The Differentiation, Analysis, and Preservation of Nitrogen and Phosphorus Forms in Natural Waters

DAVID JENKINS

Sanitary Engineering Research Laboratory, Department of Civil Engineering, Division of Hydraulic and Sanitary Engineering, University of California, Berkeley, Calif.

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

The paper describes the development and application of analytical techniques for determining the various forms of nitrogen and phosphorus at levels commonly encountered in estuarine waters. A series of techniques have been developed which allow the differentiation of suspended and soluble organic nitrogen, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, total and soluble phosphate, soluble orthophosphate, and condensed phosphate in waters whose salinity varies from that of fresh surface water to that of the ocean. In addition, the paper describes and recommends short-term and long-term preservation techniques that are satisfactory for the various forms of nitrogen and phosphorus investigated.

One of the more important reasons for determining the concentrations and the forms of nitrogen and phosphorus in natural waters is that these materials are often the nutrients that limit the growth of photosynthetic aquatic macro- and microorganisms.

The interest of the Sanitary Engineering Research Laboratory in the analysis of N and P forms in water originally stemmed from the conduct of a comprehensive investigation of the water quality of San Francisco Bay (*12*) and more recently from two studies of the analysis and preservation of these materials in estuarine waters in conjunction with the Central Pacific Basin Comprehensive Project of the FWPCA (*9, 10*). Because of this association, the separative, analytical and preservative techniques developed were tailored to suit the extremely variable conditions that

In Trace Inorganics In Water; Baker, Robert A.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

exist in an estuarine water such as San Francisco Bay. It is indeed felt that an estuarine water, with a salinity that may fluctuate between that of fresh water and that of ocean water and with a suspended matter content that may reach values of over 100 mg./liter, places a severe test on the applicability of an analytical method.

A result of this might be the fact that while standard texts are available both for the analysis of fresh surface waters (*1*) and the analysis of ocean waters (*20*), no such volume exists for estuarine waters.

Since the fertilizing effect of N and P forms is of prime interest in natural waters, several prominent biologists working in the limnological field were approached to determine their opinion of the desired analytical classification and the sensitivity and detectability of analytical techniques that would be necessary to make N and P analysis meaningful in the determination of the fertilizing potential of a water.

The opinions of the aquatic biologists (*4, 5, 15, 16*) were all qualified by two statements. These were: (1) the required sensitivity of an N or P analysis depends greatly on the biological productivity of the water, and (2) refinements in analytical differentiation of N and P forms should not exceed the present ability of the biologist to make interpretive judgments of the biological effect of the various N and P forms in the aquatic environment. Within the bounds of these qualifications it was universally thought that nitrogen should be differentiated into organic, ammonia, nitrate, and nitrite. It was generally agreed that nitrite analysis may prove to be insignificant, but that this point should be first proven for any particular water. Recommended limits of detectability were 0.1 mg. N/liter for all forms of nitrogen for more productive waters and 3 to 10 μg N/liter for all forms of nitrogen in less productive waters.

While broad agreement existed on the forms and limits of detectability required for the adequate biological interpretation of nitrogen analyses there was less meeting of the biological minds on the required attributes of phosphorus form differentiation. It was pointed out that the present ability to make interpretive judgments from phosphorus data was limited so that differentiation beyond total P, soluble P, and soluble orthophosphate was probably not justifiable. The detectable limits recommended for the analysis of P forms ranged between 3 to 10 μg. P/liter.

While most of these recommendations were met in the methods developed for use in San Francisco Bay, it should be borne in mind that in these waters the concentrations of N and P forms were significantly greater than those that would be encountered in an oligotrophic water. It is therefore imperative to consider the N and P concentration range expected before adopting any specific method for N and P forms. The methods discussed herein have all performed exceptionally in estuarine waters of highly variable salinity and high turbidity.

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

Nitrogen Forms

The forms of nitrogen determined are summarized in Table I.

Table I. Analytical Differentiation of Nitrogen Forms

Nitrogen Form	
(1) Total Unoxidized N	Kjeldahl on whole sample
(2) Soluble Unoxidized N	Kjeldahl on membrane filtered sample
(3) Ammonia N	Distill and nesslerize whole sample
(4) Soluble Organic N	(2) minus (3)
(5) Suspended Organic N	(1) minus (2)
(6) Nitrite N	Diazotize membrane filtered sample
(7) Nitrate N	Brucine method on membrane filtered sample

Ammonia Nitrogen. An attempt was made to use a direct method for the determination of ammonia nitrogen. The pyrazolone technique of Strickland and Parsons (*20*) which employs standards prepared in sea water of low NH_3–N content to combat the effect of high salinity was adapted to the analysis of estuarine waters by employing a salt-masking technique similar to that used by Jenkins and Medsker (*11*) in their brucine method for nitrate. The absorbance of the pyrazolone ammonia complex was constant for salinities in the range of 20 to 40 grams Cl^-/liter. Thus, NaCl to give 20 grams Cl^-/liter was added to eliminate salinity interference in samples containing 0–20 grams Cl^-/liter. The reproducibility and recovery of NH_3–N obtained by the method was unsatisfactory on estuarine water samples but it performed satisfactorily on distillates from such samples. However, because of the instability of the pyrazolone reagent, distillation and nesslerization were adopted for the determination of NH_3–N.

