Determination of crude protein content – Kjeldahl method

Principle

Digestion of organic matter with sulfuric acid in the presence of a catalyst, Rendering the reaction product alkaline then distillation and titration of the liberated ammonia, Calculation of the nitrogen content, Multiplication of the result by the conventional factor 6.25 to obtain the crude protein content.

Reagents and materials

- Potassium sulfate
- Copper (II) oxide (CuO)
- Copper (II) sulfate pentahydrate ($CuSO_4 5H_2O$)
- Sulfuric acid - 18 mol/l, = 1.84 g/ml
- Paraffin wax
- Saccharose
- Acetanilide, with melting point 114ºC, nitrogen content 103.6 g/kg.
- Tryptophan, with melting point 282ºC, nitrogen content 137.2 g/kg. Before use, dry the tryptophan.
- Sodium hydroxide solution, (NaOH) = 33% (m/m).
- Boric acid = 40 g/l.
- Sodium hydroxide - 0.1 mol/l or (NaOH) = 0.25 mol/l.
- Sulfuric acid - 0.05 mol/l or (H_2SO_4) = 0.125 mol/l.
- Mixed indicator, neutral point at pH 4.4 to 5.8.
- Dissolve 2 g of methyl red and 1 g of methylene blue in 1000 ml of ethanol (w(C_2H_5OH) = 95% (v/v).

Apparatus

- Analytical balance (Fig. 7.3)
- Digestion, distillation and titration apparatus

Procedure

Weigh, to the nearest 1 mg, a mass of the test sample chosen according to the expected nitrogen content so that the test portion contains between 0.005 g and 0.2 g of nitrogen and, preferably more than 0.02 g.

Note: The mass of the test portion of homogeneous air-dry samples should be between 0.5 g and 2.0 g. The mass of the test portion of wet and/ or inhomogeneous samples should be between 2.5 g and 5.0 g.

Digestion of organic matter

Transfer the test portion quantitatively into a Kjeldahl digestion flask of suitable size (usually 800 ml).

Add 15 g of potassium sulfate.

Add an appropriate quantity of catalyst as follows: 0.3 g of copper (II) oxide or 0.9 g to 1.2 g of copper (II) sulfate pentahydrate.

Fig. 7.3 Analytical balance

Add 25 ml of sulfuric acid (18 mol/l) for the first gram of dry matter of the test portion and 6 to 12 ml for each additional gram of dry matter. Mix thoroughly, ensuring complete wetting of the test portion.

Heat the flask moderately at first to prevent foam from rising into the neck of the flask or escaping from the flask.

Heat moderately, swirling from time to time, until the mass has carbonized and the foam has disappeared. Then heat more intensively until the liquid is boiling steadily.

Avoid overheating of the walls of the flask not in contact with liquid.

After the liquid has become clear with a light green-blue colour, heat for 2 h.

Leave to cool. If the digest starts to solidify, add some water and mix by swirling.

Distillation of ammonia

Carefully add 250 to 350 ml of water to dissolve the sulfates completely. If necessary, facilitate dissolving by heating the flask in warm water. Mix by swirling and allow cooling.

Add a few boiling aids.

Pipette into the collecting flask of the distillation apparatus 25 ml of the sulfuric acid (0.05 mol/l), choosing the concentration according to the expected nitrogen content of the test portion. Add 100 to 150 ml of water. Add a few drops of the mixed indicator. Proceed as follows:

Immerse the end of the condenser in the liquid contained in the collecting flask, to a depth of at least 1 cm.

Slowly pour 100 ml of sodium hydroxide solution (33 per cent) into the digestion flask along the wall.

Immediately connect the flask to the distillation apparatus.

Heat the flask in such a manner that approximately 150 ml of distillate is collected in 30 min. At the end of this time, check the pH of the distillate at the tip of the condenser using litmus paper. If the reaction is alkaline, continue distillation.

Alternatively, transfer into the collecting flask 100 to 250 ml of boric acid. Add a few drops of mixed indicator.

Titration

If sulfuric acid is used as the collecting liquid, titrate in the collecting flask, the excess sulfuric acid with sodium hydroxide solution 0.1 mol/l or 0.25 mol/l as appropriate, until the endpoint is indicated by the pH meter or until the color changes from violet to green.

If boric acid is used as the collecting liquid, titrate the ammonia with sulfuric acid 0.05 mol/l or 0.125 mol/l as appropriate, until the endpoint is indicated by the pH-meter or the color changes from green to violet (Fig. 7.4).

Blank test

Perform a blank test using about 1 g of saccharose in place of the test portion.

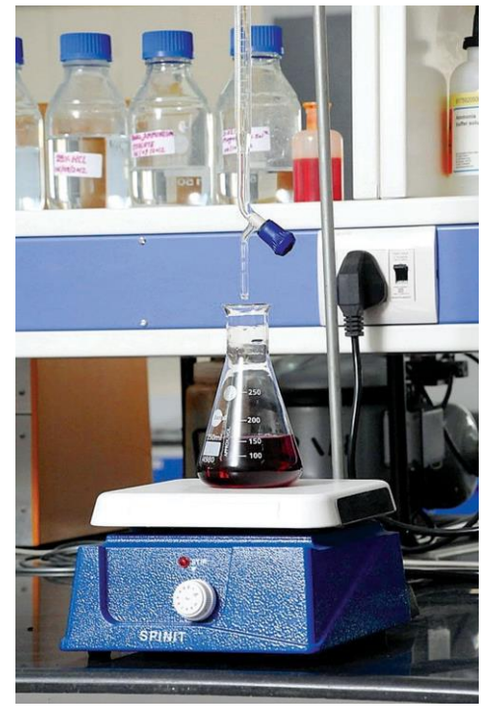

Fig. 7.4 Titration

Check test

Perform a check test by determining the nitrogen content of acetanilide or tryptophan after addition of 1 g of saccharose.

Calculation and expression of results

Distillate collected in sulfuric acid:

$$W_{N1} = \frac{(V_0 - V_1) \times C_1 \times M}{m}$$

Where,

W_{N1} is the nitrogen content, in grams per kilogram, of the test sample.

V_o is the volume, in milliliters, of the sodium hydroxide solution required for the blank test.

V_1 is the volume, in ml, of the sodium hydroxide solution required for the determination.

C_1 is the concentration, in moles per litre, of the sodium hydroxide solution used for the titrations;

M is the molar mass, in grams per mole, of nitrogen (M = 14 g/mol);

C_2 is the concentration, in moles per litre, of the sulfuric acid (4.9.2) used for the titrations;

m is the mass, in grams, of the test portion.

Distillate collected in boric acid:

$$2(V_3 - V_2) \times C_2 \times M$$

$$W_{N2} = \frac{\text{----------}}{\text{---- m}}$$

W_{N2} is the nitrogen content, in grams per kilogram, of the test sample.

V_2 is the volume, in ml, of the sulfuric acid required for the blank test.

V_3 is the volume, in ml, of the sulfuric acid required for the determination.

M is the molar mass, in grams per mole, of nitrogen (M = 14 g/mol);

m is the mass, in grams, of the test

portion. Calculation of crude protein content

Calculate the crude protein content of the test sample by the equation.

$$Wp = 6.25 \times w_N$$

Where,

Wp is the crude protein content, in grams per kilogram, of the test sample;

Wn is the nitrogen content, in grams per kilogram, of the test sample (either w_{N1} w_{N2}); or

Reference: IS 14825 : 2000, ISO 5983 : 1997