COLLECTION AND PRESERVATION OF SAMPLES

Introduction

It is an old axiom that the result of any testing method can be no better than the sample on which it is performed. It is not practical to specify detailed purposes and analytical procedures. More detailed information appears in specific methods. This section presents general considerations. Applicable primarily to chemical analyses. See appropriate sections for samples to be used in toxicity testing and microbiological or biological examinations.

The 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.

This objective implies that the relative proportions or concentrations of all pertinent components will be the same in the samples in the material being sampled, and that the sample will be handled in such a way that no significant changes in composition occur before the tests are made.

In many instances the objective of sampling is to demonstrate compliance with specific regulatory requirements. Samples are presented to the laboratory for specific determinations with the sample collector taking responsibility for the validity of the sample. Because of the increasing importance of the ability to verify the accuracy and representativeness of data, greater emphasis is placed on proper sample collection and preservation techniques.

Often, in water and wastewater work, the laboratory conducts or prescribes the sampling program, which is determined in consultation with the user of the test results. Such consultation is essential to insure selecting samples and analytical methods that provide a true basis for answering the questions that prompted the sampling.

This section addresses the collection and preservation of water and wastewater samples, although the general principles also apply to the sampling of solid or semi-solid matrices.

1. General precautions

Obtain a sample that meets the requirements of the sampling program and handle it so that it does not deteriorate or become contaminated before it reaches the laboratory. In water sampling, before filling sample bottle, rinse it two or three times with the water being collected, unless the bottle contains a preservative or dechlorinating agent.

Depending on determinations to be performed, fill container full (most organic compound determinations) or leave space for aeration, mixing, etc. (microbiological analyses). If bottle already contains preservative, take care not to overfill the bottle, as preservative may be lost or diluted. Except when sampling for analysis of volatile organic compounds, leave an air space equivalent to approximately 1% of the container volume to allow for thermal expansion during shipment.

Special precautions are necessary for samples containing organic compounds and trace metals. Because many constituents may be present at concentrations of micrograms per liter, they
may be totally or partially lost when proper sampling and preservation procedures are not followed.

Representative samples of some sources can be obtained only by making composites of samples collected over a period of time or at many different sampling points. The details of collection vary with local conditions, so specific recommendations would not be universally applicable. Sometimes it is more informative to analyze numerous separate samples of one composite so as not to obscure variability, maxima, and minima.

Because of the inherent instability of certain properties and compounds, composite sampling is not recommended where quantitative values are desired. These include acidity, alkalinity.

**BOD**, carbon dioxide, chlorine residual, iodine, hexavalent chromium, nitrate, volatile organic compounds, dissolved oxygen, ozone, and pH. In certain cases, such as for BOD, the collection of composite samples is routinely by regulatory agencies to monitor discharge limits and to take into account the relative uncertainty of analyses.

Sample carefully to insure that analytical results represent the actual sample composition. Important factors affecting results are the presence of suspended matter or turbidity, the method chosen for its removal, and the physical and chemical changes brought about by storage or aeration. Detailed procedures are essential when processing (blending, sieving, filtering) samples to be analyzed for trace constituents, especially metals and organic compounds. Some determinations, such as analyses for lead, can be invalidated by contamination from such processing.

Treat each sample individually with regard to the substances to be determined, the amount and nature of turbidity present, and other conditions that may influence the results.

The choice of technique for collecting a homogeneous sample must be defined in the sampling plan. In general, separate any significant amount of suspended matter by decantation, centrifugation, or an appropriate filtration procedure. For metals it often is appropriate filtration procedure. For metals it often is appropriate to collect both a filtered and an unfiltered sample to differentiate between total dissolved metals present in the matrix. Beforehand, determine the acid requirements to be bring the pH to <2 on a separate sample. Add the same relative amount of acid to all samples. Be sure that the dilution caused by acidifying is negligible or reproducible enough for a dilution correction factor. Filter samples in the field, or at the point of collection before preservation with acid to pH <2.0.

Often slight turbidity can be tolerated if experience shows that it will cause no interference in gravimetric or volumetric tests and that its influence can be corrected in colorimetric tests, where it has potentially the greatest interfering effect.

Sample collector must state whether or not the sample has been filtered. To measure the total amount of a constituent, do not remove suspended solids but treat them appropriately.

