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Clinical Blood Chemistry

Analysis of normal and abnormal constituents of blood is carried out by photocolorimeter (analyzer). So photocolorimeter is the basic requirement in clinical chemistry which is available in various models. Nowadays digital analyzer is available in market which is simple to operate and direct result can be noted. Commercially, available colorimeters have optical density and transmission scales which follow the Beer's and Lambert's laws.

Beer's law

It states that equal thickness of an absorbing material will absorb a constant fraction of the energy incident on it.

Lambert's law

It states that the proportion of light absorbed by an absorbing substance is independent of the intensity of the incident light. The instrument is widely used for clinical laboratories, agricultural analysis, petroleum and water pollution, etc.

Colorimetric analysis is performed for the quantitative determination coloured substance in solution. The general instruments (deflection type) comprises the following parts.

1. *Light source*: Tungsten filament lamp.
2. *Optical system*: It consists of reflector lens.
3. *Filter wheel*: For selecting appropriate wavelength complimentary to the colour of the sample.
4. *Shutter*: It permits light to fall photo cell.
5. *Cuvette*: For placing test tube containing solution being examined.
6. *Photo cell:* For detecting amount of light passing through the solution.
7. *Calibrated micrometre*: For measurement of optical density and percentage transmission.

Selection of filter

Select the filter in that region of the spectrum which gives maximum reading of optical density. In this region the rate of change of optical density is quite sensitive to the rate of change of concentration.

Operation

Make necessary power connection and switch on the instrument. Select the appropriate filter. Set the metre reading on 100% transmission (zero optical density) by using a blank solution in the test tube. It is done by adjusting the concern knob. Then keep the shutter in open position. Insert the standard and test solutions and note the optical densities of the both respectively. Calculate the concentration of test solution as follow.

Calculation

$$\text{Glucose conc. per dl} = \frac{\text{OD of test sample}}{\text{OD of standard}} \times 100$$

OD = Optical density.

Result

..... mg per dl.

Diagnostic significance

Glucose in urine or blood is reported in diabetes mellitus, hyper activity of pituitary thyroid and adrenal glands. Hypoglycemia is observed in case of insulin overdose, hypopituatrism, hypoadrenalism, etc.

Reagents

1. *O-toluidine*: Dissolve 1.5 gm of thio-urea in 200 ml of glacial acetic acid, add 60 ml of *o*-toludine. Mix and make the volume to 1 litre with glacial acetic acid. Keep in brown bottle. Store at 2–8 °C.
2. *Tri-chloroacetic acid*: 4% W/V.
3. *Standard glucose solution* (100 mg/dl): Dissolve 100 mg in 100 ml of saturated solution of benzoic acid. Store at 2–8 °C.

Calculation

$$\text{Serum creatinine in mg/dl} = \frac{\text{OD of test sample} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 3$$

Result

Normal value
Male : 0.9–1.4 mg/dl
Female : 0.8–1.2 mg/dl

Clinical significance

Increased level: Impaired renal functions.
Decreased level: Muscular dystrophy.

Reagents

1. *Alkaline picrate solution* (alkali reagent): To 10 ml of saturated solution, add 2 ml of 10% W/V of NaOH solution and mix. Store at room temperature.
2. *Creatinine standard solution* (150 mg/dl): Prepare the standard solution in 0.1 N HCl. Take pure creatinine and make the volume with 0.1 N HCl. Store at 2– 8° C.
3. *For deproteinization*: 10% W/V sodium tungstate solution and 2/3 N sulphuric acid are used in equal volume (creatinine reagent A). Store at room temperature.

Measurement of standard: Read the optical density of bilirubin standard against distilled water.

Calculation

$$\text{Total bilirubin in mg/dl} = \frac{\text{OD of T1} - \text{OD of T2}}{\text{OD of standard}} \times 10$$

$$\text{Direct bilirubin in mg/dl} = \frac{\text{OD of D1} - \text{OD of D2}}{\text{OD of standard}} \times 10$$

Indirect = Total – direct bilirubin

Normal value

Adults (total) : 0.2 – 1 mg/dl

(direct) : 0 – 0.2 mg/dl

Result

Diagnostic significance

Increase levels: Liver diseases, Coombs position anaemia, defective uptake and storage of bilirubin, defective glucuronyl transferase activity.

Reagents

1. *Diazo reagent A* (sulphanilic acid solution): Add 5 gm of sulphanilic acid to 60 ml of conc. HCl in a litre of VF and dilute to the mark with distilled water. Store at room temperature.
2. *Diazo reagent B* (sodium nitrite solution): Dissolve 20 gm sodium nitrite ($NaNO_2$) in distilled water and dilute to the volume of 100 ml with distilled water. Store at 2 to 8° C.
3. *Bilirubin standard* (10 mg/dl): Dissolve 100 mg in 100 ml of distilled water. Dilute 10 ml of the stock solution to 100 ml with distilled water. Store at 2 to 8° C.
4. *Diazo blank*: Dilute 60 ml of conc. HCl to 1 litre with distilled water. Store at room temperature.
5. *Bilirubin reagent D* (methanol): Analytical grade. Store at room temperature.