⇒ DOMINION

URINARY PROTEINS

TEST FOR PROTEINS IN URINE

Question 1 → State three methods of identifying proteins in urine (in the laboratory).

ANSWER

(1) Heat Coagulation Test.
(2) Sulphosalicyclic acid Test (SSA)
(3) Urine dip Strip Test.

Question 2 → List three Causes of proteins in urine (proteinuria)/Disease Conditions that Causes proteinuria.

ANSWER

(1) Urinary Tract infection
(2) Multiple myeloma
(3) Kidney Stone.

EXPERIMENT 1 - TEST FOR PROTEINS IN URINE

TITLE: Sulphosalicyclic acid test

AIM: To test for protein in urine.

Apparatus: Beaker, testtube, testtube rack, pasteur pipette

REAGENT: Sulphosalicyclic acid (SSA), Sample A and B

PRINCIPLE: Check manual

PROCEDURE: Take 1ml of Sample A and B and put in a test tube, add 3mls of Sulphosalicyclic acid (SSA) and check for precipitation/Turbidity which denotes the presence of protein especially albumin.

RESULTS

TEST	OBSERVATION	INFERENCE
1ml of Sample A + 3mls of SSA	Precipitation occurs	proteins are present.
1ml of Sample B + 3mls of SSA	Precipitation does not occur	Absence of proteins.

BIS

PAST QUESTIONS

(1) State/List three methods of identifying protein in urine

Answer → Already answered above.

(2) List three Causes of proteinuria / List three ~~Cause~~ diseases Conditions that lead to proteinuria

Answered above.

page 14 PRECIPITATION BY HEAT COAGULATION

TITLE : HEAT COAGULATION TEST

AIM : To test for protein in urine

APPARATUS: Test tube, test tube rack, water bath, pasteur pipette.

REAGENT: Acetic acid, Sample A and B

PRINCIPLE: Check manual

PROCEDURE: Take 5ml of Sample A and Sample B in a test tube and heat for 5 minutes in a water bath and Coagulation would occur if there is protein. Acidify both samples A and B with 3-5 drops 1% acetic acid, adding the acid drop by drop to the hot Solution. Phosphates precipitate will disapper under these conditions depending on the presence or absence of protein in the Solution.

RESULT

TEST	OBSERVATION	INFERENCE
1ml of Sample A + 5mins heat + 3 drops of acetic acid in drops	Coagulation does not occur	protein is absent
	precipitate does not dissappear	protein is absent
1ml of Sample B + 5mins heat + 3 drops of acetic drop by drop	Coagulation occurs	protein is present
	precipitate dissapears	Protein is present

PAST QUESTIONS

(1) Mention one Characteristic of Bence-Jones protein and name the disease it is a biomarker for.

ANSWER

The Characteristic property of Bence-Jones protein is that it Coagulates between 45-60°C and redissolves above 60°C.

NOTE → Normal proteins Coagulates at 60°C & don't redissolve

It is a biomarker for Multiple Myeloma

(2) How is Multiple myeloma diagnosed in the lab?

ANSWER

The presence of Bence Jones protein in urine is an important factor in the diagnosis of multiple myeloma in the laboratory.

Bence Jones protein Coagulates between 45-60°C and redissolves above 60°C.

Take up the Sample to be tested (a little quantity 5ml) and heat for about 5minutes, if the Sample Coagulates between 45-60°C and redissolves above 60°C then there is multiple myeloma

EXP 2 → PURIFICATION OF PROTEINS

TITLE:

AIM: To purify or Separate protein in a given sample

APPARATUS: Spatula, Test tube, test tube rack, beaker, pasteur pipette.

REAGENT: Ammonium Sulphate (→ Salt used for the experiment.), Sample A and B

PRINCIPLE: Check manual. Also Stated below.

PROCEDURE: To 1ml of test Solution, add little by little ammonium Sulphate and observe; Continue adding until the Solution precipitate.

