Revised material in Section 4, ***Analytical Chemistry***

Fe-01-RC: **IRON IN AQUEOUS SAMPLES - DUAL-DPM MODE LIQUID SCINTILLATION ANALYSIS**

G-04: **PREPARATION OF MICROPRECIPITATION SOURCES FOR REANALYSIS**

Fe-01-RC

IRON IN AQUEOUS SAMPLES - DUAL-DPM MODE LIQUID SCINTILLATION ANALYSIS

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APPLICATION

The procedure is applicable for the determination of ^{55}Fe in water samples prepared for the EML Quality Assessment Program (QAP, Greenlaw, 1998). The ^{55}Fe, which decays by electron capture, is determined from an aliquot of the spiked solution containing a mixture of alpha, beta and gamma emitting radionuclides. Beta emitting ^{59}Fe (E_{max} = 0.475 MeV) tracer is added to the sample as the yield determinant prior to the precipitation of $Fe(OH)_3$. Following two anion exchange separations to remove interferences, the sample activities (as $FePO_4$) are measured in a commercially available liquid scintillation counter that is operated in the dual-dpm mode. A single count, in a calibrated instrument, provides the quench corrected activity concentrations of ^{55}Fe in a sample based on recovered ^{59}Fe (Scarpitta and Fisenne, 1996). The procedure has been adapted from an ASTM (1990) method.

SPECIAL APPARATUS

1. Disposable ion exchange columns, pre-filled with AG1-X8 Resin (100 - 200 mesh) - Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547-9980, or equivalent.

2. Disposable 4.0 cm (h) x 0.6 cm (d) ion exchange columns (empty) - Bio-Rad Laboratories or equivalent.

3. Liquid scintillation counter - Packard Tri-Carb 2250CA, Packard Instrument Co., Downers Grove, IL 60515, or equivalent.

4. 20-mL low K borosilicate liquid scintillation glass vials.

5. Vortexer - Scientific Industries, Inc., Bohemia, NY 11716, or equivalent.

6. Centrifuge, IEC Clinical Model containing a four place trunnion-type horizontal rotor and 40-50 mL capacity shields, or equivalent.

SPECIAL REAGENTS

1. Anion exchange resin, AG1-X8 (50-100 mesh).

2. Cerium carrier: 1 mg mL^{-1} - $CeCl_3$ in 2M HCl.

3. Cesium carrier: 1 mg mL^{-1} - CsCl in 2M HCl.

4. Cobalt carrier: 1 mg mL^{-1} - $CoCl_2$ in 2M HCl.

5. Iron carrier: 5 mg mL^{-1} - $FeCl_3$ in 2M HCl.

6. Manganese carrier: 1 mg mL^{-1} - $MnCl_2$ in 2M HCl.

7. Strontium carrier: 1 mg mL^{-1} - $SrNO_3$ in 2M HCl.

8. Zinc carrier: 1 mg mL^{-1} - $ZnCl_2$ in 2M HCl.

9. 10M HCl - dilute 833 mL of conc HCl to1 L with water.

10. 6M HCl - dilute 500 mL of conc. HCl to1 L with water.

11. 4M HCl - dilute 333 mL of conc. HCl to1 L with water.

12. 0.5M HCl - dilute 42 mL of conc. HCl to1 L with water.

13. 0.01M HCl - dilute 20 mL of 0.05M HCl to1 L with water.

14. 8M HNO_3 - dilute 500 mL of conc. HNO_3 to1 L with water.

15. Ultima Gold liquid scintillation cocktail - Packard Instrument Co., Downers Grove, IL 60515.

16. Ammonium phosphate, 0.5M - dissolve 66 g of $(NH_4)_2HPO_4$ in 1 L of H_2O.

17. Quenching agent (e.g., chloroform, nitromethane, carbon tetrachloride, etc.).

18. ^{59}Fe tracer solution in 0.1 N HCl, about 17 Bq gm^{-1} in a dispensing bottle (1.7 Bq per sample).

19. ^{55}Fe standard solution - NIST SRM or equivalent.

20. Aerosol OT solution - 1%.

SEPARATION

1. Weigh out 1.7 Bq (~100 dpm) of ^{59}Fe tracer for each sample and reference standard.

2. Add the ^{59}Fe yield tracer, 1 mL (5 mg mL^{-1}) of Fe^{+3} carrier solution and 1 mL each (1 mg mL^{-1}) of Ce, Cs, Co, Mn, Sr and Zn holdback carriers to an aliquot of the sample in a 40-mL centrifuge tube (see **Note 1**).

