , Check your sample of gastric juice is made proper or not by following formula,

$$\text{Gastric Acid Output} = \frac{[\text{Average Reading R}] * [\text{Gastric Juice Output in one hour}]}{}$$

We require 10 ml of NaOH & give 80 ml/hr Gastric output, so our result is

Gastric Acid Output = 10 x 80/100

=8 mmol/hr ,that is our BAO.

Principle:

Acid output in stomach is measured as mmol/hour. For its measurement, amount of gastric juice output as well as amount of acid in gastric juice needs to be measured.

Amount of Gastric juice output is measured by suction of gastric juice using Ryle's tube inserted in to stomach.

Amount of acid in gastric juice is measured as follows.

Free Acidity:
Due to H+ (H3O+) ions.
Combined Acidity:
Some of the H+ in gastric juice are bound to other anions like proteins and lactic acids at low pH of Gastric Juice. These represent combined acidity.
(Proteins-).(H+) , (Lactate-).(H+)

Free Acidity + Combined Acidity = Total acidity
On addition of alkali, initially free H+ and later on combined H+ are neutralized. When not much H+ remain in solution (at pH 8.6), Phenolphthalein indicator becomes pink. The requirement of alkali is used to calculate acid output.

Procedure:

First Reading:
Take10 ml gastric juice in a flask/beaker. Add 1 drop of phenolphthalein.
(Do not mouth pipette anything)
Fill burette with 0.1 mol/L NaOH up to zero mark.
Perform as follows.
Add 1 ml of NaOH from burette, mix, and watch for pink color.
Repeat above step till pink color develops.
Suppose reading is X_1 ml of NaOH
Second Reading and third reading:
Repeat-step 1 and step-2 of above.
Add [X-1] ml of NaOH from burette, mix.
Add NaOH one drop at a time till pink color develops.
Take reading X_2 and X3. Find average(R) of second and third reading.

Calculation:

Explanation of calculation:
1 mol NaOH ≡ 1 mol HCl
R ml of 0.1 mol/L NaOH ≡ R ml of 0.1 mol/L HCl

≡ R /10 ml of 1 mol/L HCl
≡ R /(10*1000) mol HCl
≡ (R /10) mmol HCl

Thus, 10 ml of Gastric Juice will have (R/10) mmol HCl equivalents.
Thus, 1 ml of Gastric Juice will have (R/100) mmol HCl equivalents.

If Gastric Juice Output is G ml / hr
Then, Gastric acid output will be (R/100)*G mmol/hr

Result:

Thus, your **result** will be

Gastric Acid Output (mmol/hr) =

$$\frac{[\text{Average Reading R}] * [\text{Gastric Juice Output in one hour}]}{100}$$

Reference Ranges:

Fasting Gastric Juice Output: 20-100 ml /hr
Basal Acid Output (BAO): Measured in fasting state
Normal 1-6 mmol/hr
ZE Syndrome >15 mmol/hr (M)
>10 mmol/hr (F)
Maximum Acid Output (MAO): Measured after pentagastrin stimulation
Normal 5-40 mmol/hr

In pernicious anemia, both MAO and BAO are almost zero.
Above reference ranges are not universally accepted. Serum gastrin level, pH of gastric juice and other clinical finding e.g megaloblastic anemia are important to establish diagnosis.

What will you do:

Estimate gastric acid output in given sample or gastric juice. Consider Gastric juice output 80 ml/hr.

Your Reading(NaOH requirement)	ml
X1	
X2	
X3	
Average of three	

Gastric Acid Output = $\frac{[\text{Average Reading R}] * [\text{Gastric Juice Output in one hour}]}{100}$

Result : Your Gastric acid output is ----------

Comment on your result

Q-1 What is Zollinger-Ellision syndrome?

Q-2 What happens to Gastric acid output in the ZE syndrome? Why?

Q-3 Write complications of the ZE syndrome.

Q-4 Write cause of destruction of parietal cells in pernicious anemia.

Q-5 What happens to Gastric acid output in the pernicious anemia? Why?

Q-6 Which other important products are formed and secreted by parietal cells?

Q-7 Why should destruction of parietal cell lead to anemia?

Q-8 What is difference between gastrin and pentagastrin.

Q-9 Both pernicious anemia and ZE syndrome result in high serum gastrin level. Explain.

Q-10 Explain mechanism of action and use of ranitidine and omeprazole as drugs.

8.Secretion and buffering of acids by kidney.

Reagent

1 % phenolphthalein :Dissolve 0.5 gm of phenolphthalein in 50 ml of Methanol.
Neutral formalin (formaldehyde):Take 500ml of formaldehyde & add 0.1ml of phenolphthalein in solution. Then add 0.1 mol/L NaOH till colorless formaldehyde solution become slight pink coloured.
0.1mol/L NaOH : Dissolve 20 gm of NaOH & make upto 5000 ml with Water.
Urine Sample Preparation:
Urine output ml/day = U
Titrable acidity mmol/day = A

$$\text{Take } \frac{A}{U} \times 68 \text{ gm of } KH_2PO_4 \qquad \text{MW of } KH_2PO_4 = 68 \text{ gm/L}$$

Ammonia bound acidity mmol/day = B

$$\text{Take } \frac{B}{U} \times 66 \text{ gm of } (NH_4)_2SO_4 \qquad \text{MW of } (NH_4)_2SO_4 = 132 \text{ gm/L}$$

Here two NH4+ is released when 1 molecule of $(NH_4)_2SO_4$ will be dissociated.

Example
You want to give Titrable acidity = 30 mmol HCL /day &
Ammonia bound acidity = 40 mmol HCL /day , then prepare Urine sample as follow,
Urine output U = 1500 ml/day
Titrable acidity mmol/day A = 30 mmol HCL/day
= A/U x 68
=30/1500 x 68
=1.36 gm of KH_2PO_4

Ammonia Bound acidity mmol/day B = 40 mmol HCL/day
= B/U x 66
=40/1500 x 66
=1.76 gm of $(NH_4)_2SO_4$

Finally dissolve 1.36 gm of KH_2PO_4 and 1.76 gm of $(NH_4)_2SO_4$ & make upto 1000 ml with water.

Principle:

Catabolism of food substances produces H+ and OH-. In the process, there is excess of H+ over OH-. Excess H+ is excreted by kidney. NH3 and Phosphate buffer the H+ secreted by renal tubules.

$NH_3 + H^+ \longrightarrow NH_4^+$ ----(1)

$HPO_4^{2-} + H^+ \longrightarrow H_2PO_4^-$ ----(2)

You will estimate total Acids in urine and proportions buffered by ammonia and phosphate.
Correlate the experiment with theoretical concepts of renal regulation of pH learnt in the classroom.

pK of reaction (1) is 9.25.
pK of reaction (2) is 6.8.
For phenolphthalein: pH 8.2 = colorless; pH 10 = red

$$\underset{\text{colourless}}{HIn} \xrightarrow[\text{PH} > 8.2]{} \underset{\text{Red colour}}{H^+ + In^-}$$

Phenolphthalein is a weak acid, which can lose H^+ ions in solution. The phenolphthalein molecule(HIn) is colorless, and the phenolphthalein ion(In^-) is pink. When a base is added to the phenolphthalein, the molecule □ ions equilibrium shifts to the right, leading to more ionization as H^+ ions are removed

When urine, acidic in nature, is titrated with NaOH, initially reaction (2) goes towards left. When all $H_2PO_4^-$ is converted into HPO_4^{2-}, pH rises to 8.6, causing ionization of phenolphthalein .Phenolphathalein ion proceduced pink colour,sosolution turn into pink coloured. NaOH required to reach this stage represent H+ bound to phosphate, called **"Titrable Acidity".**

Neutral formalin is added to urine. We will convert formalin (Acid) to Neutral formalin, otherwise formalin(acid) itself react with NaOH when we measure H^+ of NH_4^+

Now, Formaldehyde is added to urine. Following reaction occur.

$4NH_4Cl + 6HCHO \rightarrow N_4(CH_2)6 + 6H_2O + HCl$ ----(3)

Released H+ decrease pH of urine, making phenolphthalein colorless again. Further titration with NaOH, till phenolphthalein become pink, will actually represent H+ bound with ammonia released during reaction (3). It is called **"Ammonia bound acidity".**
H+ bound to NH3 can not be titrated without adding formaldehyde. Hence, H+ bound to phosphate is called titrable acidity.

