**Government Arts and Science College (Women), Orathanadu**

**Department of Biochemistry**

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Core Course IX- Clinical Biochemistry – 16SCCBC9

Unit I

Basic units of expression

Solution

In Biochemistry laboratory, Preparation of solutions in the form of reagents is inevitable.

Solutions

It is a homogenous mixture of two or more non reacting substances having uniform properties like chemical composition, refractive index and density. Dissolved substance in a solution is known as solute where as the medium used to dissolve is known as solvent.

Solutions can be prepared by dissolving solid or liquid taken as solute and dissolving in a medium.

Expression of solution composition

It can be expressed as quantity and concentration.

* Quantity: It is any amount of substance present in a solution irrespective of the volume of the solution.
* Concentration: Exact quantity of solute present in a specific volume of solvent or solution.

Concentration of solution are expressed by the following terms.

* Mole: It is defined as the molecular weight of the compound in grams.
* Molarity (M)

The molarity of a solution is the number of moles of the solute dissolved per one litre of the solution.

Molarity = Weight of a solute in g/L of solution

Molecular weight of solute

* Molality(m)

It is defined as 1 mole of the solute dissolved in 1 kg of the solvent is known as molal solution.

Molality= Weight of a solute in g/kg of solvent

Molecular weight of solute

* Normality (N)

It is defined as the number of gram equivalents of the solute per litre of the solution.

Normality = Amount of substance in g/l of solution

Equivalent weight of substance

* **Percentage solution (%)**

**It is defined as an amount or volume of substance dissolved per 100ml of solution.**

**X% solution = X amount in gram**

**100 ml solvent**

Two types of expression of % solution

Percentage (%) weight by volume (w/v)

Percentage (%) volume by volume (v/v)

**Parts per million (ppm)**

It is defined as a gram of a solute per million grams of a solution or the gram of a solute per million ml of the solution.

ppm = mass of the component X 106

total mass of the solution

**Standard Solution**

A solution of known concentration is referred as standard solution. It can be expressed as milligram (mg) or microgram (µg)/ known volume.

Collection and Preservation of Blood Sample

Blood is collected by the following methods.

**Collection of Capillary Blood**

* Capillary blood can be collected from the fingertip or earlobe.
* The site of puncture (blood collection) is selected and sterilized with ether or methylated spirit. After the complete evaporation of ether or methylated spirit, by using a lancet or a sharp sterilized needle a smart deep prick is given. Then the fingertip is slightly pressed.
* The first drop of blood comes out is wiped off because of the presence of tissue fluids.
* Then the blood is collected in a suitable pipette held at an angle slightly downwards from the horizontal.

**Collection of Venous Blood**

* The venous blood is collected from a prominent vein. Generally a vein on the front of elbow or forearm is used for the collection of blood.
* For the process of collection, a rubber tourniquet is tied firmly a few inches above the elbow of an extended arm.
* The area of the collection site is sterilized by rubbing with spirit or ether and a fine sterile hypodermic needle fixed with the syringe of appropriate capacity (eg., 2.0, 5.0 and 10.0 ml) is inserted in to the vein, which is held steadily by the thumb of the other hand of the technician.
* After the needle enters the vein, the plunger of the syringe is withdrawn. With the appearance of blood in the syringe,the tourniquet is released, after collecting the desired amount of blood, a piece of cotton wool soaked with ether is placed on the puncture site and the needle is withdrawn. The cotton wool is held firmly for few minutes till the bleeding stops.
* The needle is then removed and the blood is transferred to a container using minimum pressure.
* If the blood is placed as such to clot, serum is obtained after the shrinking of clot. The serum can be collected after centrifugation.
* Anticoagulant is added to prevent clotting of blood. It can be centrifuged to collect the supernatant plasma. Plasma differs from serum in containing fibrinogen and anticoagulant.

Estimations in blood are carried out using whole blood, serum and plasma depends on the type of analysis.

**Collection of Arterial Blood**

This is the rarely used method for collection. Local anaesthesia is applied and the blood is obtained by inserting the needle into the radial, brachial or femoral artery. This method is generally used for blood gas determination and for studying the arterial-venous ratio for blood sugar.

