



Designation: E 258 – 67 (Reapproved 1996)<sup>ε1</sup>

## Standard Test Method for Total Nitrogen in Organic Materials by Modified Kjeldahl Method<sup>1</sup>

This standard is issued under the fixed designation E 258; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

*This standard has been approved for use by agencies of the Department of Defense.*

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<sup>ε1</sup> NOTE—Section 11, Keywords, was added editorially in March 1996.

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### 1. Scope

1.1 This test method covers the determination of total nitrogen in nitrogen-containing organic compounds. This test method is not applicable for use on materials containing N-O, N-N linkages.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

NOTE 1—Another method of restricted application is given in Test Method D 1013.

### 2. Referenced Documents

#### 2.1 ASTM Standards:

D 1013 Test Method for Total Nitrogen in Resins and Plastics<sup>2</sup>

D 1193 Specification for Reagent Water<sup>3</sup>

E 200 Practice for Preparation, Standardization, and Storage of Standard and Reagent Solutions for Chemical Analysis<sup>4</sup>

### 3. Summary of Test Method

3.1 The sample is digested in a mixture of concentrated sulfuric acid, potassium sulfate, and mercuric oxide. The amounts of organic matter, potassium sulfate, and sulfuric acid present during the digestion step are critical. The organic material is oxidized and the nitrogen converted to ammonium sulfate. Sodium sulfide is added to the digested mixture to precipitate the mercury after which the solution is made

strongly alkaline with sodium hydroxide solution and the ammonia which is liberated is distilled into a measured volume of standard acid. The amount of acid neutralized by the ammonia is determined by titrating the excess acid with standard sodium hydroxide solution.

### 4. Significance and Use

4.1 This test method may be used to determine the total nitrogen content of certain unknown organic samples, or to assay known nitrogen containing organic compounds.

4.2 This test method may be used on organic materials in which the complete conversion of nitrogen to ammonium sulfate can be accomplished by digestion in a mixture of sulfuric acid, potassium sulfate, and mercuric oxide. It cannot be used on materials containing N-O, N-N linkages.

4.3 This test method assumes that ammonia can be quantitatively measured by distillation from an alkaline solution into a measured volume of standard acid.

### 5. Apparatus

5.1 *Kjeldahl Flasks* of moderately thick, well-annealed borosilicate glass for digestion and distillation, 500 or 800-mL capacity.

NOTE 2—The usual flask and connecting bulb commercially available are made to be assembled by means of a rubber stopper. To minimize lacerations from broken flasks while assembling the apparatus, it is recommended that the flask have an outer spherical 35/25 joint and the bulb have an inner spherical 35/25 joint. This type apparatus is held together by a spring clamp.

5.2 *Connecting Bulbs* of the Willits-John type, or a scrubber bulb equally effective in preventing mechanical carry-over of the contents of the distillation flask to the condenser (Note 2).

5.3 *Digestion and Distillation Equipment* consists of an adjustable heater, water-cooled condenser, receiver support, and fume disposal unit. Any of the well-known commercial Kjeldahl digestion and distillation units are suitable. The units may be heated either electrically or by gas burner but must be adjusted after an initial warm-up period to bring 250 mL of water to a rolling boil in 4 to 6 min. Before testing the unit,

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<sup>1</sup> This test method is under the jurisdiction of ASTM Committee E15 on Industrial and Specialty Chemicals and is the direct responsibility of Subcommittee E15.01 on General Standards.

This test method has been adopted from Method 2.054 of the Official Methods of Analysis of the Association of Official Agricultural Chemists, 13th Edition, 1980.

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 06.03.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 11.01.

<sup>4</sup> *Annual Book of ASTM Standards*, Vol 15.05.

preheat for 10 min if a gas heater or 30 min if an electric heater. For the test add three to four boiling chips to prevent super heating.

5.4 *Delivery Tubes* made of moderately heavy-wall glass tubing, 6 to 8 in. (150 to 200 mm) in length, for conducting the distillate from the condenser to the receiver.

5.5 *Receiver Flask*, 500-mL capacity, wide-mouth Erlenmeyer flask.

## 6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.<sup>5</sup> Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Type II or III reagent water conforming to Specification D 1193.

6.3 *Indicator Solution* (either may be used):

6.3.1 *Methyl Purple Indicator Solution*—Commercially available.

6.3.2 *Methyl Red Indicator Solution*—Dissolve 1 g of methyl red in 200 mL of ethyl alcohol (denatured alcohol, formula 2B, 3A, or 30A of the U.S. Bureau of Internal Revenue, may be used).

