Determination of Ferrous Iron in EASTMAN Color Films, “KUL” Bleach
ECN-0007/1
ECP-0007/1

INTRODUCTION
The determination of iron(II) in EASTMAN Color Films, Process ECN-2 or Process ECP-2D, KUL Bleach is accomplished by a spectrophotometric technique. The iron(II) concentration is determined by adding a sample to deaerated 1,10-phenanthroline/sodium acetate to form a colored complex. The absorbance of this complex is then measured at 510 nm on a spectrophotometer. Bleach samples should be run as quickly as possible, because the iron(II) content of closed samples increases gradually upon standing. The 1,10-phenanthroline/sodium acetate is deaerated with nitrogen to minimize oxidation of the iron(II) in the sample as the colored complex is formed. This method is calibrated between 0.25 and 3.0 g/L iron(II).

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

PRECISION AND BIAS
Repeatability Standard Deviation, 1s, 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 will include the mean value 95 percent of the time.

To obtain the repeatability data, a single skilled analyst performed three (3) replicates on a “fresh” EASTMAN Color Films, Process ECN-2 or Process ECP-2D, “KUL” bleach and five (5) replicates on each of a “seasoned” and a “seasoned” plus standard addition EASTMAN Color Films, Process ECN-2 or Process ECP-2D, “KUL” bleach solutions during methods development:

<table>
<thead>
<tr>
<th>Sample (Process ECN-2 KUL Bleach)</th>
<th>Mean (g/L Iron(II)) (N)</th>
<th>Repeatability Standard Deviation, 1s, (g/L Iron(II))</th>
<th>95 Percent Confidence Estimate (g/L Iron(II))</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Fresh” at (1.012 g/L Iron(II))</td>
<td>1.011 3 0.003</td>
<td>0.003</td>
<td>± 0.013</td>
</tr>
<tr>
<td>“Seasoned”, As Received</td>
<td>0.367 5 0.006</td>
<td>0.006</td>
<td>± 0.017</td>
</tr>
<tr>
<td>“Seasoned” with Standard Addition</td>
<td>0.462 5 0.008</td>
<td>0.008</td>
<td>± 0.022</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample (Process ECP-2D KUL Bleach)</th>
<th>Mean (g/L Iron(II)) (N)</th>
<th>Repeatability Standard Deviation, 1s, (g/L Iron(II))</th>
<th>95 Percent Confidence Estimate (g/L Iron(II))</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Fresh” at (1.024 g/L Iron(II))</td>
<td>1.017 3 0.007</td>
<td>0.007</td>
<td>± 0.030</td>
</tr>
<tr>
<td>“Seasoned”, As Received</td>
<td>0.504 5 0.005</td>
<td>0.005</td>
<td>± 0.015</td>
</tr>
<tr>
<td>“Seasoned” with Standard Addition</td>
<td>0.656 5 0.003</td>
<td>0.003</td>
<td>± 0.009</td>
</tr>
</tbody>
</table>
Bias
Bias is a statistically significant deviation from the known level at a 95 percent confidence level. It is determined for “fresh” samples only. Bias is not determined for “seasoned” samples, since the component concentration level was not determined independent of the test method.
No statistically significant bias was found for the Process ECN-2 or Process ECP-2D “fresh” tank samples.

Recovery
Recovery is used instead of bias 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. The recovery was not statistically different from 100 percent.

Reproducibility
Customer Standard Deviation, $1_{sc}$ & 95 Percent Confidence Estimate (not including bias)
Three EASTMAN Color Films KUL bleach samples were analyzed by four trained analysts, each using different titration stations, on two different days. Duplicate analyses were performed on each sample, on each of two days. These samples were:

1. A “fresh” tank solution prepared at:
   - 0.509 g/L iron(II) for Process ECN-2
   - 0.516 g/L iron(II) for Process ECP-2D

2. An EASTMAN Color Films “seasoned” tank KUL Bleach sample analyzed, in the same manner as the “fresh” sample, as received at:
   - 0.449 g/L iron(II) for Process ECN-2
   - 0.486 g/L iron(II) for Process ECP-2D

3. The same (as in number 2, above) EASTMAN Color Films “seasoned” tank KUL Bleach sample, analyzed after making an analytically weighed, standard addition of:
   - 0.158 g/L iron(II) for Process ECN-2
   - 0.159 g/L iron(II) for Process ECP-2D

The Reproducibility or customer standard deviation ($1_{sc}$) 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.

