

SAMPLE COLLECTION, HANDLING, AND PRESERVATION

INTRODUCTION

In general, the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the analytical results. Sample preservation is difficult because almost all preservatives interfere with some of the analyses. Some determinations are more likely than others to be affected by sample storage before analysis. Regardless of the sample nature, complete stability for every constituent can never be achieved. At best, preservation methods only serve to retard the chemical and biological changes that inevitably continue after sample collection.

A most important rule to keep in mind is that the result of any analytical test can be no better than the sample on which it is performed. The primary objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and handled in the laboratory while still accurately representing the material being sampled. Specific water sampling techniques will not be discussed here but may be found in a variety of other references (APHA et al. 1980, USGS 1977). This discussion will also focus only on the chemical analyses performed by the Cooperative Central Analytical Laboratory (CCAL) as of March 1984.

The main factors affecting sample stability are: (1) the nature of the sample, (2) the sample container, and (3) the addition of preserving reagents to the sample (Wilson 1974). Strict rules for the preservation of water samples do not exist. The two primary references used by those involved in water quality measurements do not agree for many analyses. In general, it is most appropriate for a laboratory to develop a standard procedure for sample preservation in conjunction with the requirements and needs of each individual investigator.

PRESERVATION METHODS

The inhibition of biological activity in a sample is often particularly important as bacteria and algae can consume, partially or completely, a number of substances required for their growth (e.g., nitrogen, phosphorus, and silicon compounds). However, the killing of biological species may release other constituents such as orthophosphate (Wilson 1974). Biological activity is usually prevented or reduced by storing samples at low temperatures and keeping them in the dark as much as possible from the time of collection until analysis. Mackerth et al. (1978) report that ammonia and nitrogen are not affected by this treatment. Silica concentrations may be reduced in the sample (Kobayashi 1967, Burton et al. 1970) while phosphorus (Fitzgerald and Faust 1967, Philbert 1973), and alkalinity (Philbert 1973) may also be adversely affected. Philbert (1973) reports that samples from calcium rich waters may also exhibit altered calcium concentrations. Frozen samples should be thawed slowly and well-mixed before analysis. Care is still required to ensure that the sample is analyzed quickly to prevent any subsequent instability.

Often algal and bacterial activity can be reduced sufficiently simply by filtering the sample during or immediately after collection (Wilson 1974). Membrane filters (pore-size approximately 0.5 μ m) and glass-fiber filters (Whatman GF/C-pore size 1.2 μ m,

Whatman GF/F-pore size 0.7 μm) are most commonly used. The variability in pore sizes used in different laboratories renders the implied division between inorganic and organic constituents on the basis of filtration somewhat ill defined (Mackerth et al. 1978). A comparison study between polycarbonate membrane filters (Nuclepore 0.2 μm) and Whatman GF/C filter papers showed that ammonia appeared to be adsorbed in the first 50 ml filtered through the GF/C filters while no adsorption was observed for the membrane filters (Riemann and Shierup 1978). In both cases, it is advisable to remove leachable materials from all filter papers (Eaton et al. 1969) by either soaking them in distilled water for 2-3 minutes or passing at least 200 ml of distilled water through the paper and then drying it prior to use.

A number of other preservatives are often used to prevent biological or chemical reactions or both from occurring in the sample. Mercuric chloride (HgCl_2) and sulfuric acid (H_2SO_4) are considered to be primarily bacterial inhibitors (USEPA 1979). HgCl_2 was found to decrease ammonium concentrations but effectively preserve nitrite levels (Riemann and Schierup 1978). Klingaman and Nelson (1976) concur that ammonia concentrations are adversely effected by HgCl_2 in addition to nitrate levels while phosphorus remained stabilized when the samples were stored at 4°C. Chloride levels in a study by Mackerth et al. (1978) were determined to be adversely affected by HgCl_2 preservation as was nitrate.

Sulfuric acid was reported by Riemann and Schierup (1978) to change ammonia concentrations; however, nitrite levels increased in the 20 hours after preservation addition and then became stable. In general, acidification including the use of nitric and sulfuric acids was found to adversely affect nitrate, sulfate, conductivity, and alkalinity by Mackerth et al. (1978).

