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Nitrate and organic N analyses with second-derivative spectroscopy

Abstract—Simple and reliable procedures have been developed for analyses of NO_3^- , total N, and organic N in freshwaters. NO_3^- is determined by second-derivative UV spectroscopy. Total N and organic N are determined based on second-derivative analyses of NO_3^- following persulfate digestion. Resolution of organic N determinations was increased by using ion-exchange resins to remove NO_3^- from samples with high concentrations of NO_3^- prior to persulfate oxidation of the organic N.

Although numerous methods have been developed for the determination of N in aqueous samples, there is continued need for simple and reliable procedures for both inorganic and organic N analyses. The most widely accepted methods for NO_3^- analysis include Cd reduction and ion chromatography (Am. Public Health Assoc. 1989). Both methods have been adapted for automated analysis and, with proper consideration of potential interferences, both are accurate and precise. Ion chromatography has the advantage of also measuring several additional anions in a single aliquot of sam-

ple. Ion chromatography and Cd reduction, however, are relatively complicated and expensive. Samples must normally be filtered to remove suspended matter before analysis, and in the case of Cd reduction, sample color, metal ions, and phosphate are potential interferences (Olson 1980).

The principal methods for organic N determination are based on conversion of organic N to NH_4^+ or NO_3^- and subsequent analysis of these inorganic forms. Separate analysis of ambient inorganic N allows organic N levels to be calculated by difference. For freshwater samples, conversion of organic N to inorganic forms is most commonly based on either Kjeldahl digestion (U.S. EPA 1979; Am. Public Health Assoc. 1989), photo-oxidation (Armstrong et al. 1966), or persulfate digestion (Koroleff 1976; D'Elia et al. 1977; Solórzano and Sharp 1980). Photo-oxidation requires more specialized equipment and may be ineffective for some compounds (Henriksen 1970). Kjeldahl digestion is a complicated and expensive procedure and may be unsuitable for systems receiving high inorganic N loads. High concentrations of NO_3^- severely interfere with standard Kjeldahl N determinations (Schlueter 1977; U.S. EPA 1979; Am. Public Health Assoc. 1989). NO_3^- -N concentrations 10–20 times greater than those of organic N result in >90% inhibition of Kjeldahl N determinations (Schlue-

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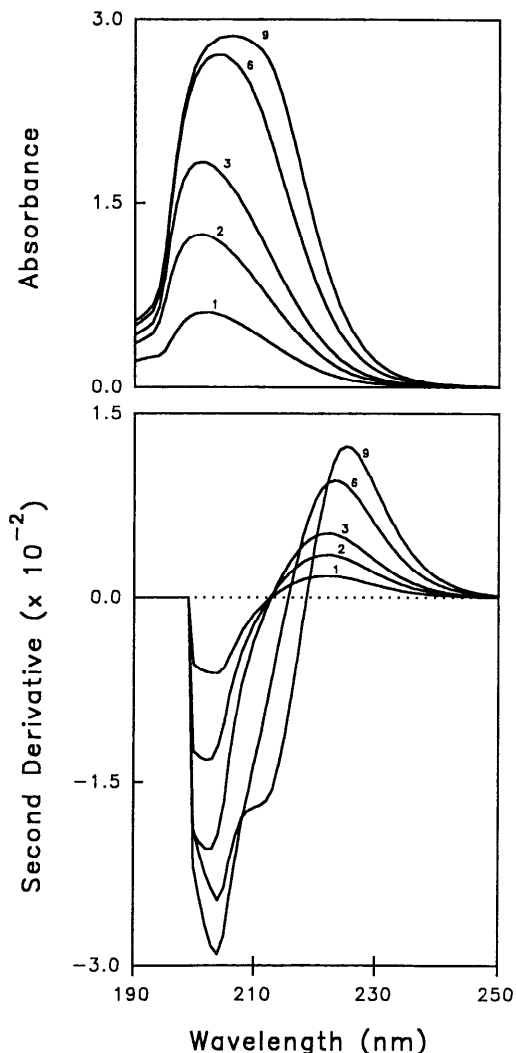


Fig. 1. Absorbance and second-derivative spectra, for NO_3^- standards having 1, 2, 3, 6, or 9 mg NO_3^- -N liter $^{-1}$.

ter 1977). We have been unable to use the Kjeldahl procedure for streams draining agricultural watersheds because of high ratios of NO_3^- to organic N (Bachmann et al. 1990). In addition, high ratios of NO_3^- to organic N limit the resolution of organic N determination based on differences between NO_3^- analyses before and after persulfate digestion.