<table>
<thead>
<tr>
<th>Samples (Process ECN-2 KUL Bleach)</th>
<th>Mean (g/L Iron(II))</th>
<th>(N)</th>
<th>Reproducibility Standard Deviation, $1_{sc}$ (g/L Iron(II))</th>
<th>95 Percent Confidence Estimate (g/L Iron(II))</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Fresh” at (0.509 g/L iron(II))</td>
<td>0.514</td>
<td>16</td>
<td>0.014</td>
<td>± 0.030</td>
</tr>
<tr>
<td>“Seasoned”, as received</td>
<td>0.449</td>
<td>16</td>
<td>0.029</td>
<td>± 0.062</td>
</tr>
<tr>
<td>“Seasoned” with standard addition</td>
<td>0.604</td>
<td>16</td>
<td>0.033</td>
<td>± 0.070</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples (Process ECP-2D KUL Bleach)</th>
<th>Mean (g/L Iron(II))</th>
<th>(N)</th>
<th>Reproducibility Standard Deviation, $1_{sc}$ (g/L Iron(II))</th>
<th>95 Percent Confidence Estimate (g/L Iron(II))</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Fresh” at (0.516 g/L iron(II))</td>
<td>0.503</td>
<td>16</td>
<td>0.031</td>
<td>± 0.066</td>
</tr>
<tr>
<td>“Seasoned”, as received</td>
<td>0.486</td>
<td>16</td>
<td>0.025</td>
<td>± 0.053</td>
</tr>
<tr>
<td>“Seasoned” with standard addition</td>
<td>0.639</td>
<td>16</td>
<td>0.036</td>
<td>± 0.077</td>
</tr>
</tbody>
</table>

APPARATUS
All volumetric glassware should meet all “Class A” specifications, as defined by American Society for Testing and Materials (ASTM) Standards E 287, E 288, and E 969, unless otherwise stated.
- Double Beam Spectrophotometer equipped with a tungsten lamp (i.e., PERKIN-ELMER Lambda 4 series)
- Two, 1-cm silica cells
- 200-µL EPPENDORF micropipet or equivalent micropipet

REAGENTS
All reagents should be ACS Reagent Grade unless otherwise specified.
- 1,10-Phenanthroline/Sodium Acetate reagent
- Ferrous Ammonium Sulfate Hexahydrate, Fe(NH₄)₂(SO₄)₂ • 6H₂O
- Water, Type I Reagent - This method was developed, and the statistical data were obtained, 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

Blank
1. Set a double-beam spectrophotometer to a wavelength of 510 nm.
2. Rinse two, clean 1.0-cm cells with reagent water, at least three times. Fill the cells with reagent water and dry the outside surfaces with a tissue.
3. Place both cells into the double-beam spectrophotometer and zero the instrument.
4. Leave the reference cell in the instrument and remove the sample cell.

Sample Preparation
1. Bubble nitrogen through the 1,10-phenanthroline/sodium acetate reagent, contained in a conical flask, for 15 minutes. Only de-gas 5 mL of reagent per sample plus 30 mL excess.
2. Using a serological pipet, add 5 mL of the deaerated 1,10-phenanthroline/sodium acetate reagent to a 100-mL volumetric flask.
3. Pipet 200-µL of sample into the flask with a micropipet (EPPENDORF or equivalent) containing the 1,10-phenanthroline/sodium acetate. Swirl the flask to mix.
4. Dilute to volume with reagent water. Invert the flask, 6-10 times, to thoroughly mix the solution.
5. Within 20 seconds, rinse the 1.0-cm cell from the spectrophotometer sample compartment, at least three times, with the above solution, and fill the cell. Rinse the outer surfaces of the cell with reagent water and dry with a tissue.
6. Place the cell into the sample compartment of the spectrophotometer. Measure and record the absorbance at 510 nm vs. reagent water.