Procedure:
First Reading:
Take 25 ml urine in a flask/beaker. Add 1 drop of phenolphthalein.
(Do not mouth pipette anything)
Fill burette with 0.1 mol/L NaOH up to zero mark.
Perform as follows.
Add 1 ml of NaOH from burette, mix, and watch for pink color.
Repeat above step (adding 1 ml NaOH) till pink color develops.
Suppose reading is X ml of NaOH
Add 10 ml of neutral formalin. Mix. The pink color disappears.
Repeat step-3. Suppose the reading is Y
Second Reading and third reading:
Repeat-step 1 and step-2 of above.
Add [X-1] ml of NaOH from burette, mix.
Add NaOH one drop at a time till pink color develops. Take reading X.
Add 10 ml of neutral formalin. Mix.
Add [Y-1] ml of NaOH from burette, mix.
Add NaOH one drop at a time till pink color develops. Take reading Y.
Find average X and Y of second and third reading.

Explanation of calculation:
Titrable acidity: reading X ml

1 mol NaOH ≡ 1 mol HCl

X ml of 0.1 mol/L NaOH	≡ X	ml of 0.1 mol/L HCl
	≡ X /10	ml of 1 mol/L HCl
	≡ X /(10*1000)	mol HCl
	≡ (X /10)	mmol HCl

As titration is done with 25 ml of urine,

Titrable acidity in 25 ml of urine	= (X /10)	mmol HCl
Titrable acidity in 1 ml of urine	= X /(10*25)	mmol HCl

If urine output per day is U ml
Excreted Titrable acidity /day = (U * X) / 250 mmol HCl

Ammonia bound acidity: reading Y ml
Ammonia bound acidity is expressed either as **mmol of HCl** or **mg of ammonia**
Ammonia bound acidity / day = (U * Y) / 250 **mmol HCl**

$H^+ + NH_3 \rightarrow NH_4^+$
1 mmol of NH_3 binds 1 mmol of H^+ to form 1 mmol of NH_4^+ ---(b)

MW of Ammonia (NH_3) = 17 gm
1 mmol NH_3 = 17 mg of NH_3 ---(a)

From (a) and (b)

Excreted Ammonia / day = (U * Y) /250 mmol NH3
= ((U * Y) /250)*(17) mg NH3
Excreted Ammonia / day = U * Y * (0.068) **mg NH3**

Reference Range:
Titrable acidity: 20-50 mmol HCl / day
Ammonia bound acidity: [30-50 mmol HCl/day] or [510-850 mg NH3/day]
Total acid excretion: 70-100 mmol/day

What will you do:

Estimate Titrable and ammonia bound acidity in given sample of urine.
Titrable acidity

No.	Initial reading(ml)	Final reading(ml)	Difference(ml)
X1			
X2			
X3			
Average X			

Ammonia bound acidity

No.	Initial reading(ml)	Final reading(ml)	Difference(ml)
1			
2			
3			
Average			

Result & conclusion

Titrable acidity =

Ammonia bound acidity =

What is the source of phosphate in urine?

What is the source of ammonia in urine?

Diabetic ketoacidosis elevate urinary ammonia. Explain.

9. Colorimetry

Colored molecule absorbs various wavelength of light passing through their solution.

Imparted light → Colored solution → immerging light

For a given wavelength of light, ratio of (immerging light intensity) to (imparted light intensity) is called Transmittance T.

$T = e^{-kct}$ c = concentration of colored molecule

t = length of light path

k = constant

$-kct = \log_e T$

$-kct = \frac{\log_{10} T}{\log_{10} e}$

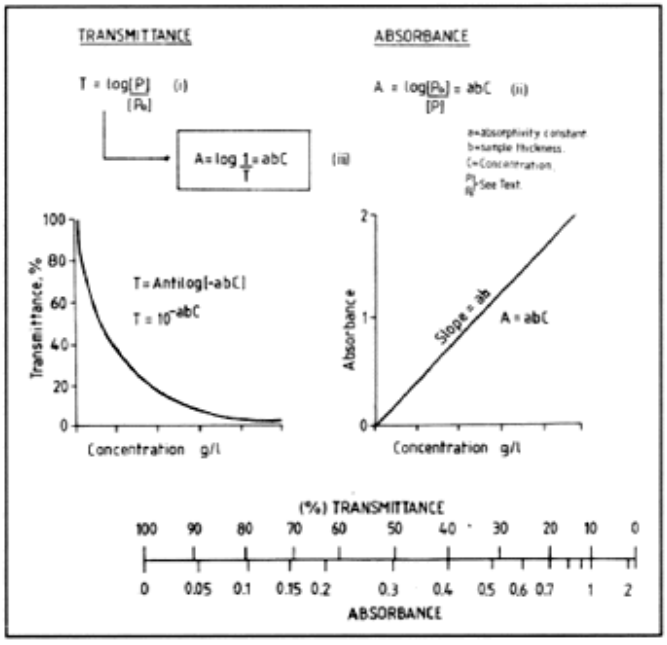
-k'ct = log T (common logarithm) k' =constant

-k'ct = log (T*100/100)

-k'ct = log (T*100) – 2

k'ct = 2 - log (T%) (T% is called percentage Transmittance)

k'ct = A (2 – logT%) is called Absorbance, denoted as A



Following graph describe relationship between T% and A.

A = k'ct. (Beer's and Lambert's law)
Hence,
$A \propto t$

Absorbance is proportional to length of light path

$A \propto c$

Absorbance is proportional to concentration of substance

Therefore, If light path is constant, for concentration (C1 and C2) and respective absorbance (A1 and A2)

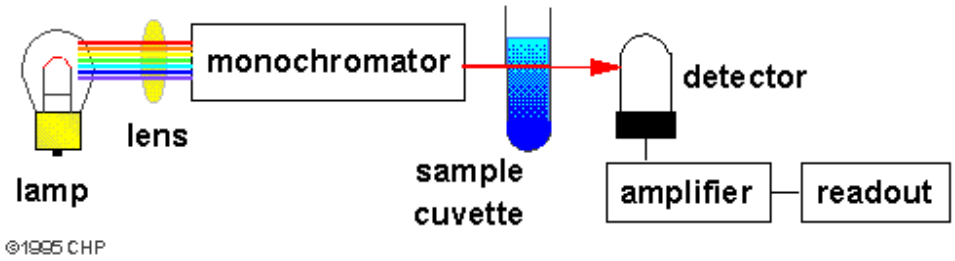
$C1/C2 = A1/A2$

If A1 and A2 is measured and C2 is known
C1= (A1/A2)*C2 can be calculated. --------(1)

This principle is utilized by biochemistry laboratory to measure various substances in biological materials.

Various instruments based on the principle are colorimeter and spectrophotometer.

Instrument:



Light source emit light of all wavelengths
Monochromator allow only certain wavelength of light to pass. (Mono + color)
Cuvette is a transparent vessel holding colored solution
Photocell converts light in to current. Current is proportional to light intensity.
Galvanometer measures current.

General procedure to use colorimeter:
Suppose concentration of Glucose in plasma is to be estimated.

Glucose is colorless, hence can not be measured directly.
Add fixed amount of Y in fixed amount of plasma. P and Q are produced

Glucose + Y → P + Q

Suppose Q is colored compound and absorbs light of a particular wavelength. Its concentration will be proportional to concentration of glucose.
Take a solution of glucose with known concentration C (it is called calibrator) and process as above in 2.
Take a water (it is called blank) and process as above in 2.
Measure absorbance of color produced by Serum and Calibrator and blank.
Blank Absorbance, amount of color produced with no glucose, needs to be deducted from absorbance of serum and calibrator.
Using equation (1)

$$\text{Glucose concentration in plasma (mg\%)} = \frac{(A_{plasma} - A_{blank})}{(A_{calibrator} - A_{blank})} * \mathbf{C}$$

What will you do:
Reagent:
Buffer:

pH	Chemical drug	Mol/L	MW	Gm/L
6.853	Na2HPO4	0.025	141.96	3.549
	KH2PO4	0.025	136.09	3.402
9.139	Na2 tetraborate	0.01	381.37	3.814

Red coloured solution: Dissolve 20 mg of Phenol red in 50 ml of 9.139 pH buffer
Blue coloured solution: Dissolve 20 mg of BCG(Bromocresol green) in 50 ml of 6.8 pH buffer.
Note: Dilution of stock coloured solution will be always done with respective Buffer.
Sample Dye solution: For both exercise
Dilute 1:30 times of red coloured stock solution with buffer of pH=9.1 by

Adding 0.1 ml of stock red coloured solution into 3000 ml of Buffer of pH=9.1

Exercise:1

You will be given a concentrated colored solution.
Dilute it in a series of test tubes as follows. Measure absorbance.

