Designation: D 4282 – 02

An American National Standard

Standard Test Method for Determination of Free Cyanide in Water and Wastewater by Microdiffusion[1]

This standard is issued under the fixed designation D 4282; 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 covers the determination of free cyanides in waters and wastewaters. Free cyanide is here defined as the cyanide which diffuses as cyanide (HCN), at room temperature, from a solution at pH 6.[2]

1.2 This test method does not include complexes that resist dissociation, such as hexacyanoferrates and gold cyanide, nor does it include thiocyanate and cyanohydrin.

1.3 This test method may be applied to water and wastewater samples containing free cyanide from 10 to 150 µg/L. Greater concentrations may be determined by appropriate dilution.

1.4 This test method has been fully validated by collaborative testing as specified by Practice D 2777.

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 applicability of regulatory limitations prior to use.* For specific hazard statements, see 8.6, 8.9, Section 9, and 12.2.1.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[3]

D 1192 Guide for Equipment for Sampling Water and Steam in Closed Conduits[3]

D 1193 Specification for Reagent Water[3]

D 2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water[3]

D 3370 Practices for Sampling Water from Closed Conduits[3]

D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water[3]

D 4210 Practice for Interlaboratory Quality Control Procedures and A Discussion on Reporting Low-Level Data[3]

D 5788 Guide for Spiking Organics into Aqueous Samples[4]

D 5789 Practice for Writing Quality Control Specifications for Standard Test Methods for Organic Constituents[4]

D 5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis[4]

E 275 Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers[5]

3. Terminology

3.1 *Definitions:*

3.1.1 For a definition of terms used in this test method refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *free cyanide*—refers to those simple cyanides or loosely held complexes of cyanide that diffuse at pH 6, at room temperature.

4. Summary of Test Method

4.1 The reactions are carried out in a microdiffusion cell.

4.2 The sample is treated with cadmium ion to precipitate the hexacyanoferrates.

4.3 The sample is buffered at pH 6 and allowed to stand for 4 h.

4.4 The HCN diffuses into sodium hydroxide solution.

4.5 An aliquot of the sodium hydroxide solution is treated with chloramine-T, and the cyanogen chloride formed is reacted with barbituric acid in pyridine. The absorbance of the color formed is measured using a spectrophotometer at a wavelength of 580 nm.

5. Significance and Use

5.1 This test method is useful in distinguishing between the potentially available free cyanide (total cyanide) and the free cyanide actually present.

5.2 This test method provides a convenient technique for making on-site free cyanide determinations.

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[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] The paper by J. M. Kruse and L. E. Thibault "Determination of Free Cyanide in Ferro- and Ferricyanides," Analytical Chemistry, 45(13): 2260–2261; 1973 Nov., recommends a diffusion at pH 7. The ANSI modification (ANSI PH 4.41-1978) uses pH 6. Using the conditions of the ANSI method, diffusion is completed within 4 hours at pH 6. Longer diffusion time was required at pH 7 on the samples analyzed.

[3] *Annual Book of ASTM Standards*, Vol 11.01.

[4] *Annual Book of ASTM Standards*, Vol 11.02.

[5] *Annual Book of ASTM Standards*, Vol 03.06.

6. Interferences

6.1 *Decomposition of Hexacyanoferrates During Diffusion*:

6.1.1 This decomposition is virtually eliminated by allowing the sample to diffuse in the dark, and by precipitating the hexacyanoferrates with cadmium ion.

6.2 *Instability of Free Cyanide in Effluents*—The reactivity of free cyanide with such chemicals as aldehydes or oxidizing agents, is not really a method interference. However, because of this instability, it is important for the diffusion to begin as soon after sampling as possible. It is beyond the scope of this test method to list all the possible cyanide reactions that may be encountered.

7. Apparatus

7.1 *Diffusion Cell*, microdiffusion cell, Conway type, 68-mm outside diameter. [6]

7.2 *Micropipets*, 0.10 mL, 1.00 mL.

