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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

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Water-Resources Investigations Report 03–4174

U.S. Department of the Interior
U.S. Geological Survey

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By Charles J. Patton and Jennifer R. Kryskalla

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Denver, Colorado
2003

U.S. DEPARTMENT OF THE INTERIOR
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CONVERSION FACTORS AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
Length		
centimeter (cm)	3.94×10^{-1}	inch
micrometer (µm)	3.94×10^{-5}	inch
millimeter (mm)	3.94×10^{-2}	inch
nanometer (nm)	3.94×10^{-8}	inch
Volume		
liter (L)	2.64×10^{-1}	gallon
liter (L)	33.81	ounce, fluid
microliter (µL)	2.64×10^{-7}	gallon
milliliter (mL)	2.64×10^{-4}	gallon
Mass		
gram (g)	3.53×10^{-2}	ounce, avoirdupois
milligram (mg)	3.53×10^{-5}	ounce, avoirdupois
Pressure		
kilopascal (kPa)	1.45×10^{-1}	pounds per square inch

Degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) by using the following equation:

$$°F = (1.8 \times °C) + 32.$$

ABBREVIATIONS AND ACRONYMS

A/D	analog-to-digital converter
ASTM	American Society for Testing and Materials
CCV	continuing calibration verification
DI	deionized water
DN_{AlkP}	alkaline persulfate dissolved nitrogen
DP_{AlkP}	alkaline persulfate dissolved phosphorus
FCA	filtered, chilled, acidified (bottle type for USGS dissolved nutrient samples)
FCC	filtered, chilled (bottle type for USGS dissolved nutrient samples)
FW	formula weight
h	hour
Hz	hertz
i.d.	inside diameter
KDN	Kjeldahl dissolved nitrogen
KDP	Kjeldahl dissolved phosphorus
KN	used collectively for KDN and KTN
KTN	Kjeldahl total nitrogen
KTP	Kjeldahl total phosphorus
lb/in^2	pounds per square inch
LRL	laboratory reporting level

mg-As/L	milligrams arsenic per liter
mg-C/L	milligrams carbon per liter
mg-N/L	milligrams nitrogen per liter
mg/L	milligrams per liter
mg-P/L	milligrams phosphorus per liter
M	molarity (moles per liter)
max	maximum
MDL	method detection limit
MPV	most probable value
N	normality (equivalents per liter)
N_{AlkP}	used collectively for DN_{AlkP} and TN_{AlkP}
NED	N-(1-Naphthyl)ethylenediamine dihydrochloride reagent
NOM	natural organic matter
NWQL	National Water Quality Laboratory
OC	organic carbon
OWQ	Office of Water Quality
o.d.	outside diameter
PC	personal computer
PBCdR	packed-bed cadmium reactor
P/N	part number
PTFE	polytetrafluoroethylene
TN_{AlkP}	alkaline persulfate total nitrogen
TP_{AlkP}	alkaline persulfate total phosphorus
QC	quality control
s	second
sp. gr.	specific gravity
SAN	sulfanilamide reagent
SLS	sodium lauryl sulfate
SOP	standard operating procedure
SRWS	U.S. Geological Survey Standard Reference Water Sample
STD CAL	standard calibration control; adjusts absorbance range of photometric detectors used in this study
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
v/v	volume per volume
w/v	weight per volume
WCA	whole water, chilled, acidified (bottle type for USGS whole-water nutrient samples)
=	equivalent to
>	greater than
<	less than
≤	less than or equal to
≈	nearly equal to
±	plus or minus

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

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Abstract

Alkaline persulfate digestion was evaluated and validated as a more sensitive, accurate, and less toxic alternative to Kjeldahl digestion for routine determination of nitrogen and phosphorus in surface- and ground-water samples in a large-scale and geographically diverse study conducted by U.S. Geological Survey (USGS) between October 1, 2001, and September 30, 2002. Data for this study were obtained from about 2,100 surface- and ground-water samples that were analyzed for Kjeldahl nitrogen and Kjeldahl phosphorus in the course of routine operations at the USGS National Water Quality Laboratory (NWQL). These samples were analyzed independently for total nitrogen and total phosphorus using an alkaline persulfate digestion method developed by the NWQL Methods Research and Development Program. About half of these samples were collected during nominally high-flow (April-June) conditions and the other half were collected during nominally low-flow (August-September) conditions. The number of filtered and whole-water samples analyzed from each flow regime was about equal.

By operational definition, Kjeldahl nitrogen (ammonium + organic nitrogen) and alkaline persulfate digestion total nitrogen (ammonium + nitrite + nitrate + organic nitrogen) are not equivalent. It was necessary, therefore, to reconcile this operational difference by subtracting nitrate + nitrite concentrations from alkaline persulfate dissolved and total nitrogen concentrations prior to graphical and statistical comparisons with dissolved and total Kjeldahl nitrogen concentrations. On the basis of two-population paired *t*-test statistics, the means of all nitrate-corrected alkaline persulfate nitrogen and Kjeldahl nitrogen concentrations (2,066 paired results) were significantly different from zero at the $p = 0.05$ level. Statistically, the means of Kjeldahl nitrogen concentrations were greater than those of nitrate-corrected alkaline persulfate nitrogen concentrations. Experimental evidence strongly suggests, however, that this apparent low bias resulted from nitrate interference in the Kjeldahl digestion method rather than low nitrogen recovery by the alkaline persulfate digestion method. Typically, differences between means of Kjeldahl nitrogen and nitrate-corrected alkaline persulfate nitrogen in low-nitrate concentration (≤ 0.1 milligram nitrate nitrogen per liter) subsets of filtered surface- and ground-water samples were statistically equivalent to zero at the $p = 0.05$ level.

Paired analytical results for dissolved and total phosphorus in Kjeldahl and alkaline persulfate digests were directly comparable because both digestion methods convert all forms of phosphorus in water samples to orthophosphate. On the basis of two-population paired *t*-test statistics, the means of all Kjeldahl phosphorus and alkaline persulfate phosphorus concentrations (2,093 paired results) were not significantly different from zero at the $p = 0.05$ level. For some subsets of these data, which were grouped according to water type and flow conditions at the time of sample collection, differences between means of Kjeldahl phosphorus and alkaline persulfate phosphorus concentrations were not equivalent to zero

at the p = 0.05 level. Differences between means of these subsets, however, were less than the method detection limit for phosphorus (0.007 milligram phosphorus per liter) by the alkaline persulfate digestion method, and were therefore analytically insignificant.

This report provides details of the alkaline persulfate digestion procedure, interference studies, recovery of various nitrogen- and phosphorus-containing compounds, and other analytical figures of merit. The automated air-segmented continuous flow methods developed to determine nitrate and orthophosphate in the alkaline persulfate digests also are described. About 125 microliters of digested sample are required to determine nitrogen and phosphorus in parallel at a rate of about 100 samples per hour with less than 1-percent sample interaction. Method detection limits for nitrogen and phosphorus are 0.015 milligram nitrogen per liter and 0.007 milligram phosphorus per liter, respectively.

INTRODUCTION

Semiautomated, batch Kjeldahl digestion methods used at the U.S. Geological Survey (USGS) National Water Quality Laboratory (NWQL) for simultaneous nitrogen and phosphorus determinations in filtered- and whole-water samples (Patton and Truitt, 1992, 2000) are rapid and robust, but they suffer from several drawbacks, including:

- health and safety risks posed by concentrated acids, toxic reagents (mercury), and high temperatures (370°C);
- environmental effects and cost associated with processing and disposing of the mercury-containing waste stream;
- propensity of acidic digests to trap and become contaminated by ammonia vapors in ambient laboratory air; and
- laboratory reporting limits (0.1 mg-N/L; 0.04 mg-P/L) higher than those of other inorganic nitrogen- and phosphorus-containing species, which limit the precision of mass balance estimates.

Alkaline persulfate digestion (Valderrama, 1981; Hosomi and Sudo, 1986; D'Elia and others, 1987; Ameel and others, 1993; D'Elia and others, 1997) provides a safer and more environmentally benign alternative to Kjeldahl digestion for routine, single-digest nitrogen and phosphorus determinations in water. Desirable characteristics of alkaline persulfate digestion compared to Kjeldahl digestion include:

- reagents that contain no mercury;
- fume hoods and acid scrubbers are not needed because digestion occurs in sealed tubes inside an autoclave;
- post-digestion contamination by ambient ammonia vapors is not a problem because all nitrogen-containing compounds are oxidized to and determined as nitrate;
- laboratory reporting limits (0.03 mg-N/L; 0.01 mg-P/L) are similar to those of inorganic nitrogen- and phosphorus-containing nutrients; and
- waste-stream processing and disposal are straightforward.

During the past 15 years, alkaline persulfate digestion methods have been widely applied for estuarine and marine water analysis in preference to Kjeldahl digestion methods. Kjeldahl digestion methods continue to be widely applied for freshwater analysis, possibly because alkaline persulfate digestion methods are not approved for National Pollution Discharge Elimination System (NPDES) and Safe Drinking Water Act (SDWA) compliance monitoring. Nonetheless, an alkaline persulfate digestion method for total nitrogen determination (method 4500-N C, which does not include determination of phosphorus) is included in the 20th Edition of *Standard Methods* (American Public Health Association, 1998b, p. 4-102 and 4-103). Note, however, that the method described in this report differs in two important respects from method 4500-N C. First, method 4500-N C states "samples preserved with acid cannot be analyzed […]." The method described in this report is applicable to acidified nutrient samples—USGS FCA (filtered, chilled, acidified) and WCA (whole water, chilled, acidified) bottle types—provided that they have been processed according to USGS field manual protocols (Wilde and others, 1998). Second, nitrogen and phosphorus are recovered quantitatively from digests prepared by the method described in this report as explained in section 2.2. Furthermore, manual post-digestion pH adjustment prior to colorimetric determinations required by other previously published alkaline persulfate digestion methods (Valderrama, 1981; Hosomi and Sudo, 1986; D'Elia and others, 1987; Ameel and others, 1993; D'Elia and others, 1997) is not necessary in the method described in this report. This modification reduces digest preparation

time substantially. Hopefully, methodological improvements and comparative data in this report in concert with publication of *Standard Methods* method 4500-N C will encourage analysts and regulators to consider potential benefits of more widespread application of alkaline persulfate digestion as an alternative to Kjeldahl digestion for nitrogen and phosphorus determinations in freshwater regimes.

This report provides complete details of the large-scale and geographically diverse study conducted by the USGS between October 1, 2001, and September 30, 2002, to evaluate and validate alkaline persulfate digestion as a more sensitive, accurate, and less toxic alternative to Kjeldahl digestion for routine determination of nitrogen and phosphorus in surface- and ground-water samples.

Purpose and Scope

This report describes USGS methods I-2650-03 and I-4650-03 for determining total nitrogen and total phosphorus in filtered and whole-water alkaline persulfate digests, respectively. All aspects of the methods are described, including sample preparation and digestion, colorimetric determinations of nitrate and orthophosphate in alkaline persulfate digests, calculation of results, bias, precision, and repeatability of results, and conventions for reporting results. These methods supplement other methods of the USGS for determination of inorganic substances in water that are described by Fishman and Friedman (1989) and Fishman (1993). Primary objectives of this study were as follows:

1. To eliminate hazards and toxic wastes associated with Kjeldahl nitrogen and Kjeldahl phosphorus determinations.
2. To ascertain if and under what conditions alkaline persulfate digestion methods can be applied to samples preserved by acidification.
3. To develop an alkaline persulfate digestion procedure that is amenable to automation and less labor intensive than existing Kjeldahl digestion procedures.
4. To achieve lower detection limits for total and dissolved nitrogen than can be achieved by typical Kjeldahl digestion methods.
5. To evaluate statistical equivalence of dissolved and total nitrogen concentrations determined by Kjeldahl and alkaline persulfate digestion methods
6. To evaluate statistical equivalence of dissolved and total phosphorus concentrations determined by Kjeldahl and alkaline persulfate digestion methods.
7. To establish guidelines for interpreting dissolved and total nitrogen and phosphorus concentrations that result from alkaline persulfate digestion in relation to those that result from Kjeldahl digestion.
8. To verify that alkaline persulfate digestion is a more sensitive, accurate, and environmentally responsible alternative to Kjeldahl digestion for routine, simultaneous determination of nitrogen and phosphorus in surface and ground water—the conclusion of several previously published, smaller scale studies—on the basis of a large, geographically and seasonally diverse data set and to demonstrate the method's applicability for compliance monitoring and water-quality assessment studies.

Acknowledgments

The authors gratefully acknowledge Carolyn Keefe and Carl Zimmermann at the Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory (NASL) in Solomons, Md., who informally reviewed a preliminary version of this report. They also kindly shared technical and operational details of NASL's long-established alkaline persulfate digestion nitrogen and phosphorus methods that have been widely applied in nutrient studies of the Chesapeake Bay. We also thank Richard Axler and John Ameel at the University of Minnesota–Duluth Center for Water and the Environment—major participants in validation of the alkaline persulfate digestion method for determination of dissolved and total nitrogen published in the 20th edition of Standard Methods—who reviewed this report. Their comments were consistently insightful and helped us focus the Introduction and Conclusions sections. Tom Maddox at the University of Georgia Stable Isotope Laboratory in Athens also provided helpful discussions and several literature citations about applying alkaline persulfate digestion methods to soil and sediment analysis.

Ron Antweiler, USGS National Research Program, provided helpful discussions about applying *t*-tests to statistical method comparison data in this report. Tom Bushly and Richard Husband, Information Technology Section at the USGS National Water Quality Laboratory (NWQL) performed several large data-base queries and designed custom reports that streamlined sample selection and data analysis. This report also benefited from technical reviews by Colleen Gupta and Mary Cast (USGS, NWQL) and Peter Rogerson (USGS, Office of Water Quality). We also thank Jon W. Raese (USGS, NWQL) for editorial review and Barbara L. Kemp (USGS, NWQL) for manuscript preparation.

ANALYTICAL METHOD

Inorganic Constituents and Parameter Codes (see table 1): Nitrogen and phosphorus, total dissolved, I-2650-03 (mg/L as N or P); nitrogen and phosphorus, total whole water, I-4650-03 (mg/L as N or P)

1. Application

These methods are intended for determination of total nitrogen (organic nitrogen + ammonium + nitrate + nitrite) and phosphorus (all forms) in filtered and whole-water samples by alkaline persulfate digestion. They were validated for determination of total nitrogen and total phosphorus in drinking water, wastewater, and water-suspended sediment. Their applicability to bottom materials was not investigated. Analytical ranges are 0.03 to 5.00 mg-N/L for dissolved and total nitrogen and 0.01 to 2.00 mg-P/L for dissolved and total phosphorus.

2. Method Summary and Analytical Considerations

2.1 Filtered and whole-water samples are dispensed into glass culture tubes, dosed with alkaline persulfate reagent, capped tightly, and digested in an autoclave at 250ºF (121ºC) and 17 lb/in^2 (117.2 kPa) for 1 hour. The alkaline persulfate digestion procedure oxidizes all forms of inorganic and organic nitrogen to nitrate and hydrolyzes all forms of inorganic and organic phosphorus to orthophosphate. Nitrate and orthophosphate in alkaline persulfate digests are determined in parallel with a 2-channel photometric, air-segmented continuous flow analyzer.