Make a record of every sample collected and identify every bottle, preferably by attaching an appropriately inscribed tag or label. Record sufficient information to provide positive sample identification at a later date, including the name of the sample collector, the date, hour, exact
location, the water temperature, and any other data that may be needed for correlation, such as weather conditions, water level, stream flow, post-sampling handling, etc. Provide space on the label for the initials of those assuming sample custody and for the time and date of transfer. Fix sampling points by detailed description in the sampling plan, by maps, or with the aid of stakes, buoys, or landmarks in a manner that will permit their identification by other persons without reliance on memory or personal guidance. Particularly when sample results are expected to be involved in litigation, use formal "chain-of-custody" procedures (see B.2 below), which trace sample history from collection on final reporting.

Cool hot samples collected under pressure while they are still under pressure.

Before collecting samples for distribution systems, flush lines with 3 to 5 pipe volumes to insure that the sample is representative of the supply, taking into account the diameter and length of pipe to be flushed and the flow velocity. As an alternative when dimensions are unavailable, flush with tap fully open for 2 to 3 min before sampling.

Collect samples from wells only after the well has been pumped sufficiently to insure that the sample represents the groundwater source. Sometimes it will be necessary to pump at a specified rate to achieve a characteristic drawdown, if this determines the zones from which the well is supplied. Record pumping rate and drawdown.

When samples are collected from a river or stream, observed results may vary with depth, stream flow, and distance from shore and from one shore to the other.

If equipment is available, take an integrated sample from top to bottom in the middle of the main channel of the stream or from side to side at mid-depth in such a way that the sample is integrated according to flow. If only a grab or catch sample can be collected, take it in the middle of the main channel of the stream and at mid-depth. Integrated samples are described further in B.1c below.

Lakes and reservoirs are subject to considerable variations from normal causes such as seasonal stratification, rainfall, runoff, and wind. Choose location, depth, and frequency of sampling depending on local conditions and the purpose of the investigation.

For certain constituents, sampling location is extremely important. Avoid areas of excessive turbulence because of potential loss of volatile constituents and of potential presence of toxic vapors. Avoid sampling at weirs because such locations tend to favor retrieval of lighter-than-water, immiscible compounds.

Generally, collect samples beneath the surface in quiescent areas and open sampling container below surface in quiescent areas and open sampling container below surface to avoid collecting surface scum. If composite samples are required, ensure that sample constituents are not lost during compositing because of improper handling of portions being composited. For example, casual dumping together of portions rather than addition to the composite through a submerged siphon can cause unnecessary volatilization.

Use only representative samples (or those conforming to a sampling program) for examination.

2. Safely Considerations
because sample constituents can be toxic, take adequate precautions during sampling and sample handling. Toxic substances can enter through the skin and, in the case of vapors, through the lungs. Ingestion can occur via direct contact of toxic materials with foods or by adsorption of vapors onto foods. Precautions may be limited to wearing gloves or may include coveralls, aprons, or other protective apparel. Always wear eye protection. When toxic vapors might be present, sample only in well-ventilated areas or use a respirator or self-contained breathing apparatus. In a laboratory, open sample containers in a fume hood. Never have food near samples or sampling locations; always wash hands thoroughly before handling food.

If flammable organic compounds are present, prohibit smoking near samples, sampling locations, and in the laboratory. Keep sparks, flames, and excessive heat sources away from samples and sampling locations. If flammable compounds are suspected or known to be present and samples are to be refrigerated, use only specially designed explosion-proof refrigerators.

When in doubt as to the level of safety precautions needed, consult a knowledgeable industrial hygienist. Samples with radioactive contaminants require other safety considerations; consult a health physicist.

Label adequately any sample known or suspected to be hazardous because of flammability, corrosivity, toxicity, or radioactivity, so that appropriate precautions can be taken during sample handling, storage, and disposal.

3. Reference


Collection of samples

1. Types Samples

a. Grab or catch samples: A sample collected at a particular time and place can represent only the composition of the source at that time and place. However, when a source is known to be relatively constant in composition over an extended time or over substantial distances in all directions, then the sample may be said to represent a longer time period or a larger volume or both than the specific time and place at which it was collected. In such circumstances, a source may be represented adequately by single grab samples. Examples are some water supplies, some surface waters, but really wastewater streams.

When a source is known to vary with time, grab samples collected at suitable intervals and analyzed separately can document

the extent, frequency, and duration of these variations. Choose sampling intervals on the basis of the expected frequency of changes which may vary from as little as 5 min to as long as 1 h or more. Seasonal variations in natural systems may necessitate sampling over months. When the source composition varies in space rather than time, collect samples from appropriate locations.

