

DETERMINATION OF VOLATILE BASIC NITROGEN (VB-N), TRIMETHYLAMINE OXIDE NITROGEN (TMAO-N) AND TRIMETHYLAMINE-NITROGEN (TMA-N) BY CONWAY'S MICRO-DIFFUSION METHOD (N/150 Hydrochloric acid and N/70 Barium hydroxide)

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INTRODUCTION

There are two types of titration in the total volatile basic nitrogen (VB-N) and trimethylamine-nitrogen (TMA-N) determinations by the Conway's microdiffusion method. The first involves an acid-alkali reaction using hydrochloric acid and barium hydroxide [HCl-Ba(OH)₂] and the second involves a buffering effect using hydrochloric acid and boric acid [HCl-H₃BO₃].

With the acid-alkali reaction method, more sensitive and accurate determinations of VB-N and TMA-N are possible. In addition the colour change at the end-point is very sharp and clear, reducing error caused by human visual judgement.

APPARATUS

1. Scalpels, knives, scissors and pincers
2. Mortars and pestles (ø: 50 - 120 mm)
3. Top loading balance (sensitivity : less than 0.1 g)
4. Filter papers (Whatman No. 1; ø : 55 - 60 mm)
5. Conway's unit
6. Bulb pipettes, 1 ml, 5 ml, 20 ml
7. Komagome pipettes, 1 ml, 2 ml
8. Micro-burette (Capacity 0.15 ml, minimum scale 0.001 ml)

REAGENTS

(Analytical grade)

1. 4% Trichloroacetic acid, TCA

Dissolve 40 g of TCA (CCl₃COOH, MW = 163.4 g) in distilled water and make up to 1000 ml.

2. N/150 Hydrochloric acid, HCl (containing Tashiro's indicator)

Pipette 10 ml of Tashiro's indicator solution into a 1000 ml measuring flask. Add 200 ml of ethyl alcohol, followed by about 200 - 300 ml of distilled water. Titrate against N/10 KOH (potassium hydroxide) till the reddish colour fades away. Then pipette 66.6 ml of N/10 HCl (it is possible to use commercial reagent) into the above measuring flask and make up to 1000 ml with distilled water.

Determine the factor of N/150 HCl and store in brown coloured reagent bottle. 1 ml of N/150 HCl has an equivalent of 0.353 mg Na₂CO₃, therefore 10 ml of N/150 HCl has an equivalent of 3.53 mg Na₂CO₃.

3. N/70 Barium hydroxide, Ba(OH)₂

Dissolve 15.8 g of Ba(OH)₂ (MW = 315.50 g) in distilled water^{*1} and make up to 1000 ml.

^{*1} Distilled water should be boiled and cooled overnight with the mouth of the flask attached to a soda lime trap to remove carbon dioxide (CO₂).

Pipette 142.9 ml of N/10 Ba(OH)₂ into 1000 ml measuring flask and make up to 1000 ml with CO₂-free distilled water. Store with paraffin seals.

4. Tashiro's indicator

Mix 200 ml of 0.1% methyl red alcohol with 50 ml of 0.1% methylene blue alcohol and store in brown coloured reagent bottle.

5. Saturated potassium carbonate solution, K₂CO₃

Dissolve 110 g of K₂CO₃ in 100 ml of distilled water and filter through Whatman No. 1 filter paper.

6. Reduced acid formaldehyde, HCHO

Mix 100 ml of 35 - 37 % formalin well with 10 g of magnesium carbonate (MgCO₃) and filter through Whatman No. 1 filter paper. The formaldehyde concentration should be more than 10%.

7. 1% Titanium (III) chloride, TiCl₃^{*2}

Pipette 6.7 ml of 15% TiCl₃ solution into 100 ml volumetric flask and make up to 100 ml with distilled water.

^{*2} If the stock TiCl₃ has been stored for sometime, a recovery test using various concentrations (%) of TiCl₃ should be carried out and the appropriate percentage yielding close to 100% recovery should be used.

8. Sealing agent

Mix white vaseline with liquid paraffin in a ratio of 2:1.

