**Designation: ~~D7237 – 15a~~ D7237 – 18**

# Standard Test Method for Free Cyanide and Aquatic Free Cyanide with Flow Injection Analysis (FIA) Utilizing Gas Diffusion Separation and Amperometric Detection[1]

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 free cyanide in an aqueous wastewater, effluent and in-stream free cyanide concentrations after mixing treated water with receiving water. The test conditions of this test 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 6. Free cyanide is determined at pH 6 at room temperature. The aquatic free cyanide can be determined by matching the pH to the water in the receiving environment in the range of pH 6 to 8. The extent of HCN formation is less dependent on temperature than the pH; however, the temperature can be regulated if deemed necessary for aquatic free cyanide to further simulate the actual aquatic environment.

1.2 The free cyanide test method is based on the same instrumentation and technology that is described in Test Method D6888, but employs milder conditions (pH 6–8 buffer versus HCl or $H_2SO_4$ 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 5 to 500 µg/L (parts per billion) 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 µg/L for chlorinated gold leaching barren effluent water and the $IQE_{10\ \%}$ is 12 µg/L in the gold processing detoxified reverse osmosis permeate waste water sample matrix.

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

1.6 This test method is not recommended for samples that contain reduced sulfur compounds such as sulfides.

1.7 *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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in 8.6 and Section 9.

1.8 *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
D1293 Test Methods for pH of Water
D2036 Test Methods for Cyanides in Water

[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 for 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~~standard's Document Summary page on the ASTM website.

***A Summary of Changes section appears at the end of this standard**

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D4658 Test Method for Sulfide Ion in 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 Cyanides with Ligand Displacement and Flow Injection Analysis (FIA) Utilizing Gas Diffusion Separation and Amperometric Detection
D7365 Practice for Sampling, Preservation and Mitigating Interferences in Water Samples for Analysis of Cyanide
D7728 Guide for Selection of ASTM Analytical Methods for Implementation of International Cyanide Management Code Guidance
E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
E1601 Practice for Conducting an Interlaboratory Study to Evaluate the Performance of an Analytical Method
E1763 Guide for Interpretation and Use of Results from Interlaboratory Testing of Chemical Analysis Methods (Withdrawn 2015)[3]

## 3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129 and Guide D6696.

3.2 *Definitions of Terms Specific to This Standard:* ~~For definitions of terms used in this test method, refer to Terminology D1129 and Guide D6696.~~

3.2.1 *aquatic free cyanide, n*—free cyanide measured when the buffer or temperature is adjusted to mimic the receiving-water environment.

3.2.2 *free cyanide, n*—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 test method at pH 6 and room temperature.

## 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 to measure aquatic free cyanide.

4.2 The sample is introduced into a carrier solution of the flow injection analysis (FIA) system via an injection valve and confluence downstream with a phosphate buffer solution at pH 6 to measure free cyanide or in the range of pH 6 to 8 to measure aquatic free cyanide. 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~~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.[4]

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

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

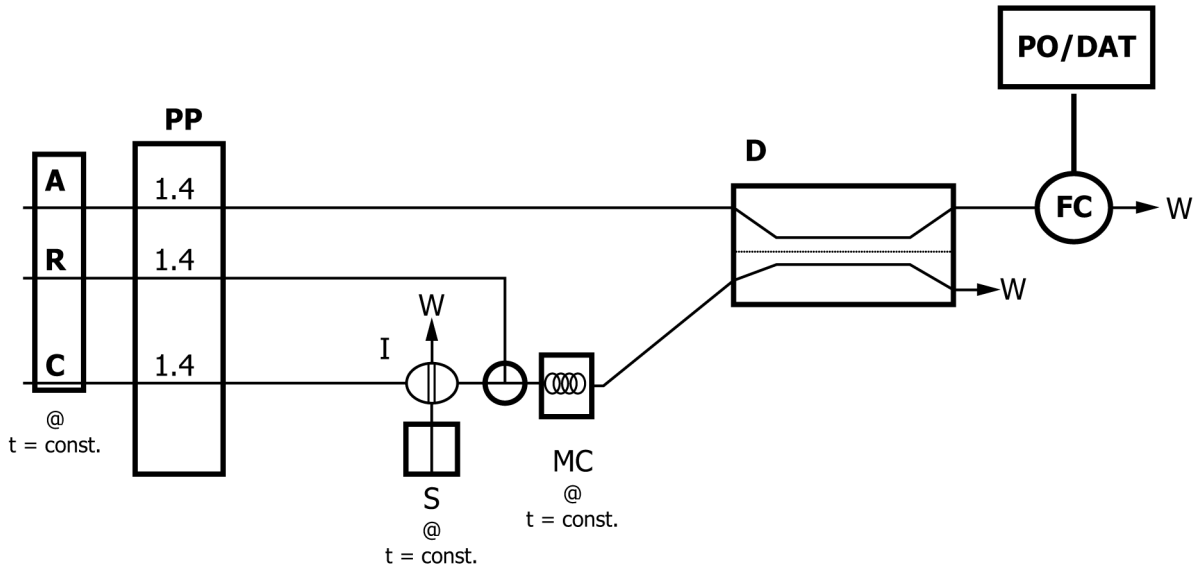
5.4 Free cyanide measured using this test method is applicable for implementation of the International Cyanide Code Guidance in accordance with Guide D7728.

## 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 11.3 for sulfide removal.~~

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

[3] The last approved version of this historical standard is referenced on www.astm.org.
[4] 40 CFR Part 136.




