Spectrophotometric Determination of Ferrocyanide in Effluents
ECN-0025-1

INTRODUCTION
This method is used to determine the concentration of ferrocyanide ion in photoprocessing solution effluents. The ion concentration is determined spectrophotometrically. The sample is diluted, if necessary, such that the ferrocyanide ion [Fe(CN)₆⁴⁻] concentration falls within a range of 0.5 to 5 mg/L. Results are reported as mg/L of potassium ferrocyanide trihydrate \( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} \). A dilution of 25 mL of effluent to 250 mL is sufficient for samples as high as 100 mg/L \( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} \) concentration. In most cases, this is adequate. However, this method can be used for samples of potassium ferrocyanide trihydrate concentrations as high as one g/L by making a second dilution of 25 mL to 250 mL.

After dilution, the sample is made alkaline to dissolve ferrocyanide. The sample is then filtered to remove any insolubles. The filtrate is acidified and ferrous/ferric reagent added. After 15 minutes, the intensity of the blue color produced is measured at 700 nm using a spectrophotometer equipped with a tungsten lamp.

Use of this method requires handling potentially hazardous chemicals. Material Safety Data Sheets (MSDS) should be consulted for each chemical before use. These can be obtained from each chemical supplier.

PRECISION AND BIAS
A. Repeatability Standard Deviation, 1\( \sigma \), and 95 Percent Confidence Estimate (not including Bias)

Repeatability standard deviation is an estimate of the variability one trained analyst should be able to obtain under favorable conditions (analyzing a sample, with one instrument, within one day).

The 95 percent confidence estimate (calculated using the repeatability standard deviation) around a single test result will include the mean value 95 percent of the time.

To obtain the repeatability data, a single skilled analyst performed five (5) replicates on each of the following solutions during methods development.

1. An effluent sample analyzed as received, at 6.85 mg/L \( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} \)

2. The same effluent sample as in number 1, above, reanalyzed after making an analytically weighed, standard addition of 2.36 mg/L \( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} \).

<table>
<thead>
<tr>
<th>Potassium Ferrocyanide, trihydrate</th>
<th>Sample</th>
<th>Mean mg/L ( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} )</th>
<th>N</th>
<th>Repeatability Standard Deviation, 1( \sigma ) mg/L ( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} )</th>
<th>95 Percent Confidence Estimate mg/L ( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent</td>
<td>6.85</td>
<td>5</td>
<td>0.123</td>
<td>± 0.34</td>
<td></td>
</tr>
<tr>
<td>Effluent plus Standard Addition</td>
<td>9.40</td>
<td>5</td>
<td>0.105</td>
<td>± 0.29</td>
<td></td>
</tr>
</tbody>
</table>

Bias
Bias is a statistically significant deviation of the mean from the known mix level at a 95 percent confidence level. It is determined for fresh samples only. Bias was not determined for this sample because the component concentration level was not determined independently of the test method.

Bias was not determined, since this is an effluent sample with an unknown level of potassium ferrocyanide trihydrate.

Recovery
Recovery is used for seasoned samples, since the component concentration level was not determined independently of the test method. It is defined as the calculated mean for the seasoned sample with a standard addition of the component minus the mean for the seasoned sample, divided by the actual amount of the standard addition. It is expressed as a percentage.

Statistically the recovery of 108.05 percent was significantly different from 100 percent at the 95 percent confidence level, but was judged not to be practically significant.
B. REPRODUCIBILITY

Customer Standard Deviation, $1s_c$ & 95 Percent Confidence Estimate (not including bias)

Reproducibility or customer standard deviation ($1s_c$) is an estimate of the variability a customer could expect when submitting a sample to any Photoprocessing Quality Services laboratory, where any trained analyst could test the sample using any instrument on any day.

The 95 percent confidence estimate (calculated using the customer standard deviation) around a single test result will include the mean value 95 percent of the time.