2.2 Digest preparation protocols and reagent formulations were adapted from previously published procedures (Valderrama, 1981; Hosomi and Sudo, 1986; Ameel and others, 1993; D'Elia and others, 1997; American Public Health Association, 1998b). Two other reports (Nydahl, 1978; Cabrera and Beare, 1993) provided insight into the potential for low nitrogen recovery in samples containing high concentrations of dissolved and particulate organic carbon.

Quantitative recovery of nitrogen and phosphorus by alkaline persulfate digestion depends critically on a

Table 1. Laboratory, parameter, and method codes for U.S. Geological Survey alkaline persulfate digestion total nitrogen and total phosphorus methods I-2650-03 and I-4650-03

[Lab, laboratory; FCC, filtered chilled container; FCA, filtered, chilled, acidified; WCA, whole water, chilled, acidified; µm, micrometer; mL, milliliter; USGS, U.S. Geological Survey]

Description	Codes			Bottle type
	Lab	Parameter	Method	
Nitrogen, total dissolved, alkaline persulfate digestion.....	2754	62854	A	FCC[1]
Nitrogen, total dissolved, alkaline persulfate digestion, acidified.....	2755	62854	B	FCA[2]
Nitrogen, total whole-water, alkaline persulfate digestion, acidified....	2756	62855	A	WCA[2]
Phosphorus, total dissolved, alkaline persulfate digestion.....	2757	00666	I	FCC[1]
Phosphorus, total dissolved, alkaline persulfate digestion, acidified....	2758	00666	J	FCA[2]
Phosphorus, total whole-water, alkaline persulfate digestion, acidified	2759	00665	H	WCA[2]

[1]FCC samples must be processed through 0.45-µm filters at collection sites.

[2]FCA and WCA samples must be amended with 1 mL of 4.5 *N* H_2SO_4 solution (USGS water-quality field supply number Q438FLD) per 120 mL of sample at collection sites.

progressive decrease in pH (initial pH >12, final pH ≤ 2.2) during the 1-hour course of the digestion (Hosomi and Sudo, 1986). These dynamic reaction conditions are achieved by formulating the digestion reagent with approximately equimolar concentrations of persulfate and hydroxide ions—0.05 ***M***, initial pH >12 after 1 + 2 dilution by samples in this method. Under these initially alkaline conditions, dissolved and suspended nitrogen in samples oxidize to nitrate. As the digestion proceeds, bisulfate ions resulting from thermal decomposition of persulfate first neutralize and then acidify the reaction mixture by the following chemical reaction:

$$S_2O_8^{2-} + H_2O \xrightarrow{\Delta} 2\,HSO_4^- + \tfrac{1}{2}O_2$$

After all of the persulfate has decomposed, the digest mixture pH approaches 2, and under these acidic conditions, dissolved and suspended phosphorus hydrolyze to orthophosphate. The foregoing discussion indicates that analysis of samples with variable and unknown acidity or alkalinity by alkaline persulfate digestion methods will be problematic. Users of this method are cautioned that amending FCA and WCA samples with concentrations of sulfuric acid other than those specified in USGS field manual protocols (Wilde and others, 1998) likely will result in undetected method failure and possible reporting of erroneous results. See section 3.1.4 of this report for additional details.

As is the case for Kjeldahl digestion, alkaline persulfate digestion converts all forms of phosphorus to orthophosphate. Thus alkaline persulfate digestion dissolved and total phosphorus (DP_{AlkP} and TP_{AlkP}) concentrations can be compared directly with Kjeldahl digestion dissolved and total phosphorus (KDP and KTP) concentrations by graphical and statistical analysis. This is not the case, however, for Kjeldahl dissolved and total nitrogen (KDN and KTN) concentrations and alkaline persulfate digestion dissolved and total nitrogen (DN_{AlkP} and TN_{AlkP}) concentrations. In principle, organic nitrogen, but not nitrate or nitrite, is reduced to ammonium during Kjeldahl digestion. Determining ammonium in Kjeldahl digests, therefore, measures organic nitrogen + ammonium. Alkaline persulfate digestion oxidizes all forms of nitrogen to nitrate. Determining nitrate + nitrite in alkaline persulfate digests, therefore, measures total nitrogen (organic nitrogen + ammonium + nitrite + nitrate). To reconcile this difference between the two methods, nitrate + nitrite concentrations were subtracted from DN_{AlkP} and TN_{AlkP} concentrations prior to graphical and statistical comparisons with KDN and KTN concentrations throughout this report. For this purpose and as a quality-control (QC) check, all filtered and whole-water samples selected for alkaline persulfate digestion also were analyzed for dissolved nitrate + nitrite, ammonium, and orthophosphate on the same day that digests were prepared. Particulates were removed from acidified, whole-water samples (WCA bottle type) by 0.45-µm filtration prior to dissolved nutrient determinations, as described in section 4.6 of this report.

2.3 A 2-channel, air-segmented continuous flow analyzer was configured for simultaneous photometric determination of nitrate + nitrite and orthophosphate in alkaline persulfate digests. Nitrate + nitrite was determined by a cadmium-reduction, Griess-reaction method (Wood and others, 1967) equivalent to U.S. Environmental Protection Agency (USEPA) method 353.2 (U.S. Environmental Protection Agency, 1993) and U.S. Geological Survey (USGS) method I-2545-90 (Fishman, 1993, p. 157) except that sulfanilamide and N-(1-naphthy)ethylenediamine reagents were separate rather than combined. The analytical cartridge diagram is shown in figure 1. Orthophosphate was determined by a phosphoantimonylmolybdenum blue method (Murphy and Riley, 1962; Pai and others, 1990), which is equivalent to the 2-reagent variants (separate molybdate and ascorbic acid reagents) of USEPA method 365.1 (U.S. Environmental Protection Agency, 1993) and USGS method I-2601-90 (Fishman, 1993). The analytical cartridge diagram is shown in figure 2.

3. Interferences

3.1 Alkaline Persulfate Digestion

3.1.1 Chloride concentrations up to 1,000 mg/L (the highest tested for this report) do not interfere. Furthermore, because good results are obtained for seawater in 2 + 1 mixture with digestion reagent (D'Elia and others, 1997), chloride concentrations of about 10,000 mg/L apparently are tolerated provided that calibrants are matrix matched. Higher chloride concentrations, however, are likely to interfere because of reaction with persulfate to form oxychlorides or chlorine that might deplete persulfate required to oxidize inorganic and organic nitrogen

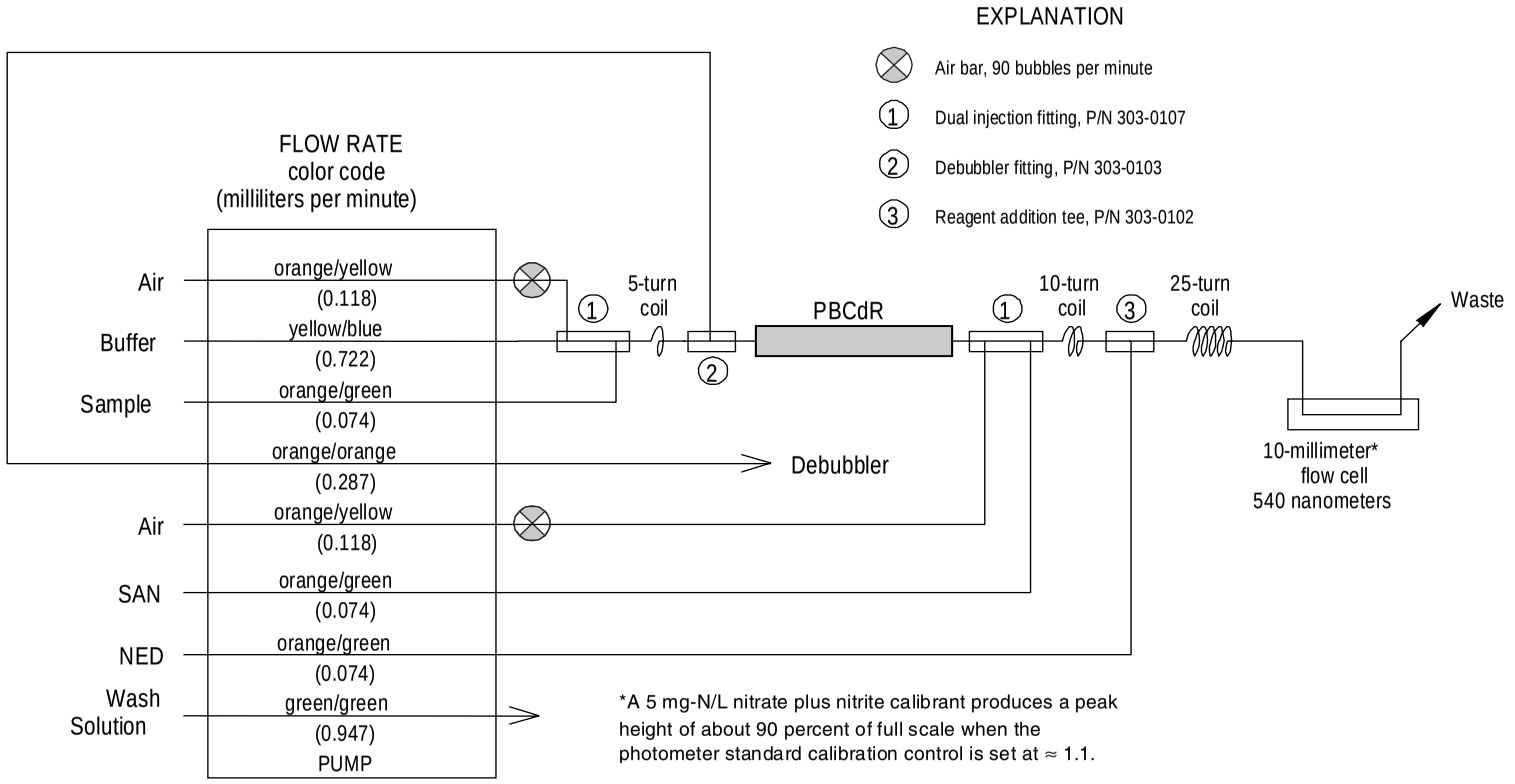

Figure 1. Analytical cartridge diagram for the air-segmented continuous flow analyzer (Alpkem RFA-300) used to automate photometric determination of nitrate + nitrite in alkaline persulfate digests with a cadmium-reduction, Griess reaction method.

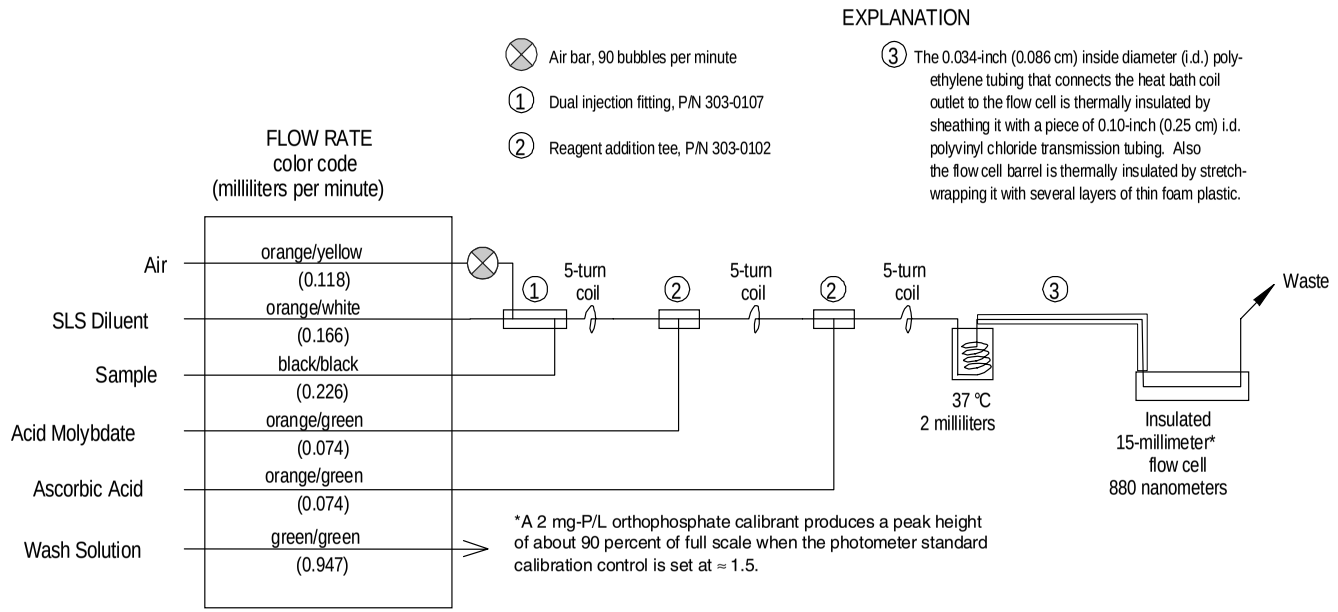

Figure 2. Analytical cartridge diagram for the air-segmented continuous flow analyzer (Alpkem RFA-300) used to automate photometric determination of orthophosphate in alkaline persulfate digests with a reduced phosphoantimonylmolybdenum blue reaction method.

species to nitrate. Resulting active chlorine species also can interfere in colorimetric reactions used to determine nitrate and orthophosphate in digests.

3.1.2 Sulfate concentrations up to 1,000 mg/L (the highest tested for this report) do not interfere.

3.1.3 Organic carbon concentrations greater than 150 mg/L interfere because of reaction with persulfate to form carbon dioxide, thus depleting persulfate required to oxidize inorganic and organic nitrogen species to nitrate.

3.1.4 Overacidification of FCA and WCA samples at collection sites can result in low recovery of inorganic and organic nitrogen at the NWQL. The possibility of overacidification can be avoided by exclusive use of the sulfuric acid field-amendment solution—one vial containing 1 mL of 4.5 *N* H_2SO_4 (One Stop Shopping number FLD-438) per 120 mL of sample—which is specified in the USGS National Field Manual (Wilde and others, 1998). See the first note in section 6.1 of this report for additional details.

3.1.5 Nitrate and nitrite do not contribute to KDN and KTN concentrations in principle, but in practice, positive and negative interferences by these ions are well known—see, for example, American Public Health Association, 1998c; Patton and Truitt, 2000. This interference can confound comparison of KN and N_{AlkP} concentrations when dissolved nitrate concentrations are greater than about 0.1 mg NO_3^--N/L.

3.1.6 Suspended particles remaining in digests must be removed by sedimentation and decantation or filtration prior to colorimetric analyses.