Calculations
Iron(II), g/L = m (A_{510}) + b

where:
- m = slope of the calibration line (in g/L/Abs. units)
- A_{510} = absorbance (ABU) of sample at 510 nm
- b = the calibration intercept of the line with the y-axis (g/L, Fe(II))

Each laboratory should establish its own calculation based on a linear regression of a set of calibration standards. APPENDIX A explains this calibration procedure. The regression line may be different for each spectrophotometer.

APPENDIX A

Calibration of Spectrophotometer For Iron(II)
Use this Appendix to recheck the spectrophotometer linear calibration for iron(II) on a regular basis of at least every 6 months. Also, use it the first time this method is performed and whenever the spectrophotometer has been repaired.

Preparation of Standards
A litre of “fresh” Process ECN-2 or Process ECP-2D, “KUL” Bleach solution should be prepared that contains all the constituents of the mix at the aim tank concentrations.

1. For the iron(II) standards in Process ECN-2 or Process ECP-2D, “KUL” Bleach, weigh out 0.088, 0.410, 0.740, and 1.065 g portions of assayed ferrous ammonium sulfate, hexahydrate (see APPENDIX B for assay procedure) to the nearest 0.0001 g.

2. Quantitatively transfer each portion of ferrous ammonium sulfate to 50-mL volumetric flasks, respectively labeled with the corresponding weights, with the “fresh” Process ECN-2 or Process ECP-2D, “KUL” Bleach mix. Swirl the flasks to dissolve the ferrous ammonium sulfate. (NOTE: the iron(II) material takes some time to dissolve.)

3. When the ferrous ammonium sulfate has dissolved, fill each volumetric to the mark with the “fresh” Process ECN-2 or Process ECP-2D, “KUL” Bleach mix. Invert each flask, 6-10 times, to thoroughly mix the solutions.

4. Run each sample in (at least) duplicate by the method described in the preceding PROCEDURE.

Determination of Iron(II)
The calculation to determine the amount of iron(II) in the standards from the weighed amount of ferrous ammonium sulfate is as follows:

\[
g_{\text{L}} \text{iron(II)} = \frac{g \text{ wt of Fe(NH}_4\text{)}_2\text{(SO}_4\text{)}_2 \cdot 6\text{H}_2\text{O}}{50 \text{ mL}} \times \frac{55.85}{391.85} \times \frac{1000 \text{ mL}}{1 \text{ L}}
\]

where:
- g wt of Fe(NH}_4\text{)}_2\text{(SO}_4\text{)}_2 \cdot 6\text{H}_2\text{O} = weight recorded in step 1 of Preparation of Standards
- 55.85 = atomic weight of iron
- 391.85 = molecular weight of ferrous ammonium sulfate, 6-hydrate
- 1000 mL/1L = conversion factor from mL to L

If the assay from APPENDIX B is not 100 percent, the result of the above calculation must be multiplied by the wt/wt%, iron (II) assay value to obtain the correct value.
Regression

The data collected may be used to construct the regression equation for the calibration line.

The reliability of the calibration line generated was done in the following manner on a SHIMADZU Model UV160U Spectrophotometer. Four “fresh” ECN-2 and ECP-2D, “KUL” bleach solutions were prepared containing 0.2515, 1.0235, 2.0296, and 3.0401 g/L iron(II). Each solution was analyzed in triplicate to create a linear regression (based on 12 data points). The average standard deviation (1s) for the linear regression was 0.007 g/L iron(II), corresponding to a 95 percent confidence estimate of ± 0.015 g/L. Each laboratory should calibrate its spectrophotometer; otherwise, an unknown bias may exist.

