

1) **Alkaline Persulfate Digestion for Total and Total Dissolved Nitrogen and Phosphorus**

a) Scope and Application

- i) This method describes the digestion procedure for the determination of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) in surface fresh and estuarine waters by the alkaline persulfate oxidation technique. The method is suitable for the determination of total nitrogen (TN) and total phosphorus (TP) with necessary precautions to ensure that particulates are fully digested.
- ii) Typical analytical ranges are 0.01 to 3.0 mg-N/L for total nitrogen and 0.01 to 0.5 mg-P/L for total phosphorus; these ranges may be extended by digesting and analyzing a diluted sample. The calibration range for nitrogen may be extended for filtered samples only (i.e., TDN).

Comment [m1]: New

b) Summary of Method

- i) The persulfate oxidation technique for nitrogen in water is performed under heated alkaline conditions, where all organic and inorganic forms of nitrogen are oxidized to nitrate. As the reaction proceeds, NaOH is consumed and the pH drops to < 2.2, which allows the oxidation of all phosphorus compounds to orthophosphate.
- 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) Some particulate nitrogen compounds in unfiltered samples may be resistant to alkaline persulfate digestion (i.e., refractory) and yield low total nitrogen results.
- ii) Samples preserved with acid will result in low recoveries of nitrogen unless the pH is adjusted to >12 prior to digestion and the reagents modified. (USGS 2003).
- iii) 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 and cause a low bias for total nitrogen.

d) Apparatus and Materials

- i) Autoclave or pressure cooker capable of maintaining 100-120 °C for at least 30 minutes.
- ii) Glass Digestion Tubes: 30 mL culture tubes with polypropylene liner-less screw-caps, or 40 mL vials with Teflon-lined screw-caps. Condition the tubes or vials prior to their first use by filling with persulfate oxidizing reagent and autoclaving at 100-120 °C for 30 minutes.

e) Reagents and Standards

- i) Reagent Water: Nitrogen-free reagent water.
- ii) Borate Buffer Solution: Add approximately 800 mL of reagent water to a two-liter volumetric flask. Quantitatively transfer 123.6 g of boric acid (H₃BO₃) and 16.16g of low-nitrogen (< 0.001% N) sodium hydroxide (NaOH) and dilute to 2.0 liters with reagent water. This solution is stable for two months at room temperature.

Comment [m2]: SM 4500-P J uses NaOH after digestion

- iii) Persulfate Oxidizing Reagent: Add 400 mL of reagent water to a one-liter volumetric flask. Quantitatively transfer and dissolve 3.0 g of low-nitrogen NaOH and 20.1 g of low-nitrogen potassium persulfate ($K_2S_2O_8$, with < 0.001% N) in the flask. Dilute to one liter with reagent water and store in a glass reagent bottle. This solution is stable for two hours at room temperature.
- iv) Instrument Wash Water

Prepare auto-analyzer wash water by mixing oxidizing reagent and reagent water in an Erlenmeyer flask, in the same proportion as added to the digestion tubes, e.g., 2:1 (v/v) ratio. Cover with foil and autoclave the solution for at least 30 min. at 100-120°C; cool and add 3N NaOH.
- 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 ($HOCOCH_2CH_2CH(NH_2)COOH$), mix, and dilute to volume with reagent water. Add 0.5 mL of chloroform (under a hood) as a preservative. This solution is stable for ten months when stored at $4 \pm 2^\circ C$.

(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)

(3) Nicotinic Acid p-toluenesulfonate Digestion Check Standard for Nitrogen – See SM 4500-P J.

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

(5) (USGS) Glycine Digestion Check stock solution (1 mL = 1.0 mg-N): Dissolve 3.98 g glycine ($C_2H_5NO_2 \cdot HCl$, 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 $4^\circ C$.

(6) (USGS) Glycerophosphate Digestion Check stock solution (1 mL = 0.4 mg-P): Dissolve 1.976 g glycerophosphate ($C_3H_7O_6PNa_2 \cdot 5H_2O$, FW=306.1) 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 $4^\circ C$.

f) Procedure

i) Preparation of Analytical Batch

- (1) Prepare a series of standard solutions covering the analytical range by diluting either the stock or standard solutions.
- (2) Add 10.0 mL of sample (or a smaller aliquot diluted to 10.0 mL) to a clean glass test tube or vial.

Use a wide-bore pipette for taking aliquots of whole-water samples. A magnetic stirrer may be necessary to obtain a representative subsample from samples with high suspended solids.

Comment [m3]: Deleted section "Sample Handling Procedures"

Comment [m4]: New

- (3) Pipette 10.0 mL of each calibration standard, method blank, LCS, CCV, etc., into a test tube.
- (4) Add reagent water to 3 test tubes as method blanks. Include two oxidizing reagent blanks which contain only the oxidizing reagent.
- (5) Pipette 5.0 mL of the persulfate oxidizing reagent to each tube. Immediately cap tightly to prevent volatilization, and then invert twice to mix.
- (6) Prepare a flask of instrument wash water (mix reagent water and persulfate oxidizing agent in a 2:1 (v/v) ratio) and cover with aluminum foil.

Comment [m5]: This is from old procedure. Purpose? Baseline Absorbance?

ii) Digestion

- (1) Autoclave the analytical batch at 100 - 120°C for at least 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 should be around 2.5 after the addition of buffer solution.
- (4) Add 3N NaOH to the digested wash water.
- (5) Remove any suspended particles remaining in digests by decantation or filtration prior to colorimetric analyses.

Comment [m6]: Discussion item - USGS validated the storage of digests at room temp for ≤ 4 days. Valderama 1 month??

Comment [m7]: Need to mix prior to adding buffer?

Comment [m8]: USGS method uses NaOH instead of buffer.

Comment [m9]: New – from USGS method

iii) Analysis – See procedures for Nitrate and Phosphate

iv) Calibration and Data Reductionions

- (1) Prepare a standard curve by plotting the absorbances or peak heights of the digested calibration standards against their known concentrations. Designate a digested method blank as having a concentration of 0.0 mg N/L and 0.0 mg P/L.

Do not select a curve-fitting function that forces a zero y-intercept. The calibration curve will have a positive y-intercept that approximates the baseline-corrected absorbance of the digestion blank.

(2) Correction for Digestion Blank

Subtract the absorbance of the digestion blank(s) from the baseline-corrected absorbance of all calibrants, quality control samples and samples in the batch. If multiple blanks are digested, calculate the average blank absorbance.

g) Quality Control

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

- iii) Method blanks: see Chapter VI, Section C.
- iv) Matrix spike samples: see Chapter VI, Section C.
- v) Laboratory duplicate: see Chapter VI, 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 as specified according to the guidelines in Chapter VI, Section C.



References

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USGS 2003. USGS WRIR 03-4174: Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water. Methods I-2650-03 (total dissolved nitrogen and total dissolved phosphorus) and I-4650-03 (total nitrogen and total phosphorus).

Valderrama, J.C., 1981. The Simultaneous Analysis of Total Nitrogen and Total Phosphorus in Natural Waters: Marine Chemistry, v. 21, p. 109-122.