PAST QUESTION

(i) State Clearly Salting-in and Salting Out (Principle)

ANSWER

When a crystal of a Soluble Salt (e.g NaCl) are added little by little to a protein Solution, the first few crystals of the Salt go into the Solution, being dissolved by water of hydration attached to the protein molecule. This phenomenon is called Salting In

On further addition of the Salt, the water of hydration is gradually lost to increasing Salt molecule. Consequently, the protein begins to precipitate. At Saturation point, all the protein will precipitate. This is called Salting-Out

EXPERIMENT 3

TITLE: Gastric acidity determination

AIM: To determine the gastric acidity content of the body.

APPARATUS: Conical flask, pipette (pasteur), Beaker

REAGENT: Thymol blue indicator. (check manual for principle.)

PROCEDURE: Take 10ml of Sample A and Sample B in your conical flask. To Sample A
- Add 3 drops of thymol blue indicator
- Titrate with 0.1M of NaOH.

Once you get the first colour change to yellow, note the amount of conc NaOH used.
- That becomes your free acidity, continue until you get permanent blue colour. Note the amount again
- The first titre value and the second titre value summed up is the total acidity.
- Do same for Sample B.

PAST-QUESTIONS

① Define pH?

pH is defined as the measure of the degree of acidity or alkalinity in a solution.

② What is pH?

In Chemistry, pH historically denoting "potential of hydrogen" (or "power of hydrogen") is a scale used to specify the acidity or basicity of an aqueous solution

③ What is the name of the indicator of gastric acid analysis?

ANSWER

Thymol blue.

NOTE => It has 2 different pH ranges that is why it is used

④ State two acids being determined in gastric juice analysis

ANSWER

(i) Free acid
(ii) Total acid

⑤ What is the significance of gastric juice analysis

ANSWER

It is used to determine peptic ulcer disease

OTHER NOTABLE POINTS

⑥ Constituents of gastric juice/acid.

① Free HCl
② Weak acid such as phosphate, protein and organic acids
③ Organic acid such as acetic acid, lactic acid.

⑦ pH ranges and Colour

pH 1.2 - 2.8 ⇒ Red-orange ⇒ yellow.

pH 8.0 - 9.6 ⇒ Yellow-Green-Blue.

⑧ There are three(3) types of acidity caused by HCl

They are:

① Free acidity [Caused by HCl]

② Combined acidity [Both HCl and other Components]

③ Total acidity [Free + Combined acidity]

RESULT

EXPERIMENT 4

TITLE : Nin hydrin Test

AIM : To determine or identify α-amino acid

APPARATUS: Beaker, test tube, test tube rack, pasteur pipette

REAGENT: Ninhydrin reagent. Check manual for principle

PROCEDURE: To 1ml of Sample A, add 2 drops of Ninhydrin reagent. Then heat for 2 minutes, watch for the purple Colouration. Do same for Sample B, C ~~and D~~ respectively. Record your result.

RESULT

TEST	OBSERVATION	INFERENCE
1ml of Sample A + 2 drops of Ninhydrin reagent + 2 mins heat	Purple Colouration	presence of amino acid.
1ml of Sample B + 2 drops of Ninhydrin reagent + 2 mins heat	purple Colouration	presence of amino acid
1ml of Sample C + 2 drops of Ninhydrin reagent + 2 mins heat	Yellow Colouration	

DISCUSSION

Ninhydrin test is a universal method that is used to assay or determine amino acid, primary amines, secondary amines, especially α-amino acids.

BIOMEDICAL IMPORTANCE

It is used for finger print detection.

⇒ Name two amino acid that does not give purple Colour in Ninhydrin test.

ANSWER

① Proline & hydroxyproline which gives a yellow Colour.
② Asparagine which gives a brown Colour.

⇒ Another name Ninhydrin reagent

ANSWER

Triketohydrindene hydrate.

⇒ The appearance of purple Colour in Ninhydrin test Indicates?

ANSWER

The presence of amino acids

⇒ State Ninhydrin principle.

EXPERIMENT 5

TITLE: Buret Test

AIM: To identify protein in a given Sample.

