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Diploma in Pharmacy 2nd Year

Biochemistry & Clinical Pathology

Experiment

To determine the calcium in blood /serum

Aim:

To determine the calcium in blood /serum

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 49 – 52

Materials Required

Ammonium oxalate, 4% solution, ammonia, 2% solution, 0.01 N potassium permanganate, 2N H₂SO₄ solution, test tube and stir rod.

Theory :

In plasma and serum, calcium can be found in three different forms:

- 1) Protein-bound
- 2) Ionised part
- 3) Coupled with other compounds like citrate

Unlike the other two, calcium that is attached to proteins is not diffusible. When it comes to extremely young children, the upper limit seems to be a little higher. During pregnancy, serum calcium levels tend to reduce slightly. One of the reasons of tetany is a decrease in serum calcium that affects the ionised calcium.

Calcium precipitates out of the serum as oxalate. The precipitate is first washed. and then it is dissolved in acid and titrated with KMnO₄ solution.

Procedure

- 1) 2ml serum should be dissolved in a test tube containing 2ml distilled water.
- 2) 4% of 1ml ammonium oxalate should be added to the above mixture and allowed to stand for 30 minutes and then centrifuged.
- 3) The supernatant fluid should be removed without disturbing the precipitate.
- 4) The test tube should be allowed to dry for 5 minutes by standing the tube inverted on a filter paper.
- 5) 3ml Ammonia solution (2%) should be added and mixed well.
- 6) It should be centrifuged again and the supernatant fluid should be poured off.
- 7) The precipitate should be mixed with 2ml H_2SO_4 (2N) and warmed by placing the beaker of almost boiling water to complete solution of the oxalate.
- 8) The mixture should be removed and kept at 60° to $70^\circ C$.
- 9) The mixture should be titrated with 0.01 $KMnO_4$ and a faint pink colour is obtained which lasts for a minute.
- 10) Calcium solution should be treated with 2ml H_2SO_4 (2N) solution at $60-70^\circ C$ and titrated with 0.01N $KMnO_4$ solution and results in faint pink colour.
- 11) 2ml of H_2SO_4 (2N) solution should be titrated to the same end-point as a blank.

Calculations

Each 1ml 0.01N $KMnO_4$ = 0.2 mg Ca"

Hence since 2ml of serum is used

Conc. of Calcium = (Titration of Test - Titration of Blank) X 0.2X

Conc. (mg%)=(Titration of Test-Titration of Blank) X 10.

Expected Values

9-11 mg/dl

Result :

Calcium concentration in blood /serum was determined.

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