Process ECN-2

<table>
<thead>
<tr>
<th>g wt of Fe(NH₄)₂(SO₄)₂ · 6H₂O</th>
<th>g/L Iron(II)</th>
<th>Absorbance at 510 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0887</td>
<td>0.2520</td>
<td>0.117</td>
</tr>
<tr>
<td>0.0887</td>
<td>0.2520</td>
<td>0.114</td>
</tr>
<tr>
<td>0.0887</td>
<td>0.2520</td>
<td>0.117</td>
</tr>
<tr>
<td>0.3562</td>
<td>1.0121</td>
<td>0.422</td>
</tr>
<tr>
<td>0.3562</td>
<td>1.0121</td>
<td>0.420</td>
</tr>
<tr>
<td>0.7101</td>
<td>2.0177</td>
<td>0.835</td>
</tr>
<tr>
<td>0.7101</td>
<td>2.0177</td>
<td>0.821</td>
</tr>
<tr>
<td>1.0658</td>
<td>3.0381</td>
<td>1.239</td>
</tr>
<tr>
<td>1.0658</td>
<td>3.0381</td>
<td>1.231</td>
</tr>
<tr>
<td>1.0658</td>
<td>3.0381</td>
<td>1.234</td>
</tr>
</tbody>
</table>

Process ECP-2D

<table>
<thead>
<tr>
<th>g wt of Fe(NH₄)₂(SO₄)₂ · 6H₂O</th>
<th>g/L Iron(II)</th>
<th>Absorbance at 510 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0885</td>
<td>0.2515</td>
<td>0.132</td>
</tr>
<tr>
<td>0.0885</td>
<td>0.2515</td>
<td>0.129</td>
</tr>
<tr>
<td>0.0885</td>
<td>0.2515</td>
<td>0.132</td>
</tr>
<tr>
<td>0.3602</td>
<td>1.0235</td>
<td>0.435</td>
</tr>
<tr>
<td>0.3602</td>
<td>1.0235</td>
<td>0.435</td>
</tr>
<tr>
<td>0.7143</td>
<td>2.0296</td>
<td>0.844</td>
</tr>
<tr>
<td>0.7143</td>
<td>2.0296</td>
<td>0.842</td>
</tr>
<tr>
<td>0.7143</td>
<td>2.0296</td>
<td>0.840</td>
</tr>
<tr>
<td>1.0665</td>
<td>3.0401</td>
<td>1.250</td>
</tr>
<tr>
<td>1.0665</td>
<td>3.0401</td>
<td>1.245</td>
</tr>
<tr>
<td>1.0665</td>
<td>3.0401</td>
<td>1.255</td>
</tr>
</tbody>
</table>

This data was then processed by a least squares linear regression to develop the line, y = mx + b. where:

\[ y = \text{grams per litre of iron(II)} \]
\[ m = \text{Slope of the line or the relation between absorbance and iron(II) concentration determined during calibration [(g/L)/absorbance]} \]
\[ x = \text{absorbance of sample at 510 nm} \]
\[ b = \text{the calibration intercept of the line with the y-axis (g/L, Fe(II))} \]

The equation generated using the above data was:

\[ \text{iron(II), g/L} = 2.49 \times (A_{510}) - 0.068 \]
APPENDIX B
This appendix contains two assay procedures for Ferrous Ammonium Sulfate. The first procedure uses Potassium Dichromate; while the alternate procedure uses Potassium Permanganate titrant.

Assay Procedure for Ferrous Ammonium Sulfate, Hexahydrate

Reagents
All reagents are ACS Reagent Grade unless otherwise stated.
- Potassium Dichromate, K₂Cr₂O₇, NIST Primary Standard, 136e, dried for 2 hours at 110°C
- Ferroin Indicator, 1,10-Phenanthroline Iron(II) Sulfate solution (0.025 M) [(C₁₂H₈N₂)₃FeSO₄]
- Sulfuric Acid, concentrated, H₂SO₄
- Ferrous Ammonium Sulfate-6-Hydrate, Fe(NH₄)₂(SO₄)₂ • 6H₂O