The same principles apply to sampling wastewater sludges, sludge banks, and muds, although these matrices are not specifically addressed in this section. Take every possible precaution to obtain a representative sample or one conforming to a sampling program.
b. Composite samples: In most cases, the term "composite sample" refers to a combination of grab samples collected at the same sampling point at different times. Sometimes the term "time-composite" is used to distinguish this type of sample from others. Time-composite samples are most useful for observing average concentrations used in calculating such variables as the loading or the efficiency of a wastewater treatment plant.

As an alternative to the separate analysis of a large number of samples, followed by computation of average and total results, the use of composite samples represents substantial saving in laboratory effort and expense. For these purposes, a composite sample representing a 24-h period is considered standard for most determinations. Under circumstances, however, a composite sample representing one shift or a shorter time period, or a complete cycle of a periodic operation, may be preferable. To evaluate the effects of variable or irregular discharges and operations, collect composite samples representing the period during which such discharges occur.

For determining components or characteristics subject to significant and unavoidable changes on storage, do not use composite samples. Make such determinations on individual samples as soon as possible after collection and preferably at the sampling point. Analyses for all dissolved gases, residual chlorine, soluble sulfide, temperature, and pH are examples of this type of determination. Changes in components such as dissolved oxygen or carbon dioxide, pH, or temperature may produce secondary changes in certain inorganic constituents such as iron, manganese, alkalinity, or hardness. Use time-composite samples only for determining components that can be demonstrated to remain unchanged under the conditions of sample collection, preservation, and storage.

Collect individual portions in a wide-mouth bottle every hour (in some cases every half hour or even every 5 min) and mix at the end of the sampling period or combine in a single bottle as collected. If preservatives are used, add them to the sample bottle initially so that all portions of the composite are preserved as soon as collected. Analysis of individual samples sometimes may be necessary.

It is desirable—and often essential—to combine individual samples in volumes proportional to flow. A final sample volume of 2 to 3 L usually is sufficient for the analysis of sewage, effluents, and wastes.

Automatic sampling devices are available; however, do not use them unless the sample is preserved as described below. Clean sampling devices, including bottles, daily to eliminate biological growths and other deposits.

C. Integrated samples: For certain purposes, the information needed is provided best by analyzing mixtures of grab samples collected from different points simultaneously, or as nearly so as possible. An example of the need for integrated sampling occurs in a river or stream that varies in composition across its width and depth. To evaluate composition or total loading, use a mixture of samples representing various points in the cross-section in proportion to their relative flows. The need for integrated samples also may exist if combined treatment is proposed for several separate wastewater streams, the interaction of which may have a significant effect on treatability or even on composition. Mathematical prediction of the interactions may be inaccurate or impossible and testing a suitable integrated sample may provide more useful information.

Both natural and artificial lakes show variations of composition with both depth and horizontal location. However, there are conditions under which neither total nor average results are especially...
useful, but local variations are more important. In such cases, examine samples separately rather than integrate them.

Preparation of integrated samples usually requires equipment designed to collect a sample from a known depth without contaminating it with overlying water. Knowledge of the volume, movement, and composition of the various parts of the water being sampled usually is required. Collecting integrated samples is a complicated and specialized process that must be described adequately in a sampling plan.

2. Chain-of-Custody procedures

It is essential to insure sample integrity from collection to data reporting. This includes the ability to trace possession and handling of the sample from the time of collection through analysis and final disposition. This process is referred to as chain of custody and is important in demonstrating sample control when litigation is involved. Where litigation is involved, chain-of-custody procedures are useful for routine control of samples.

A sample is considered to be under a persons custody if it is in the individuals physical possession the individuals sight. Secured in a tamper-proof way by that individual, or secured in an area restricted to authorized personnel. The following procedures summarize the major aspects of chain of custody. More detailed discussions are available.

a. sample labels: Use labels to prevent sample misidentification. Gummed paper labels or tags generally are adequate. Include at least the following information: sample number, name of collector, date and time of collection, place of collection, and sample preservative.

Affix tags or self-adhesive labels to sample containers before or at the time of sample collection. Use waterproof ink to provide the label information.

b. sample seals: Use sample seals to detect unauthorized tampering with samples up to the time of analysis. Use self-adhesive paper seals that include at least the following information: sample number (identical with number on sample label), collectors name, and date and time of sampling. Plastic shrink seals also may be used.