PROCEDURE

(Fig. 1)

Sample Preparation

1. Weigh 5 g of well mixed sample into the mortar.
2. Pipette in 20 ml of 4% TCA.
3. Grind well and stand for 30 minutes.
4. Filter through Whatman No. 1 filter paper. Use the filtrate as sample solution.

Analytical Procedure

1. Determination of VB-N

- 1.1 Apply sealing agent to the lid of Conway unit.
- 1.2 Pipette 1 ml of N/150 HCl containing Tashiro's indicator into the inner ring.
- 1.3 Pipette 1 ml of sample solution into the outer ring.
- 1.4 Pipette 1 ml of saturated K_2CO_3 into the outer ring, cover the unit immediately and tighten with a clip.
- 1.5 Gently mix the solutions in the outer ring well.
- 1.6 Stand for 90 mins at 37°C, or 100 mins at 30°C, or for 120 mins at 20°C.
- 1.7 Titrate the inner ring solution against N/70 $Ba(OH)_2$ with the aid of a thin glass rod. The end point of titration is reached when the inner ring solution changes from pinkish red to slightly green.

All titrations should be done in duplicate (i.e. 2 Conway's units per sample).

- 1.8 For blank, pipette 1 ml of 4% TCA instead of sample solution into outer ring.

2. Determination of TMA-N

- 2.1 Apply sealing agent to the lid of Conway unit.
- 2.2 Pipette 1 ml of N/150 HCl containing Tashiro's indicator into the inner ring.
- 2.3 Pipette 1 ml of sample solution into the outer ring.
- 2.4 Pipette 1 ml of reduced acid formaldehyde into the outer ring.
- 2.5 Gently mix the outer ring solutions well and stand for 3 - 4 mins.
- 2.6 Pipette 1 ml of saturated K_2CO_3 into the outer ring, cover the unit immediately and tighten with a clip.

- 2.7 Gently mix the solutions in the outer ring well.
- 2.8 Stand for 90 mins at 37°C, or 100 mins at 30°C, or for 120 mins at 20°C.
- 2.9 Titrate the inner ring solution against N/70 Ba(OH)₂ with the aid of a thin glass rod. The end point of titration is reached when the inner ring solution changes from pinkish red to slightly green.

All titrations should be done in duplicate (i.e. 2 Conway's units per sample).
- 2.10 For blank, pipette 1 ml of 4% TCA instead of sample solution into outer ring.

3. Determination of TMAO-N

- 3.1 Pipette 2 ml of the sample solution from Step 4 of Sample Preparation Part into a small test tube.
- 3.2 Pipette 1 ml of 1% TiCl₃ and mix well. The solution should turn violet.
- 3.3 Stand in an 80°C water bath for 90 sec. The violet colour should disappear.
- 3.4 If the violet colour persists, add saturated KNO₃ solution dropwise with shaking, until the violet colour disappears. Saturated KNO₃ is added dropwise only in cases where there is excess TiCl₃, if not it can be omitted.
- 3.5 Cool the tubes under running tap water.
- 3.6 Pipette 1 ml of the reduced sample solution into the outer ring.
- 3.7 Proceed as for TMA-N determination.

CALCULATION

$$\text{VB-N or TMA-N (mg/100 g)} = \frac{B - A}{B} \times f \times \left[\frac{14(4a + aw)}{150a} \right] \times 100$$

$$\text{TMAO-N (mg/100 g)} = \left\{ \frac{B - A}{B} \times f \times \left[\frac{14(4a + aw)}{150a} \right] \times 100 \times \frac{3}{2} \right\} - (\text{TMA-N, mg/100 g}) *$$

- where,
- B = titration value (ml) of blank test
 - A = titration value (ml) of the sample solution
 - f = factor of N/150 HCl
 - a = sample weight (g) = 5 g
 - w = moisture of sample expressed in decimal (e.g. for 80% moisture, use 0.8 for calculations)

* initial content of TMA-N (mg/100g) before reduction of sample solution.

Detection limit : 0.2 mg/100g

REFERENCE

Bysted, J., L. Swenne and H.W. Aas. (1959). Determination of trimethylamine oxide in fish muscle. *J. Sci. Food Agric.* 10:301-304.