Procedure
1. Weigh 14.0 g of ferrous ammonium sulfate, hexahydrate to the nearest 0.0001 g and record the weight.
2. Quantitatively transfer to a 200-mL volumetric flask and swirl the flask to dissolve the ferrous ammonium sulfate.
3. Dilute the solution to the mark with reagent water. Invert the flask, 6-10 times, to thoroughly mix.
4. Weigh 1.0 g of dried, potassium dichromate to the nearest 0.0001 g and record the weight.
5. Quantitatively transfer to a 100-mL volumetric flask containing 50 mL of reagent water. Swirl the flask to dissolve the potassium dichromate.
6. Dilute the solution to the mark with reagent water. Invert the flask, 6-10 times, to mix thoroughly.
7. Fill a 50-mL buret with the ferrous ammonium sulfate solution (step 3, above).
8. Add 20 mL concentrated sulfuric acid to a 150-mL beaker containing 25 mL of reagent water and a magnetic stirring bar.
9. Pipet 15.0 mL of the potassium dichromate solution into the beaker.
10. Add 2 drops of ferroin indicator to the beaker and place the beaker on a magnetic stirrer.
11. Slowly add the ferrous ammonium sulfate solution from the buret to the beaker. The solution will change colors as the titrant is added, going from a green-blue color to a reddish-brown color. The point that produced the first reddish-brown color change is the end point.
12. Record the volume of ferrous ammonium sulfate titrant consumed at the end point to the nearest 0.05 mL.
13. Repeat steps 7-12, two more times.

Calculations

N Potassium Dichromate

\[ N_{K_2Cr_2O_7} = \frac{g \text{ wt of } K_2Cr_2O_7}{100 \text{ mL}} \times \frac{1000}{294.2/6} \]

where:
- 294.2/6 = the eq. wt of K₂Cr₂O₇
- g wt of K₂Cr₂O₇ = recorded wt from step 4
- 1000 = factor to convert equivalents to milliequivalents

N of Ferrous Ammonium Sulfate

\[ N_{Fe(NH_4)_2(SO_4)_2} = \frac{(mL K_2Cr_2O_7)(N K_2Cr_2O_7)}{mL Fe(NH_4)_2(SO_4)_2} \]

where:
- mL Fe(NH₄)₂(SO₄)₂ = average volume of the three titrations required to reach the end point color change
- mL K₂Cr₂O₇ = 15.0 mL
- N K₂Cr₂O₇ = normality calculated in N Potassium Dichromate

* The average volume for the three titrations should not have a standard deviation (1s) greater than 0.50 mL.

\[ g/L, \text{ Iron(II) in Ferrous Ammonium Sulfate} \]

\[ g/L, \text{ Iron(II) (actual)} = N_{Fe(NH_4)_2(SO_4)_2} \times 55.85 \]

where:
- 55.85 = gram-equivalent weight of Iron(II) in Fe(NH₄)₂(SO₄)₂ • 6H₂O

Theoretical wt of Ferrous in Solution

\[ \frac{g/L}{\text{Iron(II)}} = \frac{\text{wt of } Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O}{200 \text{ mL}} \times \frac{55.85}{391.85} \times \frac{1000 \text{ mL}}{1L} \]

where:
- 391.85 = molecular weight of ferrous ammonium sulfate, 6-hydrate
- 55.85 = atomic weight of iron
- wt of Fe(NH₄)₂(SO₄)₂ • 6H₂O = weight recorded in step 1 of this procedure.
- 1000 mL = factor to convert mL to L

Assay Percentage

\[ \%\text{Iron(II)} = \frac{g/L \text{ Iron(II) (actual)}}{g/L \text{ Iron(II) (theoretical)}} \times 100 \]
Alternate Assay Procedure for Ferrous Ammonium Sulfate, Hexahydrate

Note: This procedure is based on the method in Reagent Chemicals, 8th Edition, Amercian Chemical Society, 1993.

Assay Procedure

Note: This procedure should be repeated in triplicate with the average of the three results used as the assay value.

1. Accurately weigh 1.6 grams of sample to the nearest milligram and dissolve in a mixture of 100 mL reagent water and 3 mL of concentrated sulfuric acid contained in a 250 mL Erlenmeyer flask.

Caution
Always add acid to water and not water to acid.

2. Titrate while stirring with standardized 0.1 N potassium permanganate (KMnO₄) from a 50 mL buret to a permanent faint pink endpoint that lasts for at least 15 seconds.

3. Repeat steps 1 and 2 without any sample. This is the blank.

Calculations

% (wt./wt.) ferrous ammonium sulfate, hexahydrate =

\[
\frac{(\text{mL KMnO}_4 \text{ sample} - \text{mL KMnO}_4 \text{ blank}) \times N \text{ KMnO}_4 \times 0.3921 \times 100}{\text{sample size in grams}}
\]