3.2 Colorimetric Nitrate + Nitrite Determination

3.2.1 Typically, concentrations of substances with potential to interfere in cadmium-reduction, Griess-reaction nitrate + nitrite methods are negligible in ambient surface- and ground-water samples. For specific details of inorganic and organic compounds that might interfere in the color reaction, see Norwitz and Keliher (1985, 1986), as well as more general information by the American Public Health Association (1998a).

3.2.2 Sulfides, which are often present in anoxic water and well known to deactivate cadmium reduction reactors, are oxidized during the alkaline persulfate digestion and are unlikely to interfere.

3.3 Colorimetric Orthophosphate Determination

3.3.1 Barium, lead, and silver can interfere by forming insoluble phosphates, but their concentrations in natural-water samples usually are less than the interference threshold (Fishman, 1993)

3.3.2 Interference from silicate, which also can form reduced heteropoly acids with molybdenum (Zhang and others, 1999), is negligible under reaction conditions used for this report.

3.3.3 Arsenate, AsO_4^{3-}—but not arsenite, AsO_3^{3-}—can interfere by forming reduced heteropoly acids analogous to those formed by orthophosphate (Johnson, 1971). Because of the possibility that arsenite might be oxidized to arsenate by persulfate, both species at concentrations up to 20 mg-As/L in deionized water were digested and analyzed. With reference to table 2, it is apparent that a major fraction of arsenite is oxidized to arsenate during alkaline persulfate digestion and that interference by either species up to 1 mg-As/L is negligible.

Table 2. Data from a study of arsenate and arsenite interference in alkaline persulfate total phosphorus determinations

[mg-As/L, milligrams of arsenic per liter; mg-P/L, milligrams of phosphorus per liter; nd, not detected; ≈, nearly equal to; ±, plus or minus]

AsO_4^{3-} added mg-As/L	PO_4^{3-} found mg-P/L	AsO_3^{3-} added mg-As/L	PO_4^{3-} found mg-P/L
0.5	nd	0.5	nd
1.0	nd	1.0	nd
2.0	≈ 0.05	2.0	nd
5.0	0.32 ± 0.01	5.0	0.29 ± 0.04
10.0	1.14 ± 0.13	10.0	0.91 ± 0.06
20.0	off scale	20.0	off scale

4. Instrumentation and Auxiliary Analyses

4.1 RFA-300™, third-generation, air-segmented continuous flow analyzers (Alpkem) were used to automate photometric determination of nitrate + nitrite and orthophosphate in alkaline persulfate digests and dissolved ammonium, nitrate + nitrite, and orthophosphate in filtered- and whole-water samples prior to digestion. Modules in these systems include 301 samplers, 302 peristaltic pumps, 313 analytical cartridge bases, 314 power modules, 305A photometers, and a personal computer (PC)-based data acquisition and processing system. Alternative instrumentation—flow injection analyzers, sequential injection analyzers, other second- or third-generation continuous flow analyzers, or automated batch analyzers—also could be used to automate photometric finishes.

4.2 Photometric data were acquired and processed automatically using FASPac™ version 1.34 software (Astoria-Pacific, Clackamas, Ore.). This software operates under Microsoft Windows on a PC platform and includes a model 350 interface box that controls the sampler and digitizes analog photometer outputs with 16-bit resolution. Other data acquisition systems could be used provided that the A/D converter has 16-bit resolution and is capable of acquiring data at frequencies ranging from 0.5 to 2 Hz, that is, from 30 points/min to 120 points/min. As a general rule, data acquisition frequencies for air-segmented continuous flow analyzers should match the roller lift-off frequency of the peristaltic pump (Patton and Wade, 1997), that is, 0.5 Hz for Technicon AutoAnalyzer II ™ and 1.5 Hz for Alpkem RFA-300 equipment. Data acquisition frequencies in the range of 2 to 5 Hz are suitable for photometric flow-injection analyzers.

4.3 Operating characteristics for this equipment are listed in table 3.

4.4 Dissolved ammonium, nitrate + nitrite, and orthophosphate in undigested samples were determined photometrically by USGS automated continuous flow methods I-2522-90, I-2545-90 (2-reagent variant), and I-2601-90 (2-reagent variant), respectively. These methods are described in Fishman (1993).

4.5 The pH of WCA samples was estimated with narrow range (0–2.5) colorimetric pH-indicating test strips to detect improperly acidified samples that had pH values outside the expected range of 1.6 to 1.9.

4.6 WCA samples were processed through 5-mL capacity UniPrep™ syringeless filters equipped with 0.45-µm nylon membranes (Whatman, Clifton, N.J.) to remove suspended solids prior to determination of dissolved ammonium, nitrate + nitrite, and orthophosphate. These syringeless filters also were used to remove suspended solids from WCA-sample digests prior to photometric analysis when simple sedimentation and decantation into analyzer cups failed to do so.

5. Apparatus

5.1 Samples were digested in an autoclave (model number STME, Market Forge Industries, Inc., Everett, Mass.) operated at 250ºF (121ºC) and 17 lb/in^2 (117.2 kPa) for 1 hour.

5.2 Filtered and chilled sample (FCC bottle type) digests were prepared robotically using a large-scale, syringe-pump-based *x-y-z* sample dispenser/diluter module (model number ML-4200, Hamilton Company, Reno, Nev.). This system is equipped with four probes and four 10-mL syringe pumps that operate in tandem under control of DOS-based *Eclipse*™ software (Hamilton Company, Reno, Nev.). Custom modifications to the ML-4200 system, including a pneumatically actuated probe expander, fixtures, and a variety of bottle and test-tube racks, were obtained from another vendor (Robotics Plus, Houston, Tex.).

5.3 Whole-water (WCA bottle type) sample digests were prepared manually using EDP Plus™

Table 3. Settings and operational details of Alpkem RFA-300 continuous flow analyzers used for this study

[nm, nanometer; mm, millimeter; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; ≈, nearly equal to; min, minute; mL, milliliter; –, not applicable; °C, degrees Celsius; s, second; h, hour]

Instrumental conditions	Nitrate + nitrite	Orthophosphate
Analytical wavelength	540 nm	880 nm
Flow cell path length	10 mm	15 mm
Calibration range	0.05 to 5.0 mg-N/L	0.01 to 2.0 mg-P/L
Standard calibration control setting	≈1.1	≈1.5
Segmentation rate (bubbles min^{-1})	90	90
Heated reaction coil volume	None used	2 mL
Heated reaction coil temperature	–	37°C
Dwell time (seconds)	140	260
Sample time (volume)	25 s (95 µL)	25 s (31 µL)
Wash time (volume)	10 s (38 µL)	10 s (12 µL)
Analysis rate, sample-to-wash ratio	≈103/h, 5:2	≈103/h, 5:2

electronic, digital pipets (Rainin Instruments, Emeryville, Calif.) equipped with a 10-mL liquid end.

5.4 Digestion vessels were 20 x 150 mm Pyrex®, screw-cap culture tubes (VWR 53283-810; Fisher 14-957-76E or 14-959-37C; or equivalent), and 18-415 linerless polypropylene caps (Comair Glass, Inc., Vineland, N.J.—Part number 14-0441-004).

6. Reagents

This section provides detailed instructions for preparing digestion and colorimetric reagents. All references to deionized water (DI) refer to NWQL in-house DI water, which is equivalent to ASTM type I DI water (American Society for Testing and Materials, 2001, p. 107–109) for nutrient analysis. All volumetric glassware and reagent and calibrant storage containers should be triple rinsed with dilute (≈5 percent v/v) hydrochloric acid and DI water just prior to use. Additionally storage containers for reagents and calibrants should be triple rinsed with small portions of the solutions before they are filled.

6.1 Digestion Reagents

NOTE: The alkaline persulfate digestion reagent for FCA and WCA samples (section 6.1.4) contains an additional amount of sodium hydroxide that is calculated to neutralize the sulfuric acid added to these samples at collection sites.

6.1.1 *Sodium hydroxide, 1.5 **M** (for FCC samples)*: Dissolve 60 g of sodium hydroxide (NaOH, FW=40.0) in about 800 mL of DI water in a 1-L volumetric flask. [**Caution:** When NaOH dissolves in water, heat is released.] After dissolution is complete, allow the resulting solution to cool and dilute it to the mark with DI water. Transfer this reagent to a plastic bottle in which it is stable at room temperature for 6 months.

6.1.2 *Sodium hydroxide, 2.3 **M** (for FCA and WCA samples)*: Dissolve 92 g of sodium hydroxide (NaOH, FW=40.0) in about 800 mL of DI water in a 1-L volumetric flask. [**See caution in 6.1.1.**] After dissolution is complete, allow the resulting solution to cool and dilute it to the mark with DI water. Transfer this reagent to a plastic bottle in which it is stable at room temperature for 6 months.

6.1.3 *Alkaline persulfate digestion reagent (for FCC samples)*: Add 18.0 g of potassium persulfate ($K_2S_2O_8$, FW=270.33) and 45 mL of 1.5 ***M*** sodium hydroxide solution to about 350 mL of DI water in a graduated 500-mL Pyrex™ media bottle (Corning number 1395-500 or equivalent). Cap the bottle, swirl its contents, and place it in an ultrasonic bath until potassium persulfate dissolution is complete (about 10 minutes). Remove the bottle from the ultrasonic bath, dry its outer surfaces, and then add enough DI water to bring the volume to 450 mL. (Make a line on the side of the bottle that indicates this volume to within ±5 mL.) Swirl the bottle to mix its contents and then divide the resulting solution among four, 125-mL clear plastic bottles used with the robotic digest preparation system. Prepare this reagent daily.

6.1.4 *Alkaline persulfate digestion reagent (for FCA and WCA samples)*: Add 18.0 g of potassium persulfate ($K_2S_2O_8$, FW=270.33) and 45 mL of 2.3 ***M*** sodium hydroxide solution to about 350 mL of DI water in a graduated 500-mL Pyrex™ media bottle (Corning number 1395-500 or equivalent). Then complete preparation of this reagent exactly as described in 6.1.3. Prepare this reagent daily.

NOTE: Reagent volumes in 6.1.3 and 6.1.4 (450 mL) are sufficient to prepare 80 digests plus a 15-percent excess for rinsing and providing a liquid level in the 125-mL bottles necessary to prevent air aspiration during robotic dispensing operations. For manual digest preparation, a 400-mL volume of digestion reagent should be sufficient.

6.2 Colorimetric Reagents

6.2.1 *Sampler wash reservoir solution (0.05 **M** sodium bisulfate)*: Dissolve 6.9 g of sodium bisulfate ($NaHSO_4 \cdot H_2O$, 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.

6.3 Orthophosphate Determination

6.3.1 *Stock potassium antimony tartrate reagent*: Dissolve 3.0 g of antimony potassium tartrate [$K(SbO)C_4H_4O_7 \cdot ½ H_2O$, FW=333.93] in about 800 mL of DI water in a 1-L volumetric flask. Dilute this solution to the mark with DI water and mix it well.

Transfer this reagent to a plastic bottle in which it is stable for 6 months at room temperature.

6.3.2 *Stock ascorbic acid reagent*: Dissolve 4.5 g of ascorbic acid ($C_6H_8O_6$, FW=176.1) in about 200 mL of DI water in a 250-mL volumetric flask. Dilute this solution to the mark with DI water, mix it well, and transfer to a 250-mL glass bottle that has been previously rinsed with 5 percent (v/v) hydrochloric acid solution and DI water. This reagent is stable for 2 weeks at 4°C.

6.3.3 *Stock sodium lauryl sulfate reagent (15 percent w/w)*: Add 340 mL of DI water to 60 g of sodium lauryl sulfate [SLS, $CH_3(CH_2)_{11}OSO_3Na$, FW=288.38] in a 500-mL Pyrex™ media bottle. Cap the bottle and place it in an ultrasonic bath until the SLS dissolves completely (about 30 minutes). Manual inversion of the bottle at 5-minute intervals speeds dissolution. Transfer this solution to a plastic bottle in which it is stable indefinitely at room temperature.

6.3.4 *Acidic molybdate-antimony reagent*: Using a graduated cylinder, cautiously add 72 mL of concentrated sulfuric acid (H_2SO_4, sp. gr. 1.84) to about 700 mL of DI water in a 1-L volumetric flask. Work in a hood and manually swirl or magnetically stir the flask during each addition of sulfuric acid. Next add 7.7 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}•4H_2O$, FW=1235.86] to the hot sulfuric acid solution. Manually swirl or magnetically stir the contents of the flask until the ammonium molybdate dissolves. Then add 50 mL of stock antimony potassium tartrate solution (6.3.1) and again mix the contents of the flask thoroughly. After the resulting solution has cooled, dilute it to the mark with DI water, mix it well, and transfer it to a clean 1-L plastic bottle in which it is stable for 1 year at room temperature.

6.3.5 *Sodium lauryl sulfate diluent reagent*: Use a 100-mL graduated cylinder to dispense 10 mL of stock SLS (6.3.3) and 90 mL of DI water into a small plastic bottle. Manually swirl the bottle to mix its contents. Prepare this reagent daily.

6.3.6 *Ascorbic acid reagent*: Use a 50-mL graduated cylinder to dispense 5 mL of the stock ascorbic acid reagent (6.3.2) and 25 mL of DI water into an amber glass reagent bottle. Manually swirl the bottle to mix its contents. Prepare this solution daily.

6.3.7 *Startup/shutdown solution*: Add 1 mL of stock SLS reagent to 100 mL of DI water in a small plastic bottle. Thoroughly rinse the bottle and prepare a fresh solution every few days or as needed.

6.4 Nitrate Determination

6.4.1 *Copper (II) sulfate reagent (2 percent w/v)*: Dissolve 20 g of copper sulfate pentahydrate ($CuSO_4•5H_2O$, FW=249.7) in about 800 mL of DI water in a 1-L volumetric flask. Dilute this solution to the mark with DI water, mix it well, and transfer it to a 1-L plastic bottle. This reagent is stable for several years at room temperature.

6.4.2 *Imidazole buffer, 0.1 **M**, (pH 7.5)*: In a hood, cautiously add 5.0 mL of concentrated hydrochloric acid (HCl, ~12 ***M***) and 1.0 mL of 2 percent copper sulfate solution to 1,600 mL of DI water in a 2-L volumetric flask. Mix the contents of the flask thoroughly and then add 13.6 g of imidazole ($C_3H_4N_2$, FW=68.08). Again swirl or shake the flask until the imidazole dissolves. Dilute the resulting solution to the mark with DI water, mix it well, and transfer it into two 1-L plastic bottles. This reagent is stable for 6 months at room temperature.

NOTE: Add 250 µL of Brij-35 surfactant to 250 mL of imidazole buffer each time its container is refilled on the continuous flow analyzer. Do not add Brij-35 to the bulk buffer solution.

6.4.3 *Packed bed cadmium reactor*: Cadmium reactors are prepared by slurry packing 40- to 60-mesh, copperized cadmium granules into 6-cm lengths of PTFE Teflon™ tubing (1.6 mm i.d. × 3.2 mm o.d.). Cadmium granules are retained in the column with hydrophilic plastic frits (40-µm nominal pore size). Detailed instructions for preparing copperized cadmium granules and packing them into columns can be found in NWQL standard operating procedure (SOP) IM0384.0 (or subsequent revisions; available on request).