7.3 *Spectrophotometer*, conforming to Practice E 275.

7.4 *Spectrophotometer Cell*, 1-cm equipped with a stopper.

7.5 *Pipet or Syringe*, adjustable (to deliver 1.30 mL).

7.6 *Calomel Reference Electrode*, with saturated KNO_3 electrolyte, or the equivalent.

7.7 *pH Meter*.

7.8 *Silver Electrode*.

8. Reagents

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society[7] where such specifications are available. Other grades may be used provided it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water conforming to Type II of Specification D 1193.

8.3 *Cadmium Chloride Solution* (10 g/L), $CdCl_2$—Dissolve 10.0 g of anhydrous cadmium chloride in 750 mL of water in a 1-L volumetric flask. Dilute to volume with water.

8.4 *Chloramine-T Reagent* (10 g/L)—Dissolve 1.00 g of chloramine-T in 50 mL of water in a 100-mL volumetric flask. Dilute to volume with water. Make this reagent fresh daily.

8.5 *Cyanide Solution, Standard* (1.00 mL = 2 µg CN^-)—Pipet 2.00 mL of cyanide stock solution (approximately 1.0 g/L CN^-) into a 1-L volumetric flask and dilute to volume with sodium hydroxide solution (2.05 g/L).

8.6 *Cyanide Solution Stock*—Dissolve 2.51 g of potassium cyanide, KCN, in 500 mL of sodium hydroxide solution (2.05 g/L) in a 1-L volumetric flask. Dilute to volume with sodium hydroxide solution (2.05 g/L). This solution contains approximately 1.0 g/L cyanide (CN^-). (**Warning—**KCN is highly toxic, avoid contact or inhalation. Prepare and standardize this solution weekly.)

8.6.1 *Standardizing Cyanide Stock Solution*:

8.6.1.1 Using a silver electrode and a reference electrode, titrate 20.0 mL of the cyanide stock solution (in a beaker also containing 50 mL of sodium hydroxide solution (2.05 g/L)) with the silver nitrate standard solution.

8.6.1.2 Record the mL of titration for use in the calculation (see Fig. 1 for an example of a typical titration curve).

8.6.1.3 Calculate the concentration of the cyanide stock solution using the following equation:

$$50 \times (\text{mL silver nitrate}) = \text{mg/L } CN^- \text{ in stock solution}$$

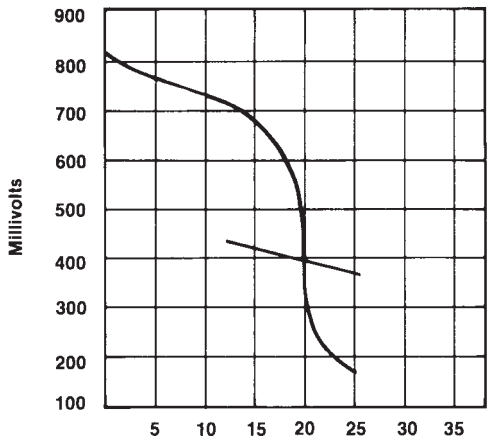
1.00 mL of silver nitrate solution is equal to 1 mg of CN^-.

8.7 *Potassium Phosphate Buffer Solution (Acidified)*—Add 8.0 mL of concentrated phosphoric acid (sp gr 1.69), H_3PO_4, to 100 mL of potassium phosphate solution.

8.8 *Potassium Phosphate Solution*, 190 g/L—Add 400 mL of water to a 2-L beaker. Add and dissolve 14.5 g of sodium hydroxide, NaOH. Add and dissolve 190 g of potassium phosphate, monobasic, KH_2PO_4. Add water to 950 mL to aid dissolution. Adjust the pH of the solution to pH 5.9 to 6.1, using 100 g/L sodium hydroxide solution. Transfer the solution to a 1-L volumetric flask, and dilute to volume with water.

