Experiment
Estimation of Creatinine in Blood (Serum) by
Jaffe's Alkaline Picrate

Aim

To estimate the concentration of **creatinine** in the given **blood (serum) sample** using **Jaffe's alkaline picrate method**.

References

K.K Pillai and J.S Qadry Biochemistry and clinical pathology. CBS Publication and Distributors Pvt. LTD, New Delhi, First edition, 136-137.

Requirements

- **Glassware Requirement:-** Test tube, Test tubes holder, Pipettes, Glass road, Colorimeter.
- **Chemical Requirement:-** Serum sample, Standard creatinine solution (2 mg/dL), Picric acid solution (saturated), Sodium hydroxide (0.75 N).

Principle

Creatinine reacts with **alkaline picrate** to form an **orange-red colored creatinine–picrate complex**. The intensity of the color formed is **directly proportional** to the concentration of creatinine present and is measured colorimetrically at **520 nm** (green filter).

Procedure

Tube	Distilled Water (mL)	Creatinine Standard (mL)	Serum Sample (mL)	Picric Acid (mL)	NaOH (mL)
Blank (B)	1.0	–	–	1.0	1.0
Standard (S)	–	1.0	–	1.0	1.0
Test (T)	–	–	1.0	1.0	1.0

Steps:

1. Label three test tubes as **Blank, Standard, and Test**.
2. Add reagents according to the table above.
3. Mix well and incubate at **room temperature for 10 minutes**.
4. Set the colorimeter at **520 nm** using the **Blank**.
5. Measure absorbance of **Standard** and **Test**.

Experimental Data (Example)

Tube	Absorbance
Blank	0.00
Standard	0.62
Test	0.93

Calculation

$$\text{Creatinine (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

$$= \frac{0.93}{0.62} \times 2$$

$$= 3.0 \text{ mg/dL (approx.)}$$

Result

The concentration of creatinine in the given serum sample **is: 3.0 mg/dL**

Normal Reference Range

- **Adult male:** 0.7 – 1.3 mg/dL
- **Adult female:** 0.6 – 1.1 mg/dL