1) **Alkaline Persulfate Digestion for TN, TDN and TDP**

**Total Dissolved Nitrogen**

### a) Scope and Application

i) This method covers the digestion procedure for the determination of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) in surface fresh and estuarine waters by the automated alkaline persulfate oxidation technique. The method may be used for the determination of total nitrogen (TN) with necessary precautions to ensure that particulates are fully digested in the range of 0.01 to 2.0 mg/L \(\text{NO}_3^- + \text{NO}_2^-\) N.

ii) Typical analytical ranges are 0.01 to 3.0 mg N/L for nitrogen and 0.01 to 0.5 mg P/L for phosphorus. These ranges may be extended by digesting a diluted sample.

### b) Summary of Method

i) This method is a persulfate oxidation technique for nitrogen where, under alkaline conditions, all organic and inorganic forms of nitrogen are oxidized to nitrate and nitrite. As the reaction proceeds, NaOH is consumed and the pH drops to < 2, which allows the oxidation of all phosphorus compounds to orthophosphate. This is followed by analysis for nitrate by an automated colorimetric method (subsection 6). The inorganic fractions can then be subtracted from the total dissolved sample to calculate the dissolved organic concentration, if desired.

ii) An aliquot of digested sample is analyzed for nitrate and orthophosphate using an automated colorimetric method (Sections D.3 & D.5) to produce total nitrogen and total phosphorus concentrations.

### c) Interferences

i) Metal ions may produce a positive nitrate error if present in sufficient concentrations. The presence of large concentrations of sulfate will cause a large loss of sensitivity to the copper-cadmium column.

ii) Turbidity and suspended sediment may interfere with the method if the sample has not been filtered.

iii) Samples preserved with acid may result in low recoveries of nitrogen. Acidified samples must be neutralized with NaOH or the reagents modified to ensure that the pH drops to < 2 for the phosphorus digestion. Samples may be frozen to -20°C as an alternative to acid preservation.

iv) Organic carbon reacts with the persulfate oxidation reagent to form carbon dioxide. Concentrations over 150 mg C/L may deplete the persulfate before all nitrogen compounds are oxidized.

### d) Apparatus and Materials

i) Autoclave or Continuous flow automated analytical system equipped with an autosampler, manifold, proportional pump, colorimeter, phototube, recorder or computer based data system, and heating bath/pressure cooker capable of maintaining 100-110 °C for 30 minutes.

ii) Glass Digestion Tubes: 30 mL culture tubes with polypropylene linerless screw-caps or Appropriate glassware. See section 1.7.40 mL vials with Teflon-lined screw-caps. Condition the tubes or vials prior to first use by filling with persulfate oxidizing reagent and autoclaving at 100-110 °C for 30 minutes.

Automator cups. See section 1.7.3
e) Reagents and Standards

i) Reagent Grade Water: Nitrogen-free reagent water see section 1.9

ii) Borate Buffer Solution: To a two liter volumetric flask, add approximately 800 mL of reagent water. Add 123.6 g of boric acid (H₃BO₃) and 16.16g of Low N sodium Hydroxide (NaOH) and dilute to two Liters with reagent water. Stable for two months at room temperature.

iii) Persulfate Oxidizing Reagent: To a one-liter volumetric flask, add 400 mL of reagent water. Add 3.0 g of low-nitrogen Baker analzyed potassium persulfate (e.g., K₂S₂O₈, with < 0.001% N), dissolve and dilute to one liter with reagent water. Store in a glass reagent bottle. This solution is only stable for two hours at room temperature.

Ammonium Chloride Preparation Water: Measure out two liters of reagent water with a graduated cylinder into a clean 2-liter Erlenmeyer flask. Add a magnetic Teflon stirring bar. While stirring the water, measure the pH. Adjust the pH to 8.5 by adding one drop of ammonium hydroxide. If the solution becomes too acidic, dilute the ammonium with water until the solution reaches a pH of 8.5.

Ammonium Chloride Reagent: In a two-liter volumetric flask, measure out approximately 1.5 L of ammonium chloride preparation water. Add 20 g of ammonium chloride (NH₄Cl) and 0.2 g of disodium EDTA, dissolve, and dilute to two liters with reagent water. Make sure the ammonium chloride and EDTA are completely dissolved. Store in a glass bottle, add 1.0 mL of Brij 35, and 5 drops of 2% copper sulfate (CuSO₄) solution.

Color Reagent: In a one-liter volumetric flask, add 500 mL of reagent water. Add 100 mL of concentrated phosphoric acid H₃PO₄ and 10 g of sulfanilamide (C₆H₈N₂O₂S), dissolve the mixture using low heat if necessary. Add 0.5 g of 1-naphthylthlyenediamine dihydrochloride (C₁₂H₁₂N₂⋅2HCl) and dissolve. Cool to room temperature then, dilute to one liter with reagent water. Pour into a plastic one-liter bottle and add 0.5 mL of Brij 35. Store in the dark at 4 ± 2°C. This solution is stable for one month at 4 ± 2°C.

