Kjeldahl Method

Basic principle

This procedure is based on the oxidation of the organic compound using strong sulphuric acid.

-As the organic material is oxidized the carbon it contains is converted to CO_2 and the H_2 is converted to water

- The nitrogen from the amine group found in the peptide bond(CO NH_2) of the polypeptide chain is converted to ammonium ion which dissolve in the oxidizing solution & can be converted to ammonia gas.

Procedure

The procedure consist of 3 steps

i) Digestion
ii) Distillation
iii) Titration & Calculation

Digestion

This is the most time consuming step in the analysis & includes the conversion of nitrogen in the sample to the ammonium ion

- This step break down the bonds that hold the polypeptide together & convert them to a simpler chemicals such as water, CO_2 and ammonia.

-Presence of catalyst (selenium,mercury,Cu) and by neutral substance such as K sulphate which raises the boiling point of the digesting acid & thus the temperature of reaction also raises.

- In this method sample is taken in the digestion flask along with concentrated H_2SO_4 acid & K_2SO_4.

- Little amount of Cu is also added & the flask is boiled ar about 370-400^{0}C until white fumes can be seen.

- The heating is continued for about 60-90 minutes.

Sample + H_2SO_4------- (NH_4) SO_4+ CO_2+SO_2+ H_2O

Distillation

It includes the conversion of ammonium ion into ammonia.

- In this method 45% NaOH solution is added to reaction mixture to increase its pH which converts ammonium into free ammonia gas.

- Nitrogen in the form of ammonia is distilled by raising the temperature to boiling point and then trapping the vapour in HCL or H_2SO_4 in 70 ml of water.

$(NH_4)\ SO_4 + 2NaOH$----------$Na_2SO_4 + H_2o + 2\ NH_3$

3) Titration & calculation

As the ammonia dissolves in the acid trapping solution it neutralizes some of the HCl and the remaining acid is back titrated with a standard known solution of base (NaOH)

- In this way amt of ammonia distilled off from the digestive solution can be calculated and hence the amount of nitrogen in the protein is determined.

Calculation

1 mole of ammonia coming from the digestion mixture will neutralize exactly 1 Mole of the acid in the trapping flask

-The first calculation is therefore to find No of moles of ammonia that have been produced and then trapped.

- Moles of acid = Molarity of acid X volume used in the flask.

-Moles of base= molarity of base Xtitre value

- Subtracting the moles of base from moles of acid gives no of moles of ammonia.

Moles of ammonia =moles of acid – moles of base (moles of nitrogen)

Gram nitrogen – moles of nitrogen X atm. Mass

Hence gram nitrogen is equal to moles of nitrogen x 14.00067

$$\% \text{ Nitrogen} = \text{gram } \frac{N_2 \times 100}{\text{Wt of sample}}$$

Or

$$\% \text{ nitrogen} = \frac{14 \times \text{normality of acid} \times \text{vol of acid used} \times 1000}{1000 \times \text{wt of sample}}$$

Limitation of Kjedahl Method

Certain heterocyclic compound produce unexceptionally low result hence another method is used for N_2 detremination

Eg diazine and triazole **(Dumas method)** is used for titration of these drugs eg triazole – flucanazole.

Diazine immidazole

- If the N_2 in the sample is present in some higher oxidation state

  Eg Azo Azoxy or nitrogrp.

The success of this method depends upon their complete reduction to ammonia

- Pyrrole & its derivative indole is necessary to heat for at least 1 Hr and none of pyrazole derivative gives satisfactory result for Kjeldahl method

- Some analysis of compound such as piperdine,quonoline and pyridine derivative shows accurate result only after a very prolong heating.

Micro Kjeldhal Method

It is the modification of Kjeldhal method for the nitrogenous compound in relatively small quantities i.e sample in which the total content of nitrogen is in the range of one to few mg .

- This method involves the use of syringe pipette, distillation in partial vacuum condition and titration with0.2cc capacity micro burette.

- Micro Kjeldhal Method is more rapid than ordinary Kjeldahl procedure.

Application of Kjeldahl Method

i) Estimation of non- protein nitrogen in blood.(micro Kjeldhal Method)

ii) Detremination of protein nitrogen in food stuff, sugar, syrup & starch.

iii) Determination of total N_2 in soil sample and fertilizers

----- .