8.9 *Pyridine-Barbituric Acid Reagent*—Add 15.0 g of barbituric acid to a 250-mL volumetric flask. Wash down the sides of the flask with just enough water to moisten the barbituric acid. Add 75 mL of pyridine and swirl to mix. Slowly add 15 mL of concentrated hydrochloric acid (sp gr 1.19) and swirl to mix. Cool the solution to room temperature. Dilute to volume and mix. It is recommended that this reagent be prepared fresh weekly and stored in a dark place. (**Warning—**Pyridine is toxic; avoid contact or inhalation. Prepare this reagent in an exhaust hood.)

8.10 *Silver Nitrate Solution, Standard* (1 mL = 1 mg of CN^-)—Weigh 3.2647 g of silver nitrate on an analytical balance. Quantitatively transfer the silver nitrate to a 1-L volumetric flask. Dissolve and dilute to volume with water. Store in a dark glass bottle.



NOTE 1—Twenty millilitres of 2.51 g/L KCN titrated with $AgNO_3$.

FIG. 1 Typical Titration Curve Standardizing KCN Solution

[6] One source of supply for these cells is Arthur H. Thomas, No. 3806-F-10.

[7] *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. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

8.11 *Sodium Hydroxide Solution* (4.1 g/L), NaOH—Add 4.10 g of sodium hydroxide to 800 mL of water in a 1-L volumetric flask. Stir until dissolved, and cool the solution to room temperature before adjusting the final volume to 1 L.

8.12 *Sodium Hydroxide Solution* (2.05 g/L), NaOH—Add 2.05 g of sodium hydroxide to 800 mL of water in a 1-L volumetric flask. Stir until dissolved, and cool the solution to room temperature before adjusting the final volume to 1 L. (An alternative preparation is to dilute 0.10 *N* sodium hydroxide solution with an equal volume of water.)

9. Hazards

9.1 *Safety Precautions*:

9.1.1 Because of the toxicity of cyanide, exercise great care in its handling. Acidification of cyanide solutions produces toxic gaseous hydrocyanide acid (HCN). Perform all manipulations in the hood so that any HCN that might volatilize is safely vented.

9.1.2 Some of the reagents used in these methods, such as cyanide solutions, are highly toxic. Dispose of these reagents and their solutions properly.

9.1.3 Do not pipet by mouth.

9.2 *Operational Precautions*—This test method requires practice and manual dexterity. The following practices have been found necessary to obtain reliable test results:

9.2.1 Keep the samples in the dark because light can dissociate complex cyanides and lead to high values.

9.2.2 Run the samples at least in duplicate.

9.2.3 Use calibrated syringes or equivalent for delivering the sample. The force of the sample ejection aids in the mixing in the microdiffusion cell.

9.2.4 Exercise great care during mixing of solutions by tilting and rotating the microdiffusion cell to avoid spilling or splashing liquid from one compartment to another.

9.2.5 Make the seal between the microdiffusion cell and lid airtight.

9.2.6 It is important to observe the specified time periods in those steps where such is noted. In particular, make the spectrophotometer measurements in the 3 to 6-min interval.

9.2.7 Full color development in the spectrophotometer cell requires that after each addition, mix the solution thoroughly without loss of material.

10. Sampling and Sample Preservation

10.1 Collect the sample in accordance with Specification D 1192 and Practices D 3370.

10.2 A satisfactory preservation technique is not available. Reactions between CN^- and aldehydes, oxidizing agents, or sulfides will continue. However, if the sample cannot be analyzed immediately, some steps can be taken to slow down the reactions taking place.

10.2.1 Adjust the sample to pH 12 or more. This minimizes CN^- losses due to vaporization.

10.2.2 Store the samples in the dark to prevent hexacyanoferrate breakdown.

10.2.3 Keep the sample cool (for example, in a refrigerator).

11. Calibration

11.1 *Calibration Standards*—Pipet 0.00 (Note 2), 5.00, 10.0, and 15.0 mL of the 2.00-mg/L cyanide standard solution into four 200-mL volumetric flasks. Dilute each of the flasks to volume with sodium hydroxide solution (2.05 g/L). These dilutions yield calibration standards that are approximately 0, 50, 100, and 150 µg/L of CN^-, respectively.