6.4.4 *Sulfanilamide reagent ("SAN")*: Use a graduated cylinder to dispense 100 mL of concentrated hydrochloric acid (HCl, 36.5–38.0 percent, ≈12 ***M***) into about 700 mL of DI water in a 1-L volumetric flask. Work in a hood and manually swirl or magnetically stir the flask during each addition of HCl. Add 10.0 g of SAN ($C_6H_8N_2O_2S$, FW=172.20) to the warm hydrochloric acid solution. Manually shake, sonicate, or magnetically stir the contents of the flask until the SAN dissolves. After the resulting solution has cooled, dilute it to the mark with DI water, mix it well, and transfer it to a clean 1-L plastic bottle in which it is stable for 1 year at room temperature.

6.4.5 *N-(1-Naphthyl)ethylenediamine dihydrochloride reagent ("NED")*: Dissolve 1.0 g

NED ($C_{12}H_{14}N_2$•2HCl, FW=259.2) in about 800 mL of DI water in a 1-L volumetric flask. Dilute the resulting solution to the mark with DI water and mix well by manually shaking the flask. Transfer this reagent to a 1-L amber glass bottle in which it is stable for 6 months at room temperature.

6.4.6 *Startup/shutdown solution*: Add 250 µL of Brij-35 surfactant to 250 mL of DI water in a plastic bottle. Thoroughly rinse the bottle and prepare a fresh solution every few days or as needed.

7. Calibrants and Quality-Control Solutions

This section provides detailed instructions for preparing calibrants, matrix spike solution, quality-control check solutions, and digestion check solution.

7.1 *Potassium nitrate stock calibrant solution, 1 mL=2.5 mg-N*: Dissolve 1.805 g of potassium nitrate (KNO_3, FW=101.1) in about 80 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock calibrant to a 100-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.2 *Potassium di-hydrogen phosphate stock calibrant solution, 1 mL=1.0 mg-P*: Dissolve 0.4394 g potassium di-hydrogen phosphate (KH_2PO_4, FW=136.09) in about 80 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock calibrant to a 100-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.3 *Sulfuric acid* ≈1.8 ***M***: Use a 25-mL graduated cylinder to dispense 10 mL of concentrated sulfuric acid (H_2SO_4, sp. gr. 1.84) into about 75 mL of DI water in a 100-mL volumetric flask. After the solution cools, dilute it to the mark with DI water, mix it well, and transfer it to a 125-mL plastic bottle. Make a new batch of this acid each time acidified working calibrants and blanks are prepared and use the remainder to prepare acidified blank solution as needed.

7.4 *Mixed stock calibrant solution, 1 mL = 1.25 mg-N and 0.5 mg-P*: Dispense equal volumes (minimum of 2 mL each) of nitrate (7.1) and phosphate (7.2) stock calibrants into a small beaker and mix them thoroughly. Prepare this solution each time working calibrants are prepared.

7.5 *Working calibrant solutions (for FCC samples)*: Use two adjustable, digital pipets (ranges 10 to 100 µL and 100 to 1,000 µL) to dispense the volumes of mixed stock calibrant (7.4) listed in table 4 into 250-mL volumetric flasks that each contain about 200 mL of DI water. Dilute the working calibrants to the mark with DI water and mix them thoroughly by manual inversion and shaking. Transfer the working calibrants to 250-mL Pyrex™ media bottles in which they are stable for 4 weeks at 4°C.

7.6 *Acidified working calibrant solutions (for FCA and WCA samples)*: Prepare these calibrants

Table 4. Volumes of mixed calibrant and amendment solution required to prepare working calibrants and blanks for determination of total nitrogen and phosphorus by the alkaline persulfate digestion method. Final volumes are 250 mL

[µL, microliter; mL, milliliter; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; ***M***, molarity (moles per liter); FCA, filtered, chilled, acidified (bottle type); WCA, whole water, chilled, acidified (bottle type)]

Calibrant identity	Mixed calibrant volume (µL)	Volume 1.8 ***M*** H_2SO_4 [1] (mL)	Nominal concentration (mg-N/L)	Nominal concentration (mg-P/L)
C1	1,000	2.5	5.00	2.00
C2	750	2.5	3.75	1.50
C3	500	2.5	2.50	1.00
C4	250	2.5	1.25	0.50
C5	100	2.5	0.50	0.20
C6	[2] 6	[2] 2.5	0.03	0.012
C7	0	2.5	0	0

[1] Add H_2SO_4 only to acidified calibrants as described in section 7.6.

[2] Prepare 1 L of C6 (24 µL of mixed calibrant and 10 mL of 1.8 ***M*** H_2SO_4, if appropriate, diluted to 1 L with DI water) to minimize dispensing error.

identically to those described in section 7.5, except add 2.5 mL of 1.8 ***M*** H_2SO_4 to each flask before diluting it to the mark with DI water.

7.7 *Check standards (for FCC samples)*: Check standards in three concentration ranges, which were designated *Low*, *High*, and *Very high*, were prepared from a concentrated commercial nutrient QC mixture (*Demand*™, Environmental Resource Associates, Arvada, Colo.), as listed in table 5. Transfer check standards to 1-L Pyrex™ media bottles in which they are stable for 2 months at 4ºC. Each of these check standards was dispensed, digested, and analyzed along with every batch of filtered and whole-water samples analyzed for this study.

7.8 *Acidified check standards (for FCA and WCA samples)*: Prepare these check standards identically to those described in section 7.7, except add 10.0 mL of 1.8 ***M*** H_2SO_4 to the flasks before diluting them to the mark with DI water.

7.9 Spike Solutions

7.9.1 *Nitrogen stock spike solution (1 mL = 0.50 mg-N)*: Dissolve 0.955 g ammonium chloride (NH_4Cl, FW=53.49) 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 the stock spike solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.9.2 *Phosphorus stock spike solution (1 mL = 0.20 mg-P)*: Dissolve 0.439 g potassium dihydrogen phosphate (KH_2PO_4, FW=136.1) 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 the stock spike solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.9.3 *Mixed spike solution (100 µL = 0.005 mg-N and 0.002 mg-P)*: Dispense 1 mL each of ammonium chloride and orthophosphate stock spike solutions into a 10-mL volumetric flask and dilute to the mark with DI water. Transfer the mixed spike solution to a 15-mL, screw-cap polyethylene centrifuge tube in which it is stable for 2 weeks at 4°C.

NOTE: An equivalent mixed spike solution can be prepared more conveniently from stock calibrants (sections 7.1 and 7.2) by diluting 500 µL of each to 25 mL in a volumetric flask.

7.10 Digest-Check Stock Solutions

7.10.1 *Glycine digest-check stock solution (1 mL = 1.0 mg-N)*: Dissolve 3.98 g glycine ($C_2H_5NO_2$•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 the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.10.2 *Glycerophosphate digest-check stock solution (1 mL = 0.4 mg-P)*: Dissolve 1.976 g glycerophosphate ($C_3H_7O_6PNa_2$•$5H_2O$, 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 the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.10.3 *Glucose digest-check stock solution (1 mL = 1.25 mg-C)*: Dissolve 1.564 g glucose ($C_6H_{12}O_6$, FW=180.2) 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 the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

Table 5. Volumes of Environmental Resource Associates (ERA) *Demand*™ nutrient concentrate used to prepare 1-liter volumes of check standards used in this study

[µL, microliter; mL, milliliter; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter]

Check standard identity	ERA *Demand*™ volume (µL)	Volume 1.8 ***M*** H_2SO_4 [1] (mL)	Nominal concentration (mg–N/L)	Nominal concentration (mg–P/L)
Low	100	10.0	0.22	0.11
High	500	10.0	1.09	0.54
Very high	1,000	10.0	2.20	1.08

[1] Add H_2SO_4 only to acidified check standards as described in section 7.8.

7.10.4 *Mixed digest-check solution (for FCC samples—nominal concentration 4 mg-N/L, 1.6 mg-P/L, and 50 mg-C/L)*: Dispense 1 mL each of glycine and glycerophosphate stock digest-check solutions and 10 mL of the glucose digest-check stock solution into a 250-mL volumetric flask that contains about 200 mL of DI water. Dilute the contents of the flask to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 250-mL Pyrex™ media bottle in which it is stable for 1 month at 4°C.

7.10.5 *Acidified mixed digest-check solution (for FCA and WCA samples)*: Prepare this digest-check solution identically to the one described in section 7.10.4, except add 2.5 mL of 1.8 ***M*** H_2SO_4 to the flask before diluting its contents to the mark with DI water. Transfer the acidified mixed digest-check solution to a 250-mL Pyrex™ media bottle in which it is stable at 4°C for 1 month.

8. Sample Preparation

8.1 Alkaline persulfate digests are prepared by dispensing samples and digestion reagent into 30-mL, screw-cap, Pyrex™ culture tubes in the volume ratio of 2 + 1. For filtered samples (FCC bottle types) that were prepared robotically, 9.5-mL volumes of samples, blanks, calibrants, and reference materials were dosed with 4.75-mL volumes of alkaline persulfate digestion reagent (see section 6.1.3). This is the maximum sample volume that could be delivered by the robotic dispenser/diluter system's 10.000-mL syringes because 0.500 mL of their capacity is expended in the creation of air gaps that minimize interaction between samples and the DI water carrier fluid. Whole-water samples (WCA bottle types) that require vigorous shaking (and in a few cases, continuous magnetic stirring) just prior to dispensing operations were prepared manually with conventional, high-precision, hand-held electronic pipets (Rainin EDP *Plus*™). Here dispensed volumes of sample and digestion reagent (see section 6.1.4) were 10.000 and 5.000 mL, respectively. After robotic or manual sample and reagent-dispensing operations are complete, 100 µL of mixed spike solution (see section 7.9.3) is added manually to the designated tube. Then all tubes are capped tightly and mixed thoroughly either by manual inversion (three times) or with a vortex mixer (3, 5-second cycles). The capped tubes positioned in a purpose-built, 80-position stainless-steel rack then are placed in an autoclave where they are digested at 121ºC and 117.2 kPa for 1 hour. Table 6 lists the rack protocol suggested for a batch of 80 tubes consisting of up to 64 samples plus six calibrants, four blanks, three quality-control (QC) check solutions, one digest-check solution, one duplicate sample, and one spiked sample. A step-by-step procedure for

Table 6. Suggested rack protocol for alkaline persulfate digest preparation

[ID, identification; QC, quality control; yyyy, year; ddd, Julian day]

Tube number	ID	Tube number	ID	Tube number	ID	Tube number	ID
1	C1	21	yyyyddd0007	41	yyyyddd0027	61	yyyyddd0047
2	C2	22	yyyyddd0008	42	yyyyddd0028	62	yyyyddd0048
3	C3	23	yyyyddd0009	43	yyyyddd0029	63	yyyyddd0049
4	C4	24	yyyyddd0010	44	yyyyddd0030	64	yyyyddd0050
5	C5	25	yyyyddd0011	45	yyyyddd0031	65	yyyyddd0051
6	C6	26	yyyyddd0012	46	yyyyddd0032	66	yyyyddd0052
7	C7 (blank)	27	yyyyddd0013	47	yyyyddd0033	67	yyyyddd0053
8	blank	28	yyyyddd0014	48	yyyyddd0034	68	yyyyddd0054
9	blank	29	yyyyddd0015	49	yyyyddd0035	69	yyyyddd0055
10	blank	30	yyyyddd0016	50	yyyyddd0036	70	yyyyddd0056
11	QC low	31	yyyyddd0017	51	yyyyddd0037	71	yyyyddd0057
12	Digest check	32	yyyyddd0018	52	yyyyddd0038	72	yyyyddd0058
13	QC high	33	yyyyddd0019	53	yyyyddd0039	73	yyyyddd0059
14	QC very high	34	yyyyddd0020	54	yyyyddd0040	74	yyyyddd0060
15	yyyyddd0001	35	yyyyddd0021	55	yyyyddd0041	75	yyyyddd0061
16	yyyyddd0002	36	yyyyddd0022	56	yyyyddd0042	76	yyyyddd0062
17	yyyyddd0003	37	yyyyddd0023	57	yyyyddd0043	77	yyyyddd0063
18	yyyyddd0004	38	yyyyddd0024	58	yyyyddd0044	78	yyyyddd0064
19	yyyyddd0005	39	yyyyddd0025	59	yyyyddd0045	79	Duplicate
20	yyyyddd0006	40	yyyyddd0026	60	yyyyddd0046	80	Spike

alkaline persulfate digest preparation is provided in NWQL SOP IM0384.0 (available on request).

NOTE: When samples contain large quantities of suspended solids, continuous stirring during sample aspiration might provide the only means of obtaining representative aliquots.

8.2 When the digestion cycle is complete and pressure and temperature gages on the autoclave indicate 0 kPa and less than 80°C, remove the alkaline persulfate digests from the autoclave and allow them to cool sufficiently to be handled comfortably. Then mix the contents of each capped digestion tube by manual inversion (three times) or with a vortex mixer (three, 5-second cycles). FCC and FCA digests can be poured into analyzer cups immediately after mixing. Wait about 1 hour after mixing WCA digests to allow suspended solids to settle. If it is not possible to decant or pipet a clear supernatant solution from digest tubes into analyzer cups, then suspended solids must be removed by 0.45-µm filtration prior to colorimetric analysis. Note that tightly capped digests can be stored at room temperature for several days (4 days was the maximum delay tested) before their nitrogen and phosphorus concentrations are determined by automated colorimetry.

9. Instrument Performance

An 80-tube batch of samples, calibrants, and reference materials can be prepared robotically and made ready for digestion in about 1 hour. Digestion time—including warm up, cool down, and postdigestion mixing—is about 2 hours. The NWQL Nutrients Unit has two autoclaves, each of which can hold two, 80-tube racks of alkaline persulfate digests. Nitrate and orthophosphate in alkaline persulfate digests can be determined simultaneously with the 2-channel air-segmented continuous flow analyzer at a rate of about 100 samples per hour with less than 1 percent interaction. Thus, using a combination of robotic and manual sample preparation, up to six racks (384 actual samples out of 480 total tubes) of alkaline persulfate digests can be prepared in an 8-hour day. This estimate assumes the use of both NWQL autoclaves and a combination of robotic (FCC samples) and manual (WCA samples) sample preparation. Likewise, up to six racks of previously digested samples can be analyzed for nitrate and orthophosphate in an 8-hour day. This production rate assumes that digest analysis can lag sample digestion by 1 to 3 days.

10. Calibration

With a second-order polynomial least-squares curve-fitting function ($y = a+bx+cx^2$, where y is the baseline and blank-corrected peak height and x is the nominal concentration), calibration plots with correlation coefficients (r^2) greater than 0.999 are achieved routinely. Typical calibration plots for nitrate and orthophosphate in alkaline persulfate digests are shown in figures 3 and 4.

NOTE: In addition to baseline drift correction, a digestion blank correction must be applied to calibrants, check standards, and samples prior to calculation of final results, as described in sections 12.3 and 12.4.

