

Aim - Estimation of Trimethylamine (TMA) Nitrogen

Principle: Volatile bases are first separated by precipitating the proteins using trichloroacetic acid (TCA) solution. The filtered TCA extract, which contains the volatile substances, is treated with strong alkali (K_2CO_3). The liberated ammonia, primary and secondary amines are fixed by formaldehyde. Only the TMA is trapped by the standard H_2SO_4 solution and the quantity absorbed is determined by titration of the remaining acid with the standard NaOH.

Materials:

- 10% Trichloroacetic acid (TCA) solution
- Conway's micro-diffusion unit
- Mortar and pestle
- Paraffin wax and Vaseline in 1:2 ratio
- 0.02 (N) H_2SO_4
- Saturated Potassium carbonate solution: 50g K_2CO_3 in 100ml distilled water by heating.
- Toshiro's indicator
- NaOH: 0.02N
- Formaldehyde: 40%, neutralized.

Procedure:

- Take 10g fish muscle and macerate by adding 20 ml of 10% TCA using mortar and pestle.
- Filter the content through filter paper in 100 ml volumetric flask.
- Repeat the extraction process twice or thrice to make the volume to 100 ml (TCA extract).
- Apply a mixture of melted paraffin wax and Vaseline this on the rims of cups.
- Add 1 ml of 0.02(N) H_2SO_4 into the inner chamber of each cup of previously wash and dried Conway cups.
- Place lid over the Conway cup covering part of the outer chamber and complete inner chamber.
- Add 1 ml of TCA extract in to the outer chamber followed by 1 ml of saturated K_2CO_3 solution.
- Add 1 ml of formaldehyde in the outer chamber.
- Close the unit with the lid and mix the contents by gently rotating the unit.

- Leave the unit overnight for reaction (it can be kept inside an incubator at 37°C for 3 hrs).
- Then, titrate the excess acid, left in inner chamber against 0.02(N) NaOH and drop of Tashiro's indiactor.
- Run a blank simultaneously with 10% TCA solution instead of TCA extract. .
- Sample preparation should be done as fast as possible, since ammonia is highly volatile).

Calculation:

TMA (mg%) =

Where,

N = Normality of H₂SO₄

A = ml of standard NaOH required for titration of samples

B = ml of standard NaOH required for blank

W = Weight of the sample