Test tube	Red Colored stock (ml)	pH=9.139 Buffer(pH=9.139) ml	Absorbance (A) on 5.5 nm Filter
0	0	1000	
1	200	800	
2	400	600	
3	600	400	
4	800	200	
5	1000	0	

Draw Graph of various Dilution of dye versus its absorbance

Result & Conclusion:

Exerscise-2

[1]:Red coloured solution (Phenol red dye)

Measure Absorbance of this red coloured solution on different filters.

Filters(nm)	Absorbance
340	
405	
450	
505	
546	
578	
630	
670	

Absorbance spectrum of phenol red dye solution (Red coloured)

[2]:Blue coloured solution [Bromocresol green dye]
Dilute 1:20 times of Blue coloured stock solution with buffer of pH=6.8 by Adding 0.1 ml of stock Blue coloured solution into 3000 ml of Buffer of pH=9.1

Measure Absorbance of these diluted Blue coloured solution on different filters.

Filters(nm)	Absorbance
340	
405	
450	
505	
546	
578	
630	
670	

Absorbance spectrum of BCG(Bromocresol green)dye solution (Blue coloured)

Note that different colored solutions absorb light at different wavelengths in different proportions.

Draw Graph of various filter versus absorption on that filter for red colored solution & Blue coloured solution.

11. Estimation of serum creatinine

Creatinine is produced from creatine present mainly in muscles. It is filtered by glomerulus of kidney. Thus, following factors affect serum creatinine concentration.
Muscle
Glomerulus of Kidney

Principle:

Picrate + OH- ----------→ activated [Picrate-OH-]* complex
[Picrate-OH-]* + creatinine ------→ Creatinine-Picrate complex + OH-
Red colored Creatinine-picrate complex, also called Janovaski complex, is measured at 505 nm.
The rate of reaction is proportional to concentration of creatinine.
The rate of reaction is also indicated by rate of rise in Absorbance (ΔA)
Thus, [creatinine] ∝ ΔA
ΔA is difference in Absorbance between 60th (A60) second and 30th (A30) second of start of reaction.
If ΔA for calibrator is ΔA_{calib} and ΔA for sample is ΔA_{sample}

$$[\text{Sample Creatinine}] = \frac{\Delta A_{sample}}{\Delta A_{calib}} \times [\text{Creatinine Calibrator}] \quad \text{-------} \quad (1)$$

Reagents

The timed measurements of Absorbance require sophisticated colorimeters with flow-through cuvette. The reaction mixture is aspirated in the cuvette and Absorbance is measured at different time.
The Laboratory technologist will help to carry out following steps:
NaOH solution :Refer to SOP for creatinine reagent
Picric acid solution : Refer to SOP for creatinine reagent

Creatinine Std.Sample
2 mg/dl Creratinine :Disolve 0.010 gm of creatinine powder in 500 ml of 0.1 mol/L Hcl solution
Creatinine Test sample**.**
4 mg/dl Creratinine :Disolve 0.020 gm of creatinine powder in 500 ml of 0.1 mol/L Hcl solution
6 mg/dl creatinine : Dissolve 0.030 gm of creatinine powder in 500 ml of 0.1 mol/L HCL solution
0.1mol/l HCL solution(11.5 molar Conc.HCL solu.) : Add 17.4 ml of Conc. HCL solution & make upto 2000 ml with DI water.

Creatinine R 1(NAOH)

1. Weigh 12gmNaOH.
2. Dissolve in approximately 500 ml DI water.
3. Add10ml of 30% brij in above mixture.
4. Weigh 1gm SDS and pour it into approximately 100 ml water in beaker.Heat the solution until SDS dissolve.
5. Add SDS containing solution in main mixture.

6. Make upto 1liter with DI water.

Creatinine R2(picric acid)

Dry picric acid between filter paper pieces

1. Weight 4.58gm dry picric acid.
2. Dissolve in approx. 300 ml water
3. Add 10 ml of 30% Brij in above mixture.
4. Remove froth with a clean object of glass or plastic dipped in capryl alcohol
5. Make 1 liter with water.

Working alkaline -picrate reagent:

mix 50 ml R1 & 50 ml R2 on the day of practical for 50 student.

Procedure
For sample and standard perform following.
Mix 0.5 ml of *Alkaline Picrate Reagent* with 0.05 ml sample
Aspirate the reaction mixture in flow-through cuvette.
Wait. The instrument will read A_{30} and A_{60} and display $\Delta A_{sample} = A_{60} - A_{30}$
For standard perform the same steps to get ΔA_{std}.

Calculation and Result:
Calculate creatinine concentration using equation (1)

Your result will be ----------
Comment on your result :

Reference Range: Male 0.7 - 1.3 mg%
Female 0.6 - 1.1 mg%

1 mmol = 1000 micromole

Creatinine Molecular weight = 113.12

What will you do:

Measure creatinine concentration in given sample of serum

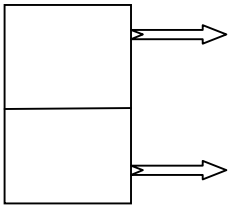
Express adult plasma creatinine reference range in **micromole/L**.

Classify conditions affecting plasma creatinine concentration based on information given in first paragraph of this page.

12. Estimation of plasma glucose

Reagent:

Glucose reagent : Dissolve 100mg of **4- Aminoantipyrine dye** in 1000ml of DI water and add 1 ml of phenol saturated water .



Water saturated Phenol

Phenol saturated water

Note :Wear goggles & Glove while taking phenol.Seniour person must be present.
Glucose test sample : Mix 2 ml of analytical grade Sodium Hypochlorite solution and 1 ml of DI water.
Glucose standard sample : Mix 1 ml of analytical grade Sodium Hypochlorite solution and 2 ml of DI water.

Principle:

Glucose + O_2 $\xrightarrow{\text{Glucose Oxidase}}$ Gluconolactone + H_2O_2

Gluconolactone + H_2O $\xrightarrow{\text{Spontaneous}}$ Gluconate

H_2O_2 + 4-aminophenazone + phenol $\xrightarrow[(505\ nm)]{\text{Peroxidase}}$ Quinonamine (red color)

Procedure

Reagents	Blank	standard	Plasma
H2O	10µl		
Glucose Calibrator		10µl	
Plasma			10µl
Glucose oxidase + Peroxidase Reagent (GOD POD reagent)	1 ml	1 ml	1 ml
Mix, incubate at room temperature for 30 min. Note: ask the lab-incharge for exact time and method for incubation. Read absorbance at 505 nm			
Absorbance	A_{blank}	$A_{calibrator}$	A_{plasma}

Calculation: (c = Standard concentration)

$$\text{Glucose concentration in plasma} = \frac{(A_{plasma} - A_{blank})}{(A_{std.} - A_{blank})} * \mathbf{C}$$

A_{blank} : ----------

A_{plasma} : ----------

$A_{calibrator}$: ----------

std conc.:----------

Glucose concentration in plasma =

Your result will be ----------

Comment on your result:

Reference Ranges:

Fasting Plasma Glucose	Interpretation	Oral Glucose Tolerance	Interpretation
<=110 mg%	Normal	<139 mg%	Normal
111-125 mg%	Impaired Fasting Glucose	140-199 mg%	Impaired Glucose Tolerance
>=126 mg%	Diabetes mellitus	>=200 mg%	Diabetes mellitus

Fasting = no food intake for at least 8 hours
Oral Glucose Tolerance = 75 gm glucose orally after 8 hrs of fasting.
Above results are valid if found on two or more occasions.
While most normal person have fasting plasma glucose >70 mg%, diagnosis hypoglycemia require consideration of many factors including age, clinical features and current treatments.

What will you do:

Measure Glucose concentration in given sample of plasma.
Draw above table again with **mmol/L** format. (Glucose MW=180 gm).