Simal et al. (1985) and Suzuki and Kuroda (1987) described a very promising method for NO_3^- analysis based on second-derivative UV spectroscopy. We have mod-

ified this method and adapted it for analyses of NO_3^- and organic N in waters with a wide range of NO_3^- :org N ratios, overcoming problems of NO_3^- interference in organic N determinations with other methods. The procedures are simple and reliable and require only small sample volumes. NO_3^- is determined by second-derivative UV spectroscopy, and total N and organic N are determined by second-derivative analyses of NO_3^- following persulfate digestion. Ion-exchange resins are used to remove NO_3^- from samples with high concentrations of NO_3^- prior to persulfate oxidation of the organic N.

Water for reagents and standards is drawn as needed from a type 1, deionized water system (Am. Public Health Assoc. 1989). Primary standards of 1,000 mg liter $^{-1}$ of NH_4^- -N, NO_3^- -N, and urea N are prepared with NH_4Cl , NaNO_3 , and urea. Working standards of 0.01–15 mg N liter $^{-1}$ are prepared by serial dilution of these primary standards. We routinely acidify our field samples to pH < 2 with 6 ml of concentrated HCl or 2 ml of concentrated H_2SO_4 per liter of sample, but acidification is not prerequisite for second-derivative analysis.

NO_3^- produces a second-derivative signature with a peak at ~ 224 nm—the wavelength shifting upward slightly at higher concentrations (Fig. 1). The amplitude of this second-derivative peak is a function of the NO_3^- concentration in the solution scanned. Cahill (1979) and Cahill and Padera (1980) discussed the general principles and application of derivative spectroscopy, including selection of instrument parameters and calculation of derivatives.

In our procedure for second-derivative NO_3^- analysis, direct absorbance spectra are obtained from scans of samples and standards between 190 and 250 nm with matched pairs of far UV, quartz cells with 10-mm optical path lengths. All scans are conducted at 120 nm min $^{-1}$ against a reference of deionized water, which is acidified or not as appropriate. We use a Perkin Elmer Lambda 3b model UV-visible scanning spectrophotometer with a spectral bandwidth of 1 nm. Minimum wavelength accuracy and repeatability are ± 0.3 and ± 0.1 nm, based on the required specification that

these ranges not be exceeded in repeated measurements at the deuterium lamp peak at 656.1 nm. The spectrophotometer is interfaced to a microcomputer which is used to calculate derivatives from absorbance. Derivatives are best calculated with a moving least-squares procedure (Savitsky and Golay 1964), although simple two-point slopes provide comparable results if signal-to-noise ratios are low.

For analyses of total and organic N, 10-ml aliquots of samples and standards are dispensed to test tubes having Teflon-lined screwcaps. Samples preserved by addition of HCl or H₂SO₄ are amended with the normal equivalent of NaOH and then 1.5 ml of oxidizing reagent is added to each tube. The oxidizing reagent is made by dissolving 6.0 g of low-N potassium persulfate in 100 ml of 1.5 M NaOH and will keep several days if stored in the dark (Solórzano and Sharp 1980). After reagent addition, the test tubes are capped tightly, shaken, and autoclaved at 121°C for 30 min. The solutions are allowed to cool, acidified to pH <2 with concentrated HCl, and analyzed for NO₃⁻ with second-derivative spectroscopy as described above. NO₃⁻-N concentration, corrected for reagent blank and dilution, is equivalent to total N for unfiltered samples and total dissolved N for filtered samples. For samples with low to moderate levels of NO₃⁻, organic N can be calculated by difference based on these estimates of total or dissolved N and analyses of inorganic N prior to digestion. For these calculations, aliquots of the original samples are analyzed for NH₄⁺ with the phenolhypochlorite method of Scheiner (1976) and for NO₃⁻ with second-derivative spectroscopy as described above.

For analyses of organic N in samples with high concentrations of NO₃⁻, ion-exchange resins are used to remove NO₃⁻ before persulfate digestion of the samples. Samples are filtered through precombusted, Whatman GF/F glass-fiber filters and then acidified to pH <2 with concentrated HCl. The acidic filtrate is pumped at 1 ml min⁻¹ through BioRad Poly-Prep columns packed with 2 ml of AG 1-X8 resin, chloride form, 100–200 mesh. AG 1-X8 resin is a strongly basic anion exchanger; because the pH of the

acidified filtrate is well below the pK for most organic N compounds, these compounds are not absorbed onto the resin. In contrast, NO₃⁻ is strongly absorbed by the resin and is removed completely under the conditions described above. Approximately the first 8 ml of sample eluent, equivalent to four bed volumes of the exchange column, is discarded. The next 10 ml of sample is collected and neutralized with 10 N NaOH. Several dozen columns can be run simultaneously. Columns are regenerated after each set of samples with 30 ml of 3 N HCl at a flow rate of 1 ml min⁻¹ and then rinsed with 30 ml of acidified, deionized water.