APPARATUS: Test tube, pasteur pipette, Test tube rack, Beaker.

REAGENT: Buret reagent, Normal Saline, plasma.

PROCEDURE:
- To 1ml of plasma, add 4ml of Buret reagent
- Allow to Stand at room temperature for 15 minutes, Record your observation
- Carry out the test on ½, ¼, ⅛, dilution of your test Solution, using Bovine Serum albumin dilution.
- Allow Colour to develop for 5 minutes and record Colour intensities at 550nm wave length in a Calorimeter.

PRINCIPLE: Compounds Containing two or more peptide bonds give a Characteristic purple Colour when treated with dilute Copper Sulphate in alkaline Solution. The name of the test Comes from the Compound buret which gives a typically positive reaction. The Colour is apparently due to the Co-ordination Complex of the Copper atom and 4 nitrogen atoms, two from each of the two peptide chains.

NOTE → Alkaline Solution is used inorder not to denature proteins

DISCUSSION

What is protein?

protein are polymers of amino acids joined together by peptide bond.

What is are amino acids?

Amino acid are organic compounds that has both NH_2 and COO group.

What colour is gotten from Biuret Test?

Violet Colour

RESULT

Serum	Neat	1/2	1/4	1/8
Plasma	1ml	1ml		
Saline	-	1ml	1ml →	1ml
Biuret	4ml	4ml	4ml	4ml
Reading	++++	+++	++	±

P. QUESTIONS

① What test can you use to identify protein in Serum.

ANSWER

Biuret Test

② List two anticoagulants used in the Laboratory

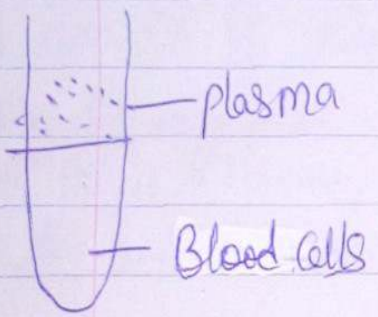
ANSWER

① Oxalates
② EDTA
③ Citrate

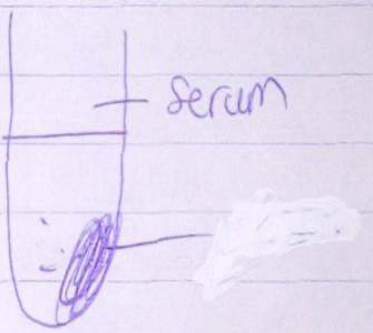
(They all remove Calcium)

③ whats is the anticoagulant of choice for Preserving blood glucose

ANSWER.



EDTA bottle



Ordinary bottle.

④ State the difference between Serum and Normal Saline.

(5) DIFFERENCE BETWEEN PLASMA AND SERUM

PLASMA	SERUM
(1) This is the liquid that remains when anticoagulant is added to prevent clotting	This is the liquid that remains after the clotting of blood
(2) Consists of 55% of the total blood volume.	less volume Compared to plasma
(3) Contains Fibrinogen	Lacks Fibrinogen.
(4) It Consists of serum and Clotting factor.	It is the part of blood without clotting factor
(5) It needs Anticoagulant before it can be obtained	Anticoagulant is not need before it can be obtained from blood Sample

(6) List the plasma proteins on the basis of their Solubility in Salt Solutions-

ANSWER

(i) Fibrinogen
(ii) Globulin
(iii) Albumin

(7) What are the Conditions that increases plasma Proteins in the body.

ANSWER

1 Dehydration
2 Diarrhoea
3 Multiple myeloma
4 Respiratory disorder
5 Vomiting excessively
6 Respiratory disorder
(7) Hemolysis
(8) Inflamatory disorder.

Conditions that decreases Plasma Proteins

(1) Malnutrition (Cas seen in kwashiorkor)
(2) Cirhosis of the Liver
(3) Malabsorption
(4) Severe burns
(5) Nephritic Syndrome.
(6)