6.4 *Mercuric Oxide* (HgO).

NOTE 3—Packaged units available commercially, containing the required weight of mercuric oxide and potassium sulfate are satisfactory.

6.5 *Potassium Sulfate* (K<sub>2</sub>SO<sub>4</sub>), anhydrous (Note 3).

6.6 *Sodium Hydroxide Solution* (450 g/L)—Dissolve 450 g of sodium hydroxide (NaOH) in water and dilute to 1 L. Alternatively a 50 % sodium hydroxide solution can be diluted with water to give a solution having a specific gravity of 1.36 or higher.

6.7 *Sodium Hydroxide, Standard Solution* (0.1 N or 0.5 N)—Prepare and standardize in accordance with Practice E 200.

6.8 *Sulfide Solution*—Dissolve 40 g of potassium sulfide or sodium sulfide in water and dilute to 1 L.

6.9 *Sulfuric Acid* (sp gr 1.84)—Concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>).

NOTE 4—In the analysis of samples containing nitrile or cyanate groups, which are easily hydrolyzed, the concentrated sulfuric acid must contain essentially no water. The acid should be taken from a freshly opened bottle, or boiled just prior to use, or preferably, a small amount of phosphorus pentoxide should be added to the acid.

6.10 *Sulfuric Acid, Standard* (0.1 N or 0.5 N)—Prepare in accordance with the Precision and Bias Section in the portion

of Practice E 200 pertaining to hydrochloric acid, 0.02 to 1.0 N. An exact standardization of this reagent is not necessary.

6.11 *Zinc*, granular.

## 7. Procedure

7.1 Place the sample, weighed to 0.0001 g, in a Kjeldahl digestion flask. The weight of sample will depend upon whether 0.1 N or 0.5 N H<sub>2</sub>SO<sub>4</sub> is used as the standard acid; if 0.1 N acid is used, the sample should contain between 14 and 56 mg of nitrogen and if 0.5 N acid is used the sample should contain between 70 and 280 mg of nitrogen, but the sample weight taken should normally be less than 2.2 g.

NOTE 5—The optimum weight of sample to be digested, if of unknown composition, must be determined by a trial determination. Since the nature and amount of the sample will affect the amount of acid consumed in the digestion, the preferred weight of sample is one that will give the largest amount of nitrogen within the prescribed range while using the smallest total weight of sample and adhering to the prescribed maximum.

7.2 To the flask add 0.7 g of HgO, 15 g of K<sub>2</sub>SO<sub>4</sub>, and 25 mL of H<sub>2</sub>SO<sub>4</sub> (sp gr 1.84) (Note 3, Note 6). Place the flask in an inclined position and heat gently until frothing ceases, then boil briskly causing the condensate to be formed approximately half way up the neck of the flask. Continue boiling until the solution clears and then for 2 h longer (Note 7).

NOTE 6—The temperature of the digestion mixture is critical and is regulated by the ratio of salt to acid (free acid at the completion of the digestion period). The ratio must be kept close to 1:1 (weight:volume) K<sub>2</sub>SO<sub>4</sub>-to-free acid in weight from the weight of acid initially added, and converting weight of acid to a volume basis. If a sample weight in excess of 2.2 g is used, increase the volume of acid by 10 mL for each 1 g in excess.

NOTE 7—For easily digested materials a digestion period of 30 min after clearing may be adequate.

7.3 Add exactly 50 mL of the standard acid (see 7.1 for concentration to be used) and 5 to 7 drops of indicator solution to the receiving flask. Connect the delivery tube to the condenser and place the receiver under the condenser with the delivery tube extending to the bottom of the flask.

7.4 Cool the digestion mixture in the Kjeldahl flask to approximately room temperature. Add approximately 200 mL of water, and swirl (Note 8). Cool the contents of the flask to below 25°C, add 25 mL of the sulfide solution to precipitate the mercury, and swirl. Add a few zinc granules to prevent bumping, tilt the flask, and add an excess (Note 9) of the NaOH solution (450 g/L) down the inclined neck of the flask without agitation to form two layers. Immediately connect the flask to the connecting bulb attached to the condenser, and mix the contents of the flask thoroughly by swirling (Note 10).

NOTE 8—Before addition of the water, the contents of the flask should have a syrup-like consistency. If the contents of the flask do not dissolve completely in the water, discard the determination (evidence of improper salt-acid ratio).

NOTE 9—To determine the required volume of alkali, dilute the same volume of acid used for the determination with water and note the volume of NaOH solution (450 g/L) required to neutralize the solution to the phenolphthalein end point. This will give an excess since acid is consumed or volatilized during digestion of the sample.