NOTE 1—The 0.00 sample can also be considered the blank.

11.2 To establish the calibration curve, analyze the calibration standards in accordance with the procedure in Section 12. Plot a calibration curve of concentrations of CN^- versus absorbance (see Fig. 2). Standards should be run daily for calibration, until it is established that the calibration curve will apply for a longer period of time. Then it is only necessary to run two standards (such as 0 and 100 µg/L CN^-) with each batch of samples as a check on the existing calibration curve.

12. Procedure

12.1 *Microdiffusion of Free Cyanide*:

12.1.1 Pipet 3.00 mL of sample or calibration standard into the outer ring of a clean, dry, microdiffusion cell (see Fig. 3).

12.1.2 Using a calibrated syringe (or adjustable pipet), pipet 1.30 mL of sodium hydroxide solution (4 g/L) into the center of the chamber of the microdiffusion cell.

12.1.3 At this time, smear the ground glass side of a glass cell cover plate with a sufficiently heavy layer of petroleum jelly or stopcock grease to achieve an airtight seal.

12.1.4 Using a micropipet, pipet 0.5 mL of 10 g/L cadmium chloride solution (10 g/L) into the sample in the outside ring of the microdiffusion cell. Tilt and rotate the cell for 15 s to ensure mixing.

12.1.5 Immediately inject 1.0 mL of potassium phosphate solution (190 g/L) into the sample in the outside ring of the microdiffusion cell, inject at an angle in order to force the solution around the chamber, and quickly seal with the greased glass plate.

12.1.6 Tilt and rotate the cell for 15 s to ensure proper mixing.

12.1.7 Keep the covered cell in the dark for a period of not less than 4 h and not more than 8 h.

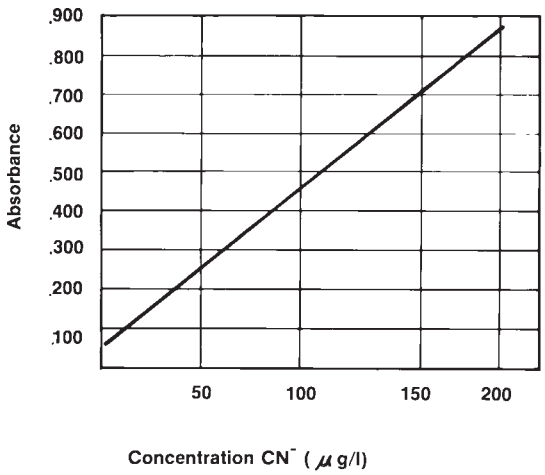
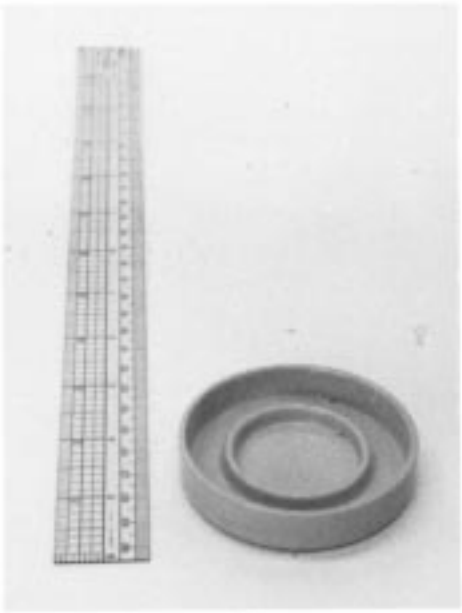
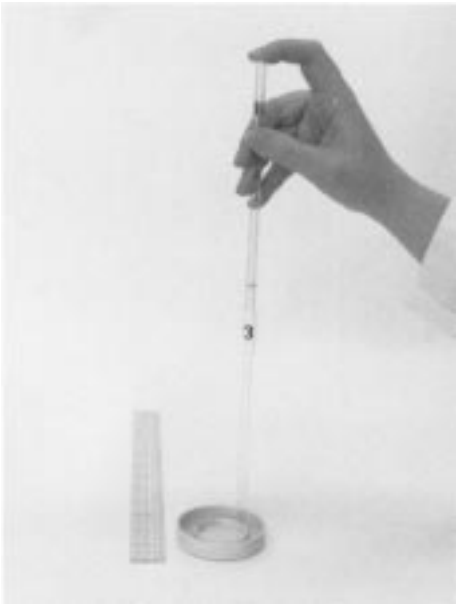


