Applied Microbiology
&
Biotechnology Laboratory

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Standard Operating Procedure

AMBL-201-A

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Bromide by Ion Selective Electrode (ISE)

METHOD SUMMARY

This SOP describes the procedure for measuring soluble bromide ion in aqueous solutions. This method is based on Method 9211 (SW-846, US EPA).

ENVIRONMENTAL HEALTH AND SAFETY

Hazards Assessment: This method involves preparation of standards using potassium bromide having a typical purity of 98% or greater. Potassium bromide is not acutely toxic and presents a low toxicity hazard. However, working with the dry chemical or solutions having high concentrations present the following hazards.

Skin Contact: Causes skin irritation.

Eye Contact: Causes serious eye irritation.

Ingestion: Can cause nausea and vomiting at high dosages.

Inhalation: May cause respiratory irritation.

Potassium bromide ionizes to potassium and bromide ions, and is harmful to aquatic organisms.

Safety Equipment and Engineering Controls: This method requires that an eyewash station and shower be located nearby during the handling of the dry chemical.

Personal Protective Equipment (PPE): This method requires the use of the following PPE.

Gloves (nitrile, PVC or neoprene)

Safety goggles or glasses

Laboratory coat

Analysis-derived Wastes and Disposal:

Waste Generated	Hazardous (Y / N)	Disposal
This procedure generates expended and unused standard solutions containing no more than 40 mg/L bromide and 20 mg/L potassium.	N	At these maximum concentrations and small volumes generated (5 mL to 100 mL), disposal in a sink is acceptable.
This procedures generates small volumes (5 mL) of environmental samples.	N	Disposal in the sink is acceptable.

METHOD DESCRIPTION

1.0 Introduction and Applicability

This method use a bromide ion selective electrode to potentiometrically measure free bromide ions in aqueous solutions. The electrode used in this method is a combination electrode with sensing and reference half-cells in a single electrode body construction. This electrode allows for bromide ion analysis in small sample volumes.

This method is applicable to all natural surface waters, groundwater, municipal drinking water, domestic and some industrial wastewater and process waters. This method is not intended for samples having interfering substances such as high concentrations of polyvalent cations or chloride ion, and sulfide, cyanide or ammonia. All of these can interfere with the measurement of bromide and if suspected, the procedures given in SOP 201B should be followed.

2.0 Apparatus

a. Bromide combination ion selective electrode (This SOP was written for the Thermo Scientific Bromide ion plus Sure-flow Solid State ISE, 9635BNWP, but should be applicable to any combination ISE.)

b. pH/mV meter capable of reading to 0.1 mV or an ISE meter

c. Analytical balance capable of weighing to nearest 0.01 mg

d. Magnetic stirrer and a micro stir bar (3 mm)

e. 18-mL (16 x 125 mm) round-bottom culture tubes

f. 1 mL, 2, mL, 5-mL, 10-mL, and 50-mL volumetric pipets

g. Pipettors, (100 µL to 1000 µL and 1mL to 5 mL)

h. 100 and 50-mL volumetric flasks

l 100-mL beaker

3.0 Reagents

a. Bromide stock calibration solution (1,000 mg/L Br or 1 mL = 1 mg Br). Dry approximately 1.0 gram of potassium bromide at 110°C for two hours and cool to room temperature (balance room temperature) in a desiccator. Weigh 0.14894 g of dried potassium bromide and dissolve in regent water to a final volume of 100 mL.

b. Bromide standard solution (100 mg/L Br or 1 mL = 0.1 mg Br). Dilute 10 mL of bromide stock solution in reagent water to a final volume of 100 mL.

c. Bromide calibration standards. Calibration standards must be prepared fresh prior to each use. Prepare five calibration solutions by diluting the 100 mg/L bromide standard solution to the desired calibration solution concentrations. These solutions should bracket the anticipated sample concentration range as narrowly as possible. The following table gives instructions for preparing typical calibration standards directly from the 100 mg/L Br standard solution.