In general, there is agreement that sample acidification is necessary for trace metal analysis. Nitric acid addition to $\text{pH} < 2$ is most frequently recommended (APHA 1980, USEPA 1979) for the analysis of total and dissolved trace metal preservation. Wilson (1974) found that acidification decreased the precipitation of Fe, Cu, Ni, Al, and Zn from water samples but cited evidence that interference in mercury determinations may occur. Zn may leach out of new plastic sample bottles if the bottles are not acid-leached and rinsed prior to use (Florence and Batley 1980).

Researchers at Coweeta Hydrologic Laboratory routinely preserve stream samples with 0.5 ppm phenyl mercuric acetate (PMA). Klingaman and Nelson (1976) found that phosphorus concentrations increased while inorganic nitrogen levels were preserved. Dan Richter (Oak Ridge National Laboratory; personal communication) indicates that in his experience PMA interferes with both nitrogen and phosphorus levels.

An additional method that is more easily applied in the laboratory than in the field is reagent addition. The objective is to begin the analysis of the sample as soon as possible after collection so that the sample is converted to a more stable form. The analyses can then be completed when convenient (Wilson 1974). If this method is adopted in the field, it may require a variety of sample bottles (and transport of reagents) when a number of different analyses are required.

The general recommendation is that the best overall sample preservation technique is storage at sub-zero temperatures (USEPA 1979, Florence and Batley 1980, Klingaman and Nelson 1976). In cases where this is not possible, filtering is highly desirable to reduce algal and bacterial activity (Wilson 1974). It is also important to keep the sample

as cool as possible and in the dark. Other preservatives may be added to the sample as required with regard to the effect of each preservative on the analyses desired.

SAMPLE HANDLING RECOMMENDATIONS

All high density polyethylene (HDPE) sample bottles supplied by CCAL are routinely acid-treated when new with a 1:1 HCl solution and well-rinsed with deionized water prior to use. They are acid rinsed after each use with 0.5 N HCl, well-rinsed with deionized water, dried, and stored with the caps on to prevent contamination. The bottle should be rinsed with sample water prior to actual sample collection.

Ideally, pH, conductivity, and temperature should be determined in the field when possible. If it is possible, alkalinity should also be measured at the time of sample collection. Samples should be placed in a cooler with ice immediately after collection, satisfying the requirements for dark and low temperatures. If a cooler is not available, storing bottles in a cold stream is preferential to no cooling at all.

It is advisable to filter samples as soon as possible to reduce algal and bacterial activity. Ideally this is done in the field as soon as the sample has been collected. (Note: if pH, alkalinity and conductivity are required, retain at least 125 ml unfiltered sample.) An easily portable field filtration system is routinely used by researchers from Oregon State University's Department of Fisheries & Wildlife Stream Team. The components of this system and likely suppliers are listed in Table 4. The total cost is about \$250. A wooden box was built to transport the entire system easily. Whatman GF/F filter papers may be substituted for GF/C filter papers. It is important to leach and dry the papers before use to remove manufacturing contaminants. The Stream Team fires the papers in a muffle oven at 400°C prior to use and stores the papers in the kit in aluminum foil. CCAL rinses filter papers with at least 500 ml of deionized water and dries them for at least 48 hours in a 55°C drying oven. CCAL currently filters most samples through Whatman GF/C glass fiber filter paper which has a particle retention size of 1.2 µm. GF/F glass fiber filters with a particle retention size of 0.7 µm are used on the H.J. Andrews long-term research samples. The filtering apparatus should either be rinsed with deionized water between uses and/or rinsed with the water from the next sample. It is important to use flat-edged filter paper forceps to avoid handling the papers and possibly puncturing the papers.