FIG. 2 Example of Calibration Curve for CN^-



Typical Cell
(a)

Filling Inner Compartment
(b)



Filling Outer Compartment
(c)

FIG. 3 Microdiffusion Cell

12.2 *Dye Formation*:

12.2.1 At the end of the diffusion period, pipet a 1.00-mL aliquot of the NaOH solution from the center chamber of the microdiffusion cell into a clean, dry, stoppered spectrophotometer cell. The stopper shall form a watertight seal. For a large number of determinations, it will be necessary to periodically clean the cells with acid-dichromate or methanol-hydrochloric acid (3 volumes of water, 1 volume of concentrated HCl [sp gr 1.19] and 4 volumes of methanol). Avoid contact with skin or eyes with these acid reagents. Methanol may be used to dry the cell, if necessary. (**Warning—**DO NOT keep methanol near a flame.)

12.2.2 Micropipet 0.1 mL of acidified potassium phosphate buffer into the spectrophotometer cell. Seal the cell and invert 4 to 5 times to mix.

12.2.3 Micropipet 0.50 mL of chloramine-T reagent into the spectrophotometer cell. Seal the cell and invert 4 to 5 times to mix. Complete mixing at this point is critical to the procedure.

12.2.4 Micropipet 1.00 mL of pyridine-barbituric acid reagent into the cell and replace the stopper. Note the time and invert the cell 8 to 10 times to mix.

12.2.5 At the end of 3 min, read the absorbance of the colored solution against air at the absorbance maximum in the region of 578 nm. The optimum wavelength may vary slightly when different bottles of barbituric acid are used.

NOTE 2—The total volume of that liquid in the cell is 2.6 mL that is normally sufficient. If it is not sufficient, the buffer volume may be increased in 12.2.2 by a standard amount. This will result in a different calibration curve.

12.2.6 The optimum wavelength may vary slightly when new bottles of barbituric acid are used.

13. Calculation

13.1 Derive the amount of CN^- in micrograms per litre from the calibration curve established in 11.2. Fig. 2 is an example of a typical calibration curve in the 0 to 150 µg/L range. For samples of greater CN^- concentration, the sample will be diluted appropriately with sodium hydroxide solution (2.05 g/L). A3.00-mL sample of the diluted solution is analyzed according to the procedure. The value read from the calibration curve is then corrected in accordance with the dilution factor.

14. Precision and Bias [8]

14.1 *Precision*—Based on the results of six operators in six laboratories, the overall and single-operator precision of this test method within its designated ranges may be expressed as follows:

$$\text{Reagent Water} \qquad S_t = 0.07x + 1.03$$

$$S_o = 0.03x + 2.94$$

$$\text{Selected Water Matrices} \qquad S_t = 0.10x - 0.98$$

$$S_o = 0.05x + 1.04$$

where:

S_t = overall precision,
S_o = single-operator precision, and
x = cyanide concentration, µg/L.

14.2 *Bias*—Recoveries of known amounts of diffusible cyanide from Type II reagent water and selected water matrices, were as shown in Table 1.

14.3 These collaborative test data were obtained on reagent water and selected matrix waters including tap water, river (natural) water, and treatment plant effluent mixed with non-contact cooling water. These data may not apply to other matrices.

