Determination of crude protein – Automated Kjeldahl method

Digestion

Operation

- Weigh 0.5 g of sample (or 5 ml of biological samples, e.g. milk, urine) into the digestion tubes. Note that if there are fewer than 20 samples per rack, then empty tubes must be placed in unused positions in the rack of digestion unit (Fig. 7.5).
- Add reagents to the tubes containing samples; for most application, it is 2 catalyst tablets and 12 ml H_2SO_4.
- The digestion block should be pre-heated to 420ºC. Check by inserting thermometer in the well in the block.
- Turn on the scrubber unit and fully open the air-flow-regulating valve.

Fig. 7.5 Digestion unit

- Remove thermometer, and fit the exhaust manifold on the tubes in the rack and place the rack in the digestion unit.
- Fit the heat shields to the rack and set the timer for 1 hour.
- After 5-7 minutes turn the valve to 1-2 stops before close-off, so as to prevent acid escaping between the tubes and the manifold. During digestion, check the temperature of the scrubber unit – it should be < 60ºC. A high temperature or excessive build-up of liquid in the acid trap probably indicates too high an airflow.
- When digestion is complete, increase the scrubber airflow and carefully remove the hot rack and manifold from the digestion unit and place on a heat resistant surface.
- Remove the heat shields and allow the tubes to cool.
- When no further fumes are evident at the top of the tubes, remove the manifold and replace on its stand.
- When the tubes are partly cooled, add 70 ml distilled water to each tube and mix. This step should be carried out while the tubes are still warm, so as to prevent the precipitation of salts.
- The tubes are now ready for distillation.

Distillation

Start-up procedure

- Check that the water drain valve on the back of Tecator is closed.
- Switch on the power to the 1030, computer, printer and interface.
- Check burette for bubbles and clear by operating TITRANT SWITCH. Note that when safety door is open, acid will go to and from the reservoir, and when closed, to and from the titration cell.
- Press REC-SOL 5-6 times to flush the line.

Fig. 7.6 Automatic distillation unit

- Turn on the cooling water.
- Connect test tube and shut door.
- Turn the STEAM switch ON and wait until steam is generated for 1-2 minutes.
- Turn the steam switch OFF.

Operation

- Select KJELDAHL program (should be set already).
- Set constants A to 00.00, B to 1.000 and blank to 0.00.
- Open safety door and press AUTO/RESET TO AUTO.
- Place the 1st test tube (usually containing = 70 ml D.I. water) to be distilled in position.
- Close door when the "CYCLE OVER" L.E.D. is ready. Distillation should begin (Fig. 7.6).
- Note the displayed value when "CYCLE OVER" lights up. Open door and remove tube with *hot hand* and repeat from step 4 (usually 2-3 times), until the blank value is satisfactory.
- Set the blank value which will be subtracted from the distillations and select the B value to be used for the display output from the Table 1.

Table 1

HCl concentration	0.05 M	0.1 M	0.2 M	0.5 M
RESULT	B=	B=	B=	B=
% P, f=5.7	0.399	0.799	1.597	3.993
% P, f=6.25	0.438	0.876	1.751	4.378
% P, f=6.38	0.447	0.894	1.788	44.69
% N, f=1.00	0.070	0.140	0.280	0.701
Ml titrant*	1.000	1.000*	1.000	1.000

B=1.000 is the preferred setting for all analysis, and blank = .06

NOTE: If the door is closed before the "CYCLE OVER" light comes on, open the door and wait until the light is steady, then close the door and start the distillation.

Shut down procedure

- Remove tube and clean plastic cover behind safety door and tube holder foot.
- Turn off power to the 1030, computer, the interface and printer.
- Fill the titration cell with D.I. water.
- Remove the drip tray from under test tube and clean.
- Wipe any spills from the chassis of the TECATOR with a solution of 3 per cent acetic acid.
- If the TECATOR is not to be used for 2-3 days, open the drain valve on the back of the unit.
- Turn off cooling water at tap.

Calculations

$$\% \text{ Nitrogen} = \frac{\text{Titrant (ml)} - \text{Blank (ml)} * 0.1\ (N) * 14.01}{\text{Sample Wt (mg)} * DM/100} \times 100$$

$$\% \text{ Protein} = \% \text{ Nitrogen} * 6.25$$

N = Normality of acid (mole/l); 14.01 = Atomic weight of nitrogen; 6.25 standard Kjeldahl factor

Testing recovery

1. Weigh 0.500 g ammonium iron (II) sulphate [$(NH_4)_2Fe(SO_4)_2.6H_2O$] into a tube.
2. Add about 75 ml distilled water.

3. Using 0.1N HCl as titrant, connect to the distilling unit and distil.
4. Calculation is as follows:

$$\% \text{ Nitrogen} = \frac{(\text{Titrant} - \text{Blank}) * N * 1.401}{\text{Sample (g)}}$$

$$\% \text{ Nitrogen} = 7.145$$

The results after the calculations are done should be = 7.145 or close to it. The closer the value, higher the accuracy of nitrogen recovery.

Reference: AOAC (1997). 976.05, 16th edition.