12. Estimation of serum cholesterol

Reagent

Cholesterol reagent : Dissolve 100mg of 4- Aminophenabenzene & 1 ml of phenol saturated water and make upto 1000ml with DI water.
Cholesterol test sample : Mix 2 ml of HOCL(analytical grade) & 1 ml of DI water
Cholesterol std. sample : Mix 1 ml of HOCL(analytical grade) & 2 ml of DI water

Principle:

Cholesterol ester $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + H_2O_2

H_2O_2 + 4-aminophenazone + phenol $\xrightarrow{\text{Peroxidase}}$ Quinonamine (505 nm)

Reagents and procedure:

Reagents	Blank	Calibrator	Plasma
H2O	10µl		
Cholesterol Calibrator		10µl	
Plasma			10µl
Cholesterol oxidase + Peroxidase Reagent (COD POD reagent)	1 ml	1 ml	1 ml
Mix, incubate at room temperature for 30 min. Note: ask the lab-incharge for exact time and method for incubation. Read absorbance at 505 nm			
Absorbance	A_{blank}	$A_{calibrator}$	A_{plasma}

Calculation : (C = std. concentration)

$$\text{Cholesterol concentration in plasma} = \frac{(A_{plasma} - A_{blank})}{(A_{std} - A_{blank})} * \mathbf{C}$$

Result: A_{blank} : ---------- A_{std} : ----------

A_{plasma} : ---------- std conc.:----------

Glucose concentration in plasma =

Your result will be ----------

Comment on your result:

Reference Ranges:

Desirable: <200 mg/dL
Borderline: 200-239 mg/dL
High: >=240 mg/dL

What will you do:
Measure Cholesterol concentration in given sample of plasma.
Rewrite reference ranges in **mmol/L** format. Cholesterol MW = 386.64 gm

13. Estimation of serum bilirubin

Reagent:

Refer to SOP for billirubine reagent:

R1

1. Dissolved 75 gm caffine in 900 ml deionised water with constant mixing
2. Add 112 gm Na Benzoate in above mixer with constant mixing
3. Add 112 gm anhydrous Na Acetate in above mixer with constant mixing
4. Add 2 gm disodium EDTA in above mixer with constant mixing
5. Make upto **2 liter** with deionised water
6. Filter if turbid
7. Store in glass container in freeze
8. If crystalline precipitation are seen at 2-8'C, bring solution to room temperature to redisolve it before use

R2a

1. Dissolve 10 gm sulfanilic acid in 900 ml deionised water
2. Add 30 ml concentrated HCL in above mixer
3. Make upto **2 liter** with deionised water
4. Store in glass container

R2b

1. Dissolve 1.25 gm Na nitrite(NaNo2) and **make upto 250 ml** with deionised water.
2. Store in brown glass container

Working_Diazo_R2

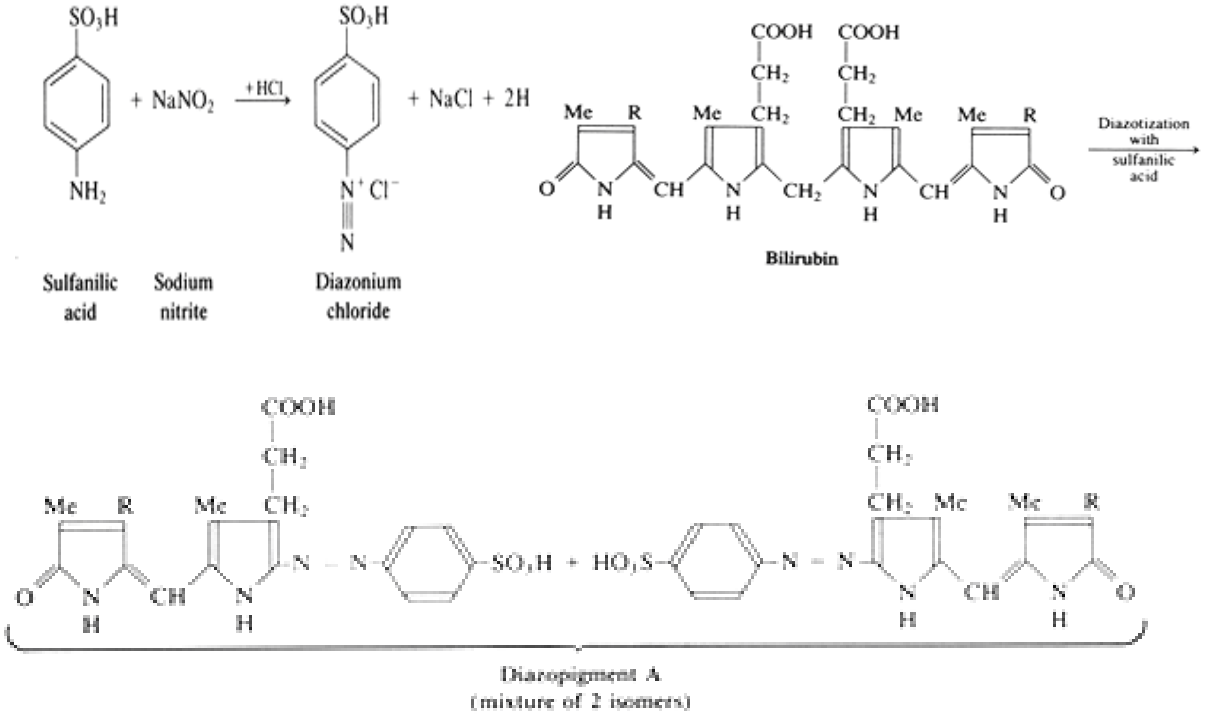
Working reagent (Diazo) made by mixing 10ml R2a and 0.3 ml R2b

Billirubin test solution :Dissolve 2 mg of billirubine powder & make upto 100 ml with DI water

Billirubin test solution :Dissolve 4 mg of billirubine powder & make upto 100 ml with DI water

Principle

One molecule of bilirubin reacts with two molecules of diazotized sulfanilic acid in an acid solution to form two purple azobilirubin molecules (560 nm). While direct bilirubin reacts in water as well as methanol medium, indirect bilirubin react only in presence of methanol.



Reagents

Reagents	Test	Test Blank	Absorbance of **std.** and **std. blank** will be given in the class
Sample	0.05ml	0.05ml	
Caffeine Reagent	0.5 ml	0.5 ml	
Incubate for 10 minute			
Diazo Reagent(Diazo A+Diazo B)	0.1 ml	---	
Diazo Blank Reagent(Diazo A)		0.1 ml	
Mix the contents and incubate in dark for 10 minutes. Read absorbance at 560 nm.			
Absorbance	As_{td}	$As_{tdblank}$	As_{erum}	$As_{erum\ blank}$

As the procedure is done with caffeine, both direct and indirect bilirubin reacts in the reaction to give Total Bilirubin in the sample.

Blanks are taken to subtract absorbance caused by hemolysis (resulting in presence of red color of hemoglobin in serum).

Diazo blank reagent does not have sodium nitrite, hence do not produce azobilirubin.

Calculation:

Std. Concentration, As_{td} and $As_{tdblank}$ will be provide in practical class.

$$\text{Total Bilirubin (mg/dL)} = \frac{(As_{erum} - As_{erumblank})}{(As_{td} - As_{tdblank})} \times \text{Std. Concentration mg\%)}$$

Result

Your result will be----------

Comment

Reference ranges: (For Adults)

Total Bilirubin	0.2-1.2 mg/dl
Direct Bilirubin	0.1-0.4 mg/dl
Indirect Bilirubin	0.2-0.7 mg/dl

Bilirubin = MW 584.67 gm

1 mmol=1000 micromole

What will you do:

Measure Total Bilirubin concentration in given sample of serum.

Enumerate causes of unconjugated hyperbilirubinemia and mixed hyperbilirubinemia.

Express Reference ranges in micromole/Liter.

Sample for bilirubin should not be exposed to light. Phototherapy is used in treatment of neonatal jaundice. Explain and correlate.

14. Estimation of serum total protein

Except **immunoglobulins**, majorities of plasma proteins are synthesized by **liver**. Various tissues **catabolize** plasma proteins. Plasma protein concentration reflects balance between their synthesis and catabolism. Under certain conditions intact proteins from plasma are also lost through **GIT, urine** and **skin**. Proteins from intravascular compartment may reach other body compartments. Protein concentration may also be affected by change in plasma water.

Reagent:

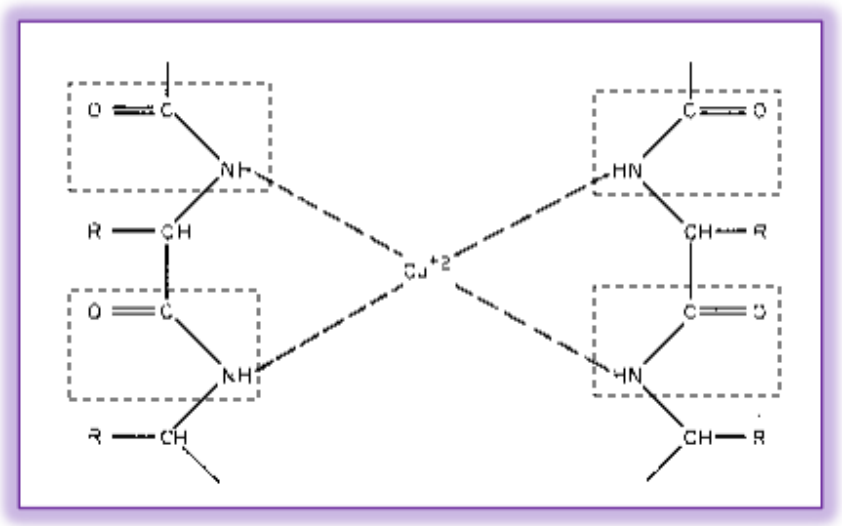
Refer to SOP for total protein.