For persulfate digestion, 10-ml aliquots of neutralized sample, standard, and blank eluent are amended with 1.5 ml of oxidizing reagent in test tubes with Teflon-lined screwcaps. The tubes then are capped tightly, shaken, and autoclaved at 121°C for 30 min. The solutions are allowed to cool, acidified to pH <2 with concentrated HCl, and analyzed for NO₃⁻ with second-derivative spectroscopy as described above. NO₃⁻-N concentration in these solutions, corrected for reagent blank and dilution, is equivalent to the sum of NH₄⁺ and dissolved organic N in the original sample. Dissolved organic N concentrations are calculated by difference from NH₄⁺ determinations prior to digestion with the phenolhypochlorite method of Scheiner (1976). Particulate N collected on precombusted glass-fiber filters can be determined separately by placing the filters in test tubes with 10 ml of deionized water and following the procedure described above for total N.

Second-derivative analysis was found to be sensitive, accurate, and precise for NO₃⁻ determination (Fig. 2). Its detection limit (Am. Public Health Assoc. 1989) is <5 μg NO₃⁻-N liter⁻¹. A typical calibration curve for NO₃⁻ demonstrates a linear relationship between the amplitude of the second-derivative peak and NO₃⁻-N concentration from 0 to 3 mg NO₃⁻-N liter⁻¹ (Fig. 3). The relationship becomes nonlinear at higher concentrations but provides sufficient resolution up to at least 15 mg NO₃⁻-N liter⁻¹. Alternately, samples can be diluted with deionized water so that their concentrations

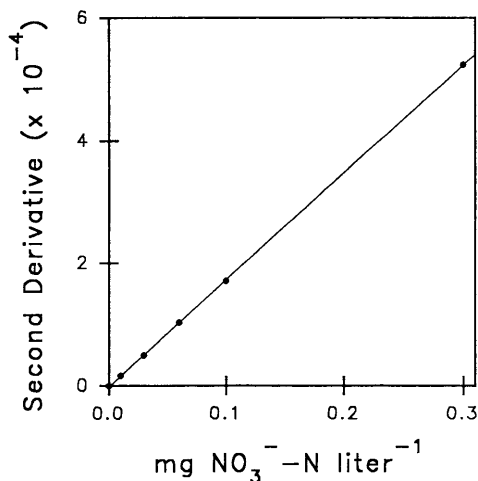


Fig. 2. NO_3^- -N calibration curve obtained with second-derivative technique for low-level standards from 0.0 to 0.3 mg NO_3^- -N liter^{-1} . The curve was fitted by least-squares linear regression ($r^2 = 0.99$).

are within the linear region of the curve, assayed, and a dilution correction calculated. Analysis of undiluted samples with a quadratic regression for higher concentrations and a linear regression for lower concentrations is simpler, however, and more precise.

Accuracy of the second-derivative technique was determined by analyzing repli-

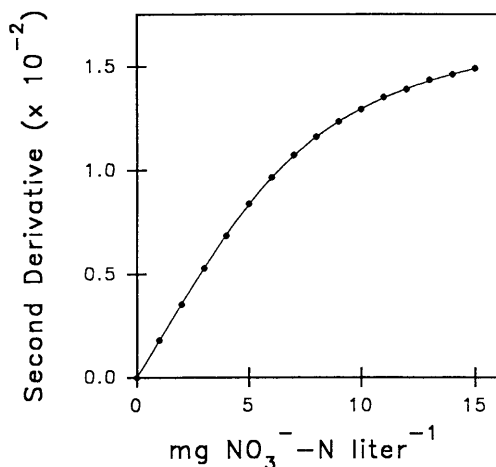


Fig. 3. NO_3^- -N calibration curve obtained with second-derivative technique for standards from 0 to 15 mg NO_3^- -N liter^{-1} . The curve was fitted by a combination of linear (0–3 mg N liter^{-1} , $r^2 = 0.99$) and quadratic (3–15 mg N liter^{-1} , $r^2 = 0.99$) regression.

