



Designation: D7237 – 06

Standard Test Method for Aquatic Free Cyanide with Flow Injection Analysis (FIA) Utilizing Gas Diffusion Separation and Amperometric Detection¹

This standard is issued under the fixed designation D7237; 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.

1. Scope

1.1 This test method is used to establish the concentration of aquatic “free” cyanide in an aqueous wastewater or effluent. The test conditions of this method are used to measure free cyanide (HCN and CN^-) and cyanide bound in the metal-cyanide complexes that are easily dissociated into free cyanide ions at the pH of the aquatic environment ranging from pH 6 to pH 8. The extent of HCN formation is less dependent on temperature than the pH; however, the temperature can be regulated if deemed necessary to further simulate the actual aquatic environment.

1.2 The aquatic free cyanide method is based on the same instrumentation and technology that is described in standard test method D6888, but employs milder conditions (pH 6-8 buffer versus HCl in the reagent stream), and does not utilize ligand displacement reagents.

1.3 The aquatic free cyanide measured by this procedure should be similar to actual levels of HCN in the original aquatic environment. This in turn may give a reliable index of toxicity to aquatic organisms.

1.4 This procedure is applicable over a range of approximately 2 to 500 $\mu\text{g/L}$ (parts per billion) aquatic free cyanide. Sample dilution may increase cyanide recoveries depending on the cyanide speciation; therefore, it is not recommended to dilute samples. Higher concentrations can be analyzed by increasing the range of calibration standards or with a lower injection volume. In accordance with Guide E1763 and Practice D6512 the lower scope limit was determined to be 9 $\mu\text{g/L}$ for chlorinated gold leaching barren effluent water.

1.5 *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 applica-*

bility of regulatory limitations prior to use. Specific hazard statements are given in Section 8.6 and Section 9.

2. Referenced Documents

2.1 ASTM Standards:²

- D1129 Terminology Relating to Water
- D1193 Specification for Reagent Water
- D1293 Test Methods for pH of Water
- D2036 Test Methods for Cyanides in Water
- D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
- D3370 Practices for Sampling Water from Closed Conduits
- D3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water
- D4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents
- D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
- D6512 Practice for Interlaboratory Quantitation Estimate
- D6696 Guide for Understanding Cyanide Species
- D6888 Test Method for Available Cyanide with Ligand Displacement and Flow Injection Analysis (FIA) Utilizing Gas Diffusion Separation and Amperometric Detection
- E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
- E1763 Guide for Interpretation and Use of Results from Interlaboratory Testing of Chemical Analysis Methods

3. Terminology

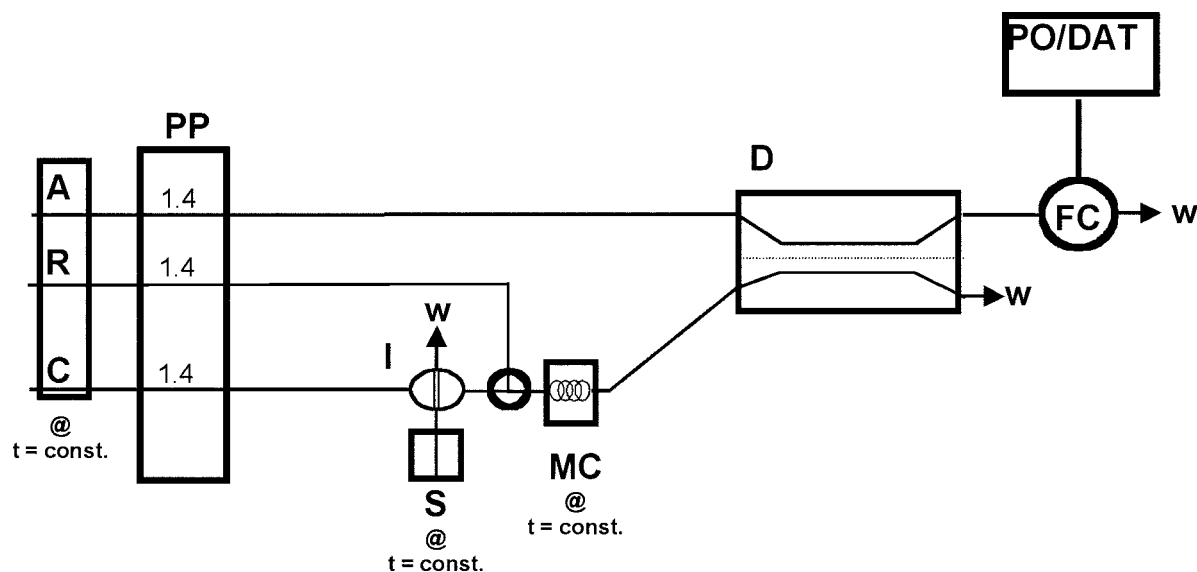
3.1 *Definitions* For definitions of terms used in this test method, refer to Terminology D1129 and Guide D6696.

