Designation: D5176 – 20

Standard Test Method for
Total Chemically Bound Nitrogen in Water by Pyrolysis and Chemiluminescence Detection[1]

1. Scope

1.1 This test method covers the determination of the total nitrogen content of water in concentrations from 0.5 to 1000 mg/L. Higher nitrogen concentrations may be determined by making the proper dilutions.

1.2 This test method does not determine molecular nitrogen (N_2).

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]
D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *total chemically bound nitrogen, n*—all inorganic and organic nitrogen in the sample, except molecular nitrogen (N_2).

4. Summary of Test Method

4.1 The sample of water is introduced into a stream of oxygen or inert/oxygen mix flowing through a quartz pyrolysis tube. Oxidative pyrolysis converts chemically bound nitrogen to nitric oxide (NO). The gas stream is dried and the NO is contacted with ozone (O_3) producing metastable nitrogen dioxide (NO_2^*). As the NO_2^* decays, light is emitted and detected by a photomultiplier tube. The resulting signal is a measure of the total chemically bound nitrogen in the sample.

5. Significance and Use

5.1 This test method is useful for the determination of total chemically bound nitrogen in wastewaters and other waters.

6. Apparatus[3]

6.1 *Pyrolysis Furnace*—An electric tube furnace capable of achieving a temperature of 1100°C. The furnace may be single or multizoned and may have common or separate and independent temperature controls.

6.2 *Pyrolysis Tube*—The pyrolysis tube must be fabricated from quartz and should be designed to ensure complete pyrolysis of a wide variety of samples.

6.3 *Chemiluminescence Detector*—The detector shall have a photomultiplier tube capable of sensing the light emission of the decaying NO_2^*. The detector shall have digital display, onboard ozone generator and analog output for data system or strip chart recorder.

6.4 *Recorder* (optional)—The recorder shall be able to accept a 1 V full scale signal and to provide a chart speed of 1 cm/min.

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[3] The apparatus described in 6.1 – 6.7 is manufactured by Antek Instruments, Inc., Houston, TX, and Dohrmann Division of Rosemount Analytical Inc., Santa Clara, CA, and was used in the validation study of this test method.

6.5 *Microlitre Syringe*—Any standard series of microlitre syringes with stainless steel needles is acceptable. See manufacturer's instructions for appropriate syringe sizes.

6.6 *Syringe Drive Mechanism*—The syringe drive shall be capable of driving the sample from a microlitre syringe at a controlled, reproducible rate.

6.7 *Sample Boat*—Samples with high concentrations of suspended matter or dissolved nonvolatile compounds may tend to plug the syringe needle upon injection into the pyrolysis tube. In this case a sample boat of quartz or platinum, with or without quartz wool, should be used, in conjunction with the appropriate pyrolysis tube. The pyrolysis tube shall allow the introduction of the sample into the boat by microlitre syringe without interrupting the gas flow system.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used. 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.[4] Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water that meets the purity specifications of Type I or Type II water, presented in Specification D1193.

7.3 *Inert Gas,* Argon (minimum purity 99.99 %).

7.4 *Oxygen* (minimum purity 99.6 %).

7.5 *Stock Solution, Pyridine* (10 000 mg N/L)—Prepare by accurately weighing 5.647 g of pyridine into a 100 mL volumetric flask and dilute to 100 mL with water.

7.6 *Pyridine Solutions, Standard* (1000, 500, 100, 50, 10, 5, 1, and 0.5 mg N/L)—Dilute ten volumes of the stock solution (see 6.5) with 90 volumes of water to prepare a 1000 mg N/L standard. Similarly, by serial dilution with water, prepare 500, 100, 50, 10, 5, 1, and 0.5 mg N/L standards.

8. Preparation of Apparatus

8.1 Assemble apparatus according to manufacturer's instructions.

9. Calibration and Standardization

9.1 Use the water that was used to prepare the standards as a zero blank standard.

9.2 A sample size of 5 to 10 µL is sufficient to cover the concentration range of this test method. The volume of the sample shall be accurately determined.

9.3 *Syringe Injection*—Fill the syringe to the 5 µL mark and retract the plunger so that the liquid meniscus is at the 1 µL mark. Note the position of the plunger. Insert the syringe needle through the inlet septum up to the syringe barrel and allow the furnace to burn all nitrogen bearing residue off the syringe needle. Reset the detector and inject the sample at a controlled rate of 1 to 2 µL/s. A syringe drive mechanism (see 6.6) is strongly recommended. When all sample has been injected, withdraw the syringe needle. Retract the plunger so that the sample meniscus is again at the 1 µL point and note the plunger position. The true amount injected is the difference between the two plunger positions.

NOTE 1—If water samples contain high concentrations of suspended matter or dissolved nonvolatile compounds, the syringe needle may tend to plug or the precision and bias of the test method may be degraded. In such a case, the sample boat system should be used (see 6.7).

9.4 *Boat Injection*—Fill the microlitre syringe to the mark and inject the sample directly into the boat while holding the needle in contact with the side of the boat or with the quartz wool.