The "Standard Methods" (*1*) technique for distillation and nesslerization includes the use of a phosphate buffering system to maintain pH at 7.4 during distillation. In the presence of the high concentrations of Ca^{2+} and Mg^{2+} ions which occur in estuarine waters, the phosphate is precipitated and further buffer must be added. This technique is unsatisfactory for low NH_3–N concentrations since the phosphate buffer is a major contributor to the blank. In the method used for estuarine waters the phosphate buffer described in "Standard Methods" (*1*) was replaced by a sodium carbonate solution (20 ml. of 10% Na_2CO_3). No interference resulted with this buffer system and satisfactory blank values were obtained. It may be argued that the use of the Na_2CO_3 buffer would produce a higher pH (~8.3) and during distillation organic nitrogen-containing materials might tend to decompose more readily. While no such decomposition was evident in the waters of San Francisco Bay (which do not

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

contain high organic nitrogen concentrations) it was only possible to offer circumstantial evidence in favor of the absence of such decomposition.

Unfiltered samples and samples filtered through 0.45 μ membrane filters had identical NH_3–N contents, showing that the suspended matter in San Francisco Bay water (which is in fact mainly inorganic in nature) did not contain any decomposable organic nitrogen. The presence of a considerable amount of membrane filterable (0.45 μ) organic nitrogen was demonstrated. This so-called 'soluble organic nitrogen' did not start to decompose during distillation until the original volume of sample had been reduced from about 350 ml. to 50 ml. and until well after a plateau had been reached in the amount of ammonia recovered by distillation. Thus complete ammonia recovery was attained after the distillation of about 150 ml. from an original volume of about 350 ml. while high and erratic results only occurred after the distillation of about 300 ml. when the increasing salt concentration in the distillation flask markedly raised its boiling point.

In further support of the lack of decomposition of organic nitrogen during distillation it may be noted that the pyrazolone method performed directly on a sample gave results that agreed semi-quantitatively with those obtained by distillation and nesslerization.

Recoveries of NH_3–N from waters varying in salinity from 120 to 14,000 mg. Cl^-/liter were quantitative at the 100 μg./liter NH_3–N level. The precision of the technique fell off for the distillation of a 200 ml. sample below NH_3–N concentrations of 100 μg./liter (Table II). Thus at 110 μg. NH_3–N/liter a coefficient of variation of 1.8% was obtained while at 35 μg. NH_3–N/liter the coefficient of variation was about 13%. It was concluded therefore that to obtain precise results with this technique below NH_3–N levels of about 60 μg./liter samples of greater than 200 ml. volume should be distilled.

Total Unoxidized Nitrogen (Total Kjeldahl Nitrogen). The classical macro-scale digestion first proposed by Kjeldahl (*13*) using a copper catalyst (added as one bag of the commercial product Kelpak) and 20 ml. conc. H_2SO_4/200 ml. sample was used. Although mercury salts are recognized to be more effective catalysts than copper salts, recoveries of Kjeldahl nitrogen from San Francisco Bay waters using copper salts was comparable to when mercury salts were used and since a shorter analytical procedure results, their use was adopted. The ammonia released was determined as previously described.

Recovery of 50 μg. NH_3–N by the total unoxidized nitrogen technique was quantitative from waters of all salinities. It would seem, therefore, that the salt that accumulates from highly saline samples when the volume is reduced during digestion has no deleterious effect on the technique.

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

In Trace Inorganics In Water; Baker, Robert A.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.

Table II. Precision of Techniques for Analysis of Forms of Nitrogen

Nitrogen Form	*Mean Concentration μg./liter*	*Standard Deviation μg./liter*	*Coefficient of Variation (%)*	*Reported Precision of "Standard Method" (1)*
Ammonia	36	4.69	13.2	± 5%
	66	4.53	6.9	
	111	2.04	1.8	
Soluble Unoxidized	340	12	3.6	—
Total	702	97	13.9	
Unoxidized	382	90	2.4	—
	265	48	18.2	
Nitrite	2.2	0.03	1.2	Standard Deviation = 30 μg./liter at 250 μg./liter level
	5.6	0.14	2.6	
	8.0	0.15	1.9	
Nitrate	290	4.8	1.7	Standard Deviation = 490 μg./liter at 1,100 μg./liter level for Standard Method

Replication of the total unoxidized nitrogen technique for unfiltered Bay water samples was generally poor (Table II). Usually, out of ten replicates, there were one or two unusually high values and all efforts to eliminate them failed, including mixing for several minutes in a high-speed Waring blender.