3.1.1 *aquatic free cyanide*—Sum of the free cyanide (HCN and CN^-) and cyanide bound in the metal-cyanide complexes that are easily dissociated into free cyanide under the test conditions described in this method.

¹ This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis of Organic Substances in Water.

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.



C = carrier (water), R = reagent buffer (variable: pH 6-8, 0.2M phosphate buffer), A = acceptor solution (0.1M NaOH), S = sample, P = peristaltic pump (flow rates in mL/min), I = injection valve (200µL sample loop), MC = mixing coil (30-60 cm × 0.5mm i.d.), positioned in optional constant temperature manifold, D = gas-diffusion cell, FC = amperometric flow cell, PO/DAT = potentiostat/data collection device running data acquisition software, W = waste flows.

FIG. 1 Example of flow injection manifold for the determination of aquatic free cyanide.

4. Summary of Test Method

4.1 The test is generally performed at room temperature, but temperature of the sample and flow injection reagents can be regulated to match the aquatic environment if necessary.

4.2 The sample is introduced into a carrier solution of the flow injection analysis (FIA) system via an injection valve and confluent downstream with a phosphate buffer solution at pH in the 6-8 range. The released hydrogen cyanide (HCN) gas diffuses through a hydrophobic gas diffusion membrane into an alkaline acceptor stream where the CN⁻ is captured and sent to an amperometric flowcell detector with a silver-working electrode. In the presence of cyanide, silver in the working electrode is oxidized at the applied potential. The anodic current measured is proportional to the concentration of cyanide in the standard or sample injected.

4.3 Calibrations and sample data are processed with the instrument's data acquisition software.

5. Significance and Use

5.1 Cyanide and hydrogen cyanide are highly toxic. Regulations have been established to require the monitoring of cyanide in industrial and domestic wastes and surface waters.³

5.2 It is useful to determine the aquatic free cyanide to establish an index of toxicity when a wastewater is introduced to the pH and temperature of the natural environment.

5.3 This test method is applicable for natural water, saline waters, and wastewater effluent.

6. Interferences

6.1 Sulfide will diffuse through the gas diffusion membrane and can be detected in the amperometric flowcell. Oxidized products of sulfide can also rapidly convert CN⁻ to SCN⁻ at a high pH. Refer to 11.2 for sulfide removal.

6.2 Refer to section 6.1 of Test Method D6888 and Test Method D2036 for elimination of cyanide interferences.

7. Apparatus

7.1 The instrument should be equipped with a precise sample introduction system, a gas diffusion manifold with hydrophobic membrane, and an amperometric detection system to include a silver working electrode, a Ag/AgCl reference electrode, and a Pt or stainless steel counter electrode. An example of the apparatus schematic is shown in Fig. 1. Example instrument settings are shown in Table 1.

NOTE 1—The instrument and settings in Fig. 1 and Table 1 are shown as examples. The analyst may modify these settings as long as performance of the method has not been degraded. Contact the instrument manufacturer for recommended instrument parameters.

7.2 An autosampler is recommended but not required to automate sample injections and increase throughput. Autosamplers are usually available as an option from the instrument's manufacturer. If the sample is to be analyzed at a constant temperature other than the temperature of the room, manual injections may be required unless the autosampler is equipped to maintain constant temperature.