9.5 Determine each calibration standard and the zero blank three times and record the net response from the average of each set of standard responses.

9.6 By injecting the same volumetric amount of sample for each determination, the only variables will be total nitrogen concentration and detector response (digital display). Construct a curve plotting milligrams of N per litre versus detector response. Check the complete calibration curve at least once per week; check one or two standards daily.

10. Procedure

10.1 Flush the microlitre syringe several times with the unknown sample. Inject the sample at a controlled rate of 1 to 2 µg/s as described in 9.3 or inject the sample into the sample boat (see 6.7) as described in 9.4.

10.2 Set instrument parameters as recommended by manufacturers. Some changes may be needed to accommodate specific kinds of samples.

11. Calculation

11.1 Determine the total chemically bound nitrogen content of the water sample in milligrams N per litre by reading off the calibration curve (see 9.6).

12. Quality Control (QC)

12.1 In order to be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when running the test:

12.2 *Calibration and Calibration Verification:*

12.2.1 *Instrument:*

12.2.1.1 Analyze at least one calibration standard containing nitrogen representing the mid-range of the calibration prior to analysis of samples to calibrate the instrument.

12.2.1.2 Verify instrument calibration daily by analyzing a standard at the concentration of one of the calibration standards.

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[4] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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.

12.2.1.3 If calibration cannot be verified, recalibrate the instrument.

12.3 *Initial Demonstration of Laboratory Capability:*

12.3.1 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability.

12.3.2 Analyze seven replicates of a standard solution prepared from an independent reference material (IRM) containing ~100 mgN/L. The matrix and chemistry of the solution should be equivalent to the solution used in the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps. The replicates may be interspersed with samples.

12.3.3 Calculate the mean and standard deviation of the seven values and compare to the acceptable ranges of precision and bias Table 1. This study should be repeated until the single operator precision and the mean recovery are within the limits given in the table. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the *F* test and *t* test in evaluating the acceptability of the mean and standard deviation.

12.4 *Laboratory Control Sample (LCS):*

12.4.1 To ensure that the test method is in control, analyze a LCS containing a nitrogen concentration representing 25–50 % of the range with each batch samples. If large numbers of samples are analyzed in the batch, analyze the LCS after every twenty (20) samples. The LCS must be taken through all of the steps of the analytical method including sample preservation and pretreatment. The result obtained for the LCS shall fall within the limits of Table 1.

12.4.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be reanalyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Precision and Bias

13.1 *Collaborative Test*—Using deionized water as the matrix, a stock solution was prepared containing ammonium sulfate, potassium nitrate, and pyridine in such proportions that each compound contributed about one-third of the total nitrogen. Dilutions were made to provide samples of accurately-known concentrations of about 0.5, 1, 10, 100, and 1000 mg N/L. This procedure was repeated, using nitrogen-containing wastewater as the matrix (the wastewater was expected to have a nitrogen content of approximately 20 mg/L, but the actual value was found to be about 150 mg/L). The ten samples plus unspiked deionized (DI) water and unspiked wastewater (as trip blanks) were sent to 13 laboratories for analysis in triplicate according to this test method. The laboratories were required to make up their own standard solutions, and to use their own laboratory water to determine their instrument blank, that they subtracted from their raw results before reporting them.

13.2 *Analysis of Results*—Only nine of the 13 laboratories submitted results for this study. The data were processed as specified in the 1986 edition of Practice D2777. The whole of one laboratory's results for the DI water matrix samples failed the outlier test. A single result from another laboratory was also rejected as an outlier. One laboratory reported only the average of its three readings, without standard deviation, and another laboratory did only duplicate runs; the results from these two laboratories were used where possible. The overall average value for nitrogen in the blank wastewater was calculated by the collaborative test organizer, who then subtracted it from the reported values for the spiked wastewater samples.

13.3 *Precision*—See Table 1.

13.3.1 *DI Water Matrix*—Single-operator precision is poor at the low levels (1 mg N/L and lower) and adequate to good at other levels. Overall precision is poor for the central values and very poor for the extremes.

13.3.2 *Wastewater Matrix*—Both measures of precision were poor to very poor over the whole range tested. This may

TABLE 1 Precision and Bias

Matrix Water	Amount Added, mg N/L	Amount Found, mg N/L	Net Amount,[A] mg N/L	Precision		Bias, mg N/L
				S_t	S_o	
DI	0.40	0.3	...	0.2	0.1	−0.1[B]
	1.01	0.8	...	0.4	0.3	−0.2[B]
	11.1	10.1	...	1.5	0.4	−1.0[B]
	106	109	...	10	1.0	+3
	1105	1289	...	298	28	+184[B]
Waste	0.50	150	−2	5	4	−2
	0.91	151	−1	4	2	−2
	10.1	161	9	4	2	−1
	95.8	257	105	45	9	+9
	1008	1336	1184	330	43	+176[B]
	...	152	0	18	3	...

[A] After subtraction of wastewater matrix's N content.
[B] Bias significant at the 5 % level.