1. Weight 3 gm Cuso4.5H2O.
2. Dissolve in approx. 500 ml water.
3. Weight 9 gm (Na K Tartrate).(4H2O) and 5 gm KI.
4. Add sequentially 9 gm (Na K Tartrate).(4H2O) and 5 gm KI in copper sulphate solution.
5. Weight 24 gm NaOH.
6. Add slowly with mixing 24 gm NaOH in 100ml of water.
7. Add slowly with mixing NaOH solution in copper sulphate solution.
8. Make upto 1 liter with water .

Principle :

Two or more peptide bonds of proteins form coordination complex with one cu^{2+} in alkaline solutions to form a colored product. The absorbance of the product is determined spectrophotometrically at 540 nm.

Cu^{2+} - peptide complex



Procedure:

Reagents	Blank	Std.	Sample
H_2O	0.02 ml	-	-
Protein standard	-	0.02 ml	-
Sample	-	-	0.20 ml
Biuret reagent	1 ml	1 ml	1 ml
Mix the contents, and incubate at 37^0 C temperature for 30 min. Note: ask the lab incharge for exact time and method for incubation. Read Absorbance at 540 nm			
Absorbance	A_{blank}	$A_{std.}$	A_{sample}

Calculation and result: (C = Standard concentration)

$$\text{Total Protein concentration in plasma} = \frac{(A_{serum} - A_{blank})}{(A_{std} - A_{blank})} * \mathbf{C}$$

Your result will be ----------

Comment :

Reference ranges:

Serum proteins	6.0-8.0 g/dL
Albumin	3.5-5.5 g/dL
Globulins	2.0-3.6 g/dL
Fibrinogen	0.2-0.6 g/dL

What will you do:

Measure total proteins in given sample of serum or plasma.

Q-1 Serum protein reference ranges are lower than that of plasma. Explain.

Q-2 Why reference ranges for plasma proteins can not be expressed in mmol?

QA-3 Enumerate conditions affecting plasma protein level.

15. Estimation of serum albumin

Different disorders affect different plasma proteins differently. Thus, it is useful to know albumin and globulin concentration in serum, in addition to total protein. Once total protein and albumin (as shown below) are estimated, serum globulin can be calculated.

Reagent :

BCG reagent : Refer SOP for Albumin reagent preparation.

Add 42mg BCG(MW=698) in approx. 250 ml DI water.

Add 5.9 gm succinic acid (MW=118.09 ,pKA1=4.2 ,pKA2 = 5.6) in above mixer while constantly mixing.

Add 1.8 ml of 30% Brij-35 In above mixer while constantly miximg.

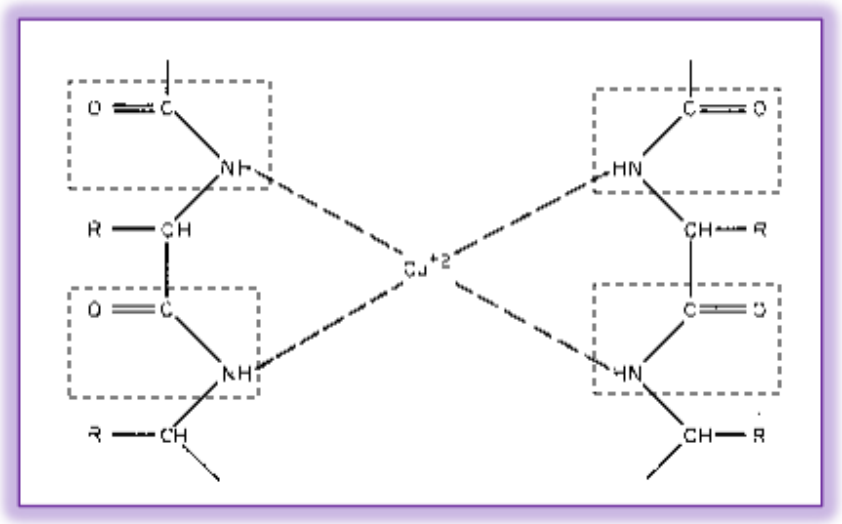
Add 1 gm NAOH in above mixter while constatly mixing

Add 200 mg Na azide in above mixer while constatly mixing.

If required adjust pH to 4.2

Make upto 1000 ml with volumetric flask with deionised water.

Cu^{2+} - peptide complex



Principle:

BCG = BromoCresol Green

At pH 4.2: [Albumin$^+$] + [BCG$^-$] → [Albumin$^+$ BCG$^-$ complex]

At pH 4.2 BCG is yellowish, while Albumin$^+$ BCG$^-$ complex is greenish. The green color is measured at 630 nm.

Procedure:

Reagents	Blank	Calibrator	Sample
H_2O	10µl	-	-
Albumin Calibrator	-	10µl	-

Sample	-	-	10µl
BCG reagent	1 ml	1 ml	1 ml
Mix, and read immediately at 630 nm.			
Absorbance	A_{blank}	A_{calib}	A_{sample}

Calculation and result: (C = calibrator concentration)

$$\text{Albumin concentration in serum} = \frac{(A_{serum} - A_{blank})}{(A_{calibrator} - A_{blank})} * \mathbf{C}$$

Your Result will be ----------
Comment on your result

Reference ranges: (see experiment on Serum total proteins)

What will you do:
Measure albumin in given sample of serum.
Using serum total protein values given to you by lab incharge, find serum globulin level in the serum.

Enumerate conditions where ratio of albumin to globulin is significantly altered.

16.Estimation of Cerebrospinal fluid protein.

Cerebrospinal fluid is not freely permeable to plasma proteins. Hence, its concentration is almost 1/100 times the plasma. Some proteins are synthesized by the pia matter itself. Under various **CNS inflammatory conditions,** CSF protein is increased due to increased permeability of pia matter as well as due to increased synthesis by it.

Reagent:

Pyrogallol Red reagent:Refer to SOP for Pyrogallol red reagent

PR(Pyrogallol red)

Making Reagent

1. Dissolve pyrogallol red 60 mg in 100 ml of methanol.
2. Store in plastic container.

MB(molybabdate)

Making Reagent

1. Dissolve disodium molybdate 0.24 gm in 100 ml of deionized water.
2. Store in plastic container.

Final microprotein Reagent

Making Reagent

1. Dissolve succinic acid 5.9 gm in 900 ml of deionized water.
2. Add sodium oxolate 0.14 gm in above mixture with constantly mixing.
3. Add sodium banzoate 0.5 gm in above mixture with constantly mixing.
4. Add PR(Pyrogallol red) 40 ml in above mixture with constantly mixing.Discard other 60 ml PR(Pyrogallol red).
5. Add (molybabdate) 4 ml in above mixture with constantly mixing.Discard other 96 ml (molybabdate).
6. Calibrate PH meter and if required adjust PH to 2.5.
7. Make up to above mixture 1 L with deionized water.

CSF Protein Calibrator: Take 0.1ml of serum protein & make upto 10 ml with DI water

CSF protein Sample :Take 0.2 ml of serum Protein & make upto 10 ml with DI Water

Principle:

pyrogallol red-molybldate complex combine with protein and give colour which is mesure at 630 nm.

Reagents and Procedure:

Reagents	Blank	Calibrator	Sample
H_2O	20µl	-	-
CSF Protein Calibrator	-	20µl	-
CSF	-	-	20µl
Pyrogallol Red reagent	1 ml	1 ml	1 ml
Mix, wait for10 min, mix before reading at 630 nm.			
Absorbance	A_{blank}	A_{calib}	A_{sample}

$$\text{Protein concentration in CSF} = \frac{(A_{serum} - A_{blank})}{(A_{calibrator} - A_{blank})} * \mathbf{C}$$

Your result & comment

Reference ranges: 15-45 mg%

What will you do:
Measure protein in given sample of CSF.
Using **bold words** used in the top paragraph, enumerate conditions affecting CSF protein level.

17. Estimation of plasma uric acid

Uric acid is formed by catabolism of **purines**. Uric acid is excreted by **kidney**.

Reagent:

Refer to Practical of estimation of blood glucose. Reagent ,standard, test will be made by same method.