7.3 If aquatic free cyanide at a temperature other than room temperature is required, a constant temperature bath capable of maintaining the temperature of the aquatic environment within ± 0.5°C should be used to regulate the temperature of the flow injection reagents and samples.

7.4 *Data Acquisition System*—Use the computer hardware and software recommended by the instrument manufacturer to control the apparatus and to collect data from the detector.

7.5 *Pump Tubing*—Use tubing recommended by instrument manufacturer. Replace pump tubing when worn, or when precision is no longer acceptable.

7.6 *Gas Diffusion Membranes*—A hydrophobic membrane which allows gaseous hydrogen cyanide to diffuse from the

³ 40 CFR Part 136.

TABLE 1 Flow Injection Analysis Parameters

<i>FIA Instrument Parameter</i>	<i>Recommended Method Setting</i>
Pump Flow Rates	0.5 to 2.0 mL/min
Cycle period (total)	Approximately 120 seconds
Sample load period	At least enough time to completely fill the sample loop prior to injection
Injection valve rinse time between samples	At least enough time to rinse the sample loop
Peak Evaluation	Peak height or area
Working Potential	0.0 V vs Ag/AgCl

donor to the acceptor stream at a sufficient rate to allow detection. The gas diffusion membrane should be replaced when the baseline becomes noisy, or every 1 to 2 weeks.

7.7 Use parts and accessories as directed by instrument manufacturer.

8. Reagents and Materials

8.1 *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 American Chemical Society, where such specifications are available.⁴ 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.

8.2 *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 **D1193**.

8.3 *Sodium Hydroxide Solution (1.00M NaOH)*—Dissolve 40 g NaOH in laboratory water and dilute to 1 L.

8.4 *Sodium Hydroxide and Acceptor Solution (0.10M NaOH)*—Dissolve 4.0 g NaOH in laboratory water and dilute to 1 L.

8.5 *Carrier*—Water, as described in section 8.2.

8.6 *Stock Cyanide Solution (1000 µg/mL CN⁻)*—Dissolve 2.51 g of KCN and 2.0 g of NaOH in 1 L of water. Standardize with silver nitrate solution as described in Test Methods **D2036**, section 16.2. Store the solution under refrigeration and check concentration approximately every 6 months and correct if necessary.⁵ (**Warning**—Because KCN is highly toxic, avoid contact or inhalation.)

8.7 *Intermediate Cyanide Standards:*

8.7.1 *Intermediate Standard 1 (100 µg/mL CN⁻)*—Pipette 10.0 mL of stock cyanide solution (see 8.6) into a 100 mL volumetric flask containing 1 mL of 1.0 M NaOH (see 8.3). Dilute to volume with laboratory water. Store under refrigeration. The standard should be stable for at least 2 weeks.

8.7.2 *Intermediate Cyanide Solution 2 (10 µg/mL CN⁻)*—Pipette 10.0 mL of Intermediate Cyanide Solution 1 (see 8.7.1) into a 100 mL volumetric flask containing 1.0 mL of 1.00 M NaOH (see 8.3). Dilute to volume with laboratory water. The standard should be stable for at least 2 weeks.

8.8 *Working Cyanide Calibration Standards*—Prepare fresh daily as described in 8.8.1 and 8.8.2 ranging in concentration from 2 to 500 µg/L CN⁻.

8.8.1 *Calibration Standards (20, 50, 100, 200, and 500 µg/L CN⁻)*—Pipette 20, 50, 100, 200, and 500 µL of Intermediate Standard 1 (see 8.7.1) into separate 100 mL volumetric flasks containing 1.0 mL of 0.10 M NaOH (see 8.4). Dilute to volume with laboratory water.

8.8.2 *Calibration Standards (2, 5, and 10 µg/L CN⁻)*—Pipette 20, 50, and 100 µL of Intermediate Cyanide Solution 2 (see 8.7.2) into separate 100 mL volumetric flasks containing 1.0 mL of 0.10 M NaOH (see 8.4). Dilute to volume with laboratory water.