To obtain this concentration of Br calibration solution	Dilute this volume of 100 mg/L Br standard solution to 50 mL.
30 mg/L	15.0
20 mg/L	10.0
10 mg/L	5.00
5 mg/L	2.50
4 mg/L	2.00
2 mg/L	1.00
1 mg/L	0.500
0.5 mg/L	0.250
0.3 mg/L	0.150

Alternately, prepare calibration standards using a serial dilution method. The following Table is provided as a guide.

To obtain this standard solution	Dilute this volume of specified solution to final volume given
40 mg/L	20.0 mL of 100 mg/L to 50 mL
20 mg/L	10.0 mL of 100 mg/L to 50 mL
10 mg/L	10.0 mL of 100 mg/L to 100 mL
8 mg/L	10.0 mL of 40 mg/L to 50 mL
2 mg/L	10.0 mL of 20 mg/L to 100 mL
1 mg/L	10.0 mL of 100 mg/L to 100 mL
0.2 mg/L	10.0 mL of 2 mg/L to 100 mL

d. Bromide ISE equilibration solution (3 mg/L Br). Dilute 1.50 mL of 100 mg/L Br standard solution to a final volume of 50 mL with reagent water.

e. Electrode filling solution. Use solution recommended by the electrode manufacturer.

f. 4M potassium chloride (KCl) electrode storage solution. Dissolve 29.82 g of KCl in 80 mL of reagent water. Add 0.3 mL of 100 mg/L Br standard solution and dilute to a final volume of 100 mL. Store in a glass or plastic container.

g. 5M sodium nitrate (5 M NaNO3) ionic strength adjusting (ISA) solution. Dissolve 42.5 g of NaNO3 in approximately 50 mL of reagent water and dilute to 100 mL. Store in a glass or plastic container.

h. Reagent water, deionize or distilled.

4.0 Procedure for Preparing a Calibration Curve

a. Allow prepared calibration standards to equilibrate to the same temperature as the samples, equilibrating both to the temperature of the room measurements are conducted.

b. Determine the mV readings for each standard in the exact way described for measuring samples (see Section 5.0), and begin these measurements with the least concentration and end with the highest concentration.

c. In the Excel file provided (this file may be downloaded from https://www.cefns.nau.edu/~teb/ambl/ambl_SOPs.html), enter the standard concentration values in the “Concentration” column and the mV readings for each concentration in the “mV Reading” column. This Excel file automatically calculates the log concentration (LogC) values, and the slope, intercept and r-squared values from linear regression analysis of the calibration data. A calibration curve and a basic calculator that uses the calibration curve equation for calculating sample concentration are also provided in this file.

5.0 Procedure for Measuring Standards and Samples

a. The bromide combination ISE may be stored in the storage solution for periods of no longer than 1 week between measurements. If stored longer than 1 week between measurements is necessary, drain the filling solution from the electrode, place the protective shipping cap over the sensing element, and store the electrode dry. Follow the electrode manufacturer's instructions for preparing the stored electrode for use.

b. Before using, check the electrode filling solution and if necessary add filling solution to the bottom of the filling hole. The filling solution in the electrode must be at least 1 inch (2.54 cm) above the level of the sample and the filling hole must be open to the atmosphere when conducting a measurement.

c. Before conducting measurements, equilibrate the bromide ISE for approximately 1 hour in bromide equilibration solution.

d. Allow all samples and standard solutions being measured to equilibrate to the same temperature of the room measurements are conducted.

e. Turn on the meter and set to mV mode.

f. Add 5 mL of sample (or standard solution) into a dry clean 18-mL tube, followed by 0.10 mL (100 µL) of the ISA solution. The ISA solution must be added to all samples and standards. If necessary, tilt and rotate the tube to ensure that the ISA solution is actually added to the sample rather than being retained on the wall of the tube.

g. Insert a micro stir bar and secure the tube just above the surface of the magnetic stirrer.