11. Procedure and Data Evaluation

Set up the continuous flow analyzer analytical cartridges as shown in figures 1 and 2. Turn on electrical power to all system modules and put fresh sampler wash reservoir solution and reagents on-line. After about 10 minutes, verify that the sample and reference outputs of both photometers are set at about 5 volts. A suggested sampler tray protocol for automated determination of nitrate and orthophosphate in alkaline persulfate digests is listed in table 7.

NOTE: To minimize errors that result from contaminated analyzer cups, rinse them several times with the solution they are to contain before placing them on the analyzer sampler tray.

NOTE: The full-scale absorbance range control (STD CAL) of photometers should not require daily adjustment. Between-analysis/between-day variations in baseline-absorbance level and calibration curve slope of about ±5 percent are acceptable. Adjustment of the STD CAL control to compensate for larger variations in sensitivity or baseline (reagent blank) levels will only mask underlying problems, such as incipient light source failure, partially clogged flow cells, or contaminated or improperly prepared reagents, any of which could compromise analytical results.

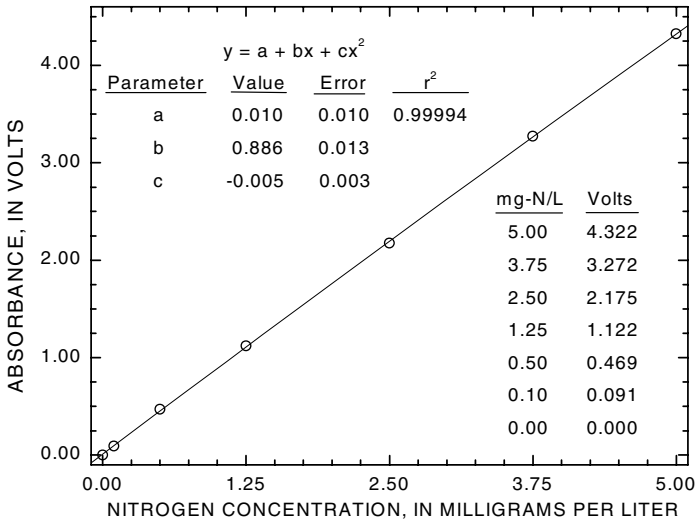

Figure 3. Typical calibration graph for total nitrogen determined as nitrate in alkaline persulfate digests.

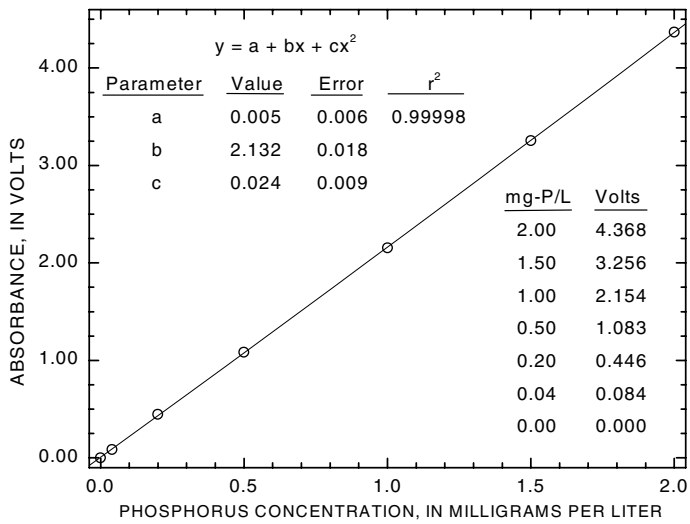

Figure 4. Typical calibration graph for total phosphorus determined as orthophosphate in alkaline persulfate digests.

12. Calculations

12.1 Instrument calibration requires preparing a set of solutions (calibrants) in which the analyte concentration is known. These calibrants are digested along with samples and used to establish a calibration function that is estimated from a least-squares fit of nominal calibrant concentrations (x) in relation to peak absorbance (y). A second-order polynomial function ($y = a+bx+cx^2$) usually provides improved concentration estimates at the upper end of the calibration range than a more conventional linear function ($y = a+bx$). Accuracy is not lost when a second-order fit is used, even if the calibration function is strictly linear, because, in this case, the value estimated for the quadratic parameter c will approach zero.

12.2 Before the calibration function can be estimated, the baseline absorbance component of measured peak heights, including drift (continuous increase or decrease in the baseline absorbance during the course of an analysis), if present, needs to be removed. Baseline absorbance in continuous flow analysis is analogous to the reagent blank absorbance in batch analysis. Correction for baseline absorbance is an automatic function of most data acquisition and processing software sold by vendors of continuous flow analyzers.

NOTE: These correction algorithms are based on linear interpolation between initial and intermediate or final baseline measurements, and so they do not accurately correct for abrupt, step-changes in baseline absorbance that usually indicate partial flow-cell blockage. It is prudent, therefore, to reestablish baseline absorbance at intervals of 20 samples or so.

Table 7. Suggested analyzer sample tray protocol for automated determination of nitrate and orthophosphate in alkaline persulfate digests

[#, number; ID, identification; SYNC, synchronization peak; CO, carry-over peak; W, wash; UB, undigested blank; DB, digested blank; CCV, continuing calibration verification; QC, quality control; yyyy, year; ddd, Julian day]

Cup #	ID	Cup #	ID	Cup #	ID	Cup #	ID
1	SYNC	24	yyyyddd0006	47	yyyyddd0029	70	yyyyddd0050
2	CO (C6)	25	yyyyddd0007	48	yyyyddd0030	71	yyyyddd0051
3	(C6)	26	yyyyddd0008	49	yyyyddd0031	72	yyyyddd0052
4	W	27	yyyyddd0009	50	yyyyddd0032	73	yyyyddd0053
5	C1	28	yyyyddd0010	51	UB	74	yyyyddd0054
6	C2	29	yyyyddd0011	52	W (DB)	75	yyyyddd0055
7	C3	30	yyyyddd0012	53	yyyyddd0033	76	yyyyddd0056
8	C4	31	yyyyddd0013	54	yyyyddd0034	77	yyyyddd0057
9	C5	32	yyyyddd0014	55	yyyyddd0035	78	yyyyddd0058
10	C6	33	yyyyddd0015	56	yyyyddd0036	79	yyyyddd0059
11	C7	34	yyyyddd0016	57	yyyyddd0037	80	yyyyddd0060
12	W	35	yyyyddd0017	58	yyyyddd0038	81	yyyyddd0061
13	CCV	36	yyyyddd0018	59	yyyyddd0039	82	yyyyddd0062
14	UB[1]	37	yyyyddd0019	60	yyyyddd0040	83	yyyyddd0063
15	QC low[2]	38	yyyyddd0020	61	yyyyddd0041	84	yyyyddd0064
16	Digest check[3]	39	yyyyddd0021	62	yyyyddd0042	85	duplicate
17	QC high[2]	40	yyyyddd0022	63	yyyyddd0043	86	Spike
18	QC very high[2]	41	yyyyddd0023	64	yyyyddd0044	87	UB
19	yyyyddd0001	42	yyyyddd0024	65	yyyyddd0045	88	CCV
20	yyyyddd0002	43	yyyyddd0025	66	yyyyddd0046	89	UB
21	yyyyddd0003	44	yyyyddd0026	67	yyyyddd0047	90	W (DB)
22	yyyyddd0004	45	yyyyddd0027	68	yyyyddd0048		
23	yyyyddd0005	46	yyyyddd0028	69	yyyyddd0049		

[1]Undigested blank (sampler wash reservoir solution, see section 6.2.1).
[2]NWQL Check Standard, see sections 7.7 and 7.8.
[3]Digest-check sample; see sections 7.10.4 and 7.10.5.

12.3 After peaks are baseline corrected, they need to be digestion-blank corrected.

This correction can be applied in several ways:

1. Subtract the baseline-corrected absorbance of the digestion blank—compute an average concentration if multiple digested blanks are included in each block—from the baseline-corrected absorbance of all calibrants, check standard, and samples in the block. Then estimate regression parameters (a, b, and c terms) for the calibration function by using a second-order polynomial least-squares algorithm. For second and higher order calibration functions, use the Newton-Raphson successive approximations algorithm (Draper and Smith, 1966; Swartz, 1976, 1977, 1979) to convert corrected peak heights into concentrations.
2. Designate digestion blanks as a calibrant with a nominal concentration of zero. In this case the resulting calibration function will have a positive *y*-intercept that approximates the baseline-corrected absorbance of the digestion blank. If this method is used, be sure that the curve-fitting algorithm does not force a zero *y*-intercept by including one or more "dummy" (0,0) points in the data set used for calibration.
3. Designate digested blanks as baseline correction samples—that is, "W" in the FasPac™ software used to acquire and process data at the NWQL. In this case initial, intermediate (if included), and final baselines are interpolated between digested blank peak maxima. Thus, baseline and digestion blanks are corrected in a single operation.

NOTE: Digestion blanks were corrected for data in this report by using method 3. However, analytical results calculated by the other two methods should be equivalent. Regardless of the blank correction algorithm chosen, make sure that it is documented in the SOP and that analysts understand it. The SOP for these methods must be updated whenever any changes in data acquisition and processing software or in calculation algorithms are implemented.

12.4 Most software packages provide a data base for entering appropriate dilution factors. Usually these factors can be entered before or after samples are analyzed. If dilution factors are entered, reported concentrations will be compensated automatically for the extent of dilution. The dilution factor is the number by which a measured concentration must be multiplied to obtain the analyte concentration in the sample prior to dilution. For example, dilution factors of 2, 5, and 10 indicate that sample and diluent were combined in proportions of 1+1, 1+4, and 1+9, respectively.

13. Reporting Results

Total nitrogen (lab codes 2754, 2755, 2756)

- 2 decimal places for concentrations up to 5.00 mg-N/L
- 2 significant figures for concentrations greater than 5.00 mg-N/L

Total phosphorus (lab codes 2757, 2758, 2759)

- 2 decimal places for concentrations up to 2.00 mg-P/L
- 2 significant figures for concentrations greater than 2.00 mg-P/L

14. Detection Levels, Bias, and Precision

14.1 Method detection limits (MDL) for composited, low-concentration FCC and WCA samples (five of each) were estimated using the U.S Environmental Protection Agency (1997) protocol—see table 8. Target concentrations for nitrogen and phosphorus in FCC and WCA composite samples were 0.05 mg-N/L and 0.02 mg-P/L, respectively. The MDL for nitrogen was 0.015 mg-N/L and for phosphorus was 0.007 mg-P/L. Laboratory reporting levels (LRL) will be about twice the MDL concentrations.

14.2 Table 9 lists the average and standard deviation of 9987L, 9987H, and 9987VH QC check solutions that were included in every rack of alkaline persulfate digests. Most probable values (MPVs) and standard deviations in table 9 were published by the USGS Branch of Quality Systems for the 2002 water year (12-month period ending September 30 each year is called the "water year"). In all cases, total nitrogen and total phosphorus concentrations determined for these reference materials by the alkaline persulfate digestion method were tightly centered around published MPVs and well within published control limits.

Table 8. Data and calculations used to estimate method detection limits (MDL) for nitrogen and phosphorus in unacidified (FCC) and acidified (WCA) samples following alkaline persulfate digestion. Low-concentration FCC and WCA samples (five of each) were composited for these determinations

[mg-N (-P)/L, milligrams nitrogen (or phosphorus) per liter; %, percent; MDL, method detection limit]

Target concentration [mg-N (-P)/L]	Concentration found (mg-N/L or mg-P/L)			
	Dissolved nitrogen (unacidified)	Total nitrogen (acidified)	Dissolved phosphorus (unacidified)	Total phosphorus (acidified)
0.05 (0.02)	0.064	0.041	0.026	0.033
0.05 (0.02)	.078	.042	.024	.029
0.05 (0.02)	.072	.035	.026	.029
0.05 (0.02)	.066	.035	.029	.027
0.05 (0.02)	.067	.032	.026	.029
0.05 (0.02)	.066	.039	.023	.027
0.05 (0.02)	.071	.026	.022	.026
0.05 (0.02)	.063	.035	.026	.026
Average	**.068**	**.035**	**.025**	**.028**
Standard deviation	.005	.005	.002	.002
Number of values	8	8	8	8
Degrees of freedom	7	7	7	7
t-value (1-sided, 99%)	2.998	2.998	2.998	2.998
MDL	.015	.015	.007	.007

Table 9. Most probable values and standard deviations for reference samples 9987L, 9987H, and 9987VH along with averages and standard deviations of these reference materials that were included in every rack of alkaline persulfate digests

[ID, identification of reference sample; MPV, most probable value; FCC, filtered, chilled (bottle type); WCA, whole water, chilled, acidified (bottle type); mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; ±, plus or minus]

ID	MPV	High-flow samples		Low-flow samples	
		FCC[1]	WCA[2]	FCC[3]	WCA[4]
	Alkaline persulfate dissolved and total nitrogen concentration (mg-N/L)				
9987L	0.22 ± 0.08	0.21 ± 0.03	0.21 ± 0.03	0.19 ± 0.03	0.20 ± 0.02
9987H	1.09 ± 0.15	1.09 ± 0.03	1.09 ± 0.03	1.06 ± 0.08	1.04 ± 0.04
9987VH	2.20 ± 0.24	2.27 ± 0.05	2.18 ± 0.06	2.16 ± 0.07	2.13 ± 0.06
	Alkaline persulfate dissolved and total phosphorus concentration (mg-P/L)				
9987L	0.108 ± 0.008	0.105 ± 0.004	0.104 ± 0.004	0.107 ± 0.006	0.105 ± 0.004
9987H	0.54 ± 0.02	0.54 ± 0.01	0.55 ± 0.02	0.57 ± 0.02	0.54 ± 0.01
9987VH	1.08 ± 0.05	1.13 ± 0.02	1.10 ± 0.03	1.13 ± 0.03	1.09 ± 0.02

[1]Number of points: $n = 19$; [2]$n = 21$; [3]$n = 21$; [4]$n = 18$.

14.3 Spike Recoveries

Median, 90th and 10th percentiles of percent spike recoveries measured in samples collected during high-flow and low-flow conditions are listed in table 10. Median spike recoveries for nitrogen (0.5 mg-N/L as glycine) ranged from about 92 to100 percent and for phosphorus (0.2 mg-P/L as glycerophosphate) from about 86 to 108 percent.

14.4 Duplication of Results

Median, tenth percentiles, and ninetieth percentiles for concentration differences for duplicate samples collected during the nominally high- and low-flow conditions are listed in table 11. Median concentration differences between duplicate analyses are about the same as the MDLs. Larger tenth-percentile differences for whole-water samples that were collected during nominally high-flow conditions in relation to those of filtered water samples likely reflect the difficulty of obtaining reproducible aliquots from samples that contain large amounts of suspended solids. Such samples were purposely chosen as duplicates to assess "worst-case" digest-preparation sampling precision.