Principle:

Uric acid yields allantoin and H2O2 on action by uricase. Peroxidase use hydrogen peroxide to oxidize various colorless dyes to red colored quinonimine like dyes measured at 505 nm by absorption photometry .

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow[(505\ nm)]{\text{Peroxidase}} \text{Quinonamine (red color)}$$

Procedure:

	Test	Standard	Blank
Serum	20 ul		
Calibrator		20 ul	20 ul
Water			
Reagent	1 ml	1 ml	1 ml
Measure absorbance at 505 nm			
Absorbance	A_{Serum}	$A_{Std.}$	A_{Blank}

Calculation (C = Std. concentration)

$$\text{Uric acid concentration in plasma} = \frac{(A_{Serum} - A_{blank})}{(A_{std.} - A_{blank})} * \mathbf{C}$$

Result & Comment:

Reference ranges: 3.6 - 7.7 mg/dL (214 to 458 micromole/L) for males
2.5 – 6.8 mg/dL (149 to 405 micromole/L) for females

What will you do:

Measure uric acid in given sample of plasma.
Using **bold words** used in the top paragraph, enumerate conditions affecting plasma uric acid level.

Calculate molecular weight of uric acid from reference ranges given.

18.Electrophoresis

Reagent: Refer to Sop for Serum & Hb electrophoresis

Principle:

Electrophoresis is a refers to the migration of charged molecules under electrical field.

Procedure:

Prepare thin 1 % Agarose gel in appropriate buffer.
Apply appropriate sample in thin line over agarose gel.
Keep gel with sample applied in electrophoretic chamber & connect the gel with buffer through strips of filter paper and apply appropriate voltage.
After sample run is completed, switch off the power supply and remove slide from chamber.
Denature proteins in methanol and dry the gel with heating.
Stain slide with appropriate stain.

Clinical Applications:
Diagnosis of sickle trait and sickle disease.
Diagnosis of multiple myeloma

Questions:

What is agarose? Why it is used to prepare gel.

Name other electrophoresis support media.

How much agarose is required to prepare 15 ml of 1% agarose?

Which sample is used for hemoglobin electrophoreis?

What was valtage, current and duration of electrophoresis demonstrated to you?

What are major hazard of electrophoresis procedure? What precautions must be taken to avoid them.

What is difference between electrophorogram of serum protein and plasma protein?

Name stain used during demonstration.

Draw hemoglobin electrophoretic patten in normal, sickle trait and sickle cell disease patients. Explain its biochemical basis.

Draw hemoglobin electrophoretic patten in HbC and HbD carrier patients. Explain its biochemical basis.

Draw serum protein electrophoretic patten in multiple myeloma. Explain its biochemical basis.

What is monoclonal antibody?

19. Chromatography

Principle : Chromatography is a process in which components of a mixture are separated by differential distribution between a mobile phase and a stationary phase. Components with greater distribution into the stationary phase are retained and move through the system more slowly.

Requirements :

Amino acid standard : 1% amino acid standard
Mobile phase : 12(Butanol):3(Glacial acetic acid):5 (Di water)
Sample Type : Serum,Urine .
Equipments & consumables :Chromatography chamber(air tight), Glass road, clips, Whatman fiter paper, Gloves, pencil, scale, centrifuge, pipettes
Stain : Ninhydrin solution (0.25 %)(250 mg of Ninhydrin powder in 100 ml of methanol/acetone)

Procedure :
Clean hands throughly with soap.
Wear gloves before handling filter paper.
Take a Whatman filter paper ,make a horizontal line at one end of filter paper,around 1.5-2 cm above from the edge of the paper.
Put marking at 3.5 cm apart for each sample for sample application.
Repeat sample & standard application for twice once previous sample gets dried .
Take 500 ml of mobile phase reagent in reagent chamber.
Clip the dried filter paper on glass rod,Make sure that distance between each sample & road is equal.
Put glass rod in chromatography chamber, make sure that sample application sites do not get dipped in the solvent.
Close the chamber air tight .Note the time & allow the separation for 4 hours.
Remove the paper from chamber after 4 hours, allow the paper to dry at room temperature.
Take 0.25% Ninhydrin solution in shallow plastic container big enough to accommodate the entire filter paper. Dip paper in it for few seconds.
Put the paper in incubator at 100^{o}c for 20-25 minute/ till purple bands are seen
Preserve the paper in dark room for latter use.

Calculate Rf value

$$Rf = \frac{\text{Distance from application point to solute center}}{\text{Distance from application point to solvent front}}$$

QUESTIONS :

Name stationary phase in the experiment. Is it mainly hydrophobic or mainly hydrophilic? Explain.

Name mobile phase in the experiment. Is it mainly hydrophobic or mainly hydrophilic? Explain.

List hydrophobic amino acids used in the experiment.

List hydrophobic amino acids used in the experiment.

Comment why some amino acids move faster and other slower during the chromatography.

Why wearing gloves is essential in the experiment?

Why wearing protective eye glass is essential in the experiment?

Name few conditions where abnormal amount of some amino acids are lost in urine. Explain their biochemical basis.

20. Case - Cancer Chemotherapy

A 7 years old boy had fever and difficult breathing. X-ray revealed pneumonia. On laboratory investigation, Total WBC was 200000/mm^3. Peripheral smear suggested diagnosis of leukemia.

Chemotherapy was started with Vincristine, methotrexate, arabinocyl-cytosine and dexamethasone. One of the many drugs given to the patient was allopurinol. Patient's uric acid level was monitored every other day.

Why serum uric acid level should be monitored in the patient? (5)

Write principle of the method used for estimation of serum uric acid. (5)

Estimate serum uric acid in the given serum sample of the patient.

Draw table showing the procedure (3)
Write Absorbances for Blank, standard and test. (3)
Calculate patient's serum uric acid level. (3)

Write reference range for serum uric acid. Is your result within reference range? Explain (3)

Explain rationale for using Allopurinol in the patient. (3)

21.Case - Diabetes Mellitus

30-years-old female is brought to the emergency room, in semi-comatose state. On examination, Respiratory rate 30/ min., BP 80/50 mm of Hg, heart rate 112/ min. was found. She was a known case of diabetes mellitus.

Laboratory investigations performed on admission were:

Random Plasma Glucose	?????	P_aco_2	18 mm Hg
Urine Glucose	?????	P_ao_2	98 mm Hg
Urine Ketone Bodies	?????	P_H	7.2
Plasma Urea	60 mg%	Serum Na^+	147 mmol/l
		Serum K^+	3.4 mmol/l

Physician started treatment with insulin and IV electrolytes.

Perform "?????" marked investigations in given sample of Plasma and Urine. Re-write the investigation report using the results found by you. (10+3+3+2).

Correlate your results with the patient's history and clinical findings.(5)

Explain the arterial blood gas and P_H results of the patient.(5)
Why the plasma used for estimation of plasma Glucose is preserved with Fluoride? Explain its biochemical basis. (4)
What is difference between FBS and RBS terms? (3)
Why should the glycated Hb be measured in the patient ?(3)
How would you correlate elevated plasma urea with carbohydrate metabolism in the patient?(2)

22. Case - Nephrotic Syndrome

A 8 years old girl attended by a pediatrician in Out-patient-department, had fever and generalized pitting edema. X-Ray investigations suggested pneumonia as a possible cause of fever. She was admitted for pneumonia four times in last six months. Her laboratory reports were as follows.

Investigation	Report	Investigation	Report
Urine Proteins	5 gm/day	Plasma Proteins	3 gm/dl
Urine RBC	Absent	Plasma Albumin	1.5 gm/dl
Urine Pus cells	Absent	Fasting Plasma Glucose	78 mg/dl
Urine casts	Absent	Plasma cholesterol	250 mg/dl

Provisional diagnosis of Nephrotic syndrome was made. The girl was treated by antibiotics and IV fluids. Later on glucocoticosteroid was also given. Nutritional advice was also given to improve her protein intake. The edema improved. The specimen of urine and plasma given to you was collected after few weeks of treatment.

Perform qualitative tests for detection of urinary protein in given sample of urine. Write observations and inferences.(4)
Estimate Total protein and Albumin in given sample of plasma. Write Absorbance, calculations and results. (16)
Explain principle of method for plasma protein estimation. (4)
Tabulate your results. Correlate your results with normal values, previous laboratory reports and clinical features. (10)
Explain biochemical basis for repeated infections in patients of nephrotic syndrome. (3)
Give normal values for plasma cholesterol and fasting plasma glucose.(3)

23. Case - Physiological Jaundice

A 6 days old premature neonate developed yellowish discoloration of skin and sclera. The pediatrician asked parents not to become anxious and ordered total serum bilirubin for the neonate in addition to other tests.