NOTE 10—The digestion flask must be connected to the connecting bulb and the rest of the apparatus immediately after the alkali has been added and layered, but before swirling to mix the concentrated acid and

<sup>5</sup> *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

alkali. Should any mixing occur before the digestion flask is connected, the heat of neutralization may be sufficient to volatilize some of the ammonia which would be lost, yielding low recoveries.

7.5 Promptly heat the flask and collect the distillate in the receiver containing the standard acid solution (Note 11). Continue the distillation until all of the ammonia has been collected (a minimum of 150 mL of distillate).

NOTE 11—Heat must be applied promptly to prevent sucking the standard acid into the condenser if the digestion solution cools. The initial distillation rate must not be too rapid since most of the ammonia is distilled during the first few minutes and if too large an amount is present it may not be completely trapped in the standard acid.

7.6 Remove the receiver and delivery tube from the apparatus (preferably just before stopping the distillation). Rinse the delivery tube with water, collecting the rinsings in the receiver.

7.7 Titrate the excess acid in the receiver with the standard NaOH solution, using the same (approximate) concentration as the acid used in the receiver. Record the volume to the nearest 0.02 mL.

7.8 Conduct a blank determination in parallel, using the same volume of reagents as used in the determination.

NOTE 12—Water from the same lot should be used in the blank as in the determination since reagent water tends to contain free ammonia which would affect the results of samples having a low nitrogen content.

## 8. Calculation

8.1 Calculate the nitrogen content as follows:

$$\text{Nitrogen, \%} = \frac{(A - B)N \times 0.01401}{C} \times 100 \quad (1)$$

where:

- A* = standard NaOH solution required for titration of the blank, mL,
- B* = standard NaOH solution required for titration of the sample, mL,
- N* = normality of the NaOH solution,
- C* = sample used, g, and
- 0.01401 = meq weight of nitrogen.

## 9. Report

9.1 Report the nitrogen content to the nearest 0.01 % if the content is below 1.0 % and to the nearest 0.1 % if the content is greater than 1.0 %.

9.2 Results from duplicate determinations that agree within 0.2 % *absolute* are acceptable for averaging.

## 10. Precision and Bias

10.1 The following criteria should be used for judging the acceptability of the results (Note 13):

10.1.1 *Repeatability*—The average difference between two results (each the average of duplicate determinations) obtained by the same analyst on different days will approximate 0.1 % *absolute*. Two such values should be considered suspect (95 % confidence level) if they differ by more than 0.2 % *absolute*.

10.1.2 *Reproducibility*—The average difference between two results (each the average of duplicate determinations) obtained by analysts in different laboratories will approximate 0.2 % *absolute*. Two such values will be considered suspect (95 % confidence level) if they differ by more than 0.5 % *absolute*.

10.2 The bias of this test method has not been determined.

NOTE 13—The precision of various Kjeldahl procedures for determination of nitrogen content has been evaluated in several independent interlaboratory studies. The precision estimates are based on studies: (a) conducted by the Association of Official Agricultural Chemists in 1955 and 1956 in which ten or eleven laboratories analyzed one sample each of nicotinic acid, a urea-formaldehyde resin, a mixed fertilizer of 8-6-4 composition, and a mixed fertilizer of 14-14-14 composition,<sup>6</sup> and (b) conducted by ASTM Committee D-1, Subcommittee XI, in 1958, in which five laboratories analyzed two samples of a urea-formaldehyde resin, two samples of a melamine-formaldehyde resin, one sample each of melamine and of acetanilide (Method D 1013). The precision statements given above should be interpreted as guides rather than as specific values which will apply in all cases. They should be approximately applicable to materials containing 10 to 40 % nitrogen, where sample size is controlled to contain an amount of nitrogen in the upper portion of the ranges specified in 7.1. ASTM committees planning to use this method for specific classes of materials are urged to develop their own precision estimates.

## 11. Keywords

11.1 modified Kjeldahl method; nitrogen; organic materials

<sup>6</sup> Miles, S. R., and Quackenbush, F. W., "Reliability of Chemical Analyses for Fertilizers and Feeds," *Journal of the Association of Official Agricultural Chemists*, JOACA Vol 38, 1955, p. 108.

Davis, H. A., and Miles, S. R., "Report on Nitrogen in Fertilizers," *ibid*, Vol 39, 1956, p. 550.

Davis, H. A., and Miles, S. R., "Report on Nitrogen in Fertilizers," *ibid*, Vol 40, 1957, p. 675.

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