Since the total unoxidized nitrogen technique gave excellent reproducibility for membrane filtered Bay water, it would seem that the poor reproducibility of the technique for unfiltered samples is because of inhomogeneity caused by uneven distribution of suspended matter.

Soluble Unoxidized Nitrogen. This form of nitrogen was determined by a Kjeldahl digestion (then distillation and nesslerization) on a sample filtered through a washed 0.45 μ Millipore membrane filter. Membrane filters used for separations of this type must be thoroughly washed with distilled water since it has been found that they contain soluble organic material that contributes significantly to the N content of the filtrate.

Excellent replication of the technique (coefficient of variation = 3.6%) was obtained at the 340 μg./liter N level (Table II).

A promising recent technique that could possibly be applied to the determination of soluble oxidized nitrogen is the photooxidation of organic matter by ultraviolet radiation (*3*).

Nitrite Nitrogen. The standard diazotization and coupling procedure with sulfanilic acid and α-naphthylamine was used for nitrite determination (*1*). It should be emphasized that the presence of even

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

small amounts of suspended solids drastically hinders the recovery of NO_2–N. For example the excellent recoveries obtained in 0.45 μ membrane filtered estuarine waters was reduced to a recovery of 86% by the presence of 12 mg./liter suspended solids and to 53% when 20 mg./liter suspended solids were present. Chloride concentrations varying between 150–12,300 mg. Cl^-/liter had no effect on the excellent performance of the method.

Excellent precision was found down to NO_2–N concentrations of 2 μg./liter where the method yielded a coefficient of variation of never greater than 2.6% (Table II).

Nitrate Nitrogen. The brucine method of Jenkins and Medsker (*11*) was used for the estimation of nitrate. This method is designed for the measurement of intermediate to low NO_3–N concentrations, such as those found in relatively unpolluted estuarine or offshore waters. Its limit of detectability is 0.01 mg. NO_3–N using a 10 ml. sample and with this sample volume the range of the technique is from 0.01 to 0.9 mg. NO_3–N/liter, while higher nitrate concentrations can be determined by prior dilution of the sample. The method would therefore not be sensitive enough for application in highly oligotrophic waters or in the open ocean. For these situations the cadmium reduction method of Strickland and Parsons (*20*) is recommended especially since in these situations large fluctuations in salinity are not generally encountered. The recommended brucine technique differs from the brucine method given in "Standard Methods" (*1*) in several important respects.

The sulfuric acid solution used in the "Standard Methods" (*1*) technique (500 ml. conc. H_2SO_4 + 75 ml. water) was found to give erratic results but satisfactory results were obtained when an acid mixture of 500 ml. conc. H_2SO_4 and 125 ml. water was used. Since the brucine-nitrate color development depends greatly on the amount of heat generated during the reaction, the modified method seeks to control the heat by altering the order of reagent addition to: sample, acid, and then brucine solution. This cooled mixture is then heated in a boiling water bath for exactly 20 minutes, following which the mixture is cooled rapidly to room temperature before reading the developed color (Table III).

Contrary to the statement in "Standard Methods" (1), it has been found that high concentrations of chloride ion produce a decrease in brucine-nitrate color in the standard brucine technique. The modified technique only gives a constant response with chloride concentrations between 27 to 50 grams Cl^-/liter. In our modification the effect of variable chloride concentrations is masked out by adding a large amount of sodium chloride to the reaction mixture before color development (Table III).

The recovery of 0.2 mg./liter NO_3–N from waters varying in chlorosity from 10 mg./liter to 16 grams/liter was quantitative using brucine

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

Table III. Comparison of Brucine Techniques for Nitrate Determination

	"Standard Methods" (1)	*Modified* [*Jenkins and Medsker* (11)]
Acid Mixture	500 ml. H_2SO_4 } 75 ml. H_2O }	500 ml. H_2SO_4 } 125 ml. H_2O }
Reagent Addition Sequence	Sample Brucine Acid	Sample Acid Brucine
Heat Control	None or water bath	Water bath and order of reagent addition
Chloride Effect	Decrease in response at high Cl^-	Chloride masking used to prevent chloride interference
Standard Curve	Non-linear	Linear between 0.05 mg./liter and 0.9 mg./liter NO_3–N

sulfate reagents purchased from three different manufacturers. At a level of 0.29 mg.NO_3–N/liter the coefficient of variation of the test was about 1.7% (Table II).