TABLE 1 Recovery and Precision Data

Amount Added, µg CN^-/L	Amount Found, µg CN^-/L	*n*	S_t	S_o	Bias	%Bias	Statistical Significance, 95 % CL
			Reagent Water				
32	31.4	18	3.77	4.1	−0.6	−1.9	no
80	76	18	5.76	5.2	−4	−5.0	yes
144	138	18	11.5	7.6	−6	−4.2	yes
			Matrix Water				
32	30.7	18	2.56	2.6	−1.3	−4.0	no
80	74	18	6.01	4.9	−6	−7.5	yes
144	130	18	13.3	8.1	−14	−9.7	yes

15. Quality Assurance / Quality Control

15.1 Before this test method is applied to the analysis of samples of unknown cyanide concentration, the analyst must establish quality control by the procedures recommended in Practice D 4210 and Guide D 3856.

15.2 A duplicate sample and known standard must be run each day that an analysis is performed. The duplicate and standard shall meet satisfactory limits as established by the control chart before a determination is considered satisfactory.

15.3 A blank and spiked sample shall be run each day that an analysis is performed. The spike shall be in accordance with that outlined in 11.11 of Guide D 3856. The blank shall be low enough that it will not unduly influence the data.

15.4 One standard must be run with every 10 samples or with each batch, whichever results in the greater frequency. The results must meet the limits established in Section 14 of this test method before the data for that batch or set of 10 samples are acceptable.

15.5 Analysts performing this test method will be required to measure their performance against the performance level achieved in the interlaboratory study of the test method.

15.6 *Verification of Colorimetric Procedure*

15.6.1 Prepare a series of cyanide standards including zero (blank), based on the expected concentration range of the samples, and follow the standardization each time new reagents are prepared or every six months.

15.6.2 The slope (*m*) of the standard curve should check the theoretical value (1.0–cm cell, 0.22 – 0.24 mg CN/L/a; 5.0–cm cell, 0.044 – 0.048 mg CN/L/a; 10.0–cm cell, 0.022 – 0.024 mg CN/L/a).

15.6.3 At least one standard solution and one blank should be checked each time the procedure is used.

15.7 *Demonstration of Analyst Proficiency*

15.7.1 Demonstrate the competence of the analyst before this method is used to generate reportable data (Practice D 5789, Section 9).

15.7.2 Verify the procedure to be used by analyzing standard solutions in the expected range.

15.7.3 Analyze in duplicate six samples of known or nearly the same concentration by the method.

15.7.4 Calculate the standard deviation of the data (D 3856, D 4210, D 5789, and D 5847). If the value obtained is within that given in the procedure for single operator precision, the analyst can be considered "competent." See Note 3.

NOTE 3—If this is the first data generated in the laboratory, construct a preliminary control chart (D 3856, D 4210).

15.8 *Demonstration of Laboratory Proficiency*

15.8.1 Initially analyze five or six samples in duplicate to obtain a crude estimate of population standard deviation. For

[8] Supporting data for the precision and bias statement have been filed at ASTM Headquarters as RR:D19-1091.

methods used routinely, continue to accumulate additional data until at least 40 data points are accumulated for the procedure (D 4210, Section 5).

15.8.2 Construct a control chart with upper and lower limits from the data obtained (D 3856, Section 11 and D 4210, Section 9).

15.8.3 To monitor precision and bias, analyze the following in duplicate: a standard solution, a sample of known value, a spiked sample (D 5788), a field blank, and a method blank each day (or every 20 routine samples).

15.8.4 Calculate the relative range value (*R*) for each set of duplicate analyses. If the *R*'s are greater than the upper control limit, the precision is judged out–of–control, and analyses should be discontinued until the problem is resolved.

15.8.5 Calculate the percent recovery (*P*) for the standard and the spiked sample. If the recoveries are not within 100 ± 10 %, the analyses should be discontinued until the reason is found.

16. Keywords

16.1 free cyanide; hexacyanoferrate; instability; microdiffusion; micropipet; pyridine-barbaturic acid; spectrophotometer; wastewater