ANALYTICAL PERFORMANCE AND COMPARATIVE RESULTS

This section documents analytical performance of the alkaline persulfate digestion method (I-2650-03/4650-03) developed and adapted for use at the NWQL as an alternative to USGS Kjeldahl digestion methods for nitrogen (I-2515-91/4515-91) and phosphorus (I-2610-91/4610-91). It also provides statistical and graphical analysis of data and interpretation of results for about 2,100 dissolved and whole-water samples that were collected during nominally high- and low-flow conditions and analyzed by alkaline persulfate and Kjeldahl digestion methods.

Table 10. Spike recoveries of glycine and glycerophosphate in randomly selected high-flow and low-flow samples that were included in every rack of alkaline persulfate digests

[*n*, number of samples; DN_{AlkP}, alkaline persulfate dissolved nitrogen; TN_{AlkP}, alkaline persulfate total nitrogen; DP_{AlkP}, alkaline persulfate dissolved phosphorus; TP_{AlkP}, alkaline persulfate total phosphorus]

	High-flow samples				Low-flow samples			
	n	Percent recovery			*n*	Percent recovery		
		Median	90th	10th		Median	90th	10th
DN_{AlkP}	18	100.3	108.6	90.1	18	95.0	103.2	88.7
TN_{AlkP}	22	95.1	103.1	84.0	18	92.1	101.7	83.0
DP_{AlkP}	18	97.9	112.9	86.5	17	108.3	119.2	93.4
TP_{AlkP}	22	85.8	93.3	69.5	18	99.6	107.5	91.4

Table 11. Concentration differences between selected samples prepared and analyzed in duplicate in each block of alkaline persulfate digests

[*n,* number of samples; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; DN_{AlkP}, alkaline persulfate dissolved nitrogen; TN_{AlkP}, alkaline persulfate total nitrogen; DP_{AlkP}, alkaline persulfate dissolved phosphorus; TP_{AlkP}, alkaline persulfate total phosphorus]

	High-flow samples				Low-flow samples			
	n	Concentration difference for mg-N/L or mg-P/L (percentile)			*n*	Concentration difference for mg-N/L or mg-P/L (percentile)		
		Median	90th	10th		Median	90th	10th
DN_{AlkP}	20	0.011	0.050	-0.023	20	-0.023	0.008	-0.109
TN_{AlkP}	20	-0.007	0.052	-0.296	20	-0.024	0.027	-0.093
DP_{AlkP}	20	0.000	0.009	-0.024	20	-0.002	0.010	-0.034
TP_{AlkP}	20	0.000	0.015	-0.040	20	-0.004	0.006	-0.023

Analytical Performance

Prior to beginning the large-scale evaluation and validation study with samples collected during nominally high- and low-flow conditions, preliminary experiments were performed to establish performance of the alkaline persulfate digestion method. Recoveries of nitrogen and phosphorus from individual nitrogen- and phosphorus-containing compounds that were prepared in deionized water are listed in tables 12

Table 12. Recovery of inorganic and organic nitrogen from representative compounds

[mg-N/L, milligrams nitrogen per liter; ±, plus or minus]

Nitrogen compound	Nominal concentration (mg-N/L)	Found (mg-N/L)	Recovery (percent)
Ammonia	2.5	2.51 ± 0.05	100.5
Urea	2.5	2.50 ± 0.06	100.0
Nicotinic acid	2.5	2.47 ± 0.04	98.7
Glycine	2.5	2.50 ± 0.07	97.9

and 13. Inspection of table 12 reveals greater than 95-percent recovery of nitrogen for compounds tested. The somewhat lower recoveries obtained for phosphorus compounds listed in table 13 result from the lower purity of test compounds (phenyl phosphate and phytic acid ≤95 percent according to vendor labels; ATP was purchased and first opened in 1991). Comparable, though slightly higher, phosphorus recoveries for these compounds by acid persulfate digestion (USEPA method 365.1—the generally accepted reference method for total phosphorus determinations), which also are shown in table 13, substantiate this assertion. Other researchers (Ebina and others, 1983; Hosomi and Sudo, 1986; Ameel and others, 1993), who used alkaline persulfate digestion methods similar to the one developed at the NWQL, reported phosphorus recoveries greater than 95 percent for a variety of phosphorus-containing compounds, including ATP.

Results from an experiment to assess nitrogen recovery in the presence of organic carbon (OC) are shown in figure 5. In these experiments a series of solutions containing 2.5 mg NH_4^+-N/L and increasing concentrations of OC (as glucose) were digested and analyzed for nitrogen. Data plotted in figure 5 indicate that complete oxidation of ammonium to nitrate was achieved for OC concentrations up to 150 mg/L. Similar results have been reported previously (Langer and Hendrix, 1982; Cabrera and Beare, 1993). OC in surface- and ground-water samples analyzed at the NWQL rarely exceeds 150 mg/L. Note, however, that nitrogen recovery in Kjeldahl digests is quantitative at OC concentrations 10 to 20 times greater than the 150-mg/L limit typical for alkaline persulfate digestion methods (Ebina and others, 1983).

During preliminary validation work, the cause of low nitrogen recovery in about 10 WCA samples was traced to overacidification at collection sites. When these samples were dosed with alkaline persulfate reagent, the resulting pH was less than 7. As discussed previously in section 2.2, an initial pH greater than 12 is necessary for complete oxidation of ammonium and organic nitrogen to nitrate. For this reason the pH of all WCA samples was checked with narrow range colorimetric test strips during the large-scale evaluation and validation study. Nitrogen

Table 13 Recovery of organic phosphorus from representative compounds by alkaline persulfate and low-level acid persulfate digestion methods

[mg-P/L, milligrams phosphorus per liter; ±, plus or minus]

Compound	Nominal concentration (mg-P/L)	Alkaline persulfate method		Acid persulfate method	
		Found (mg-P/L)	Recovery (percent)	Found (mg-P/L)	Recovery (percent)
Adenosine tri-phosphate (ATP)	0.200	0.166 ± 0.000	83.0	0.176 ± 0.001	88.0
	1.000	0.87 ± 0.03	86.8		
Glycerophosphate	0.200	0.196 ± 0.007	100.2	0.204 ± 0.002	102.1
	1.000	1.019 ± 0.008	101.9		
Phenyl Phosphate	0.200	0.168 ± 0.004	84.2	0.179 ± 0.000	89.5
	1.000	0.872 ± 0.002	87.2		
Phytic Acid	0.200	0.177 ± 0.002	88.4	0.180 ± 0.001	91.8
	1.000	0.906 ± 0.009	90.6		

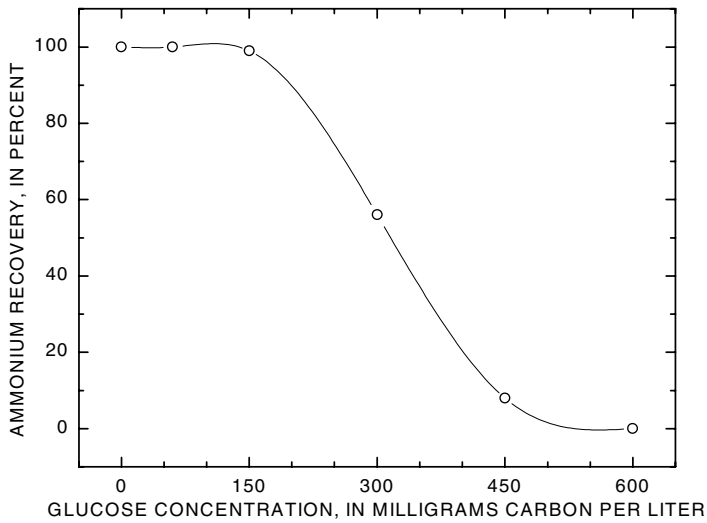

Figure 5. Percent recovery of nitrogen from a series of 2.5 mg NH_4^+-N solutions that contained increasing concentrations of organic carbon (OC) as glucose. See text for additional details.

concentration results for WCA samples with pH outside the expected range of 1.6 to 1.9 were disqualified. For more information, see section 3.1.4. About 150 results for samples with medium codes other than 6 (ground water) and 9 (surface water)—specifically, Q (quality-assurance sample, artificial), R (quality-assurance sample, surface water), S (quality-assurance sample, ground water), 2 (leachate), and 5 (elutriation)—also were not included in graphical and statistical analyses.

In Kjeldahl digestion procedures, digests are evaporated to near dryness and then resolvated with DI water prior to analytical determinations. Variation in the postdigestion volume of DI water added to each tube—and therefore in estimated mass-per-unit volume nitrogen and phosphorus concentrations in resolvated digests—is a function of the DI water dispenser precision, typically 2 to 3 percent. Alkaline persulfate digests, in contrast, are tightly capped and lose little water during digestion. After digestion, nitrogen and phosphorus are determined directly without volume adjustment. It was of interest, therefore, to assess the variation in liquid loss during alkaline persulfate digestion. To this end, pre- and postdigestion weights for one, 80-tube batch of prepared alkaline persulfate digests were measured to the nearest 0.01 g and recorded. A weight of 15 g (10 mL of sample + 5 mL of digestion reagent) was assumed in percent weight-loss calculations using equation 1 below. The results from this experiment, which indicate a weight loss of 3 percent or less for 85 percent of all tubes, are shown in figure 6. The maximum percent weight loss observed was 6 percent.

Comparative Results for Nitrogen

In discussions that follow, the designations KDN and KTN apply to Kjeldahl digestion dissolved nitrogen (ammonium + organic nitrogen determined in filtered-water digests) and Kjeldahl digestion total nitrogen (ammonium + organic nitrogen determined in acidified, whole-water digests), respectively. When filtered- and whole-water samples are considered

$$Percent\ weight\ loss = \frac{Digest\ weight_{initial} - Digest\ weight_{final}}{15g} \times 100 \quad (1)$$

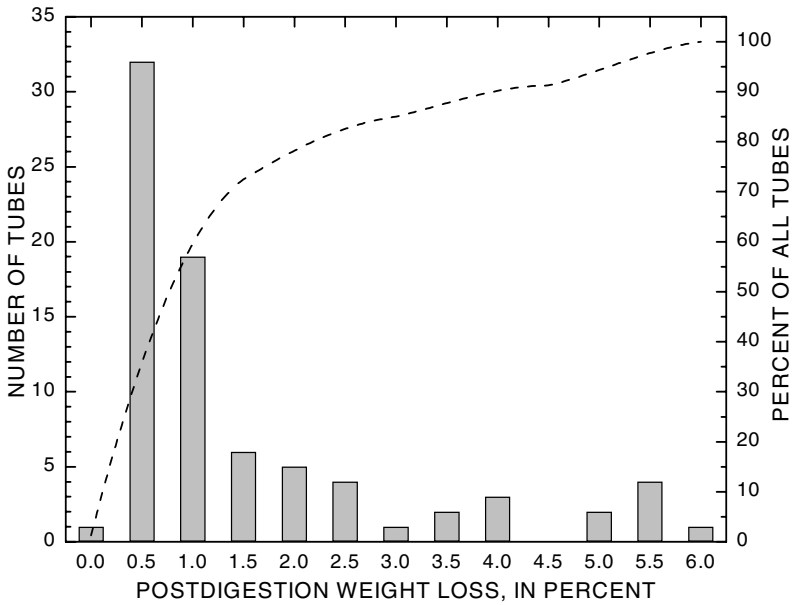

Figure 6. Histogram of postdigestion weight loss expressed as a percent of initial digest weight for one batch of 80 alkaline persulfate digests. Weights of capped digest tubes, each containing precisely dispensed volumes of sample and reagent, were weighed before and after digestion. Left and right *y*-axes relate to gray bars and the dashed line, respectively. Additional details can be found in supporting text.

together, the designation Kjeldahl nitrogen (KN) is applied. The designations alkaline persulfate digestion dissolved nitrogen (DN_{AlkP}), alkaline persulfate digestion total nitrogen (TN_{AlkP}), and alkaline persulfate nitrogen (N_{AlkP}) are applied analogously. Note also that *nitrate-corrected* DN_{AlkP}, TN_{AlkP}, and N_{AlkP} concentrations are those from which nitrate + nitrite concentrations have been subtracted to make them operationally equivalent to KDN, KTN, and KN concentrations.

A logarithmic scatter plot of nitrate-corrected N_{AlkP} concentrations and KN concentrations around a unity slope line—that is, the line of equal relation—for paired data combined from the large-scale validation experiments is shown in figure 7. Despite the large scatter between individual data pairs, linear regression analysis of these data indicate good correlation between nitrate-corrected N_{AlkP} and KN concentrations—$KN = (1.023 \pm 0.003)\ N_{AlkP} + 0.038 \pm 0.004$, $r^2 = 0.976$. The positive y-intercept and slightly greater than unity slope of the regression line indicate low bias for nitrate-corrected N_{AlkP} in relation to KN, which might be interpreted as low nitrogen recovery for the alkaline persulfate digestion method. An alternate interpretation—that KN concentrations are biased high because a small fraction of nitrate present in samples is reduced to ammonium during Kjeldahl digestion—also could account for observed concentration differences. Interference by nitrate during Kjeldahl digestion (American Public Health Association, 1998c, p. 4-123; Patton and Truitt, 2000) is well known.

To explore this alternative interpretation further, differences between nitrate-corrected N_{AlkP} and KN concentrations (*y*-axes) were plotted as a function of nitrate concentrations (*x*-axes) in the four panels of figure 8. In this figure panels A and B relate to data for filtered surface- and ground-water samples; panels C and D relate to data for whole-water acidified surface- and ground-water samples. Nitrate concentrations are plotted on logarithmic scales to provide equal linear spacing for each decade of nitrate concentration. Lines of zero concentration difference were added to facilitate visual interpretation of data. With the exception of some unexplained outliers, differences between nitrate-corrected DN_{AlkP} and KDN concentrations in filtered samples (fig. 8A and 8B) tend to scatter symmetrically about the lines of zero difference up to nitrate concentrations of about

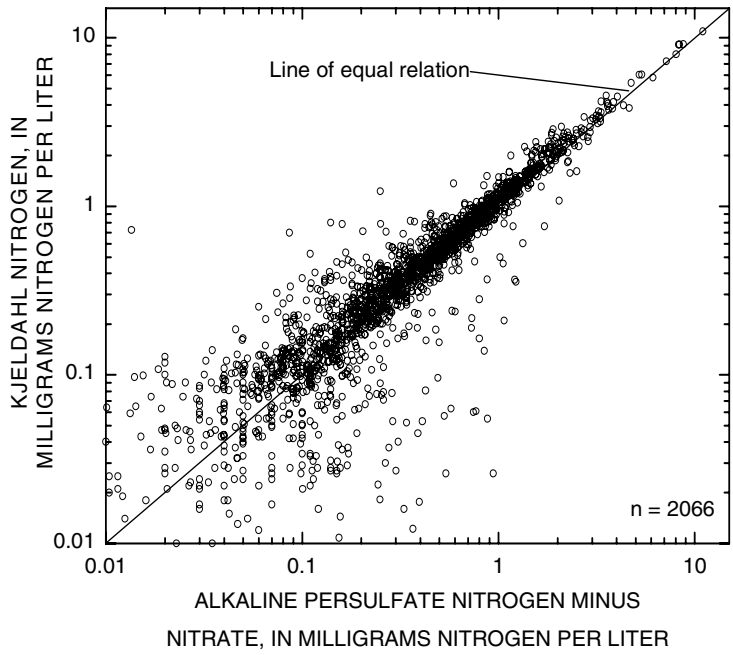

Figure 7. Logarithmic scatter plot of nitrate-corrected N_{AlkP} concentrations (x-axis) and KN concentrations (*y*-axis) around the line of equal relation for 2,066 data pairs combined from large-scale validation experiments. The linear regression equation for these data is $y = (1.023 \pm 0.003)\ x + 0.038 \pm 0.004$, with a correlation coefficient (r^2) of 0.976.