Four analysts analyzed two effluent samples, on two different days. Duplicate analyses were performed on each sample, on each of the two days. These samples were:

1. An effluent sample was analyzed as received as 6.57 mg/L $K_4Fe(CN)_6\cdot3H_2O$.

2. The same effluent sample, as in number 1, above, analyzed in the same manner, after making a standard addition of 2.68 mg/L $K_4Fe(CN)_6\cdot3H_2O$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/L $K_4Fe(CN)_6\cdot3H_2O$</th>
<th>N</th>
<th>Reproducibility Standard Deviation, $1s_r$ mg/L $K_4Fe(CN)_6\cdot3H_2O$</th>
<th>95 Percent Confidence Estimate mg/L $K_4Fe(CN)_6\cdot3H_2O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent</td>
<td>6.57</td>
<td>16</td>
<td>0.207</td>
<td>± 0.44</td>
</tr>
<tr>
<td>Effluent plus Standard Addition</td>
<td>9.67</td>
<td>16</td>
<td>0.340</td>
<td>± 0.72</td>
</tr>
</tbody>
</table>

**Bias**

Bias is a statistically significant deviation of the mean from the known mix level at a 95 percent confidence level. It is determined for fresh samples only. Bias was not determined for this sample because the component concentration level was not determined independently of the test method.

Bias was not determined, since this is an effluent sample with an unknown level of potassium ferrocyanide trihydrate.

**Recovery**

Recovery is used for seasoned samples, since the component concentration level was not determined independently of the test method. It is defined as the calculated mean for the seasoned sample with a standard addition of the component minus the mean for the seasoned sample, divided by the actual amount of the standard addition. It is expressed as a percentage.

Statistically, the recovery of 115.67 percent was significantly different from 100 percent at the 95 percent confidence level, however it was judged not to be practically significant.
APPARATUS
- Pipet (40-mL)
- Graduated Cylinder (100 mL)
- (2) Beakers (150 mL)
- Conical Flask (250-mL)
- Filter apparatus

All volumetric glassware should meet all Class A specifications, as defined by American Society for Testing and Materials (ASTM) Standards E 287, E288, and E969, unless otherwise stated.

REAGENTS
All reagents should be ACS Reagent Grade unless otherwise specified.
- 2.5 N Sodium hydroxide, NaOH
- Concentrated Hydrochloric acid, HCl
- Ferrous/Ferric Reagent
- Water, Type I Reagent - This method was developed using reagent water equivalent to or purer than Type I grade, as defined in ASTM Standard D 1193. Other grades of water, e.g., reverse osmosis (RO), demineralized, or distilled water, may give equivalent results, but the effects of water quality on method performance have not been studied.

PROCEDURE
1. Pipet (wipe the pipet before leveling) 25.0 mL of effluent sample (Solution A) into a 250 mL volumetric flask. Dilute to volume with reagent water; stopper and invert the flask several times to mix (this is solution B).
2. Add, from a graduated cylinder, 100 mL of the diluted sample (Solution B) from step 1 to a 250 mL conical flask, and make it alkaline by adding 10 drops of 2.5 N sodium hydroxide.
3. Mix thoroughly and filter the solution through Whatman 2V filter paper.
4. Make the filtrate acid by the dropwise addition of concentrated hydrochloric acid. (Use 0-14 pH indicating paper as an indicator. The paper will turn red when solution is acidic.)
5. Pipet (wipe the pipet before leveling) 40.0 mL of the filtrate into each of two 150 mL beakers. (One will be the sample and the other will be the blank.)
6. Add 2 drops of ferrous/ferric reagent to the first beaker (sample); allow both beakers to stand for 15 minutes.
7. If a blue color is apparent, rinse and fill a 1-cm silica cell with solution from the first 150 mL beaker (step 6). Rinse the outer faces of the silica cell with reagent water and wipe dry with a clean tissue. Measure the absorbance of the sample vs. air at 700 nm on a spectrophotometer equipped with a tungsten lamp. Record this reading as $A_{700\text{ spl}}$.

Note: If no blue color is produced by Solution B in the first beaker in Step 6, repeat Steps 2 through 8 using the undiluted effluent sample (Solution A).
8. Rinse and fill the 1-cm silica cell from step 7 with the filtrate in the second 150 mL beaker from step 5. Measure the absorbance of this solution as in step 7. Record this reading as $A_{700\text{ blk}}$. 
Calculations

\[ y = mx + b \]

Definition of the equation is found in the Regression section of Appendix A.

\[ \text{mg/L Fe(CN)}_6^{4-} = m (A_{700 \text{ spl}} - A_{700 \text{ blk}}) + b \]

Where:

- \( m \) = slope of the calibration line
- \( A_{700 \text{ spl}} - A_{700 \text{ blk}} \) = absorbance of sample at 700 nm minus the absorbance of blank at 700 nm
- \( b \) = the intercept of the calibration line with the y-axis (mg/L Fe(CN)_6^{4-})