3. Prepare a reagent blank by adding the Fe^{+3} carrier and the holdback carriers in a 40-mL centrifuge tube, but **without** ^{59}Fe tracer.

4. Add 1.7 Bq of ^{59}Fe tracer and 1 mL of Fe^{+3} carrier to a 20 mL glass scintillation vial labeled "^{59}Fe Reference Tracer." Set the "^{59}Fe Reference Tracer" aside until Step 18.

5. Add NH_4OH to the samples and blank until the pH is >10 to precipitate the combined hydroxides. Digest in a hot water bath with occasional stirring for ~ 20 min. Cool,

add several drops of 1% Aerosol solution and centrifuge at 2000 rpm for approximately 5 min. Decant and discard the supernate.

6. Add 10 mL of water and breakup the precipitate with a stirring rod. Add several drops of 1% aerosol solution and centrifuge at 2000 rpm for ~ 2 min. Decant and discard the wash.

7. Dissolve the hydroxide precipitate in 3 mL of HCl and heat in a hot water bath with occasional stirring until the precipitate is completely dissolved, then cool the solution to room temperature.

8. Condition an ion-exchange column containing 3 mL of Bio-Rad AG1-X8 (100-200 mesh) resin by passing 30 mL of conc. HCl through the column. Tap the column to speed the flow and to assure the packing of the resin.

9. Pass the sample solution from Step 7 through the column. Collect the effluent in a 50- mL beaker.

10. Wash the column with 10 mL volumes of each of the following acids: 10M HCl; 6M HCl; and 4M HCl to remove Mn, Cs, Ce, Sr, and Co. Collect the three washes in the 50-mL beaker. Discard the effluent and combined washes.

11. Replace the 50-mL beaker with a clean 50 mL beaker. Elute the iron with exactly 10 mL of 0.01M HCl. The solution should be yellow.

12. Add 10 mL of HNO_3 to the eluted sample from Step 11 to produce an 8M HNO_3 solution.

13. Prepare a second ion-exchange column containing 3 mL of Bio-Rad AG1-X8 (50-100 mesh) resin and condition the resin by passing 30 mL of 8M HNO_3 through the column.

14. Pass the 8M HNO_3 sample solution through the second column. Collect the effluent in a 100 mL labeled beaker. Wash the column with10 mL of 8M HNO_3. Collect the wash with the eluent (the wash should completely remove the iron from the column

as evidenced by the absence of the yellow color in the effluent). Discard the column which contains plutonium, uranium and zinc.

15. Add 10 mL of 0.5M $(NH_4)_2HPO_4$ to the beaker containing the iron eluate and add NH_4OH until the pH is ~3.0. Gently heat the beaker on a hot plate for ~ 20 min with occasional stirring to completely precipitate iron as $FePO_4$.

16. Remove the beaker from the hot plate, cool, and transfer the mixture to a 40 mL centrifuge tube using H_2O to rinse the contents of the beaker into the centrifuge tube. Centrifuge at 2000 rpm for ~ 2 min. Decant and discard the supernate. Add 10 mL of H_2O, break up the precipitate with a stirring rod, and heat in a hot water bath for ~ 10 min. Cool, transfer the precipitate and wash to solution to a 20 mL scintillation vial whose cap has been labeled with the sample identification number. Use H_20 to rinse the contents of the centrifuge tube into the scintillation vial. Cap the vial and centrifuge the scintillation vial for ~ 2min (see **Note 2**). Decant and discard the wash.

17. Dissolve the precipitate in the scintillation vial in 2 mL of 0.5M HCl (the total volume should **not** exceed 2 mL of 0.5M HCl) and gently heat on a hot plate to obtain a clear solution.

18. Add 1 mL of 0.5M HCl to the ^{59}Fe reference tracer from Step 4 to bring the total volume to 2 mL, then add 6 drops of conc. phosphoric acid.

19. Dispense 15 mL of Ultima Gold liquid scintillation cocktail to each vial, cap, and vortex for 20 sec. Wipe the external surface of each vial with an alcohol soaked tissue. Allow the samples to dark adapt in the refrigerated liquid scintillation counter for 30 min prior to counting.

20. Proceed to **Determination**.

Notes:

1. If the sample aliquot volume is greater than the capacity of the centrifuge tube, use a beaker of appropriate capacity to accommodate the sample. Add the carriers and ^{59}Fe

yield tracer, evaporate on a hot plate to approximately 30 mL, transfer to a 40-mL centrifuge tube, then proceed to Step 5.

2. Centrifuge the sample in the scintillation vial using the specified centrifuge. The vial may be lifted from the shield with forceps.