Write principle of the method for estimation of total serum bilirubin. (5)
Estimate Total serum bilirubin in the given serum sample of the patient.
Draw table showing the procedure (3)
Write Absorbances for Blank, standard and test. (3)
Calculate patient's serum bilirubin level. (3)

Note: Assume that patient's majority of bilirubin is indirect bilirubin

Explain biochemical basis for your result (8)
(Hint: Explain physiological jaundice, its relation with prematurity etc.)
If neonate bilirubin is very high e.g 30mg/dl, it is dangerous. Explain.
(Hint: Explain kernicterus) (3)

24. Medical Biochemistry -What should you study?

Medical Biochemistry encompasses any topic of biochemistry relevant to human health and diseases. As medicine is an ever expanding body of knowledge, Medical Biochemistry syllabus is continuously expanding.

At bare minimum, you are expected to get integrated knowledge of theoretical and practical aspects of following in context of the field of Medicine. In addition, newer advances in the field of medical biochemistry needs to be studied.

Carbohydrates:
Chemistry, Nutrition, Digestion, Absorption, Transport, metabolism and biochemical basis of related diseases, their treatment and prevention.

Amino acids and Proteins:
Chemistry, Nutrition, Digestion, Absorption, Transport, metabolism and biochemical basis of various diseases, their treatment and prevention.
Enzymes
Hemoglobin and Heme metabolism
Plasma proteins
Collagen, elastin and extracellular matrix proteins

Lipids:
Chemistry, Nutrition, Digestion, Absorption, Transport, metabolism and biochemical basis of various diseases, their treatment and prevention.
Prostaglandins
Alcohol metabolism

Nucleic Acids:
Chemistry, Nutrition, Digestion, Absorption, Transport, metabolism and biochemical basis of various diseases, their treatment and prevention.
Genetics
DNA and RNA structure and functions
Genome and Chromatin
Replication, Transcription, Genetic code and Translation
DNA Damage and repair
Mutations
Recombinant DNA Technology
Cell cycle and its regulation
Biochemistry of cancer
Biochemical basis of genetic diseases, their treatment and prevention.

Integration of metabolism:
Bioenergetics
Cellular Respiration
Interrelationship among metabolic pathways.
Biochemical basis of related diseases, their treatment and prevention.

Vitamins:
Chemistry, Nutrition, Digestion, Absorption, Transport, metabolism and biochemical basis of various diseases, their treatment and prevention.

Minerals:
Chemistry, Nutrition, Digestion, Absorption, Transport, metabolism and biochemical basis of various diseases, their treatment and prevention.

Water and pH:
Water biochemistry and biochemical basis of related disorders.
Blood buffers, regulation of blood pH and biochemical basis of related disorders.

Xenobiotics:
Chemistry, Metabolism and excretion of xenobiotics.
biochemical basis of related disorders

Tools for study of Biochemistry:
Colorimetry
Chromatography
Electrophoresis
ELISA
RIA
PCR and blotting techniques

Biochemistry of supramolecular structures (overlapping above topics):
Biochemical characteristics of various organelles, cells, tissues and organs e.g. Mitochondria, perioxisomes, general cell structure, RBC, Liver, Brain, Heart, Skeletal muscles etc.

25. Subject distribution and paper style

Subject distribution:

Paper 1: Chemistry, digestion, absorption and metabolism of *Carbohydrate, Lipid, Water, pH, Minerals*

Paper 2: Chemistry, digestion, absorption and metabolism of Protein(including hemoglobin, plasma proteins and enzymes), Nucleic acids including genetics, Vitamins, Xenobiotics

Note: Overlapping common topics are acceptable in any paper e.g integration of metabolism, nutrition, tissue and organ biochemistry, biochemistry laboratory techniques, biochemistry of microorganisms (e.g HIV), environmental biochemistry and Cancer.

Paper style(paper 1 and 2)

Section 1

Q-1 Short notes (2 out of 3)	*08 marks*
Q-2 Describe in brief (4 out of 6)	*12 marks*

Section 2

Q-3 Case with 5 questions	10 marks
Q-4 Answer in few lines(5 out of 7)	*10 marks*

MCQ

Q-5 MCQ (no negative marking)	10 marks

MCQ shall have separate question paper of 10 minutes and answer sheet shall be of OMR type

Chemical required for UG practical

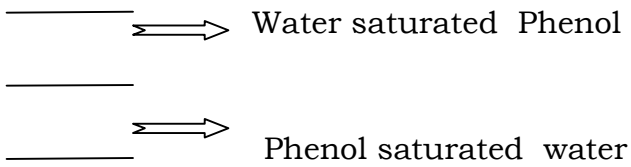
No.	Chemical	Quantity
1	glucose powder	
2	starch powder	
3	Sucrose powder	
4	Maltose powder	
5	Fructose powder	
6	alfa-Naphthol:	
7	methanol	
8	Conc.H_2SO_4	
9	sodium citrate,	
10	sodium carbonate(Na2CO3)	
11	cupric sulphate pentahydrate.	
12	Glucose strip	
13	cupric acetate monohydrate	
14	glacial acetic acid	
15	Resorcinol	
16	Concentrated hydrochloric acid	
17	NaOH pellet	
18	potassium iodide crystals	
19	Egg albumin	
20	Peptone powder	
21	Casein Powder	
22	Gelatin powder	
23	Ninhydrine powder	
24	Concentrated HNO3	
25	Formaldehyde solution	
26	sodium nitrite powder	
27	mercuric sulphate(HgSO4) powder	
28	5-6 % Analytical grade Sodium hypochloride	
29	Lead acetate powder	
30	Ammonium sulphate [(NH4)2SO4] power	
31	Any oil (Ground nut oil, coconut oil)	

32	Acetone	
33	sodium deoxycholate Powder	
34	Sodium phosphate dibasic or monobasic	
35	Pottasium dihydrogen phosphate	
36	Urea powder	
37	Creatinine powder	
38	Uric acid powder	
39	Calcium carbonate/Calcium chloride	
40	NaCl	
41	Phenolphthalein powder	
42	AgNO3	
43	ammonium oxalate powder	
44	Ammonium Molybdate powder	
45	Barium chloride	
46	Urease powder	
47	sodium tungstate powder	
48	phosphoric acid	
49	30% brij	
50	SDS	
51	picric acid powder	
52	bovin albumin	
53	Sulphosalisylic acid powder	
54	Sodium Nitroprusside	
55	liquor ammonia	
56	Bile salt powder	
57	Sulphur powder	
58	Vitamin C tablet or powder	
59	cephalosporin drug	
60	KH2PO4	
61	Na2HPO4	
62	Na2 tetraborate	
63	Phenol red dye	
64	Bromocresol green dye	
65	4- Aminophenabenzene dye	
66	Phenol Crystal	

67	billirubine powder	
68	caffine	
69	Na Benzoate	
70	anhydrous Na Acetate	
71	disodium EDTA	
72	sulfanilic acid	
73	Na K Tartrate	
74	KI	
75	pyrogallol red dye	
76	disodium molybdate power	
77	succinic acid	
78	sodium oxolate	
79	sodium banzoate	

Perform Estimation of Glucose,Cholesterol,Uric acid

Glucose reagent : Dissolve 100mg of 4- Aminoantipyrine dye & 1 ml of phenol saturated water and make upto 1000ml with DI water.



Note :Wear goggles & Glove while taking phenol.Seniour person must be present.
Glucose test sample : Mix 2 ml of analytical grade Sodium Hypochlorite solution and 1 ml of DI water.
Glucose standard sample : Mix 1 ml of analytical grade Sodium Hypochlorite solution and 2 ml of DI water.

Perform estimation of Albumin in given sample

BCG reagent : Refer SOP for Albumin reagent preparation.
Add 42mg BCG(MW=698) in approx. 250 ml DI water.
Add 5.9 gm succinic acid (MW=118.09 ,pKA1=4.2 ,pKA2 = 5.6) in above mixer while constantly mixing.
Add 1.8 ml of 30% Brij-35 In above mixer while constantly miximg.

Add 1 gm NAOH in above mixter while constatly mixing
Add 200 mg Na azide in above mixer while constatly mixing.
If required adjust pH to 4.2
Make upto 1000 ml with volumetric flask with deionised water.

Albumin test sample: Prepare serum pool approximate 5 ml each day for 50 student
Albumin standard sample:Prepare serum pool approximate 3 ml each day and dilute 1:3 times(2 part pooled serum & 1 part DI water.)