Each laboratory should establish its own regression equation based on a set of calibration standards. Appendix A explains this calibration procedure. The regression equation may be different for each spectrophotometer. A typical regression equation line for the effluent is described by the following equation:

\[ \text{mg/L Fe(CN)}_6^{4-} = 28.942 (A_{700 \text{ spl}} - A_{700 \text{ blk}}) - 0.2072 \]

\[ \text{mg/L K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} = \text{mg/L Fe(CN)}_6^{4-} \times 1.99 \]

\[ \text{mg/L Na}_4\text{Fe(CN)}_6\cdot10\text{H}_2\text{O} = \text{mg/L Fe(CN)}_6^{4-} \times 2.28 \]

Example of calculations:

Spectrophotometric readings: \( A_{\text{spl}} = 0.146 \quad A_{\text{blk}} = 0.020 \)

\[ \text{mg/L Fe(CN)}_6^{4-} = 28.942 (0.146 - 0.020) - 0.2072 \]

\[ = 28.942 (0.126) - 0.2072 \]

\[ = 3.44 \]

\[ \text{mg/L K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} = 3.44 (1.99) \quad = 6.85 \]

\[ \text{mg/L Na}_4\text{Fe(CN)}_6\cdot10\text{H}_2\text{O} = 2.44 (2.28) \quad = 7.84 \]

Appendix A

Calibration of Spectrophotometer for Ferrocyanide in Effluents

This Appendix should be used to establish the initial calibration equation, whenever the instrument has been adjusted, or to recheck the calibration (at least every six months)

A. Preparation of Standards

A 4.0 g/L Fe(CN)_6^{4-} stock standard solution is prepared by dissolving 8.0 grams of potassium ferrocyanide trihydrate (K_4Fe(CN)_6·3 H_2O) in a 1 liter volumetric flask and diluting to the mark with reagent water.

1. Pipet 10.0 mL of this stock standard solution to a second 1 liter volumetric flask and dilute to volume with reagent water. This solution contains 0.040 g/L of Fe(CN)_6^{4-}.

2. Label five 100 mL volumetric flasks as follows: 0.0 mg/L; 1.0 mg/L; 2.5 mg/L; 5.0 mg/L; and 10.0 mg/L.

3. Using a 25 mL buret, add the following amounts of 0.040 g/L ferrocyanide standard solution to the flasks as indicated:

<table>
<thead>
<tr>
<th>Volumetric Flask Identification</th>
<th>Volume of Standard Solution, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mg/L</td>
<td>0.0</td>
</tr>
<tr>
<td>1.0 mg/L</td>
<td>2.5</td>
</tr>
<tr>
<td>2.5 mg/L</td>
<td>6.3</td>
</tr>
<tr>
<td>5.0 mg/L</td>
<td>12.5</td>
</tr>
<tr>
<td>10.0 mg/L</td>
<td>25.0</td>
</tr>
</tbody>
</table>

4. Dilute to volume with reagent water.
B. Analysis of Standards

1. Run each sample by the method described in the preceding Procedure.

2. Table of Data gathered from Analysis of Standards

<table>
<thead>
<tr>
<th>Standard (g/L Fe(CN)$_6^{3-}$)</th>
<th>ABS$_{spl}$</th>
<th>ABS$_{blk}$</th>
<th>Net ABS@700 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.038</td>
<td>0.038</td>
<td>0.000</td>
</tr>
<tr>
<td>1.0</td>
<td>0.078</td>
<td>0.038</td>
<td>0.040</td>
</tr>
<tr>
<td>2.5</td>
<td>0.133</td>
<td>0.038</td>
<td>0.095</td>
</tr>
<tr>
<td>5.0</td>
<td>0.236</td>
<td>0.038</td>
<td>0.198</td>
</tr>
<tr>
<td>10.0</td>
<td>0.380</td>
<td>0.038</td>
<td>0.342</td>
</tr>
</tbody>
</table>

C. Regression

1. This data was processed by a least squares linear regression to develop the line represented by equation, $y = mx + b$:

   $$y = mg/L \text{Fe(CN)}_6^{3-}$$

2. The equation generated using the above data was:

   $$\text{Fe(CN)}_6^{3-}, \text{mg/L} = 28.942 \times (\text{Net ABS@700 nm}) - 0.2072$$

3. The calibration equation was done in the following manner on a (SHIMADZU Model UV 160 U) spectrophotometer. Five fresh solutions were prepared (see Step #1 of Preparation of Standards). Each solution was analyzed to create a linear regression, based on 5 data points, for each spectrophotometer being used. Each laboratory should calibrate their spectrophotometer, otherwise an unknown bias may exist.