Phosphorus Forms

General Scheme of Phosphorus Analysis. In the analysis of natural waters the measurement of all forms of phosphorus (P) can be divided into two parts: (1) a preliminary treatment in which the P form of interest is separated and converted to orthophosphate, and (2) the colorimetric analysis of orthophosphate (usually by the formation of phosphomolybdic acid followed by its reduction with one of a variety of reducing agents to molybdenum blue). The forms of P in natural waters identified in this study are enumerated in Table IV.

Table IV. Analytical Differentiation of Phosphorus Forms

(1) Soluble P	Persulfate oxidation on 0.45 μ membrane filtered sample then colorimetry
(2) Soluble Ortho P	Colorimetry on 0.45 μ membrane filtered sample
(3) Soluble Organic P	(1) minus (2)
(4) Suspended P	Perchloric acid digestion of solids retained by 0.45 μ membrane filter followed by colorimetry
(5) Soluble Condensed P	Acid hydrolysis of 0.45 μ membrane filtered sample followed by colorimetry

A chemical classification into orthophosphate, polyphosphate, and organic P is made as well as the physical separation of these three chemical forms into "soluble" and "insoluble" components by filtration through

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

an 0.45 μ membrane filter. While this classification of soluble and insoluble forms is somewhat arbitrary, (Rigler (*19*) has shown that the percentage of soluble organic P in three lake waters decreased from 42% to 18% of the total P as the pore size of the filters was reduced from 5 μ to 0.1 μ), the 0.45 μ size was chosen since it provided a good compromise between slow rates of filtration and poor retention of particles. In turbid estuarine or offshore waters it should be noted that when a 200 ml. sample is filtered through a 47 mm. filter a considerable solids pack builds up and after the start of filtration particles much smaller than the nominal pore size of the filter may be removed.

Millipore filters (47 mm. diameter) were found to contain about 1.3 μg.P/filter of which about 1 μg. P could be washed out. It is therefore necessary thoroughly to wash membrane filters prior to their use in separations involved in P analysis.

In the waters of San Francisco Bay condensed phosphates were not found in any significant concentrations and hence the analytical scheme does not include a method for their determination. Should condensed phosphate be present the use of the American Association of Soap and Glycerine Producers (AASGP) method (*2*) is recommended on both a whole and a membrane-filtered sample.

Orthophosphate. The method used on San Francisco Bay water is a modification of the technique suggested by the AASGP (*2*) in which a membrane-filtered sample reacts with ammonium molybdate in acid solution and the resulting phosphomolybdic acid complex is extracted into an isoamyl alcohol/benzene mixture. Stannous chloride is used to reduce the extracted phosphomolybdic acid to molybdenum blue which is measured colorimetrically.

The ascorbic acid reduction method of Murphy and Riley (*18*) can also be used for orthophosphate analyses in estuarine waters since hydrolysis of polyphosphates and salt effects are absent in this technique.

It is extremely important that orthophosphate analyses on natural waters containing large amounts of suspended particulate material be carried out on membrane-filtered or clarified samples. In the investigation of the orthophosphate technique it was impossible to obtain consistent results if samples were not filtered.

The amount of orthophosphate which could be recovered from a sample seemed to depend largely on how long and how vigorously the sample was shaken during the analysis. It appeared that orthophosphate which was either adsorbed on or associated with the sediment was being released into solution. Since it was also found that salinity variations affected color development and that large turbidity corrections that were necessary when samples high in suspended solids were analyzed, it was decided that only a soluble orthophosphate determination was possible.

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

The solvent used for extraction of the phosphomolybdate complex was changed from the 1:1 benzene/isobutyl alcohol recommended by AASGP (2) to a 1:1 mixture of benzene/isoamyl alcohol. The change in solvent composition produced a slight (10 to 15%) increase in sensitivity but of far greater importance was the fact that isoamyl alcohol is about one-fifth as soluble in water as isobutyl alcohol. It was therefore not necessary to bring up the volume of the organic phase following extraction (as is necessary when isobutyl alcohol is used and large (200 ml.) aqueous samples are analyzed).

The method gave results that were slightly affected by salinity. The depression of absorbance caused by 20 grams Cl^-/liter varied with the amount of orthophosphate present. Thus, 20 grams Cl^-/liter produced depressions of 4.5, 2.8, and 0.5% respectively for sample contents of 5 μg., 20 μg., and 50 μg. orthophosphate–P. These depressions represent approximately the same absolute amount of PO_4–P.

Since 200 ml. samples of sea water contain about 20 μg. orthophosphate–P, depressions of 2 to 3% may be expected in water of a salinity equivalent to about sea water concentration. While a deviation of this magnitude can be tolerated, further studies of the technique showed that depressions were only about 1 to 2% when a solvent mixture of 25 ml. isoamyl alcohol + 30 ml. benzene was used instead of the 1:1 solvent mixture. It is probable that further investigation could yield a solvent mixture that would be completely devoid of a salt effect.