DETERMINATION

A. Instrument window settings (see Notes 1 and 2).

1. Prepare unquenched ^{55}Fe and ^{59}Fe standards in the 20 mL scintillation vials.

2. Set the instrument energy regions for ^{55}Fe (Region A), ^{59}Fe (Region B) using the guidance found in the applicable liquid scintillation counter manual.

3. Optimize the energy regions using the guidance found in the instrument operating manual. Alternately, use Steps 4 to 6.

4. Perform a spectral analysis using the ^{55}Fe standard and visually adjust the energy range of Region A (0-7 keV, ^{55}Fe) to maximize the count rate and to minimize the width of the energy range. (**Note:** A balance between the count rate and the energy range should be obtained).

5. Perform a spectral analysis using the ^{59}Fe standard, and visually adjust the energy range of Region B (8-500 keV, ^{59}Fe) to maximize the width of the energy range (Note: A balance between the count rate and the energy range should be obtained).

6. Utilizing the full window, set a third region, Region C (0-2000 keV).

B. ^{55}Fe Calibration, Efficiency as a function of Quenching (see Notes 1, 2 and 3).

1. Weigh an aliquot (0.1 g) of an ^{55}Fe standard solution in a 20 mL scintillation vials. The aliquot of the tracer standard should contain sufficient activity (i.e., 17 Bq) to give a counting error of < 1% for a 15 min counting period.

2. Pipette various volumes of a quenching agent (i.e., nitromethane) into separately labeled vials, each containing the tracer standard, to obtain a range of quenching from high to low efficiency. (**Note:** The following are examples of quenching agent volumes: 0.01, 0.02, 0.03, 0.04, 0.06, 0.08 mL.) Record standard information (including: identification number, aliquot weight, activity concentration, reference date, etc.).

3. Prepare a vial labeled "zero" that does **not** contain a quenching agent.

4. Dispense enough scintillation cocktail into each glass vial to make up the final volumes equal to the sample set to be analyzed (e.g., 17 mL).

5. Cap and vortex the labeled scintillation vials for 10 to 20 sec. Clean the exterior of the vials by wiping with ethanol and a paper towel, and refrigerate in the dark for at least 15 min.

6. Load the six aqueous quench standards from Step 2 into the counting rack with the first sample labeled as "zero." Select the dual-dpm mode from the main program menu. Key in: a) the number of standards per set for ^{55}Fe, b) the activity per sample (dpm), c) the reference date of the standards as MM/DD/YY, d) the half-life of ^{55}Fe = 2.73 years, 23915 h, and e) low-level count mode (optional).

7. Set the instrument window for ^{55}Fe and ^{59}Fe as established in "instrument window settings."

8. Count the quench standards, typically for 15 min (or until 1% counting statistics are obtained), in each of the three regions selected on the liquid scintillation counter.

9. The net count rate for each quench standard vial is calculated automatically by subtracting the background (zero) count rate in Region A from the total count rates in Region A.

10. The efficiency (Eff) (see **Note 4**) is also determined automatically for each quenched sample in units of counts min^{-1}/disintegration min^{-1} by dividing the net activity

measured in count min^{-1} by the calculated activity added in dpm. Also, the uncertainty in the efficiency, sigma (Eff), should be estimated for each vial.

11. The efficiency curve is generated by the instrument which plots Ln(Eff) versus the QIP.

12. A least squares fit on the plot may be performed. The coefficients (a) and (b) or the equation

$$Eff = a \exp[b * QIP]]$$

(obtained from the intercept [Ln a] and the slope [b]), and the fitting coefficient, R^2, should be recorded.

13. A complete instrument calibration for ^{55}Fe should be performed annually **(Note 3).**

C. ^{59}Fe Calibration, Efficiency as a function of Quenching (see Notes 1, 2 and 3)

1. Calibrate the instrument for ^{59}Fe in an identical fashion to ^{55}Fe (Steps B1 to B12), except that a tracer solution of ^{59}Fe is used instead of the ^{55}Fe standard solution, and count rates are measured in Region B rather than Region A.

2. Establish a curve of the ratio of the count rate of each standard of ^{59}Fe in Region A (0-7 keV) to the count rate in Region B (8-500 keV) to QIP. This is to adjust the sample count rate of the ^{55}Fe in Region A because of spectral overlap of the ^{59}Fe into Region A.

3. Steps 6 to 11 of the ^{55}Fe calibration above should be followed, with the exception that the half-life of ^{59}Fe is used.