Perform estimation of Total Protein in given sample.

Reagent:
Refer to SOP for total protein.

- Weight 3 gm Cuso4.5H2O.
- Dissolve in approx. 500 ml water.
- Weight 9 gm (Na K Tartrate).(4H2O) and 5 gm KI.
- Add sequentially 9 gm (Na K Tartrate).(4H2O) and 5 gm KI in copper sulphate solution.
- Weight 24 gm NaOH.
- Add slowly with mixing 24 gm NaOH in 100ml of water.
- Add slowly with mixing NaOH solution in copper sulphate solution.
- Make upto 1 liter with water .

Protein sample: Prepare serum poolapproximate 5 ml each day for 50 student
Protein std.sample:prepare serum pool approx. 3 ml each day and dilute 1:3 times(2 part pooled serum & 1 part DI water.)

Perform estimation of Creatinine in given sample
Creatinine Std.Sample
2 mg/dl Creratinine :Disolve 0.010 gm of creatinine powder in 500 ml of 0.1 mol/L Hcl solution
Creatinine Test sample.
4 mg/dl Creratinine :Disolve 0.020 gm of creatinine powder in 500 ml of 0.1 mol/L Hcl solution
6 mg/dl creatinine : Dissolve 0.030 gm of creatinine powder in 500 ml of 0.1 mol/L HCL solution
0.1mol/l HCL solution(11.5 molar Conc.HCL solu.) : Add 17.4 ml of Conc. HCL solution & make upto 2000 ml with DI water.
Reagent
Creatinine R 1(NAOH)
Weigh 12gmNaOH.
Dissolve in approximately 500 ml DI water.
Add10ml of 30% brij in above mixture.
Weigh 1gm SDS and pour it into approximately 100 ml water in beaker.Heat the solution until SDS dissolve.
Add SDS containing solution in main mixture.
Make upto 1liter with DI water.

Creatinine R2(picric acid)
Dry picric acid between filter paper pieces
Weight 4.58gm dry picric acid.
Dissolve in approx. 300 ml water
Add 10 ml of 30% Brij in above mixture.
Remove froth with a clean object of glass or plastic dipped in capryl alcohol
Make 1 liter with water.
Working alkaline -picrate reagent:
mix 50 ml R1 & 50 ml R2 on the day of practical for 50 student.

Find out Abnormal constitute in given sample of Urine
Solution A:Glucose + Protein
Add 3 gm of Glucose powder & one egg in 1 liter tap water for 20 student.
Solution B : Glucose + ketone
add 3 gm of Glucose powder & 5 ml of acetone in 1 liter of tap water for 20 student.
Reagent
GOD Strip test-cut strip in 2 halfs for both protein &glucose test.
Benedict's reagent
Benedict's Reagent:One liter of Benedict's solution contains ,
173 grams ----------> sodium citrate,
100 grams ----------> sodium carbonate
17.3 grams ----------> cupric sulphate pentahydrate.

With the help of heat,dissolve 173 gm of sodium citrate & 100 gm of sodium carbonate in 800 ml of water.Dissolve 17.3 gm cupric sulphate pentahydrate in 100 ml of water in different container.
Pour cupric sulfate solution in carbonate- citrate solution with constant stirring& make upto 1000ml.
Sulphosalisylic Test
30% Sulphosalisylic acid :Add 150 gm of sulphosalisylic acid powder & make upto 500 ml with DI water.

Rothera's powdered reagent : Sodium Nitroprusside 2 gm
Sodium carbonate 40 gm
Ammonium sulphate 40 gm
Mix & grind all in fine particales & stored in Tight container.

Rothera's test, liquid reagent
Ammonium sulphate powder
Small crystals of sodium nitroprusside
liquor Ammonia
Sulphur powder

Estimate titrable acidity & ammonia bond acidity in given urine sample.

Reagent

1 % phenolphthalein :Dissolve 0.5 gm of phenolphthalein in 50 ml of Methanol.

Neutral formalin (formaldehyde):Take 500ml of formaldehyde & add 0.1ml of phenolphthalein in solution. Then add 0.1 mol/L NaOH till colorless formaldehyde solution become slight pink coloured.

0.1mol/L NaOH : Dissolve 20 gm of NaOH & make upto 5000 ml with Water.

Urine Sample Preparation:

Urine output ml/day = U

Titrable acidity mmol/day = A

$$\text{Take } \frac{A}{U} \times 68 \text{ gm of } KH_2PO_4 \qquad \text{MW of } KH_2PO_4 = 68 \text{ gm/L}$$

Ammonia bound acidity mmol/day = B

$$\text{Take } \frac{B}{U} \times 66 \text{ gm of } (NH_4)_2SO_4 \qquad \text{MW of } (NH_4)_2SO_4 = 132 \text{ gm/L}$$

Here two NH4+ is released when 1 molecule of $(NH_4)_2SO_4$ will be dissociated.

Example

You want to give Titrable acidity = 30 mmol HCL /day &
Ammonia bound acidity = 40 mmol HCL /day , then prepare Urine sample as follow,

Urine output U = 1500 ml/day

Titrable acidity mmol/day A = 30 mmol HCL/day

= A/U x 68

=30/1500 x 6

=1.36 gm of KH_2PO_4

Ammonia Bound acidity mmol/day B = 40 mmol HCL/day

= B/U x 66

=40/1500 x 6

=1.76 gm of $(NH_4)_2SO_4$

Finally dissolve 1.36 gm of KH_2PO_4 and 1.76 gm of $(NH_4)_2SO_4$ & make upto 1000 ml with water.

Estimate hourly Gastric acid output in given Gastric juice sample.

Reagent

0.1 mol/L NaOH : Dissolve 20 gm of NaOH in 5000 ml of water

1 % phenolphthalein : Dissolve 1 gm of phenolphthalein in 100 ml methanol
Sample preparation
Gastric juice Sample : 0.1mol/L HCL solution
How 0.1 mol/L HcL will be prepared?
1000ml of HCL solution contain=11.5 mol H^+
??????? =0.08 mol H^+
=1000x0.1/11.5
=8.6 ml
So add 17 ml of concentrated HCL & make upto 2 liter with water.
Examples
Example-1:
If you want your result will be Gastric Acid Output (mmol/hr) = 5 mmol/hr and You give Fasting Gastric juice output in 1 hour =100 ml/hr then prepare gastric juice sample as follow,

Fasting Gastric juice output =100 ml/hr
BAO = 5 mmol/L

100 ml of fasting gastric juice contain = 5 mmol/L HCL
1000 ml of fasting gastric juice contain = ???

$$= \frac{1000 \times 5}{100}$$

= 50 mmol/L HCL
= 0.05 mol/L HCL
Now We use fixed 10 ml of Gastric juice sample & titrate with fixed 0.1 mol/L NaOH

10 ml of 0.05 mol/L HCL =-------ml of 0.1 mol/L NaOH
V1=10 ml of Gastric juice V2=???? ml of NaOH
NI=0.05 mol/L HCL N2=0.1 mol/L NaOH

V2=10 x 0.05/0.1
=5 ml of 0.1 mol/L NaoH
Thus 5 ml of 0.1 mol/L NaOH is required to titrate 10 ml of 0.05 mol/L HCL.

Required Glassweres

Items	Quantity
Test tubes large 15 ml	300
Test tubes small 10 ml for chemistry	200
Test tube holder	60
Burner & lighter or machish	1
Test tube racks large	50
Test tube racks small for chemistry	50
Pipettee 10 ul	2
Pipettee 1 ml	2
Pipette 500 ul	2
Pipette 50 ul	1
100 ml lebeled beakers for reagent filling	5
Plain vacuttee for std.	4
Small tips with box	2 box
Large tips with box	2 box
Gastric titration 50 ml /student for gastri juice filling	40 beakers (100ml)
Urine titration 90 ml/student	40 flasks(250 ml)
Abnormal urine	40 beakers (100ml or 50 ml)
Gastric titration for doing titration	40 flasks(50 ml)
Phenolphthelein bottals	2
eppendrofs	For filling test sample
500 ml beakers for Filling Gastric titration 50 ml /student for gastri juice filling 0.1 N NaOH for titration& neutral formaldehyde	4
10 ml cylinders or measuring flask	5
25 ml measuring cylinder or volumetric flask	2
glass pipette for taking reagnts	8

Reagents put on side of laboratory

Reagent	Quantity
Sulphur powder	2
Ammonium sulphate powder	2
Small crystals of sodium nitroprusside	2
liquor Ammonia	2
Rothera powder	2
Benedicts reagent	2
30 % sulphosalisylic acid solution	2
1 % acetic acid	2