As a general policy, the laboratory recommends that the samples be filtered by the investigator as soon as possible after collection. If the sample can be transported to the laboratory within 24 hours, it should not be frozen or preserved but kept cold and in the dark. If the time between sample collection and submission to the laboratory is greater than 24 hours, the filtered sample should immediately be frozen and not allowed to thaw until it arrives at the laboratory. Be careful, when freezing samples, to leave at least 10% of the bottle volume as head space to accommodate expansion of the sample. If the sample is to be analyzed for silicon, an unfrozen aliquot must be submitted. Unfrozen samples should contain little or no head space.

Unfiltered samples should be frozen until arrival at the laboratory. It is recommended that no acids be added to unfiltered samples as the acidity may cause leaching of the sediments and/or degradation of biological constituents.

If other preservation methods are desirable to the investigator, this should first be discussed with the laboratory prior to sample submission.

Investigators who desire trace metal and/or cation analyses should discuss this also with the laboratory. If total metal concentrations are required, it may be to the investigators advantage to add nitric acid (2 ml/100 ml) to a 100 ml subsample immediately after collection. Dissolved cation determinations are not routinely performed by this laboratory on acidified samples. An alternate method for preserving trace metal or cation samples is to keep the filtered samples frozen until immediately prior to analysis.

The volume of sample submitted to the laboratory is dependent on the types of analyses required. Table 5 shows the minimum volume required for each analysis and the recommended volume to allow for rinsing graduated cylinders, sample tubes and bottles in order to reduce possible contamination. In general, most investigators should submit at least 1 liter of sample if possible, even if that great a volume is not necessary for the requested analyses. Investigators who wish to have samples analyzed for Kjeldahl nitrogen should submit at least 2 liters of sample. Additional sample may be used for quality control duplicate analyses or repetition of anomalous sample results. New investigators should discuss sample collection volume with the laboratory prior to submitting samples.

Investigators are strongly encouraged to participate in the laboratory quality control program by submitting one duplicate sample in every set of 10 samples submitted. This duplicate will be analyzed at no charge and the results of the analyses returned with the sample data. If investigators wish to participate in the duplicate sample program, the laboratory must be informed prior to sample submission for investigators to qualify for the analyses at no charge. Also, the laboratory must be informed which samples are duplicated before sample data will be released. Sample submission, however, should be on a blind basis. Please discuss the duplicate sample program with the laboratory supervisor prior to sample submission.

CCAL Analytical Detection Levels

Analysis	Level of Detection		Precision [@]	
Alkalinity	0.2	mg/l	+/- 0.02	mg/l ¹
Ammonia-nitrogen*	0.010	mg/l ²	+/- 0.002	mg/l
Calcium	0.06	mg/l	+/- 0.065	mg/l ³
Carbon, dissolved organic	0.1	mg/l	+/- 0.1	mg/l
Chloride	0.02	mg/l	+/- 0.085	mg/l
Magnesium	0.001	mg/l	+/- 0.0006	mg/l ³
Nitrate-nitrogen	0.001	mg/l ²	+/- 0.001	mg/l
Nitrogen, total Kjeldahl	0.01	mg/l ⁴	+/- 0.006	mg/l
Phosphate, ortho	0.001	mg/l ³	+/- 0.001	mg/l
Phosphorous, total	0.001	mg/l ³	+/- 0.002	mg/l
pH	0 – 14	pH units	+/- 0.1	pH unit ⁵
Potassium	0.03	mg/l	+/- 0.015	mg/l ³
Silica	0.20	mg/l ²	+/- 0.05	mg/l
Sodium	0.01	mg/l	+/- 0.005	mg/l ³
Specific conductance	0.4	us/cm	+/- 2% ⁵	
Sulfate	0.01	mg/l	+/- 0.025	mg/l

Table 1. Levels of Detection and Precision for CCAL Analyses.

[@]Precision evaluated by repeated analysis of near detection level standard solutions.

¹Titration precision and accuracy evaluated by comparison with Gran titration results.

²Method specified detection level as yet unconfirmed, but evaluated as reasonable by monitoring sample response at this concentration level.

³Evaluated by low concentration detection level analysis.

⁴Estimate based on low concentration standards analyzed on a continuing basis.

⁵Limitation of instrument scale on instrument currently in use.

