

Aim - Estimation of Crude Protein using Microkjeldahl System

Principle: The digestion of protein to release the N_2 content of the taken sample by strong H_2SO_4 in the presence of digestion mixture. The release of N_2 collected in the solution of Boric Acid (4%). This collected N_2 gives green colour in the presence of Mixed indicator. This N_2 is titrated with N/50 H_2SO_4 or N/50 HCl and the quantity of used H_2SO_4 or HCl is the measurement of total Nitrogen by multiplying factor (6.25).

Materials:

- Digestion mixture- Take K_2SO_4 and $CuSO_4 \cdot 5H_2O$ in 9:1 ratio. Mix it properly and store it in a reagent bottle.
- 40% NaOH - Take 40 gm of NaOH crystals in 100 ml of distilled water and dissolve it properly.
- 4% Boric acid- Take 4 gm of Boric acid crystals in 100 ml of distilled water and mix it properly.
- Mixed indicator- Take 0.5 gm of Bromocresol green and 0.1 gm of Methyl red in 100 ml of ethanol and mix it properly.
- N/50 H_2SO_4 or HCl solution for titration.
- Weighing balance, Microkjeldahl unit.
- Pipette, Pipette pump, Conical flask, Measuring cylinder, Funnel, Burette, Burette stand.

Method:

Digestion:

- Take 0.5 to 1 gm of sample in digestion tube.
- Pour 10ml of conc. H_2SO_4 in digestion tube.
- Add a pinch or 1.5gm of Digestion mixture to digestion tube.
- Place the digestion tube on digestion unit at $450^\circ C$ for 1.5 hours.

Distillation:

- Cool the digested sample properly.
- Add 10 ml of distilled water to digested sample in each tube.
- Place the digestion tube in Distillation unit and on the other side place the conical flask containing 25 ml of 4% Boric acid and 2-3 drops of Mixed indicator.

- Switch ON the Power Button.
- Allow the Ready Button to blow.
- Now, switch ON the Alkali Button and allow the sample colour change to brown colour.
- Then switch OFF the Alkali Button and switch ON the Process Button and allow the conical flask to fill upto 120 to 150 ml.
- Then switch OFF the Process Button.

Titration:

- Titrate the solution of conical flask with N/50 H₂SO₄ or N/50 HCl.
- Note the reading of end point.
- Completion of titration will be characterised by turning Bluish colour to slight Pink colour.

Calculation:

Total Crude Protein content = Total Nitrogen 6.25