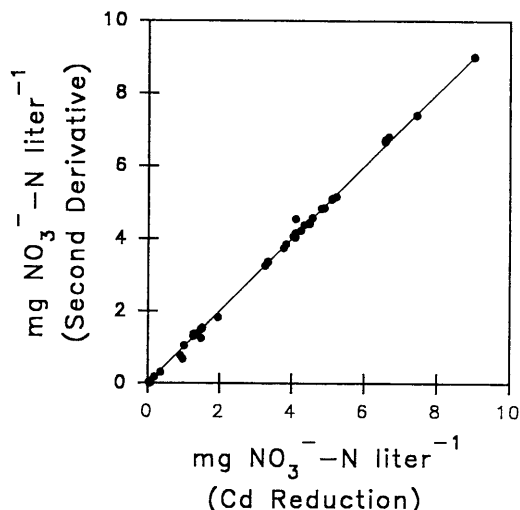


Fig. 4. Comparison of NO_3^- -N concentrations for 55 surface water samples as determined by second-derivative spectroscopy and by automated Cd reduction ($r^2 = 0.99$). A paired sample *t*-test showed no significant difference between the two techniques (see text).

cate aliquots of a range of surface-water samples including rivers, reservoirs, and wetlands for NO_3^- (Fig. 4) with both the second-derivative procedure and automated Cd reduction (U.S. EPA 1979; Am. Public Health Assoc. 1989). A paired-sample *t*-test showed no significant difference between the two techniques ($n = 55$, mean difference = 0.005, SE = 0.014, $t = 0.363$). Selected samples were also spiked with known amounts of NO_3^- and recoveries were calculated. Average recovery for known additions was 102% for second-derivative analysis and 102.3% for automated Cd reduction. The average C.V. of duplicate measures with the second-derivative technique was <1%.

A typical calibration curve for urea standards illustrates the precision and capabilities of this technique for organic N analyses (Fig. 5). Reagent blanks average ~ 0.1 mg N liter^{-1} with commercially available, low-N potassium persulfate. For applications requiring greater sensitivity, recrystallized potassium persulfate can be used. Solórzano and Sharp (1980) reported that recrystallization of potassium persulfate reduced reagent blanks for N analysis by 50–90%. The

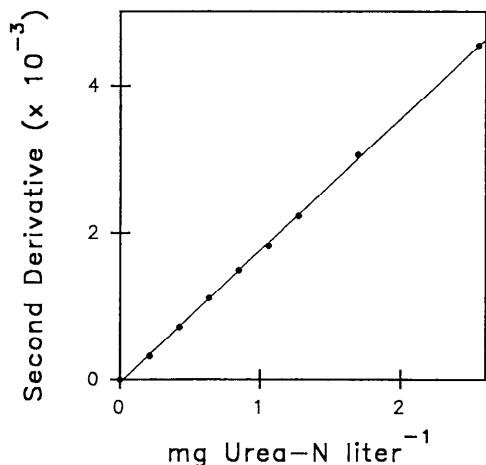


Fig. 5. Urea-N calibration curve obtained with persulfate oxidation and second-derivative spectroscopy. The curve was fitted by least-squares linear regression ($r^2 = 0.99$).

accuracy of the organic N procedure and the effects of the ion-exchange treatment on organic N estimates were tested by determining the recovery of various organic compounds after ion exchange. Replicate aliquots of urea, glycine, glutamic acid, sulfanilamide, and methionine standards containing 1 mg N liter⁻¹ were assayed with persulfate oxidation and second-derivative spectroscopy. The results were then compared with identical assays of these same standards after ion exchange as described above, and percent recoveries were calculated for each compound. Ion exchange removed NO₃⁻ effectively from acidified solutions with no detectable effects on the recovery of organic N. Ion exchange reduced NO₃⁻ concentrations of 10 mg N liter⁻¹ to background levels, while recoveries of dissolved organic N after ion exchange ranged between 96.9 and 105.8% (Table 1).

Interference from bromide and possibly other compounds makes the method as described here unsuitable for seawater samples and possibly for highly saline inland waters. In addition, total and organic N determinations based on the second-derivative analysis of NO₃⁻ following persulfate digestion obviously are only as good as the persulfate procedure itself. Any problems related to refractory compounds or to interference with persulfate digestion remain.

Table 1. Percent recovery of organic N following ion exchange and persulfate oxidation of standards.