8.9 *Cyanide Electrode Stabilization Solution (Approximately 5 ppm as CN⁻)*—Pipette 500 µL of Stock Cyanide (see 8.6) into a 100 mL volumetric flask containing 1.0 mL of 0.10M M NaOH (see 8.4). Dilute to volume with laboratory water. The solution should be stored under refrigeration.

8.10 *Acetate Buffer*—Dissolve 410 g of sodium acetate trihydrate (NaC₂H₃O₂·3H₂O) in 500 mL of laboratory water. Add glacial acetic acid (approximately 500 mL) to yield a pH of 4.5.

8.11 *Buffer Solution A, 2M Sodium phosphate monobasic solution*—Weigh 276 g sodium phosphate monobasic monohydrate (NaH₂PO₄·H₂O) in a 1 L volumetric flask. Dissolve and dilute to volume with water.

8.12 *Buffer Solution B, 2M Sodium phosphate dibasic solution*—Weigh 284 g sodium phosphate dibasic, anhydrous (Na₂HPO₄) in a 1 L volumetric flask. Dissolve and dilute to volume with water. If necessary, warm to approximately 40°C on a hot plate and stir to completely dissolve the sodium phosphate dibasic into the water. Allow the solution to cool prior to use.

8.13 *1M Phosphate Buffer pH 7.0 Stock Solution*—Add 97.5 mL Buffer Solution A and 152.5 mL Buffer Solution B to a 500 mL volumetric flask. Dilute to volume with water.

8.14 *0.2 M Phosphate Buffer pH 7.0*—In a 1 L volumetric flask, add 200 mL 1M Phosphate Buffer Solution pH 7.0 and dilute to volume with water. The pH should be pH 7.0 ± 0.1. Verify the pH as described in **D1293** (Test Method A) and adjust if necessary with dilute sodium hydroxide or sulfuric acid. This buffer solution is to be used in the FIA system when aquatic free cyanide is to be determined at pH 7.0.

8.15 *1M Phosphate Buffer pH 6.0 Stock Solution*—Add 219.25 mL Buffer Solution A and 30.75 mL of Buffer Solution B to a 500 mL volumetric flask. Dilute to volume with water.

8.16 *0.2 M Phosphate Buffer pH 6.0*—In a 1 L volumetric flask, add 200 mL 1M Phosphate Buffer Solution pH 6.0 and dilute to volume with water. The pH should be pH 6.0 ± 0.1. Verify the pH as described in **D1293** (Test Method A) and adjust if necessary with dilute sodium hydroxide or sulfuric acid. This buffer solution is to be used in the FIA system when

⁴ *Reagent Chemicals, American Chemical Society Specifications*, Am. Chemical Soc., 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*.

⁵ Commercial Solutions of Stock Cyanide may be substituted.

aquatic free cyanide is to be determined at pH 6.0 or if the pH of the aquatic environment has not been specified.

8.17 *1 M Phosphate Buffer pH 8.0 Stock Solution*—Add 10.0 mL Buffer Solution A and 240 mL Buffer Solution B to a 500 mL volumetric flask. Dilute to volume with water.

8.18 *0.2 M Phosphate Buffer pH 8.0*—In a 1 L volumetric flask, add 200 mL 1 M Phosphate Buffer Solution pH 8.0 and dilute to volume with water. The pH should be $\text{pH}=8.0 \pm 0.1$. Verify the pH as described in **D1293** (Test Method A) and adjust if necessary with dilute sodium hydroxide or sulfuric acid. This buffer solution is to be used in the FIA system when aquatic free cyanide is to be determined at pH 8.0.

8.19 *Ag/AgCl Reference Electrode Filling Solution*—Fill the reference electrode as recommended by the instrument manufacturer.

9. Hazards

9.1 **Warning**—Because of the toxicity of cyanide, great care must be exercised in its handling. Acidification of cyanide solutions produces toxic hydrocyanic acid (HCN). All manipulations must be done in the hood so that any HCN gas that might escape is safely vented.

9.2 **Warning**—Many of the reagents used in these test methods are highly toxic. These reagents and their solutions must be disposed of properly.

9.3 All reagents and standards should be prepared in volumes consistent with laboratory use to minimize the generation of waste.