The hydrolysis of condensed phosphates during the orthophosphate analysis was virtually eliminated by minimizing contact time between the acidic reagents and the sample. Negative results were obtained when concentrated solutions of sodium tripolyphosphate were analyzed by this technique, however in the reactive phosphate technique of Strickland and Parsons (*20*)—a widely used oceanographic method—considerable hydrolysis of the polyphosphate took place.

Silica (50 mg. SiO_2/liters) did not interfere, neither did arsenate (500 μg. as arsenic). The absence of arsenate interference may be caused by conditions of molybdate concentration and pH unfavorable for the formation of arsenomolybdate or possibly insolubility of the arsenomolybdate in the organic phase.

Copper and iron which may be released into solution by the digestion of suspended sediment material in the analysis of total insoluble phosphorus, did not interfere in concentrations (200 μg. Cu^{2+} and 3 mg. Fe^{3+}/sample) several times greater than those found in typical San Francisco Bay sediments.

Mercuric chloride (40 mg. Hg^{2+}/liter) and chloroform (5 ml. $CHCl_3$/liter) which are commonly used sample preservatives, did not

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

significantly interfere with the method although in the presence of $CHCl_3$ a small decrease in orthophosphate recovery was observed.

It was possible to recover quantitatively 5 μg. and 20 μg. PO_4–P added to Bay water containing approximately 19 grams Cl^-/liter. Excellent reproducibility was obtained down to levels of about 50 μg. P/liter (the lowest tested) in waters with salinities varying from fresh water to ocean water. Coefficients of variation never exceeded 1.5% (Table V).

Table V. Precision of Techniques for Analysis of Forms of Phosphorus

Phosphorus Form	*Mean Concentration μg./liter*	*Standard Deviation μg./liter*	*Coefficient of Variation (%)*	*Reported Precision of Standard Method*
Soluble Ortho	48	0.56	1.2	Standard Deviation = 20 μg/liter
	76	0.74	0.97	
	94	1.30	1.4	
Total Soluble	49.6	0.59	1.2	—
	79.6	0.52	0.66	
	99.5	0.79	0.80	
Total Insoluble	113	2.7	2.4	—
	65	0.8	1.2	
	14	0.4	2.9	

Total Soluble Phosphorus. Total soluble phosphorus includes soluble orthophosphate (soluble condensed phosphate if present) and soluble organic phosphorus. A membrane-filtered sample is treated to release phosphorus as orthophosphate from combination with organic matter and condensed phosphates by some form of oxidation (and hydrolysis).

Several oxidants and techniques have been suggested for this pretreatment, *viz.* ignition (*14*), digestion with 30% H_2O_2 (*8*), acid treatment at 30 to 40 psi (*7*), nitric acid/sulfuric acid digestion (*2*), perchloric acid digestion (*6*), digestion with a perchloric acid/nitric acid mixtures (*2*), and digestion with potassium persulfate (*17*).

Each of the above digestion techniques (with the exception of acid hydrolysis at 30 to 40 psi) were studied for their applicability to estuarine waters. With the exception of the persulfate digestion, all methods were unsatisfactory or impractical for the analysis of samples with high dissolved solids contents. Any digestion technique that causes a significant volume reduction (i.e., ignition, perchloric acid digestions, nitric acid/sulfuric acid digestion) causes the precipitation of large quantities of salt. For example 200 ml. of sea water (which is the volume required for the determination of low amounts of total soluble phosphorus) will produce about 7 grams residue when evaporated to dryness.

In perchloric acid and nitric acid/sulfuric acid digestions, salts precipitate out as the digestion reduces the volume of solution. Danger exists

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

that dry spots may be formed in a heavy crystal mass during perchloric acid digestions and create an explosion hazard. In perchloric acid digestions chloride ion is replaced by perchlorate and the acidity of the solution decreases. Since the subsequent colorimetric orthophosphate technique relies on the presence of a precise amount of acid, it is necessary to adjust the volume of perchloric acid used according to the salinity of the sample. This is a tedious procedure that does not lend itself well to the routine analysis of large numbers of samples.

Digestion by boiling in the presence of 30% hydrogen peroxide (Jackson (*8*)) yielded incomplete recoveries of total soluble phosphorus from all the estuarine water samples tested and from organic media such as Difco tryptone and peptone. Moreover, the method failed to yield quantitative recovery of an orthophosphate standard carried through the test procedure.

The low result for the orthophosphate standard was probably caused by the presence of unreacted peroxide which during the subsequent colorimetric procedure reacted with molybdic acid to form permolybdic acid (Table VI).