h. Remove the electrode from equilibration solution, thoroughly rinse it with reagent water and gently shake of all excess water. You may pat the sides of the electrode with a soft tissue to remove excess water, but do not use a tissue on the sensing surface.

i. Insert the electrode into the tube and ensure that the bottom of the electrode does not touch or interfere with the stir bar.

j. Turn on the magnetic stirrer and stir the solution at a uniform and moderate rate.

k. Record the mV reading as soon as it is stable, but do not exceed 5 minutes.

l. Remove the electrode from the tube and again thoroughly rinse it with reagent water and gently shake of all excess water.

m. If additional samples (or standards) will be measured, secure the electrode while preparing the next sample for measurement.

n. Remove the micro stir bar from the tube, thoroughly rinse it with reagent water and dry with a soft tissue.

o. Continue with steps f through n until all samples (or standards) have been measured.

p. When finished, return the rinsed electrode to the storage solution or prepare it for dry storage.

q. Turn off the meter.

6.0 Procedure for Checking the Electrode Operational Slope

a. The electrode slope is determined as the observed change in millivolts (mV) for every tenfold change in concentration. An acceptable change is from -54 to -60 mV difference between two measurements when sample temperature is between 20 and 25°C. If the slope is outside this mV range, refer to the troubleshooting information found in the electrode's user guide.

b. Prepare the first electrode slope check sample by adding 0.1 mL of bromide stock calibration solution to a 100-mL beaker containing 50.0 mL of reagent water (1.96 mg/L bromide).

c. Prepare the second electrode slope check sample by adding 1.0 mL of bromide stock calibration solution to a 100-mL beaker containing 50.0 mL of reagent water (19.6 mg/L bromide).

d. Determine the mV reading for each slope check sample in the exact way described for measuring samples (see Section 5.0), beginning with the lowest concentration.

e. Calculate the change between the two mV readings and compare against the acceptable difference given above.

7.0 Calculations

Sample bromide ion concentration is calculated using the calibration curve equation as follows.

$$L = \frac{R - b}{m}$$

where,

L = Log of bromide ion concentration

R = mV reading

b = intercept from regression analysis

m = slope from regression analysis

and

$$C = 10^{L}$$

where,

C = bromide in concentration, mg/L

8.0 Quality Control

a. ISA solution must be added to all environmental samples, quality control samples, and calibration standard samples at a ratio of 50:1 sample to ISA solution. With this method, 0.1 mL of ISA is added to each 5-mL of sample volume.

b. Continuing Calibration Verification (CCV) sample. Analyze a CCV sample with each batch of 10 or fewer sample samples. The CCV sample used with this method is prepared to contain a bromide concentration of 10.0 mg/L. The result of this measurement should be between 9 and 11 mg/L. If not, either determine and correct the source of your error, check the operating slope of the electrode, or recalibrate the electrode.

c. Reagent water blank. Analyze a reagent water blank (with ISA added) after each CCV sample. The result of the reagent blank measurement must be less than the most recently determined method detection limit. If not, the source of error must be found and corrected and all samples since the previous acceptable reagent blank must be reanalyzed.

d. Duplicate sample. Analyze at least one sample in duplicate for each batch of 20 or fewer samples. If a single sample is being analyzed, this sample must be analyzed in duplicate. Acceptance criteria for duplicate analysis using this method has not yet been determined.

e. Slope verification to check electrode operation. The procedure for measuring the electrode slope is presented in Section 6.0. Verify the electrode slope at least once each week during use or prior to each use if the electrode has been stored dry.

9.0 Bibliography

1. SW-846 Method 9211: Potentiometric Determination of Bromide in Aqueous Samples with Ion-Selective Electrode (December 1996) in *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods (SW-846)*, US PA OSWER, Washington, D.C.

2. Bromide Ion Selective Electrode User Guide, 25404-001, Rev. A 10-08. Thermo Fisher Scientific, Beverly, MA, 2008.