10. Sample and Sample Preservation

10.1 Collect the sample in accordance with Practices **D3370** and **D3856**. Usually 100 mL sample volume is sufficient.

10.2 The sample must be stabilized at time of collection with the addition of sodium hydroxide. Add 1 mL of 0.1 M NaOH to 100 mL of the sample or until the sample is pH 11.

10.3 See section 11 if oxidizing agents or sulfide are suspected to be present in the sample.

10.4 Samples should be stored in dark bottles that minimize exposure to ultraviolet radiation and refrigerated at 4°C.

10.5 Synthetic samples have been shown to be stable for at least 14 days, but in actual samples the cyanide concentrations may decrease significantly prior to this holding time if there are undetectable traces of chlorine, reduced sulfur species, or hydrogen peroxide present. Analyze the sample as soon as possible to avoid degradation. Holding times can be estimated in accordance with Practice **D4841**.

11. Elimination of Interferences

11.1 *Oxidizing Agent*—Test for the presence of oxidizing agents. Add a drop of the sample to acidified KI starch test paper (acidify KI starch paper with acetate buffer, see 8.10) as soon as the sample is collected; a blue color indicates the need for treatment. If oxidizing agents are present, add 0.1 g/L sodium arsenite to the sample to avoid degradation of cyanide.

11.2 *Sulfide*—Test for sulfide by placing a drop of sample on lead acetate paper previously moistened with acetate buffer solution (see 8.10). If the paper turns black, sulfide is present. Add lead acetate, or if the sulfide concentration is too high, add

powdered lead carbonate to avoid significantly reducing the pH. Repeat this test until a drop of treated sample no longer darkens the acidified lead acetate test paper. The supernatant containing cyanide must be filtered immediately to avoid the rapid loss of cyanide due to the formation of thiocyanate.

NOTE 2—Lead acetate test strips may not be sensitive enough to detect sulfide concentrations below approximately 50 mg/L; therefore, treatment may be performed on samples where sulfide is suspected. Interference can be confirmed by analyzing the sample with and without treatment. If the measured cyanide in the untreated sample is significantly higher, sulfide is likely present and treatment described in section 11.2 should be performed to remove sulfide.

12. Calibration and Standardization

12.1 Turn on the power to the apparatus and the autosampler (if equipped). Start the data acquisition system.

12.2 Clamp the pump tube platens in place and start pumping reagents in the flow injection system. Allow the system to warm up at least 15 min or until a stable baseline is achieved. Take care not to over-tighten the pump tube platens as this greatly reduces the lifetime of the tubing.

12.3 If recommended by the instrument manufacturer, aspirate the Cyanide Shocking Solution (5 ppm CN^-) from 8.9. After at least 30 s, inject the shocking solution into the apparatus and record the amperometric response (pA value) after the cycle period has completed. Repeat this procedure until the peak responses are less than 2 % RSD. This process will ensure that the electrode system has stabilized.

12.4 After the electrode system has stabilized, aspirate the highest working standard (see 8.8) into the flow injection apparatus. Follow the instrument manufacturer's instructions to store the retention time window for cyanide using the data acquisition software.

12.5 Select the buffer to be used for instrumental analysis of the sample, which is the closest pH to that of the receiving water for the sample.

12.6 Inject each working standard and a reagent blank into the apparatus and record the amperometric response with the data acquisition system. Plot the response versus the cyanide concentration with a straight line or a quadratic fit curve depending on the instrument and data acquisition system employed. If the calibration model is polynomial, it may be no more than third order. A second order polynomial is recommended.

12.7 Prepare a new calibration curve at least once daily.

13. Procedure

13.1 If samples were stored under refrigeration, allow the samples to stand at room temperature or place the samples in a constant temperature bath (section 7.3) until a constant temperature is achieved. Record the temperature to the nearest 0.1°C.

13.2 Inject each sample into the flow injection apparatus, and inspect for irregular peak shapes, disturbances, or detector overloads.

14. Data Analysis and Calculations

14.1 Report the aquatic free cyanide in $\mu\text{g/L}$ at the pH of the buffer solution along with the temperature of samples and