Table VI. Comparison of Several Techniques for the Analysis of Total Soluble Phosphorus (Based on Recovery)

		Method of Analysis			
Type of Sample	*Unit of Expression*	H_2SO_4/ HNO_3 *Digestion* (2)	*Ignition 800°* (14)	H_2O_2 *Digestion* (8)	*Persulfate Digestion* (17)
Orthophosphate standard 16.3 μg. PO_4–P	μg.	16.3	16.3	14.6	16.3
Orthophosphate standard 16.3 μg. PO_4–P in 20 gram Cl^-/liter	μg.	—	—	—	16.3
Difco Tryptone 3–30 μg.; 0.97% P[a]	%	0.92	0.97	0.66	0.97
Difco Tryptone 3–30 μg.; 0.97% P[a] in 20 gram Cl^-/liter	%	—	—	—	0.97
Difco Peptone 15–100 mg.; 0.22% P	%	—	0.26	0.18	—
Bay Water, Pittsburg	μg. P/liter	47.0	56.1	38.5	51.9
Bay Water, Crockett	μg. P/liter	80.0	90.5	66.0	79.5
Bay Water, Fort Baker	μg. P/liter	97.6	116	72.5	99.3

[a] Values for Peptone and Tryptone supplied by Difco Company.

The persulfate digestion technique carried out by autoclaving the sample in the presence of persulfate at 15 to 20 psi for one hour (*17*)

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

gave excellent results and had a simplicity in execution that made it especially attractive for routine analysis. Complete recovery of orthophosphate, of soluble organic phosphorus in tryptone (both in the presence and absence of 20 grams Cl^-/liter and in samples from three points in San Francisco Bay were obtained (Table VI).

Menzel and Corwin (*17*) have reported excellent recoveries of organic phosphorus from several resistant organic phosphorus-containing compounds (lecithin, phosphorocholine, and 5-adenylic acid) as well as from zooplankton tissue using the persulfate digestion. During the present study the ability of persulfate to cleave P–C bonds was tested by performing the total soluble phosphorus test on phenylphosphoric acid and phenylphosphorous acid. Quantitative recovery of phosphorus from each of these materials was obtained at the 50 μg. P level.

The precision of the persulfate digestion technique for the determination of total soluble phosphorus was tested on estuarine water samples of widely differing salinity at total soluble phosphorus concentrations varying between 60–100 μg. P/liter. The maximum coefficient of variation of the technique was 1.2% (Table V).

Total Insoluble Phosphorus. A drastic digestion and oxidation technique is necessary to release all phosphorus associated with the particulate material present in estuarine waters such as those of San Francisco Bay. Although most soil analyses for total phosphorus involve the use of perchloric acid digestions (*8*) there is a widespread acceptance that, however drastic the digestion, some P-containing materials will not be released. This conclusion was substantiated for the suspended solids in San Francisco Bay water where it was found that, by using a sodium carbonate fusion technique, P yields of 2 to 5% higher than those from nitric acid/perchloric acid digestions were obtained. Results obtained for total insoluble phosphorus by a perchloric acid digestion method will be slightly lower than the actual total phosphorus content.

The method used for the analysis of total insoluble phosphorus was a nitric acid/perchloric acid digestion performed on the suspended material retained on a washed membrane filter and followed by a colorimetric estimation of the orthophosphate released by digestion. By performing the analysis on the filtered suspended matter it was possible to obtain suspended material from large volumes of water and carry out a direct analysis of suspended P. The method eliminated the previously mentioned difficulties encountered in performing perchloric acid digestions on large volumes of highly saline sample material.

A perchloric acid volume of 21 ml. reagent grade 70 to 72% $HClO_4$ and a digestion time of 10 to 15 minutes following the evolution of the dense white fumes of perchloric acid were found to produce a satisfactory digestion and a final digestion mixture with the correct acidity to proceed

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

directly to the analysis of orthophosphate by the AASGP technique (2) using 25 ml. of 10% neutral ammonium molybdate in place of the 50 ml. of acidified ammonium molybdate used in the orthophosphate determination. Acid concentrations varied no more than ±10 to 12% after digestion so that of the original 21 ml. perchloric acid introduced at the start of digestion, 19 ml. ±1 ml. remained at the end of the digestion procedure.

Replicate analyses of total insoluble P on the suspended solids from samples of estuarine water gave results with a coefficient of variation of less than 3% in the range of total insoluble P concentration of between 15 μg. P/liter and 110 μg. P/liter (Table V).

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

Preservation of Nitrogen and Phosphorus Forms

A study was made to determine the length of time that samples could be stored prior to analysis in the presence of various preservatives. Large volumes of bay water were collected and mixed well before splitting them into aliquots for preservative treatment. A separate plastic container was used for each day that the stored samples were analyzed, so that quiescent storage conditions could be maintained.