4. A complete instrument calibration for ^{59}Fe should be performed annually (see **Note 3**).

Notes:

1. Before counting the samples and blanks, the appropriate window settings are determined for each of the two regions of interest (i.e., ^{55}Fe, ^{59}Fe). Steps A1 to A6 are

performed once, after which the appropriate parameters are manually set in the liquid scintillation counting instrument.

2. Check the liquid scintillation counter's stability before running the standards and samples. Measure the instrument background and the ^{3}H and ^{14}C standards provided by the instrument manufacturer using the "SN" (system normalization) counting plug. The instrument software contains an appropriate spreadsheet program that compares the count rate of these standards with previously determined standard data according to criteria currently in use at EML.

3. A complete instrument recalibration is required if any major component of the instrument is replaced.

4. The efficiency curve is a plot of the counting efficiency as a function of the quench index parameter (QIP). The QIP is determined internally by the instrument, using a ^{133}Ba source, and is also known as the automatic external standardization (AES) number or the transformed spectral index of the external standard (t/SIE). A sample aliquot is prepared with a measured volume of a scintillation cocktail that is then placed in a programmed liquid scintillation analyzer, which is operated in the dual-dpm counting mode.

PREPARATION OF SAMPLE LIQUID SCINTILLATION COUNTING VIALS

1. Mark the appropriate glass liquid scintillation vials with the sample identification on the cap.

2. Dispense an appropriate amount (e.g., 15 mL) of the scintillation cocktail into each scintillation vial containing the dissolved sample from Step 17 (**Separation**). A separate vial containing 2 mL of water serves as "background" (**Note:** Check the samples for phase separation. If phase separation is evident, solubilizing or complexing agents will be required to produce a stable solution.). Clean the exterior of the vials by wiping with ethanol and a paper towel.

3. Load the samples into the counting rack with the the reagent blank from Step 3 (**Separation**) as the first sample in position “zero”.

4. Use the Packard Instrument counting protocol plug No. 1 which is delegated for $^{55}Fe/^{59}Fe$ counting.

5. Select the “use curve” option from the count mode menu under the dual-dpm mode when counting samples for ^{55}Fe.

CALCULATIONS

Calculate the ^{55}Fe activity in the sample on the reference date using the following equation:

$$^{55}\text{Fe Activity, Bq L}^{-1} = \frac{\left[a - \left(b * c\right)\right] * 1000}{E * W * D * R * 60}$$

where:

a = net count rate in the ^{55}Fe region of interest, cpm (Region A)

b = net count rate in the ^{59}Fe region of interest, cpm (Region B)

c = spectral overlap factor obtained from ^{59}Fe (Region A)/^{59}Fe (Region B) versus the quench curve

E = ^{55}Fe counting efficiency obtained from the ^{55}Fe quench curve

W = sample weight, g

D = decay factor, $e^{-0.693t/T}$, where

t = elapsed time from collection

T = half live of ^{55}Fe, 2.73 years

e = base of natural logarithms

R = ^{59}Fe recovery (dpm ^{59}Fe of sample/dpm ^{59}Fe of the reference tracer)

Alternately, when using the “dual-isotope” counting mode, calculations may be performed internally by the liquid scintillation software and the results are given in dpm.

LOWER LIMIT OF DETECTION (LLD)		
Counter Efficiency	(% Max)	47
Counter Background	(cps)	0.157 (0 - 7.0 keV)
Yield (^{59}Fe)	(%)	85
Blank (cps)		
LLD (60 min)	(Bq)	0.08

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G-04

PREPARATION OF MICROPRECIPITATION SOURCES FOR REANALYSIS

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APPLICATION

This procedure is applicable for the reanalysis of microprecipitation sources on Metricel filters (see Procedure G-03).

A 25 mm Metricel filter is removed from the sample mount and dry ashed in a temperature programmed oven that is held at a final temperature of 475°C for 6 h. The residue is redissolved in preparation for additional treatment.

SPECIAL APPARATUS

1. Temperature programmed oven.

2. Sample mounts.

SPECIAL REAGENTS

Analytical grade concentrated nitric acid.

SAMPLE PREPARATION

1. Remove the 25 mm Metricel filter from the mount using a pair of tweezers and place it in a small Pyrex beaker.

2. Place the beaker in a programmable oven.

3. Program the oven to ramp at 1.5°C min^{-1} to a final temperature of 475°C. Hold that temperature for 6 h.

4. Allow the sample to cool to room temperature before treating it any further.

5. Treat the residue with several additions of concentrated nitric acid (~5-10 mL), evaporating each addition (1-2 mL).

6. Redissolve the residue in the appropriate solution (based on the nuclide of interest).

7. The sample is now ready for further treatment or analysis (based on the nuclide of interest).