Copper Sulfate Solution, 2% (w/v): In a one-liter volumetric flask, add 500 mL of reagent water. Add 20.0 g of CuSO₄, dissolve, and dilute to volume with reagent water. Store in a plastic bottle at room temperature. This solution is stable for two months.

Phosphoric Acid, 10%: In a 500-mL volumetric flask, add 400 mL of reagent water. Add 50 mL of concentrated phosphoric acid H₃PO₄, dissolve, and dilute to volume with reagent water. Add 0.25 mL of Brij 35. Store in a ground glass-stoppered glass bottle at room temperature.

Artificial Sea Water: see section 1.7

Wash Water: Dilute substitute ocean water to the average salinity of the samples being analyzed. Add 4 mL of Brij 35 per liter. Make this solution daily.

iv) Instrument Wash Water

SM 4500-P J: Prepare autosampler wash water in Erlenmeyer flask by adding oxidation reagent to reagent water in same proportion as in digestion tubes. Autoclave samples and wash water for 55 min at 120°C. After digestion and cooling, add 3N NaOH to the digested wash water.

USGS: Dissolve 6.9 g of sodium bisulfate (NaHSO4•H2O, FW=138.08) in about 800 mL of DI water in a graduated 1-L Pyrex™ media bottle. Dilute this solution to the mark with DI water, mix it well, and store it tightly capped at room temperature. NOTE: This solution matches the matrix of sample digests. Use it as the matrix for continuing calibration verification (CCV) solutions and any other undigested check samples.
v) Digestion Check Standards

(1) Stock Glutamic Acid Standard: In a 500-mL volumetric flask, add 400 mL of reagent water. Add 0.3705 g of glutamic acid (HOCOCH2CH(NH2)COOH), mix, and dilute to volume with reagent water. Add 0.5 mL of chloroform (under a hood) as a preservative. This solution is only stable for ten months when stored at 4 ± 2°C.

(2) Working Glutamic Acid Standard: In a 100-mL volumetric flask, add 50 mL of wash water solution. Add 0.5 mL of stock standard solution, mix, and dilute to volume with wash water solution. This solution should be prepared on the day of analysis. (1.0 mL = .3528 mg N/L)

v) Nicotinic Acid p-toulenesulfonate Digestion Check Standard

Stock Nitrate Standard: In a one-liter volumetric flask, add 500 mL of reagent water. Add 0.7218 g of potassium nitrate (KNO₃) that has been dried overnight at 103 ± 2°C (stored in a desiccator), mix, and dilute to volume with reagent water. Add 2 mL of chloroform (under a hood) as a preservative. This solution is only stable for six months at 4 ± 2°C.

Stock Nitrite Standard: In a 500-mL volumetric flask, add 400 mL of reagent water. Add 0.6160 g of sodium nitrite (NaNO₂) that has been dried overnight at 103 ± 2°C (stored in a desiccator), mix, and dilute to volume with reagent water. Add 2 mL of chloroform (under a hood) as a preservative. This solution is only stable for one month at 4 ± 2°C. For Nitrogen – See SM 4500-P J.

vi) Adenosine triphosphate Digestion Check Standard for Phosphate – See SM 4500-P J.

vii) (USGS) Column preparation:

Treatment of cadmium: Approximately 7 g of cadmium are poured into a 125 mL Erlenmeyer flask. Add enough 1N HCl to the flask to cover the cadmium, and swirl for 10 seconds (do not exceed this allotted time). Quickly rinse the cadmium with ASTM type II water until the solution is non-acidic (test with pH paper). Next, add 100 mL portions of 2% CuSO₄ solution and swirl for 30 to 40 seconds. The cadmium will start to look very dark, the blue color will fade, and a brown colloidal precipitate forms. Pour off the 2% CuSO₄. Wash the cadmium-copper with reagent water (at least ten times) to remove all precipitated copper. The color of the cadmium so treated should be black. Add ammonium chloride reagent to the flask so that it covers the cadmium. The cadmium can be capped tightly and stored this way or used immediately to make a column.

NOTE: When a cadmium column significantly loses its efficiency, or the ammonium chloride the cadmium column is stored in appears cloudy, the column can be reconditioned with acid and recoupled with copper sulfate according to the above directions, except that it is only exposed to the copper sulfate for 15 seconds.

Packing the column: Disconnect the end of the plastic tubing which connects the cadmium column to the second set of mixing coils on the cartridges (farthest from the column). Tape the end high enough so the ammonium chloride reagent will not leak out of the column. Unscrew the column from the column, fill the column with ammonium chloride, pack the column by adding small amounts of the treated cadmium to it and tapping the column so the cadmium is packed without allowing the air into the column.