1 mg-N/L. At higher nitrate concentrations, differences between nitrate-corrected DN_{AlkP} and KDN concentrations increase about the line of zero difference—erratically for filtered ground water and negatively for filtered surface water. In contrast, concentration differences between nitrate-corrected TN_{AlkP} and KTN for unfiltered, acidified samples (fig. 8C and 8D) are predominately negative with differences becoming more negative as nitrate concentrations increase. This trend is particularly evident for unfiltered, acidified surface water.

The result of sorting data from each panel in figure 8 according to nominal flow conditions at the time of sample collection and recasting them as box plots is shown in figure 9. In this figure, concentration differences between nitrate-corrected N_{AlkP} and KN for samples collected during nominally high-flow (HF) and low-flow (LF) conditions are grouped into three nitrate concentration ranges: NO_3^--N ≤ 0.1 mg/L, 0.1 mg/L < NO_3^--N ≤ 1.0 mg/L, and NO_3^--N > 1.0 mg/L. Figure 9 further substantiates the hypothesis that concentration differences between nitrate-corrected N_{AlkP} and KN likely result from the well known, though poorly characterized, high-temperature reactions between nitrate and natural organic matter (NOM) that can produce positive (reduction of nitrate to ammonium) or negative (oxidation of ammonium to nitrous oxide) interference in Kjeldahl nitrogen determinations (see section 3.1.5 and Patton and Truitt, 2000). Positive nitrate interference in KN concentrations predominates for surface-water samples and is greater for whole-water samples than for filtered-water samples. This result is consistent with typically larger NOM concentrations in whole-water samples than in filtered-water samples. Nitrate appears to interfere positively and negatively in KN concentrations for ground-water samples, although the trends are less clear than for surface-water samples. In general, differences between nitrate-corrected N_{AlkP} and KN concentrations were least for samples with nitrate concentrations less than 0.1 mg NO_3^--N/L—a finding consistent with nitrate interference during Kjeldahl digestion. Complete two-population, paired *t*-test results for subsets of nitrate-corrected DN_{AlkP} and

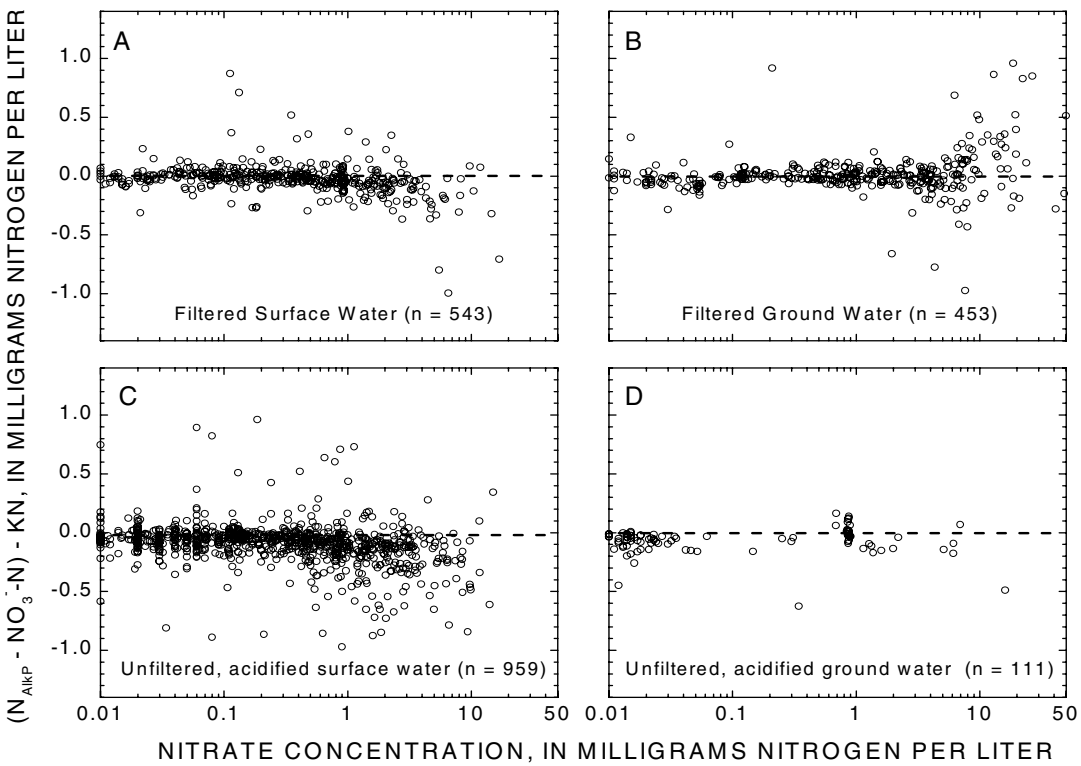

Figure 8. Concentration differences between nitrate-corrected alkaline persulfate digestion nitrogen (N_{AlkP}) and Kjeldahl digestion nitrogen (KN) plotted about the line of zero difference as a function of nitrate concentration.

KDN concentrations in filtered-water samples are listed in table 14. Nitrate-corrected TN_{AlkP} and KTN concentrations in acidified whole-water samples are listed in table 15.

Comparative Results for Phosphorus

A logarithmic scatter plot of P_{AlkP} concentrations (*x*-axis) and KP concentrations (*y*-axis) around a unity slope line for 2,093 data pairs combined from high- and low-flow phases of validation experiments is shown in figure 10. This plot reveals good correlation among phosphorus concentrations determined by the P_{AlkP} and KP digestion methods. The slope and *y*-intercept of the linear least squares regression of these data—KP = (0.994 ± 0.002) P_{AlkP} + 0.0003 ± 0.0005; correlation coefficient (r^2) = 0.994 closely approximate 1 and 0. A two-population, paired *t*-test confirmed the null hypothesis that the difference between means of phosphorus concentrations for these 2,093 paired results determined by the P_{AlkP} and KP digestion methods were not significantly different from zero at the p = 0.05 level. Differences between means of alkaline persulfate phosphorus and Kjeldahl phosphorus concentrations for some subsets of these data, which were grouped according to water type and flow conditions at the time of sample collection, were statistically different from zero at the p = 0.05 level. In all such cases, however, differences between means were less than method detection limits—0.007 mg-P/L for P_{AlkP} and 0.02 mg-P/L for KP—and therefore were not analytically significant. Complete results for these *t*-tests are listed in table 16.

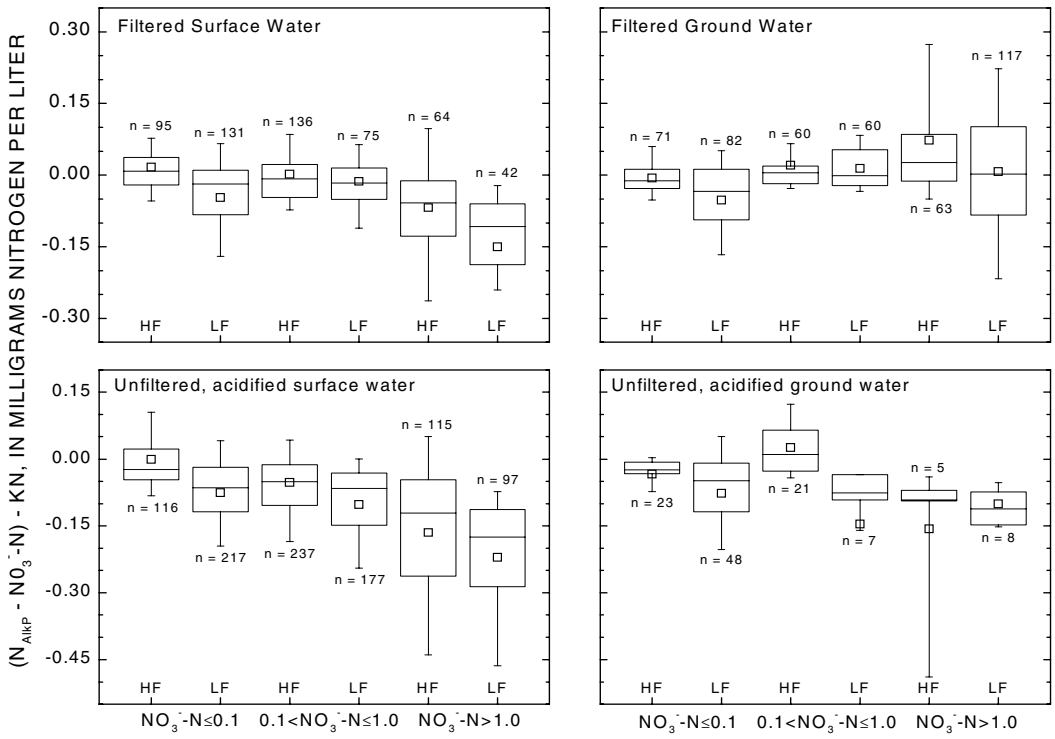

Figure 9. Boxplots of concentration differences between nitrate-corrected alkaline persulfate nitrogen (N_{AlkP}) and Kjeldahl nitrogen (KN) for surface- and ground-water samples collected during nominally high- and low-flow conditions. Data in each panel are grouped according to nominal flow conditions (HF = high-flow; LF = low-flow) at the time of sample collection and dissolved nitrate concentrations (milligram nitrogen per liter). In each boxplot, open squares, hinges, gates and whiskers indicate average, median, 75th and 25th percentiles, and 90th and 10th percentiles for differences between nitrate-corrected N_{AlkP} and KN.

SUMMARY AND CONCLUSIONS

An alkaline persulfate digestion method and automated colorimetric finishes for simultaneous nitrogen and phosphorus determinations in filtered and whole-water acidified water samples were developed and validated. This method is more sensitive, accurate, and uses less toxic reagents than Kjeldahl digestion methods, such as U.S. Geological Survey (USGS) I-2515/4515-91 and U.S. Environmental Protection Agency (USEPA) 351.2 for nitrogen and USGS I-2610/4610-91 and USEPA 365.4 for phosphorus. Data in this report result from about 2,100 filtered and whole-water samples that were analyzed for alkaline persulfate dissolved and total nitrogen (DN_{AlkP} and TN_{AlkP}), Kjeldahl dissolved and total nitrogen (KDN and KTN), alkaline persulfate dissolved and total phosphorus (DP_{AlkP} and TP_{AlkP}), and Kjeldahl dissolved and total phosphorus (KDP and KTP). All filtered and whole-water samples analyzed by the alkaline persulfate digestion method also were analyzed for dissolved nitrate + nitrite, ammonium, and orthophosphate on the same day that digests were prepared. Results of these analyses were compared by statistical and graphical methods. About half the data

Table 14. Results of paired *t*-tests for nitrate-corrected alkaline persulfate dissolved nitrogen and Kjeldahl dissolved nitrogen concentrations determined in filtered-water samples. Distributions of nitrate concentrations for each subgroup are shown in the rightmost three columns

[mg-N/L, milligrams nitrogen per liter; DN_{AlkP}, alkaline persulfate digestion dissolved nitrogen; KDN, Kjeldahl digestion dissolved nitrogen; *n*, number of samples; max, maximum; 75th, 75th percentile; <, less than; ≤, less than or equal to; >, greater than]

Filtered ground water	Mean (mg-N/L)		Difference (mg–N/L)	Variance (mg-N/L)		*n*	Significance[1]			Nitrate (mg-N/L)		
	DN_{AlkP}	KDN		DN_{AlkP}	KDN		p_{calc}	$p_{0.05}$	$p_{0.01}$	Median	Max	75th
All	0.340	0.333	0.007	2.387	2.397	453	0.386	no	no	0.513	50	3.289
High-flow												
All	0.415	0.390	0.025	5.153	5.179	194	0.012	yes	no	0.212	50	1.875
$NO_3^- < 0.1$	0.446	0.456	-0.010	0.526	0.535	71	0.341	no	no	0	0.094	0.007
$0.1 < NO_3^- \leq 1.0$	0.125	0.106	0.019	0.029	0.020	60	0.229	no	no	0.372	0.984	0.795
$NO_3^- > 1.0$	0.657	0.588	0.069	15.278	15.375	63	0.004	yes	yes	3.536	50	6.27
Low-flow												
All	0.283	0.290	-0.007	0.319	0.321	259	0.540	no	no	0.674	48	3.96
$NO_3^- < 0.1$	0.466	0.494	-0.027	0.708	0.698	82	0.018	yes	no	0.02	0.084	0.04
$0.1 < NO_3^- \leq 1.0$	0.107	0.100	0.007	0.096	0.099	60	0.303	no	no	0.467	0.988	0.628
$NO_3^- > 1.0$	0.245	0.244	0.000	0.124	0.127	117	0.993	no	no	4.222	48	8.6
Filtered surface water												
All	0.41	0.435	-0.025	0.191	0.226	543	<0.001	yes	yes	0.182	16.88	0.883
High-flow												
All	0.385	0.393	-0.008	0.078	0.088	295	0.21	no	no	0.358	16.88	0.922
$NO_3^- < 0.1$	0.365	0.34	0.025	0.082	0.088	95	0.041	yes	no	0.016	0.096	0.053
$0.1 < NO_3^- \leq 1.0$	0.403	0.401	0.002	0.073	0.081	136	0.836	no	no	0.329	1.547	0.541
$NO_3^- > 1.0$	0.388	0.456	-0.068	0.084	0.096	64	0.002	yes	yes	2.111	16.88	3.199
Low-flow												
All	0.44	0.484	-0.044	0.325	0.387	248	<0.001	yes	yes	0.084	9.76	0.475
$NO_3^- < 0.1$	0.436	0.462	-0.026	0.226	0.232	131	<0.001	yes	yes	0.009	0.098	0.027
$0.1 < NO_3^- \leq 1.0$	0.359	0.374	-0.015	0.14	0.128	75	0.342	no	no	0.236	0.967	0.468
$NO_3^- > 1.0$	0.599	0.748	-0.149	0.95	1.279	42	<0.001	yes	yes	2.238	9.757	3.819

[1] p_{calc} is the probability that population means of nitrate-corrected DN_{AlkP} and KDN concentrations are the same—that is, difference between the population means is statistically equivalent to zero—on the basis of calculated paired *t*-tests. Difference between population means is significant at the 95-percent confidence level ($p_{0.05}$) when p_{calc} is less than 0.05 and at the 99-percent confidence level ($p_{0.01}$) when p_{calc} is less than 0.01.