Attach seal in such a way that it is necessary to break it to open the sample container. Affix seal to container before sample leaves custody of sampling personnel.

c. Field log book: Record all information pertinent to a field survey or sampling in a bound log book. As a minimum, include the following in the log book: purpose of sampling, location of sampling point, name and address of field contact, producer of material being sampled and address, if different from location, type of sample and method of preservation if applicable. If sample is wastewater, identify process producing waste stream, also provide suspected sample composition, including concentrations, number and volume of sample taken, description of sampling point and sampling method, date and time of collection, collectors sample identification number(s), sample distribution and how transported: references such as maps or photographs of the sampling site, field observations and measurements, and signatures of personnel responsible for observations. Because sampling situations vary widely, it is essential to record sufficient information so that one could reconstruct the sampling event without reliance on the collectors memory. Protect the log book and keep it in a safe place.
d. Chain-of-custody record: Fill out a chain-of-custody record to accompany each sample or group of samples. The record includes the following information: sample number; signature of collector; date, time, and address or collection; sample type; signatures of persons involved in the chain of possession; and inclusive dates of possession.

e. Sample analysis request sheet: The sample analysis request sheet accompanies sample to the laboratory. The collector completes the field portion of such a form that includes most of the pertinent information noted in the log book. The laboratory portion of such a form is to be completed by laboratory personnel and includes: name of person receiving the sample, laboratory sample number, date of sample receipt, and determinations to be performed.

f. Sample delivery to the laboratory: Deliver sample(s) to laboratory as soon as practicable after collection, typically within 2 d. Where shorter sample holding times are required, make special arrangements to insure timely delivery to the laboratory.

Where samples are shipped by a commercial carrier, include the waybill number in the sample custody documentation. Insure that samples are accompanied by a completed chain-of-custody record and a sample analysis sheet. Deliver sample to sample custodian.

g. Receipt and logging of sample: In the laboratory, the sample custodian inspects the condition and seal of the sample, reconciles label information and seal against the chain-of-custody record, assigns a laboratory number, logs sample in the laboratory log book, and stores it in a secured storage room or cabinet until it is assigned to an analyst.

Assignment of sample for analysis: The laboratory supervisor usually assigns the sample for analysis. Once sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.

3. Sampling Methods

a. Manual sampling: Manual sampling involves minimal equipment but may be unduly costly and time-consuming for routine or large-scale sampling programs.

b. Automatic sampling: Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may provide the means for more frequent sampling. And are used increasingly. Be sure that the automatic sampler does not contaminate the sample. For example, plastic components may be incompatible with certain organic compounds that are soluble in the plastic parts. If sample constituents are generally known, contact the manufacturer of an automatic sampler regarding potential incompatibility of plastic components. Manual sampling with a glass container and in accordance with appropriate safety procedures may be best. Manually collect certain samples, such as waters containing oil and grease.

Program an automatic sampler in accordance with sampling needs. Carefully match pump speeds and tubing sizes to the type of sample to be taken.

4. Sample containers

The type of sample container used is of utmost importance.
Containers typically are made of plastic or glass, but one material may be preferred over the other. For example, silica and sodium may be leached from glass but not plastic, and trace levels of metals may sorb onto the walls of glass containers. For samples containing organic compounds, avoid plastic containers except those made of fluorinated polymers such as polytetrafluoroethylene (TFE).

Some volatile organic compounds in samples may dissolve into the walls of plastic containers or may even leach substances from the plastic. Container failure due to breakdown of the plastic is possible. Some organic compounds are compatible with certain plastics (see manufacturer's literature). However, even if compatibility is assured, recognize that the walls of a plastic container can be porous to volatile organic substances. Use glass containers for all organics analyses such as volatile organics, semi-volatile organics. Pesticides. PCBs, and oil and grease. Avoid plastics wherever possible because of potential contamination from phthalate esters contained in the plastic, also can be a problem. Use foil or TFE liners. Serum vials with TFE-lined rubber or plastic septa are useful.

In certain situations it may be necessary to sample a waste in containers that were not specifically prepared for use, or were unsuitable for the particular situation. Thoroughly document deviations from recommended protocols to validate the representativeness of the results obtained. Documentation should include type and source of container, preparation technique, i.e., acid washed with reagent water rinse. For critical situations, the inclusion of a bottle blank may be adequate to demonstrate freedom from interference.

**Sample Preservation**

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, or natural waters, is a practical impossibility. Regardless of the sample nature, complete stability for every constituent never can be achieved. At best, preservation techniques only retard chemical and biological changes that inevitably continue after sample collection.

**1. Sample storage before analysis**

a. Nature of sample changes: Some determinations are more likely than others to be affected by sample storage before analysis. Certain cations are subject to loss by adsorption on, or ion exchange with, the walls of glass containers. These include aluminum, cadmium, chromium, copper, iron, lead, manganese, silver, and zinc, which are best collected in a separate clean bottle and acidified with nitric acid to a pH below 2.0 to minimize precipitation and adsorption on container walls.