Compound	Recovery \pm 1 SD ($n = 3$)
Urea	101.1 \pm 1.5
Glycine	99.2 \pm 0.6
Glutamic acid	105.8 \pm 2.3
Sulfanilamide	96.9 \pm 2.4
Methionine	101.4 \pm 0.7

There are no interferences in the second-derivative analysis of NO₃⁻, however, that would prevent application in most freshwater systems. The broad absorption band characteristic of dissolved organic matter does not produce a significant second-derivative signal in the wavelength range for NO₃⁻ analysis and would present problems only if absorbance by organics was extremely high. Suspended solids do have a significant effect, especially at low concentrations of NO₃⁻. A paired-sample *t*-test showed a significant difference between second-derivative analyses of unfiltered samples of river water having suspended solids concentrations between 5 and 115 mg liter⁻¹ and Cd reduction analyses of samples after filtration through GF/F glass-fiber filters ($n = 88$, mean difference = 0.111, SE = 0.264, $t = 3.96$). Concentrations for the samples compared above ranged between 1.09 and 6.76 mg NO₃⁻-N liter⁻¹, and the average difference between filtered and unfiltered samples was only 3.3%. The difference between filtered and unfiltered samples is small enough to ignore for many applications. At lower NO₃⁻ concentrations, however, analyses of filtered and unfiltered samples differ by comparable absolute amounts so that percentage differences are much higher. For greatest sensitivity and accuracy, samples should be filtered.

The effects of suspected chemical interferences were determined by amending NO₃⁻ standards with various concentrations of Na₂HPO₄, KNO₂, NaHCO₃, FeCl₃, CuCl₂, and CuSO₄. These solutions were then analyzed for NO₃⁻ with second-derivative analysis and percent recoveries were calculated for each. Simal et al. (1985) reported interference from HCO₃⁻ at concentrations >0.5 mg liter⁻¹ and recommended

acidification to eliminate this interference. In repeated tests of unacidified samples and standards at pH 8.3, however, we found no interference from HCO_3^- at concentrations of 500 mg HCO_3^- liter $^{-1}$ or less. Although we acidify for preservation of field samples, it is not necessary to acidify samples for the second-derivative analysis. As reported by Simal et al. (1985), we found no interference from phosphate at a concentration of 20 mg P liter $^{-1}$, but both Fe and Cu interfered at this same concentration. For an NO_3^- standard of 2 mg N liter $^{-1}$, there was no significant interference from 2 mg liter $^{-1}$ of Fe or Cu in either acidified or unacidified solutions. For acidified solutions of this same standard, 20 mg liter $^{-1}$ of Fe caused a 43% underestimate of NO_3^- and 20 mg liter $^{-1}$ of Cu caused a 27% overestimate. For unacidified solutions, the interference from 20 mg liter $^{-1}$ of Fe or Cu was even greater.

The concentrations of tested compounds that interfere with second-derivative analysis of NO_3^- are well above those found in most surface waters. As with any assay technique, the effect of interferences would be greatest at very low concentrations of NO_3^- and it is prudent to check for effects of interfering compounds by standard addition. For samples where concentrations of interfering compounds are relatively constant, it would usually be possible to remove their effect by scanning samples against a reference solution containing the same concentrations of the interfering compounds. This approach may, for example, be a way to adapt the method for analysis of seawater samples.

NO_2^- has a second-derivative signature very similar to that of NO_3^- . It thus adds to the amplitude of the second-derivative peak for NO_3^- (Simal et al. 1985; Suzuki and Kuroda 1987). Per molar equivalent, however, NO_2^- contributes only ~15% as much as NO_3^- to the second-derivative peak at 224 nm, and NO_2^- that is converted to nitrous acid does not interfere. In most surface waters, NO_3^- concentrations are much higher than those of NO_2^- , and NO_2^- interference would be insignificant. In samples where NO_2^- might contribute significantly to the second-derivative peak, NO_3^-

can be measured with somewhat less resolution but without NO_2^- interference based on the second-derivative at the isobestic point for NO_2^- at ~223.2 nm (Suzuki and Kuroda 1987). NO_2^- can be assayed based on the second-derivative at the isobestic point for NO_3^- at ~215.8 nm (Suzuki and Kuroda 1987). Samples for NO_2^- analysis cannot be preserved with acid, however, and must be assayed promptly to avoid conversion of NO_2^- to NO_3^- or NH_4^+ (Am. Public Health Assoc. 1989).

We have used the procedures described above routinely over the past 3 yr for surface and groundwaters draining agricultural watersheds. The high NO_3^- :org N ratios encountered in these systems precluded the use of standard Kjeldahl procedures. The procedures for NO_3^- , total N, and organic N have proven to be simple and reliable and have the additional advantage of requiring only small volumes of sample. These advantages are significant when it is necessary to analyze large numbers of samples as in mass-balance studies or large-scale synoptic studies.

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