Table 15. Results of paired *t*-tests for nitrate-corrected alkaline persulfate total nitrogen and Kjeldahl total nitrogen concentrations determined in acidified whole-water samples. Distributions of nitrate concentrations for each subgroup are shown in the rightmost three columns

[mg-N/L, milligrams nitrogen per liter; TN_{AlkP}, alkaline persulfate digestion total nitrogen; KTN, Kjeldahl digestion total nitrogen; *n*, number of samples; max, maximum; 75th, 75th percentile; <, less than; ≤, less than or equal to; >, greater than]

Unfiltered ground water	Mean (mg-N/L)		Difference (mg-N/L)	Variance (mg-N/L)		*n*	Significance[1]			Nitrate (mg-N/L)		
	TN_{AlkP}	KTN		TN_{AlkP}	KTN		p_{calc}	$p_{0.05}$	$p_{0.01}$	Median	Max	75th
All	0.514	0.577	-0.063	0.319	0.362	111	<0.001	yes	yes	0.022	16.04	0.859
High-flow												
All	0.509	0.53	-0.021	0.178	0.179	48	0.135	no	no	0.498	16.04	0.868
$NO_3^- < 0.1$	0.242	0.277	-0.035	0.162	0.181	23	0.001	yes	yes	0.010	0.052	0.017
$0.1 < NO_3^- \leq 1.0$	0.821	0.795	0.026	0.034	0.032	21	0.096	no	no	0.864	0.904	0.872
$NO_3^- > 1.0$	0.414	0.593	-0.179	0.135	0.285	4	0.185	no	no	4.164	16.04	8.613
Low-flow												
All	0.518	0.613	-0.095	0.431	0.503	63	<0.001	yes	yes	0.017	6.962	0.052
$NO_3^- < 0.1$	0.596	0.679	-0.083	0.51	0.613	48	<0.001	yes	yes	0.015	0.062	0.022
$0.1 < NO_3^- \leq 1.0$	0.429	0.575	-0.146	0.114	0.097	7	0.127	no	no	0.344	0.885	0.866
$NO_3^- > 1.0$	0.092	0.211	-0.119	0.062	0.062	8	<0.001	yes	yes	1.797	6.962	5.376
Unfiltered surface water												
All	0.728	0.820	-0.092	0.911	1.007	959	<0.001	yes	yes	0.215	61.65	0.874
High-flow												
All	0.677	0.744	-0.067	0.608	0.694	468	<0.001	yes	yes	0.345	11.74	0.993
$NO_3^- < 0.1$	0.654	0.655	-0.001	0.560	0.517	116	<0.001	no	no	0.020	0.095	0.054
$0.1 < NO_3^- \leq 1.0$	0.573	0.625	-0.052	0.331	0.380	237	<0.001	yes	yes	0.349	0.999	0.585
$NO_3^- > 1.0$	0.914	1.080	-0.166	1.160	1.385	115	<0.001	yes	yes	2.119	11.74	3.125
Low-flow												
All	0.778	0.892	-0.114	1.197	1.297	491	<0.001	yes	yes	0.134	61.65	0.807
$NO_3^- < 0.1$	0.898	0.974	-0.076	1.303	1.374	217	<0.001	yes	yes	0.034	0.099	0.057
$0.1 < NO_3^- \leq 1.0$	0.588	0.689	-0.101	0.299	0.395	177	<0.001	yes	yes	0.330	0.974	0.557
$NO_3^- > 1.0$	0.855	1.076	-0.221	2.524	2.681	97	<0.001	yes	yes	2.380	61.65	4.648

[1] p_{calc} is the probability that population means of nitrate-corrected TN_{AlkP} and KTN concentrations are the same—that is, difference between the population means is statistically equivalent to zero—on the basis of calculated paired *t*-tests. Difference between population means is significant at the 95-percent confidence level ($p_{0.05}$) when p_{calc} is less than 0.05 and at the 99-percent confidence level ($p_{0.01}$) when p_{calc} is less than 0.01.

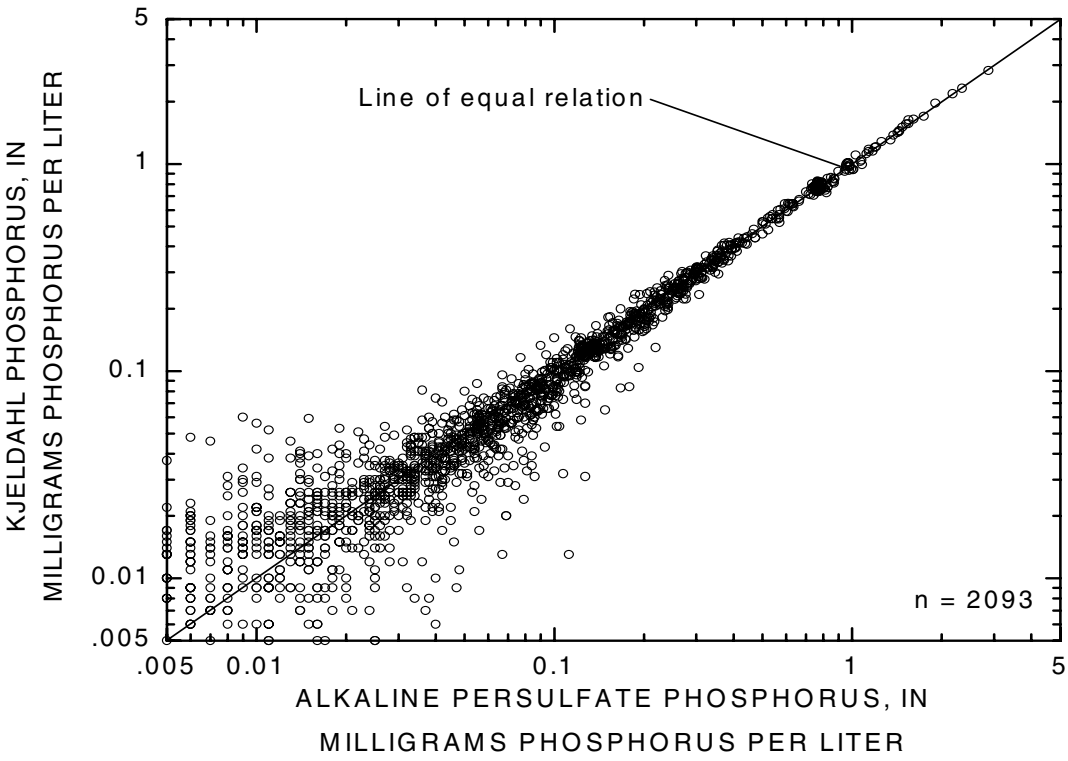

Figure 10. Logarithmic scatter plot of P_{AlkP} concentrations (*x*-axis) and KP concentrations (*y*-axis) around the line of equal relation for 2,093 data pairs combined from large-scale validation experiments. The linear regression equation for these data is KP = (0.994 ± 0.002) P_{AlkP} + 0.0003 ± 0.0005, with a correlation coefficient (r^2) of 0.994.

in this report were obtained from samples collected during nominally high-flow (April–June 2002) conditions, and the other half were collected during nominally low-flow (August–September 2002) conditions. Numbers of filtered and acidified whole-water samples were about equal. This report provides details of alkaline persulfate digest preparation as well as complete operational information, including interferences and analytical figures of merit for the automated colorimetric methods developed to determine nitrate and orthophosphate in alkaline persulfate digests. Primary conclusions of this report follow:

1. Hazards to analysts and toxic wastes are substantially less for alkaline persulfate digestion methods than for Kjeldahl digestion methods.
2. Alkaline persulfate digestion methods described in this report can be applied successfully to acidified samples (USGS FCA and WCA bottle types) provided that samples are acidified at collection sites using supplies and protocols specified in the USGS field manual (Wilde and others, 1998).
3. Alkaline persulfate digestion methods described in this report are amenable to automation and should prove substantially less labor intensive than the existing Kjeldahl digestion methods. For example, filtered-water sample digests can be prepared robotically, and the manual

Table 16. Results of paired *t*-tests for dissolved and total phosphorus concentrations determined in filtered and acidified whole-water samples by alkaline persulfate digestion (P_{AlkP}) and Kjeldahl digestion (KP) methods

[mg-P/L, milligrams phosphorus per liter; *n*, number of samples; FCC, filtered, chilled (bottle type for samples); WCA, whole water, chilled, acidified (bottle type for samples); MC 6, ground water medium code; MC 9, surface water medium code; HF, high flow; LF, low flow; <, less than]

Water type	Mean (mg-P/L)		Difference (mg-P/L)	Variance (mg-P/L)		*n*	Significance[1]		
	P_{AlkP}	KP		P_{AlkP}	KP		p_{calc}	$p_{0.05}$	$p_{0.01}$
All	0.134	0.133	0.001	0.061	0.060	2,093	0.242	no	no
All FCC	0.100	0.103	-0.003	0.063	0.062	1,115	<0.0001	yes	yes
All WCA	0.171	0.167	0.004	0.055	0.056	978	<0.0001	yes	yes
All MC 6	0.084	0.087	-0.003	0.045	0.045	645	<0.0001	yes	yes
All MC 9	0.155	0.154	0.002	0.066	0.066	1,448	<0.0005	yes	yes
High-flow									
All	0.123	0.123	0.000	0.045	0.046	990	0.640	no	no
FCC, MC 6	0.068	0.069	-0.001	0.026	0.028	204	0.557	no	no
FCC, MC 9	0.126	0.131	-0.006	0.059	0.061	320	<0.0001	yes	yes
WCA, MC 6	0.296	0.297	-0.001	0.124	0.125	49	0.787	no	no
WCA, MC 9	0.127	0.123	0.004	0.028	0.029	417	<0.0001	yes	yes
Low-flow									
All	0.143	0.142	0.001	0.075	0.073	1,103	0.043	yes	no
FCC, MC 6	0.046	0.050	-0.004	0.023	0.022	337	<0.0005	yes	yes
FCC, MC 9	0.166	0.166	0.000	0.141	0.135	254	0.833	no	no
WCA, MC 6	0.190	0.193	-0.003	0.116	0.119	55	0.140	no	no
WCA, MC 9	0.196	0.190	0.006	0.062	0.063	457	<0.0001	yes	yes

[1] p_{calc} is the probability that population means of P_{AlkP} and KP concentrations are the same—that is, difference between the population means is statistically equivalent to zero—on the basis of calculated paired *t*-tests. Difference between population means is significant at the 95-percent confidence level ($p_{0.05}$) when p_{calc} is less than 0.05 and at the 99-percent confidence level ($p_{0.01}$) when p_{calc} is less than 0.01.

post-digestion, pH adjustment step typical in previously reported alkaline persulfate digestion procedures (Ameel and others, 1993; D'Elia and others, 1997) has been eliminated.

4. Method detection limits (MDLs) for the alkaline persulfate digestion dissolved and total nitrogen (0.015 mg-N/L) and phosphorus (0.007 mg-P/L) are substantially less than those of USGS methods I-2515/4515/91 for dissolved and total Kjeldahl nitrogen (0.05 mg-N/L) and USGS methods I-2610/4610/91 for dissolved and total Kjeldahl phosphorus (0.02 mg-P/L) methods. The lower nitrogen and phosphorus MDLs of alkaline persulfate digestion methods described in this report improve the precision of nutrient-mass balance estimates.
5. On the basis of two-population, paired *t*-test statistics, the means of all nitrate-corrected alkaline persulfate digestion nitrogen (N_{AlkP}) and Kjeldahl digestion nitrogen (KN) concentrations (2,066 paired results) were significantly different from zero at the p = 0.05 level. Statistical and graphical analyses of experimental data indicate that concentration differences between nitrate-corrected N_{AlkP} and KN result from nitrate interference in the Kjeldahl digestion method rather than incomplete recovery of nitrogen by the alkaline persulfate digestion method. Alkaline persulfate digestion, therefore, provides more accurate estimates of total nitrogen concentrations in samples that contain nitrate concentrations greater than about 0.1 mg NO_3^--N/L. For some subsets of these data, the means were not different from zero at the p = 0.05 level, typically in ground-water samples or in surface-water samples with nitrate concentrations less than 0.1 mg-N/L.
6. On the basis of two-population, paired *t*-test statistics for 2,093 paired results, the means of all Kjeldahl digestion phosphorus concentrations determined by USGS method I-2610/4610-91 (similar to USEPA method 365.4) and those determined by the alkaline persulfate digestion method reported here were not significantly different from zero at the p = 0.05 level. For some subsets of these data, the means were different from zero at the p = 0.05 level, but in such cases differences were less than the method detection limit (0.007 mg-P/L) for the alkaline persulfate digestion method and were not analytically significant. Changing from Kjeldahl digestion to alkaline persulfate digestion, therefore, does not affect comparisons with historical dissolved and total phosphorus concentrations.
7. Data and analysis provided in this report establish guidelines necessary to interpret total and dissolved nitrogen and phosphorus concentrations that result from alkaline persulfate digestion methods in relation to those that result from Kjeldahl digestion methods. Specifically—
   a. Systematic differences between DN_{AlkP} /KDN and TN_{AlkP} /KTN concentrations are expected for samples with dissolved nitrate concentrations greater than or equal to 0.1 mg-N/L.
   b. Concentration differences between N_{AlkP} and historical KN data are likely to increase in proportion to dissolved nitrate concentrations in samples. Whether concentration differences are positive or negative depend on water type in ways that were not possible to describe fully.
      (1) Negative differences between KN and N_{AlkP} were found most often for surface-water samples and unfiltered ground-water samples.
      (2) Differences between KN and N_{AlkP} in filtered ground water are as likely to be negative as positive.
   c. Samples with organic carbon (OC) concentrations greater than about 150 mg/L are not amenable to N_{AlkP} determinations unless OC concentrations are diluted below this threshold prior to digestion.
   d. As nitrate concentrations increase, N_{AlkP} digestion provides better estimates of total and dissolved nitrogen than KN digestion, which suffers from positive and negative interference by nitrate. On the other hand, estimating organic nitrogen concentrations as the small difference between two large numbers when dissolved nitrate, and therefore N_{AlkP}, concentrations are large also can be problematic.
   e. Systematic concentration differences between P_{AlkP} and historical KP data are not expected.
8. One major conclusion of this report—that alkaline persulfate digestion is a more sensitive, accurate, and environmentally responsible alternative to

Kjeldahl digestion for routine, simultaneous determination of nitrogen and phosphorus in surface and ground water—is consistent with previously published studies that are cited throughout this report. In comparison to these earlier studies, however, conclusions of this report are based on a much larger and geographically diverse sample population collected during high-flow and low-flow conditions. Furthermore, samples were collected, preserved, stored, and analyzed by rigorously controlled protocols established and documented by the USGS. In these respects, this report describes the most comprehensive study to date supporting applicability of the alkaline persulfate digestion method as a superior alternative to the time honored, but operationally flawed, Kjeldahl digestion method.

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