Glycine Digestion Check stock solution (1 mL = 1.0 mg-N): Dissolve 3.98 g glycine (C2H5NO2•HCl, FW=111.5) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer to a 500-mL reagent bottle in which it is stable for 6 months at 4°C.

viii) (USGS) Glycerophosphate Digestion Check stock solution (1 mL = 0.4 mg-P): Dissolve 1.976 g glycerophosphate (C₃H₇O₆PNa₂•5H₂O, FW=306.1) in about 400 mL of DI water in a 500-mL

Alkaline Persulfate Digestion Page 3
volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer to a 500-mL reagent bottle in which it is stable for 6 months at 4°C.

f) Sample Handling

i) Samples are stored at -20 ± 2°C for a maximum 28 days.

ii)

g) Procedure

i) Sample Preparation of Analytical Batch

(1) Prepare a series of standard solutions covering the concentration range of the samples by diluting either the stock or standard solutions.

(2) To each 30 mL screw cap test tube add 10.0 mL of filtered sample (or a smaller aliquot diluted to 10.0 mL) to a clean glass test tube or vial. Use a magnetic stirrer while subsampling unfiltered, whole water samples. Pipet 10.0 mL of each calibration standard, method blanks, LCS, CCV, etc., into test tubes.

Add reagent grade water to 3 test tubes as reagent blanks.

(3) Pipet 5.0 mL of the persulfate oxidizing reagent to all three tubes. Immediately cap test tubes tightly and very quickly due to prevent volatilization, and then invert twice to mix.

Include two oxidizing reagent blanks which contain only the oxidizing reagent.

SM 4500-P J says to prepare autoanalyzer wash water in an Erlenmeyer flask by adding oxidation reagent to reagent water in same proportion as in digestion tubes. Autoclave samples and wash water for 55 min at 120°C. After digestion and cooling, add 3N NaOH to the digested wash water.

NOTE: A precipitate will form with seawater samples which will not form with saline standards prepared with NaCl. If standards are made with substitute ocean water the precipitate will form.

ii) Digestion

(1) Autoclave the analytical batch samples at 100 - 110°C (between 3-4 psi) for 30 minutes on the liquid automatic cycle. No volatilization occurs after this point.

(2) Remove tubes from autoclave and cool to room temperature (samples can be refrigerated for several days at this point if necessary to delay analysis).

(3) Add 1.0 mL of buffer solution to each tube and mix. The pH of the sample
iii) Analysis - See procedures for Nitrate and Phosphate

Analytical sequence: The samples and associated QC samples and standards should be run according to the following sequence:

- Two high concentration calibration standards.
- Two medium concentration calibration standards.
- Two low concentration calibration standards.
- Two method blanks.
- Ten-twenty CBP samples.
- One matrix spike sample.
- One medium concentration calibration standard.
- One method blank.

Steps 8.7.7.5 - 8.7.7.8 are repeated until all samples are analyzed or QC samples indicate that the system is out of control and recalibration is necessary.

- One high concentration calibration standard.
- One medium concentration calibration standard.
- One low concentration calibration standard.

Switch sample line from distilled water to sampler and begin analysis.

Prepare appropriate standard curve by plotting peak heights of processed standards against known concentrations. Compute concentration of the samples by comparing sample peak heights with standard curve.

**NOTE:** Subtract the blank background response from the standards before preparing the standard curve.

Test the cadmium column efficiency by analyzing 2 cups of the 0.4 mg NO$_3$-N/L standard followed by one cup of the 0.4 mg NO$_2$-N/L standard. The column efficiency must be 90-110%. To ensure that the nitrate standards are correct, also analyze 2 cups of the EPA standard. Complete loading of sampler tray with quality control and unknown samples.

Record the stabilized potential of each unknown sample and convert the potential reading to the TDN concentration using the standard curve.

h) Quality Control

i) Samples with results greater than the highest calibration standard must be diluted and re-digested.

ii) Digestion Check Standards Calibration

Linear calibration range: Calibration standards should bracket the range of CBP samples.

(2) Correlation coefficient: The correlation coefficient must be 0.99 or better for the calibration curve to be used.

ii) Method blanks: see Chapter IVI, Section C.

iv) Matrix spike samples: see Chapter IVI, Section C.

v) Laboratory duplicates: see Chapter IVI, Section C.
vi) Reference materials: The laboratory must analyze a standard reference material once a year, as available. Laboratory Control Samples: see Chapter VI, Section C.

Vii) Method detection limits (MDL): Method detection limits should be established using the guidelines in Chapter VI, Section C.

i) References