Preservation of Nitrogen Forms. The preservation methods investigated were (1) Storage at 4°C., (2) Storage at −10°C., (3) Storage at 4°C. with addition of 2 ml./liter 5% H_2SO_4, and (4) Storage at 4°C. with addition (as $HgCl_2$) of 40 mg. Hg^{2+}/liter.

In evaluating the results (Figure 1) it should be remembered that they apply to the relatively unpolluted waters of San Francisco Bay and that somewhat different results might be obtained if the biological activity in the samples was increased by larger amounts of organic materials.

The erratic nature of the soluble and insoluble organic nitrogen concentrations is partly attributed to the difficulty of preparing an homogenous distribution of the initial large sample into aliquots and partly to the variation inherent in the analysis. For all forms of nitrogen the best preservation method seems to be storage at 4°C. in the presence of 40 mg. Hg^{2+}/liter, although even this method does not satisfactorily maintain the initial levels of insoluble organic nitrogen and nitrite nitrogen over long periods (30 days). It is however the only preservation technique of those investigated that satisfactorily maintained the nitrite nitrogen concentration for about one week. Nitrate and ammonia levels were maintained satisfactorily for one month by the 4°C. acid treatment, however the use of a sulfuric acid preservative cannot be recommended if nitrite nitrogen measurements are to be made. The acid treatment produced an immediate and rapid decrease in nitrite nitrogen, as may be predicted from the Van Slyke reaction:

$$H^+ + NO_2^- + R\text{–}NH_2 \rightarrow N_2 \uparrow + ROH + H_2O.$$

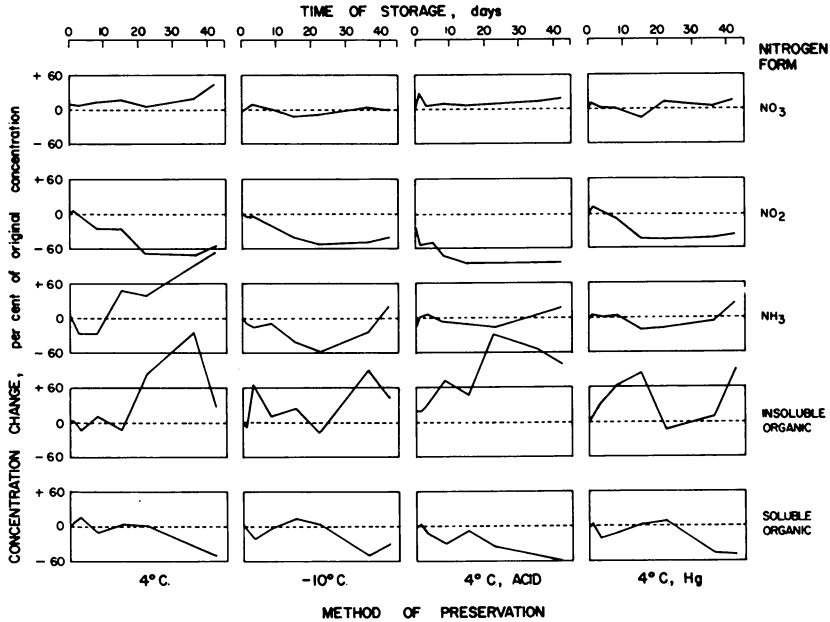

Figure 1. Preservation of nitrogen forms in estuarine water

Preservation of Phosphorus Forms. Because of the good results obtained in the study of the preservation of nitrogen forms, with an Hg^{2+} preservative, it was decided to test the efficacy of mercury preservation for P forms at both 4°C. and −10°C. Acid preservation was not considered because of the extreme lability of condensed phosphates in acid conditions. Since chloroform has been widely used as a preservative for waters to be later analyzed for forms of P, its preservative properties were investigated. Glass containers were used in this preservation study. The preservative treatments were as follows: (1) Storage at 4°C., (2) Storage at 4°C. with 5 ml. $CHCl_3$/liter, (3) Storage at 4°C. with 40 mg. Hg^{2+}/liter, (4) Storage at −10°C., and (5) Storage at −10°C. with 40 mg. Hg^{2+}/liter.

The results of the preservation study (Figure 2) show that for long periods (one month) the best preservation technique was storage at −10°C. with 40 mg. Hg^{2+}/liter. For storage periods of a few days, any of the methods, except storage at 4°C. with $CHCl_3$, were satisfactory. The chloroform treatment produced a dramatic reduction in soluble orthophosphate and an accompanying rise in total insoluble phosphorus.

Summary

A complete set of analytical techniques for the analysis and differentiation of various forms of nitrogen and phosphorus has been presented.