**Anticoagulants**

The blood has the tendency to clot after collection. The clotting of blood is prevented by adding anticoagulants. Anticoagulants are the chemicals, which prevent the clotting of the blood when mixed with blood in proper proportions.

Different types of anticoagulants are as follows:

**1.Double oxalate solution**

It consists of 3 parts of ammonium oxalate and 2 parts of potassium oxalate. These salts are combined together to balance the swelling effect of ammonium oxalate and shrinking effect of potassium oxalate on the red blood cells. The solution of double oxalate is prepared by mixing 2.4g of ammonium oxalate, 1.6g of potassium oxalate in 100 ml of distilled water.

0.2 ml of anticoagulant is needed to prevent clotting. It is added to the blood collection container and heated to 60 to 80° C in a incubator for an hour. The oxalate in anticoagulant combines with calcium in blood and form an insoluble precipitate of calcium oxalate.

**2.EDTA-Ethylene diamine tetraacetic acid disodium salt**

EDTA anticoagulant solution is prepared by mixing 4 mg EDTA to 100 ml distilled water. 0.2 ml of the solution can be used to prevent coagulation of 3.0 to 4.0 ml of blood. It is used as disodium or dipotassium salts.

**3.Trisodium citrate**

3.8 g trisodium citrate is added to 100 ml of distilled water. From this 0.2 to 0.4 ml of the solution is taken for ESR and prothrombin time determination.

**4.Heparin**

Heparin is used in a concentration of 0.1 to 0.2 mg / ml of blood.

**5.Acid citrate dextrose**

It is used in the blood banks for collecting blood for transfusion. It is a mixture of citric acid, sodium citrate and glucose.

**Preservation**

For storage of blood, it should be collected under aseptic conditions. One method of storing is by separating serum or plasma as soon after collection and placing in a refrigerator. A mixture of 3 parts of potassium oxalate and 1 part of sodium fluoride is used as a preservative and can be used for blood sugar estimation.

**Collection and preservation of Urine**

Single specimen of urine can be used for qualitative tests, but for quantitative tests 24 h specimen are used.

Urine should be collected in clean, well washed container.

When urine specimen is kept for sometime, urate and uric acid will deposit to the bottom since they are less soluble in urine. Before doing estimations, any deposits in urine to be mixed thoroughly with urine. As soon after collection it should be transported to laboratory.

**Preservatives for Urine**

If urine to be kept, preservatives to be added to prevent the changes. The main change may be due to bacterial action.

10 ml of concentrated hydrochloric acid can be added to 24 h urine specimen or 50 ml of 2N hydrochloric acid can be used.

Chloroform, formalin, toluene, light petroleum and thymol are also other preservatives. But these preservatives have some disadvantages.

**Collection of faeces**

Faeces are composed of not absorbed digested product, undigested or undigestible part of food, intestinal secretions, intestinal cells, water, metabolic products, bacteria and foreign bodies.

It is usually collected in bed pan. It should be transported to the laboratory within a short time after being passed in a waxed carton. It must not be contaminatedwith urine. For some tests portion of the stool is sufficient, but for quantitative determination whole stool should be sent to the laboratory.

The stool collected after enema should be avoided.

**Preservation of faeces**

Mostly preservation to be avoided and should be tested soon after collected. It can be homogenized in water and a portion can be placed in a refrigerator.

If to be kept, mixed with 200 ml of water and uniformly mixed , taking a portion and stirred with equal volume of concentrated sulphuric acid and can be kept in refrigerator for analysis.

Formalin can be added in small amount for preservation of faeces.

**Transportation of specimen**

The collected sample in an appropriate clean container should be labeled with the name of patient, date, time and the place of collection and sealed in a bag. In a transport box, a layer of perforated sponges will be placed at the bottom, and the specimen container is placed above in an upright manner. It was covered over with the layer of perforated sponges. Un-perforated sponges are placed above that and covered and sealed the box. It is labeled as room temperature

Samples on transport box and sent immediately to the laboratory. For refrigerated samples pre frozen gel packs to be placed below and above the perforated sponges covering the specimen.

**References**

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