Temperature changes quickly: pH may change significantly in a matter of minutes: dissolved gases (oxygen, carbon dioxide) may be lost. Because changes in such basic water quality properties may occur so quickly, determine temperature, pH, and dissolved gases in the field immediately after taking sample.

Changes in the pH-alkalinity-carbon dioxide balance may cause calcium carbonate to precipitate, decreasing the values for calcium carbonate to precipitate, decreasing the values for calcium and total hardness.

Iron and manganese are readily soluble in their higher oxidation states: therefore, these cations may precipitate or they may dissolve from a sediment, depending on the redox potential of the sample. Microbiological activity may be responsible for changes in the nitrate-nitrite-ammonia
content, for decreases in phenol concentration and in BOD, or for reducing sulfate to sulfide. Residual chlorine is reduced to chloride. Sulfite, ferrous iron, iodide, and cyanide may be lost through oxidation. Color odor, and Turbidity may increase or change in quality sodium silica and boron may be leached from the glass container Hexavalent chromium may be reduced to chromic ion.

Biological changes taking place in a sample may change the oxidation state of some constituents. Soluble constituents may be converted to organically bound materials in cell structures or cell lysis may result in release of cellular material into solution. The well-known nitrogen and phosphorus cycles are examples of biological influences on sample composition.

Zero head-space is important in preservation of samples with volatile organic compounds. Avoid loss of volatile by collecting sample in a completely filled container. Achieve this by carefully filling so that top of meniscus is above the top of the bottle rim. Take care not to dilute preservatives by overfilling. Serum vials with septum caps are particularly useful in the a sample portion for analysis can be taken through the cap by using a syringe, although the effect of pressure reduction in the head-space must be considered.

b. Time interval between collection and analysis: In general, the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the analytical results. For certain constituents and physical values, immediate analysis in the field is required. For compositied samples it is common practice to use the time at the end of composite collection as the sample collection time.

It is impossible to state exactly how much elapsed time may be allowed between sample, the analyses; this depends on the character of the sample, the analyses to be made, and the conditions of storage. Changes caused by growth of microorganisms are greatly retarded by keeping the sample in the dark and at a low temperature (<4°C but above freezing). When the interval between sample collection and analysis is long enough to produce changes in either the concentration or the physical state of the constituent to be measured, follow the preservation practices given in Table 1060: 1. Record time elapsed between sampling and analysis, and which preservative, if any, was added.

2. Preservation Techniques

To minimize the potential for volatilization or biodegradation between sampling and analysis, keep samples as cool as possible without freezing. Preferably pack samples in crushed or cubed ice or commercial ice substitutes before shipment. Avoid using dry ice because it will freeze samples and may cause glass containers to break. Dry ice also may effect a pH change in samples.

Keep composite samples cool with ice or a refrigeration system set at 4°C during compositing. Analyze samples as quickly as possible on arrival at the laboratory. If immediate analysis is not possible, storage at 4°C is recommended for most samples.

Use chemical preservatives only when they are shown not to interfere with the analysis being made. When they are used, add them to the sample bottle initially so that all sample portions are preserved as soon as collected. No single method of preservation is entirely satisfactory: choose the preservative with due regard to the determinations to be made. Because a
preservation method for one determination may interfere with another one, samples for multiple
determinations may need to be split and preserved separately. All methods of preservation may be
inadequate when applied to suspended matter. Because formaldehyde affects so many chemical
analyses, do not use it as a preservative.

Methods of preservation are relatively limited and are intended generally to retard
biological action, retard hydrolysis of chemical compounds and complexes, and reduce volatility
of constituents.

Preservation methods are limited to pH control, chemical addition, the use of amber and
opaque bottles, refrigeration, filtration, and freezing. Table 1060:1 lists preservation methods by
constituent.

The foregoing discussion is by no means exhaustive and comprehensive. Clearly it is
impossible to prescribe absolute rules for preventing all possible changes. Additional advice will
be found in the discussions under individual determinations, but to a large degree the dependability
of an analytical determination rests on the experience and good judgment of the person collecting
the sample.

3. Reference

1. WATER POLLUTION CONTROL FEDERATION, 1986. Removal of Hazardous Wastes in

4. Bibliography

1- Standard methods for the examination of water and wastewater, 21th Ed, 2005

2- KEITH, L.H., ed. 1988. principles of Environmental Sampling. ACS Professional Reference
Book, American Chemical Soc. Bo9