*Please note that for ammonia-nitrogen that the laboratory has been able to produce data with the same precision as stated above at a DL of 0.005 mg/l.

CCAL Analytical Methodology

Analysis	*Method # with Specifications and Modifications
Alkalinity	403, procedure 4c, titrate to pH 4.5. Modifications: Use 0.02N Na_2CO_3 and 0.02N H_2SO_4 .
Ammonia	417F.
Calcium	303A; flame atomic absorption spectroscopy. Modifications: nitrous oxide/acetylene flame. Addition of 1 ml 50,000 mg/l lanthanum oxide to 10 ml sample to control ionization.
Carbon, Dissolved Organic	5310B.
Chloride	4110B.
Specific Conductance	205; Wheatstone bridge.
Magnesium	303A; flame atomic absorption spectroscopy.
Nitrate	418F. Technicon industrial method 100-70W; different formulations for color and ammonium chloride reagents.
Nitrogen, Total Kjeldahl	Kjeldahl digestion: H_2SO_4 , CuSO_4/KCl , Nessler finish.
pH	423; Calomel reference electrode, glass pH electrode, temperature compensator.
Phosphate, Ortho	424F. Modifications: Ascorbic acid reagent 2g/100 ml.
Phosphorous, Total	424C, 424F. Modifications: microwave digestion 60 minutes, 50 ml analysis volume, ascorbic acid reagent 2g/100 ml.
Potassium	303A; flame atomic absorption spectroscopy.
Silica	Technicon industrial method 105-71W/B.
Sodium	303A; flame atomic absorption spectroscopy.
Sulfate	4110B.

Table 2. Analytical Methodology for CCAL.

*Method numbers refer to *Standard Methods for the Examination of Water and Wastewater* 15th Edition, 1980, except sulfate, chloride and dissolved organic carbon *Standard methods for the Examination of Water and Wastewater* 17th Edition, 1989.

CCAL Analytical Instrumentation

Analysis	Instrumentation
Alkalinity	Radiometer type TTT 1c auto-titrator with glass pH electrode, calomel reference electrode and temperature compensator electrode.
Ammonia	Technicon Auto-Analyzer II.
Calcium	Varian SpectrAA 220 atomic absorption spectrophotometer.
Carbon, Dissolved Organic	Shimadzu TOC-5000A
Chloride	Dionex 4000i Ion Chromatograph.
Specific Conductance	YSI model 31 conductivity bridge.
Magnesium	Varian SpectrAA 220 atomic absorption spectrophotometer.
Nitrate	Technicon Auto-Analyzer II.
Nitrogen, Total Kjeldahl	Milton-Roy 601 spectrophotometer with 2.54 cm pathlength cell. Analyze at 425 nm.
pH	Radiometer type TTT 1c auto-titrator with glass pH electrode, calomel reference electrode and temperature compensator electrode.
Phosphate, Ortho	Milton-Roy 601 spectrophotometer with 10 cm pathlength.
Phosphorous, Total	Milton-Roy 601 spectrophotometer with 10 cm pathlength.
Potassium	Varian SpectrAA 220 atomic absorption spectrophotometer.
Silica	Technicon Auto-Analyzer II.
Sodium	Varian SpectrAA 220 atomic absorption spectrophotometer.
Sulfate	Dionex 4000i Ion Chromatograph.

Table 3. Analytical Instrumentation Used at CCAL.

Vendor:

Markson Science
7815 S. 46th St.
Phoenix, AZ 85040
800-528-5114

Nalgene Vacuum Pump
Catalog # 083709
\$61.60

Millipore Corp.
Order Service Dept.
448 Grandview Drive
South San Francisco, CA 94080
800-227-0234

