**Lab 5 Determination of Protein**

**Kjeldahl nitrogen determination (Kjeldahl method )**

This method also requires specialized equipment and large amounts of ammonium sulfate. The ammonium solution is acidified and the amount of ammonia released is determined by a titration. This is not used routinely in biochemistry.

**Principle of Method**

The Kjeldahl procedure measures the nitrogen content of a sample. The protein content then, can be calculated assuming a ratio of protein to nitrogen for the specific food being analyzed. The Kjeldahl procedure can be basically divided into three parts:

**(1) digestion,**

**(2) distillation,**

**(3) titration**

In the digestion step, organic nitrogen is converted to an ammonium in the presence of a catalyst at approximately 370°C. In the distillation step, the digested sample is made alkaline with NaOH and the nitrogen is distilled off as NH3. This NH3 is “trapped” in a boric acid solution. The amount of ammonia nitrogen in this solution is quantified by titration with a standard HCl solution. A reagent blank is carried through the analysis and the volume of HCl titrant required for this blank is subtracted from each determination.

**Chemicals**

**Boric acid Solution (H3BO3) .**

**Mixed indicator (Methyl red +Bromocresol green) .**

**Ethanol, 95% Highly flammable.**

**Hydrochloric acid,conc. (HCl) .**

**Sodium hydroxide Solution (NaOH) 50% W/V.**

**Sulfuric acid, conc. (H2SO4) .**

**Catalyst /Salt Mixture (Kjeldahl digestion tablets )contain Potassium sulfate (K2SO4) ,Cupric sulfate and Titanium dioxide (TiO2) .**

*Note*: There are several types of Kjeldahl digestion tablets that contain somewhat different chemicals.

**Tris (hydroxymethyl) aminomethane (THAM) Solution (0.01N) Irritant .**

**Supplies**

(Used by students ●● Corn flour (not dried) ●● 5 Digestion tubes

●● 5 Erlenmeyer flasks, 250 ml ●● Spatula ●● Weighing paper

**Equipment**

1● Analytical balance 2● Automatic titrator 3● Kjeldahl digestion and distillation system

**Procedure**

(Instructions are given for analysis in triplicate. Follow manufacturer’s instructions for specific Kjeldahl digestion and distillation system used. Some instructions given here may be specific for one type of Kjeldahl system.)

**I. Digestion**

1. Turn on digestion block and heat to appropriate temperature.

2. Accurately weigh approximately 0.1 g corn flour. Record the weight. Place corn flour in digestion tube. Repeat for two more samples.

3. Add one catalyst tablet and appropriate volume (e.g., 7 ml) of concentrated

sulfuric acid to each tube with corn flour. Prepare duplicate blanks: one catalyst tablet + volume of sulfuric acid used in the sample + weigh paper (if weigh paper was added with the corn flour samples).

4. Place rack of digestion tubes on digestion block. Cover digestion block with exhaust system turned on.

5. Let samples digest until digestion is complete. The samples should be clear (but neon green), with no charred material remaining.

6. Take samples off the digestion block and allow to cool with the exhaust system still turned on.

#### 7. Carefully dilute digest with an appropriate volume of add water. Swirl each tube.

#### DIGESTION

**catalysts→  
(1)** **n - C -NH2** **+mH2SO4****CO2**  **(NH4)2** **SO4** **+ SO2  
protein                                 heat→**

**II. Distillation**

1. Follow appropriate procedure to start up distillation system.

2. Dispense appropriate volume of boric acid solution into the receiving flask. Place receiving flask on distillation system. Make sure that the tube coming from the distillation of the sample is submerged in the boric acid solution.

3. Put sample tube in place, making sure it is seated securely, and proceed with the distillation until completed. In this distillation process, a set volume of NaOH solution will be delivered to the tube and a steam generator will distill the sample for a set period of time.

4. Upon completing distillation of one sample, proceed with a new sample tube and receiving flask.

5. After completing distillation of all samples, follow manufacturer’s instructions to shut down the distillation unit.

**NEUTRALIZATION AND DISTILLATION**

**(2)** (NH2)SO4 + 2 NaOH          →          2NH3 + Na2SO4+ 2H2O  
**(3)** NH3 + H3BO3 (boric acid)         →          NH4 + H2BO3- (borate ion)

**III. Titration**

1. Record the normality of the standardized HCl solution as determined by the teaching assistant.

2. If using an automated pH meter titration system, follow manufacturer’s instructions to calibrate the instrument. Put a magnetic stir bar in the receiver flask and place it on a stir plate. Keep the solution stirring briskly while titrating, but do not let the stir bar hit the electrode. Titrate each sample and blank to an end point pH of 4.2. Record volume of HCl titrant used.

3. If using a colorimetric end point, put a magnetic stir bar in the receiver flask, place it on a stir plate, and keep the solution stirring briskly while titrating. Titrate each sample and blank with the standardized HCl solution to the first faint gray color. Record volume of HCl titrant used .

#### TITRATION

**Borate anion (proportional to amount of nitrogen) is titrated with standarized** **HCl (or H2SO4)** **:**

**(4)** **H2BO3-****+ H+             →****H3BO3**

**Data and Calculations**

Calculate the percent nitrogen and the percent protein for each of your duplicate or triplicate corn flour samples, then determine average values.

**(A-B)×N×1.4007×6.25**

**Protein(%) =**

**W**

**Where :**

**A = Volume (ml) of 0.2N HCl used sample titration .**

**B = Volume (ml) of 0.2N HCl used in blank titration .**

**N = Normality of HCl .**

**W = Weight (g) of sample .**

**14.007 = atomic weight of nitrogen .**

**6.25 = the protein nitrogen conversation factor**

**Flow Chart for Total Kieldahl Nitrogen**

**Digestion**

**Homogenous sample**

**Titration**

**Distillation**