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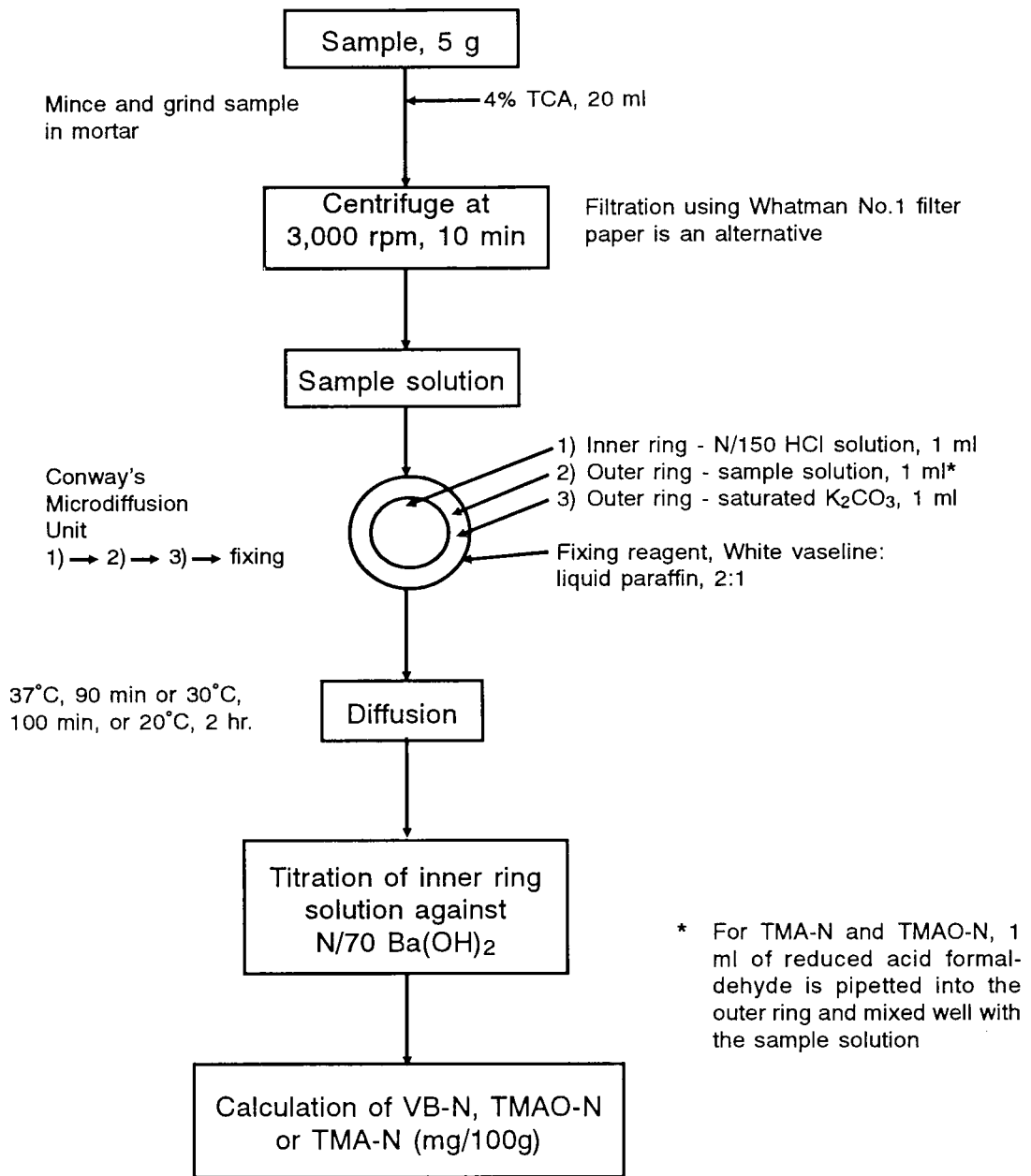


Fig. 1 Analytical procedure for VB-N, TMAO-N, TMA-N analysis