LABORATORY EXPERIMENT 6

The Determination of Amine Nitrogen by the Kjeldahl Method Using NH_4^+-selective Electrode

Discussion

These directions are suitable for the Kjeldahl determination of protein in materials such as blood meal, wheat flour, pasta products, dry cereals, and pet foods. A simple modification permits the analysis of unknowns that contain more highly oxidized forms of nitrogen. In the Kjeldahl method, the organic sample is digested in hot concentrated sulfuric acid, which converts amine nitrogen in the sample to ammonium sulfate. After cooling, the sulfuric acid is neutralized by the addition of an excess of concentrated sodium hydroxide. The ammonia liberated by this treatment is then distilled into a measured excess of a standard solution of acid; the excess is determined by back-titration with standard base.

Quantitative collection of ammonia requires the tip of the condenser to extend into the liquid in the receiving flask throughout the distillation step. The tip must be removed before heating is discontinued, however. Otherwise, the liquid will be drawn back into the apparatus.

Two methods are commonly used to collect and determine the ammonia liberated from the sample. In one, the ammonia is distilled into a measured volume of standard acid. After the distillation is complete, the excess acid is back-titrated with standard base. An indicator with an acidic transition range is required because of the acidity of the ammonium ions present at equivalence. A convenient alternative, which requires only one standard solution, involves the collection of the ammonia in an unmeasured excess of boric acid, which retains the ammonia by the reaction

$$H_3BO_3 + NH_3 + H_2O \rightarrow NH_4^+ + B(OH)_4^-$$

The dihydrogen borate ion produced is a reasonably strong base that can be titrated with a standard solution of hydrochloric acid.

Consult with the instructor on sample size. *If the unknown is powdered* (such as blood meal), weigh samples onto individual 9-cm filter papers. Fold the paper around the sample and drop each into a Kjeldahl flask. (The paper keeps the samples from clinging

to the neck of the flask.) *If the unknown is not powdered* (such as breakfast cereals or pasta), the samples can be weighed by difference directly into the Kjeldahl flasks. Add 12 mL of concentrated H_2SO_4, 5 g of powdered Na_2SO_4, and the catalyst (around 0.1 g $CuSO_4$) to each flask.

Digestion

Clamp the flasks in a slanted position in a hood or vented digestion rack. Heat carefully to boiling. Discontinue heating briefly if foaming becomes excessive; never allow the foam to reach the neck of the flask. Once foaming ceases and the acid is boiling vigorously, the samples can be left unattended; prepare the distillation apparatus during this time. Continue digestion until the solution becomes colorless or faint yellow; 2 to 3 hr may be needed for some materials. If necessary, cautiously replace the acid lost by evaporation. When digestion is complete, discontinue heating, and allow the flask to cool to room temperature; swirl the flask if the contents show signs of solidifying. Cautiously add 50 mL of water to each flask and again allow the solution to cool to room temperature.

Distillation of Ammonia

Arrange a distillation apparatus similar. Pipet 50 mL of 4% boric acid into the receiver flask. Clamp the flask so that the tip of the adapter extends below the surface of the standard acid. Circulate water through the condenser jacket. Transfer the liquid to the 3-neck distillation flask. Wash the Kjeldahl flask five times with 5-mL portions of distilled water, and pour the washings into the distillation flask. Gently introduce about 30 mL of 50% (w/v) NaOH solution, taking care to minimize mixing with the solution in the flask. *The concentrated caustic solution is highly corrosive and should be handled with great care* (If any sodium hydroxide solution comes into contact with your skin, wash the affected area *immediately* with copious amounts of water). Add several pieces of granulated zinc (Granulated zinc is added to minimize bumping during the distillation; it reacts slowly with the base to produce small bubbles of hydrogen that prevent superheating of the liquid) and several drops of phenolphthalein. Cautiously mix the contents by gentle swirling. The color should be red after mixing is complete, indicating that the solution is basic.

Bring the solution to a boil, and distill at a steady rate until one half to one third of the original volume remains. Control the rate of heating to prevent the liquid in the receiver flask from being drawn back into the distillation flask. After distillation is judged complete, lower the receiver flask to bring the adapter well clear of the liquid.

Determination of Ammonia.

Transfer all the liquid from the receiving flask (should be slightly less than 100 mL) and make it to mark in a 100-mL volumetric flask. Take a 25 mL aliquote of the resulting solution into a 50 mL volumetric flask, add 10 mL of 0.5 M NaOH, and make it to the mark. Measure the concentration of ammonia in the resulting solution using a pre-calibrated NH_4^+-sensitive electrode.

Calculate N% in a sample.