C = carrier (water), R = reagent buffer (variable: pH 6 for free cyanide and pH ~~6–8~~6–8 for aquatic free cyanide, 0.2 M phosphate buffer), A = acceptor solution (0.1 M NaOH), S = sample, ~~P~~PP = peristaltic pump (flow rates in mL/min), I = injection valve (200 µL sample loop), MC = mixing ~~cool~~coil (30–60 cm × 0.5 mm ~~i.d.),~~ID), 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**

6.1 ~~Residual~~ Sulfide, as $H_2S$, will diffuse through the gas diffusion membrane and can be detected in the amperometric flowcell. Also, residual flotation reagents have been shown to interfere, which is indicated by failure of the amperometric signal to return to baseline compared to the standards. This effect is attributed to the formation of volatile carbon disulfide. If sulfides are suspected, confirm by means of Test Method D4658~~this interference is encountered, verify by comparing with analysis using Test Method~~. Aside from this method, ion chromatography may be used as a reliable means for ~~D6888 including bismuth nitrate~~determining sulfide interference. See Appendix X1~~in the acidification reagent on a solution without sodium hydroxide preservation, which should provide confirmation due to lower results~~. For National Pollutant Discharge Elimination System reporting, however, the user is advised to analyze the sample using Test Method D6888 for samples containing reduced sulfur compounds.

6.2 Oxidants can continue to react with free cyanide, if present, lowering the concentration, if not immediately treated with a reducing reagent during sampling.



## 7. Apparatus

7.1 The instrument must 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~~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 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.

**TABLE 1 Flow Injection Analysis Parameters**

| FIA Instrument Parameter | Recommended Method Setting |
|---|---|
| ~~FIA Instrument Parameter~~ | ~~Recommended Method Setting~~ |
| ~~Pump Flow Rates~~ | ~~0.5 to 2.0 mL/min~~ |
| 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~~ |
| Peak evaluation | Peak height or area |
| ~~Working Potential~~ | ~~0.0 V vs. Ag/AgCl~~ |
| Working potential | 0.0 V versus Ag/AgCl |

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.[5] 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 Specification D1193.

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

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

NOTE 2—Acceptor solution concentration of 0.025 M NaOH has also been found to be acceptable.

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

NOTE 3—Carrier solution containing 0.025 M NaOH has also been found to be acceptable.

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, 16.2. Store the solution under refrigeration and check concentration approximately every 6 months and correct if necessary.[6] (**Warning—**Because KCN is highly toxic, avoid contact or inhalation.)

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

[6] Commercial Solutions of Stock Cyanide may be substituted.

8.7 *Intermediate Cyanide Standards:*

8.7.1 *Intermediate Standard 1 (100 µ g/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 ($NaC_2H_3O_2 \cdot 3H_2O$) 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 ($NaH_2PO_4 \cdot H_2O$) in a 1 L volumetric flask. Dissolve and dilute to volume with water.

8.12 *Buffer Solution B, 2 M Sodium phosphate dibasic solution*—Weigh 284 g sodium phosphate dibasic, anhydrous ($Na_2HPO_4$) 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.

8.12.1 Alternatively, prepare a 1 M solution by dissolving 142 g sodium phosphate dibasic, anhydrous in 1 L.

8.13 *1 M 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.

8.13.1 Alternatively, substitute 305 mL of 1 M sodium phosphate dibasic for the 152.5 mL of Buffer Solution B.

8.14 *0.2 M Phosphate Buffer pH 7.0*—In a 1 L volumetric flask, add 200 mL 1 M 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 Test Methods 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 *1 M 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.

8.15.1 Alternatively, substitute 61.5 mL of 1 M sodium phosphate dibasic for the 30.75 mL of Buffer Solution B.

8.16 *0.2 M Phosphate Buffer pH 6.0*—In a 1-L volumetric flask, add 200 mL 1 M 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 Test Methods 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 free cyanide or 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 ~~pH=8.0~~ pH = 8.0 ± 0.1. Verify the pH as described in Test Methods 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 latest version of Practice D7365. This practice is applicable for the collection and preservation of water samples for the analysis of cyanide. Responsibilities of field sampling personnel and the laboratory are indicated. Usually 100 mL sample volume is sufficient. Samples must be collected and stored in dark (amber or low actinic) containers to minimize reactions of ultra violet light.

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 must be stored in dark bottles that minimize exposure to ultraviolet radiation and refrigerated.

NOTE 4—Practice D7365 recommends refrigeration by storing the sample between its freezing point and 6°C.

10.5 Synthetic samples have been shown to be stable for at least 14 days and up to 30 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,~~ Caro's acid, reduced sulfur, or hydrogen peroxide present.[7] ~~Analyze~~ Treat, filter and measure 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 *Sulfides*—~~Practice~~ Test for sulfides 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. An odor of "rotten eggs" also indicates the presence of sulfide. Lead acetate test strips may not be sensitive enough to detect sulfide concentrations below approximately 50 mg/L. If the presence of sulfide below concentrations of 50 mg/L is suspected, confirm using Test Method ~~D7365~~D4658 ~~specifies mitigation of interference procedures~~. Mitigate using Test Method D6888 ~~for testing water samples for cyanide.~~to measure available weak and dissociable cyanide without sulfide interference. Although also effective in overcoming sulfide interference, ion chromatography is not an approved test procedure for determining free and aquatic free cyanide in accordance with 40 CFR 136.3.

11.2 *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 and mix until a drop of sample on the test strip shows no blue color, to avoid degradation of cyanide.

~~11.3 *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 5—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 11.3 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 Stabilization Solution (5 ppm $CN^-$) from 8.9. After at least 30 s, inject the stabilization 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~~manufacturer's instructions to store the retention time window for cyanide using the data acquisition software.

[7] Reduced sulfur samples will cause high results due to direct interference, or low results through the formation of SCN.