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

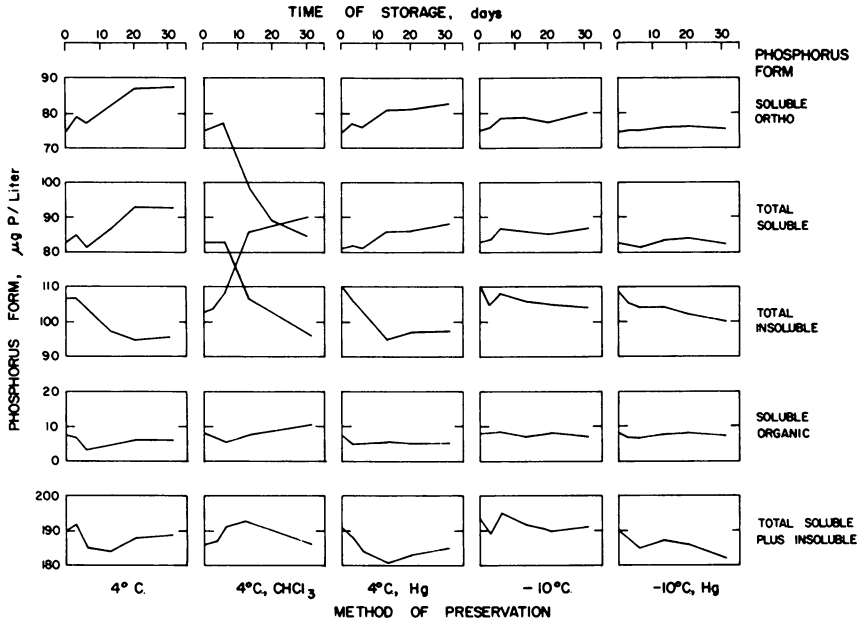

Figure 2. Preservation of phosphorus forms in estuarine water

The techniques are suitable for the analysis of estuarine waters whose high and variable salinity and suspended matter content creates considerable difficulties in analytical techniques designed for use in ocean waters and fresh waters. The precision and accuracy of the developed techniques has been determined under the entire range of conditions encountered in San Francisco Bay. Several methods of sample preservation have been investigated and methods that are reliable in preserving nitrogen and phosphorus forms in San Francisco Bay water are recommended.

Literature Cited

(1) Am. Public Health Assoc., "Standard Methods for the Examination of Water and Wastewater," 12th ed., Am. Public Health Assoc., New York, 1965.
(2) Am. Soap and Glycerine Producers Comm. *J. Am. Water Works Assoc.* **50,** 1563 (1958).
(3) Armstrong, F. A. J., Williams, P. M., Strickland, J. D. H., *Nature* **211,** 481 (1966).
(4) Davis, Ernst, Univ. of Texas (Private Communications).
(5) Goldman, C., Dept. of Zoology, Univ. of Calif. (Davis) (Private Communication).

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

(6) Hansen, A. L., Robinson, R. J., *J. Marine Res. (Sears Found. Marine Res.)* **12,** 31 (1953).
(7) Harvey, H. W., *J. Marine Biol. Assoc. U. K.,* **27,** 337 (1948).
(8) Jackson, M. L., "Soil Chemical Analysis," Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1958.
(9) Jenkins, D., *Univ. Calif. (Berkeley) Sanit. Eng. Res. Lab. Rept.* **65,** 13 (1965).
(10) *Ibid.,* **16,** 18 (1965).
(11) Jenkins, D., Medsker, L. L., *Anal. Chem.* **36,** 610 (1964).
(12) Jenkins, D., Selleck, R. E., Pearson, E. A., *Univ. Calif. (Berkeley) Sanit. Eng. Res. Lab. Rept.* **65,** 7 (1965).
(13) Kjeldahl, J., *Zeits. Anal. Chem.* **22,** 366 (1883).
(14) Legg, J. O., Black, C. A., *Soil Sci. Soc. Am. Proc.* **19,** 139 (1955).
(15) Mackenthun, K. M., Federal Water Pollution Control Admin., Cincinnati, Ohio (Private Communication).
(16) Martin, C. V., Calif. State Dept. of Water Resources (Private Communication).
(17) Menzel, D. W., Corwin, N., *Limnol. Oceanog.* **10,** 28 (1965).
(18) Murphy, J., Riley, J. P., *Anal. Chim. Acta* **27,** 31 (1962).
(19) Rigler, F. H., *Limnol. Oceanog.* **9,** 511 (1964).
(20) Strickland, J. D. H., Parsons, T. R., *Bull., Fisheries Res. Board Can.* 125 (1960).

RECEIVED April 24, 1967.

Downloaded by MICHIGAN STATE UNIV on January 23, 2016 | http://pubs.acs.org
Publication Date: June 1, 1968 | doi: 10.1021/ba-1968-0073.ch016

In Trace Inorganics In Water; Baker, Robert A.;
Advances in Chemistry; American Chemical Society: Washington, DC, 1968.