Pyrex Filter Holder
Catalog # XX10 047 00
\$105.00

1 Litre Filtering Flask
Catalog # XX10 047 05
\$31.00

Filter Forceps
Catalog # XX62 000 06
\$11.00

VWR Scientific
P.O. Box 3551
Seattle, WA 98124
800-333-6336

Whatman GF/C Filter Paper
4.7 cm diameter
Catalog # 28497-696
pkg/100 \$35.10

Black Rubber Tubing

Total Cost \$243.70

Table 4. Example Filtration System

	Minimum Volume	Recommended Volume
Alkalinity, pH, conductivity:	125 ml	250 ml
Carbon, Dissolved Organic	50 ml	70 ml
Cations – Na, K, Ca, Mg:	40 ml	50 ml
Chloride:	30 ml	50 ml
Color:	50 ml	100 ml
Nitrogen –		
Total Kjeldahl Nitrogen:	500 ml	1000 ml
Grouped Analyses –		
Ammonia,		
Nitrate + Nitrite,		
Silica:	30 ml	60 ml
Phosphorous –		
Ortho Phosphorous:	55 ml	110 ml
Total Phosphorous:	55 ml	110 ml
Total Dissolved Solids:	110 ml	220 ml
Sulfate:	30 ml	50 ml

Table 5. Required Sample Volumes

LITERATURE CITED

- American Public Health Association and others. Standard methods for the examination of water and wastewater (15th ed.). Washington, DC; 1980. 1134 p.
- Burton, J.D., T.M. Leatherland, and P.S. Liss. *Limnol. Oceanogr.* 15, 473. 1970.
- Eaton, John S., Likens, Gene E., and Bormann, F.H. Use of membrane filters in gravimetric analyses of particulate matter in natural waters. *Water Resources Res.* 5: 1151-1156; 1969.
- Fitzgerald, G.P., and Faust, S.L. *Limnol. Oceanogr.* 12, 332. 1967.
- Florence, T.M., and G.E. Batley. Chemical speciation in natural waters. *CRC Critical Review in Analytical Chemistry.* 9: 219-296; 1980.
- Klingaman, E.D., and Nelson, D.W. Evaluation of methods for preserving the levels of soluble inorganic phosphorus and nitrogen in unfiltered water samples. *J. Environ. Qual.* 5: 42-46; 1976.
- Kobayashi, J. Chemical environment in the aquatic habitat. In: Golterman, H.L. and Clymo, R.S. (eds.). *Royal Netherlands Acad. Sci., Amsterdam.*
- Latterell, J.J., Timmons, D.R., Holt, R.F., and Sherstad, E.M. Sorption of orthophosphate on the surface of water sample containers. *Water Resources Res.*: 865-869; 1974.
- Mackerth, F.J., Heron, J., and Talling, J.F. Water analysis: some revised methods for limnologists. *Fresh Water Biological Assoc. The Ferry House, Ambleside, Cumbria, LA 220LP.* 120 p.
- Peden, M.E., Skowron, L.M., and McGurk, F.F. Precipitation sample handling analysis, and storage procedures. *Urbana, IL: Illinois State Water Survey; 1979; Res. Rep. 4.* 71 p.
- Philbert, F.J. A comparative study of the effect of sample preservation by freezing prior to chemical analysis of Great Lakes waters. In: *Proceedings of the 16th Conf. Great Lakes Res.* 282-293.
- Riemann, Bo, and Schierup, Hans-Henrik. Effects of storage and conservation on the determination of ammonia in water samples from four lake types and a sewage plant. *Water Res.* 12: 849-853; 1978.
- Ryden, J.C., Syers, J.K., and Harris, R.F. Sorption of inorganic phosphate by laboratory ware: implications in environmental phosphorus techniques. *Analyst.* 97: 903-908; 1972.

U.S. Environmental Protection Agency. Methods for chemical analysis of water and wastes. Cincinnati: EPA-600/4-79-020; 1979.

U.S. Geological Survey. Techniques of water resources investigations of the United States Geological Survey: quality assurance practices for the chemical and biological analysis of water and fluvial sediments. Book 5, Chapter A6; 1982. 181 p.

U.S. Geological Survey. National handbook of recommended methods for water-data acquisition. Reston, VA; 1977.

Wilson, A.L. The chemical analysis of water: general principles and techniques. The Chemical Society. Burlington House, London; 1974. 188 p.

Special thanks to Deborah Coffey for gathering and compiling this information.