Osazone Test

This is a test for reducing carbohydrates. Reducing disaccharides and many monosaccharides can be identified by this test.

Reagents

(i) Osazone mixture: This is prepared by mixing thoroughly one part of phenylhydrazine hydrochloride and two parts of sodium acetate by weight.
(ii) Glacial acetic acid.

Procedure: It is convenient to carry out this test simultaneously on all the reducing carbohydrates detected by the earlier tests. Take 5 ml each of glucose, fructose, maltose and lactose solutions in correspondingly labelled test tubes. Add about 0.3 gm of osazone mixture and 5 drops of glacial acetic acid into each test tube. Warm gently to dissolve the solids. Keep all the test tubes in a boiling water-bath. Yellow crystals will appear in the tubes containing glucose and fructose within 5 minutes. Remove these tubes from the water-bath and allow them to cool spontaneously. Remove tubes containing maltose and lactose after 45 minutes and let them cool. With the help of a glass rod, take out some crystals on a glass slide, cover them with a cover-slip and observe under a microscope.

Glucose and fructose form needle-shaped osazone crystals lying singly or in sheaves. Maltosazone crystals are sunflower-shaped, and lactosazone crystals are puff-shaped (Fig. 2.1).

Fig. 2.1: Osazone crystals

Principle: When the reducing carbohydrates are treated with phenylhydrazine at 100°C and pH 4.3, a series of reaction take place resulting in the formation of osazones of the respective carbohydrates.

2. Conjugated proteins
3. Derived proteins.

1. Simple Proteins

The proteins made up of only amino acids are known as simple proteins. They can be sub-divided on the basis of their solubility and heat-coagulability into alubmins, globulins, glutelins, prolamins, protamines, histones and albuminoids.

2. Conjugated Proteins

They are made up of amino acids and a non-protein part which is known as the prosthetic group. Depending upon the nature of the prosthetic group, the conjugated proteins may be sub-divided into glycoproteins and mucoproteins, lipoproteins, nucleoproteins, phosphoproteins, chromoproteins and metalloproteins.

3. Derived Proteins

They are derived from native (naturally occurring) proteins. They can be divided into primary derived proteins and secondary derived proteins. The primary derived proteins are formed due to some intramolecular changes not involving hydrolysis, and include proteans, metaproteins, denatured proteins and coagulated proteins. The secondary derived proteins are formed from hydrolysis of native proteins, and include primary proteoses, secondary proteoses and peptones.

TESTS FOR PROTEINS

The proteins which are generally provided for practical exercises are albumin, casein, gelatin and peptone. Albumin usually contains a small amount of globulin as contaminant. The following tests may be done with 1–2% solutions of these proteins.

Biuret Test

Reagents

(i) Sodium hydroxide: 10% solution in water.
(ii) Copper sulphate: 0.5% solution in water.

Procedure: Take 2 ml of the protein solution in a test tube. Add 2 ml of sodium hydroxide, mix, add copper sulphate solution

6 Blood

Blood is a suspension of cells, erythrocytes, leucocytes and thrombocytes in a fluid, plasma. The cellular fraction constitutes 45% of the volume of blood. Chemically, blood is a very complex fluid containing a large variety of substances, some of which are known and many perhaps yet to be identified.

COLLECTION OF BLOOD

Venous blood is collected usually from the antecubital vein or some other prominent vein on the forearm. The patient is seated comfortably and asked to extend his arm. A tourniquet is tied firmly around the upper arm. A suitable vein is selected and the skin over it sterilized by rubbing spirit over it with a pad of cottonwool. A sterile hypodermic needle attached to a syringe is introduced into the vein and the tourniquet removed. The desired amount of blood is drawn into the syringe by gently withdrawing the plunger. A pad of cottonwool soaked in spirit is placed on the skin where the needle was introduced and the needle is withdrawn. The blood is transferred into a suitable container after removing the needle from the syringe.

Arterial blood is required rarely. This may be collected from the radial, brachial or femoral artery in the same way as venous blood.

Capillary blood may be collected from the tip of the thumb or a finger or from the ear lobe. The site of collection is sterilized with spirit and pricked with a sterile needle. The blood is allowed to flow into a suitable container or directly aspirated into a pipette.

SEPARATION OF SERUM AND PLASMA

If blood is allowed to stand, it coagulates in a few minutes. After some time, a clear, straw-coloured fluid known as serum

strong ammonia, and heat until the colour changes to brown. Add 4–5 ml of 95% ethyl alcohol and mix.

On spectroscopic examination, a diffuse band is seen in the red region at 600 nm.

Haemochromogen

To the above solution of alkali haematin, add a pinch of sodium bisulphite, mix very gently. The colour changes to pink.

On spectroscopic examination, a sharp band is seen about midway between the D and E lines with its centre at 558 nm and a relatively faint band is seen at 526 nm.

The characteristic features of different derivatives of haemoglobin are summarized in Table 6.3.

Table 6.3: Different derivatives of haemoglobin

Haemoglobin derivative	*Colour*	*State of iron*	*Position of bands*	*Position of bands*
Haemoglobin	Purple	Ferrous	One	565 nm
Oxyhaemoglobin	Bright red	Ferrous	Two	578 nm 540 nm
Carboxyhaemoglobin	Cheery red	Ferrous	Two	573 nm 535 nm
Methaemoglobin	Brownish red	Ferric	Three	633 nm 578 nm 540 nm
Sulphaemoglobin	Dirty brown	Ferrous	Three	618 nm 580 nm 540 nm
Acid haematin	Brown	Ferric	One	650 nm
Alkali haematin	Brown	Ferric	One	600 nm
Haemochromogen	Pink	Ferrous	Two	558 nm

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