SAMPLE COLLECTION, HANDLING, AND PRESERVATION

Information dated, but still very relevant; reflects practices still in place at CCAL. Prices and part numbers have been updated for 2006.
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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 Chemical 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 0.5um) and glass-fiber filters (Whatman GF/C-pore size 1.2 um, Whatman GF/F-pore size 0.7 um) 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 um) 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 ($HgC1_2$) and sulfuric acid (H_2SO_4) are considered to be primarily bacterial inhibitors (USEPA 1979). $HgC1_2$ 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 $HgC1_2$ 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 $HgC1_2$ 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 $pH < 2$ 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 0.5 N HC1 solution and well-rinsed with deionzied water prior to use. They are acid rinsed after each use with 0.5 N HC1, 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. For unfiltered nutrients analyses, see volume requirements in the table below.) 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 $400. 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 5 days in a 80°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 um. GF/F glass fiber filters with a particle retention size of 0.7 um 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. See minimum volume requirements for each analysis below, 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. 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 released. Sample submission, however, should be on a blind basis. Please discuss the duplicate sample program with the laboratory supervisor prior to sample submission.

Example of items needed for field filtration kit. Information provided as an example only; not intended for product or vendor endorsement.

VWR Scientific
800-932-5000

Nalgene Vacuum Pump
Catalog # 54903-819
Each $147.01

Whatman GF/C Filter Paper
4.7 cm diameter
Catalog # 28497-696
100/pk $73.93

	Kontes Ultra-Ware Glass Filter Funnel/Support Assembly, 47mm Catalog # KT953755-0000 Each $121.88 Filter Flask, 1L Catalog # KT953760-0000 Each $45.79 Nalgene Filter Forceps Catalog # 25729-118 Each $21.63 Black Rubber Tubing
Total Cost	$410.24

Volume Requirements for Various CCAL Analyses

	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 persulfate Nitrogen:	50 ml	100 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

Suspended Sediment	1000 ml	1000 ml

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