# Analytical chemistry

Lecture №9

## Processing the sample for analysis

Theory is when you know everything but nothing works.

Practice is when everything works but no one knows why.

In our lab, theory and practice are combined: nothing works and no one knows why.

# Steps in an analysis

## 1 Define the Problem

Factors

- What is the problem—what needs to be found? Qualitative and/or quantitative?
- What will the information be used for? Who will use it?
- When will it be needed?
- How accurate and precise does it have to be?
- What is the budget?

## 2 Select a Method

Factors

- Sample type
- Size of sample
- Sample preparation needed
- Concentration and range (sensitivity needed)
- Selectivity needed (interferences)
- Accuracy/precision needed
- Tools/instruments available

## 3 Obtain a Representative Sample

Factors

- Sample type/homogeneity/size
- Sampling statistics/errors

## 4 Prepare the Sample for Analysis

Factors

- Solid, liquid, or gas?
- Dissolve?
- Ash or digest?
- Chemical separation or masking of interferences needed?
- Need to concentrate the analyte?
- Need to change (derivatize) the analyte for detection?
- Need to adjust solution conditions (pH, add reagents)?

## 5 Perform Any Necessary Chemical Separations

- Distillation
- Precipitation
- Solvent extraction
- Solid phase extraction
- Chromatography (may include the measurement step)
- Electrophoresis (may include the measurement step)

## 6 Perform the Measurement

Factors

- Calibration
- Validation/controls/blanks

## 7 Calculate the Results and Report

- Statistical analysis (reliability)
- Report results with limitations/accuracy information

A chemical analysis uses only a small fraction of the available sample, the process of sampling is a very important operation.

Knowing how much sample to collect and how to further subdivide the collected sample to obtain a laboratory sample is vital in the analytical process. Statistical methods are used to aid in the selection of a representative sample.

The analytical sample must be processed in a dependable manner that maintains sample integrity without losing sample or introducing contaminants. Many laboratories use the automated sample handling methods.

***Types of Samples and Methods***

*Quantitative methods are traditionally classified as*

- gravimetric methods,
- volumetric methods, and
- instrumental methods.

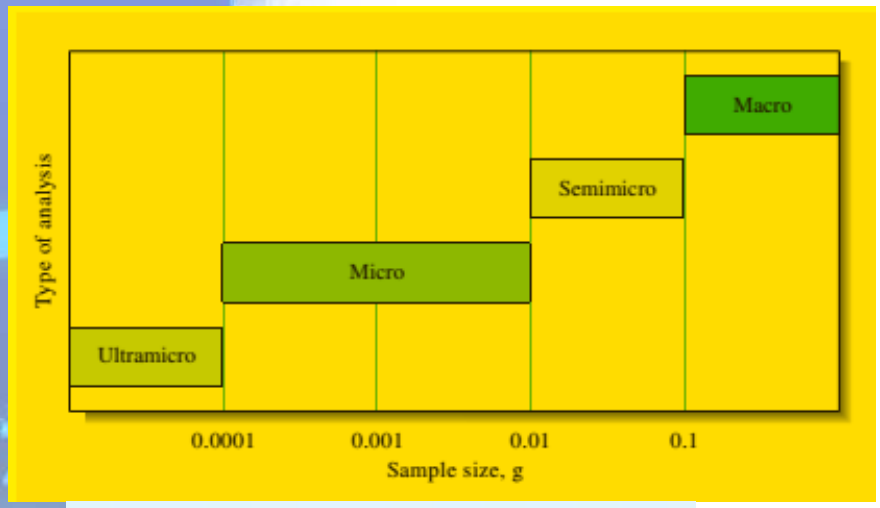
Other methods are based on the ***size of the sample*** and the ***level of the constituents.***

*Sample Size*

Techniques for handling very small samples are quite different from those for treating macro samples.

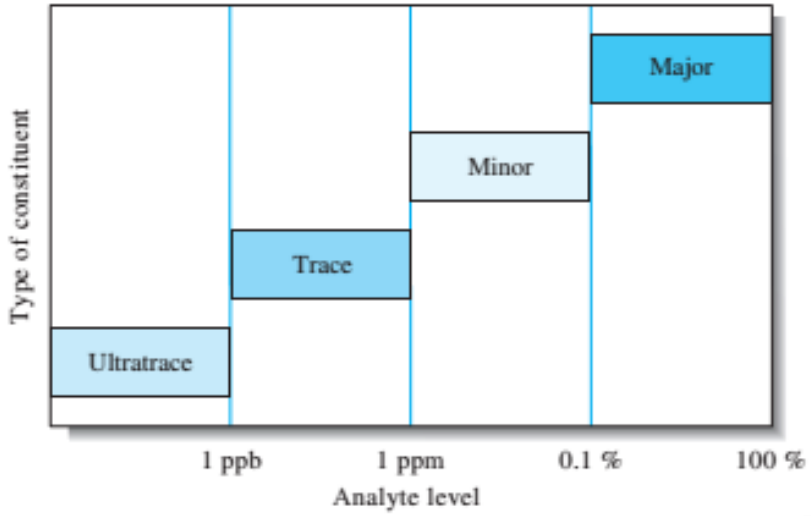
# Sample Size

## Classification of analyses by sample size




| Sample Size | Type of Analysis |
|---|---|
| > 0.1 g | Macro |
| 0.01 to 0.1 g | Semimicro |
| 0.0001 to 0.01 g | Micro |
| $< 10^{-4}$ g | Ultramicro |

## Classification of constituent types by analyte level




| Analyte Level | Type of Constituent |
|---|---|
| 1 to 100% | Major |
| 0.01 (100 ppm) to 1% | Minor |
| 1 ppb to 100 ppm | Trace |
| < 1 ppb | Ultratrace |

In some cases, analytical methods are used to determine *major constituents, which are those present in the range of 1 to 100% by mass.* Species present in the range of 0.01 to 1% are usually termed *minor constituents.* Those present in amounts between 100 ppm (0.01%) and 1 ppb are called *trace constituents.* Components present in amounts lower than 1 ppb are usually considered to be *ultratrace constituents.*

## Real Samples

• The analysis of real samples is complicated by the presence of the sample matrix.
• The matrix can contain species with chemical properties similar to the analyte.
• Matrix components can react with the same reagents as the analyte, or they can cause an instrument response that is not easily distinguished from the analyte. These effects interfere with the determination of the analyte.
• If the interferences are caused by extraneous species in the matrix, they are often called **matrix effects**. Such effects can be induced not only by the sample itself but also by the reagents and solvents used to prepare the samples for the determination.

Samples are analyzed, but constituents or concentrations are determined.

**A chemical analysis is most often performed on only a small fraction of the material of interest, for example a few milliliters of water from a polluted lake. The composition of this fraction must reflect as closely as possible the average composition of the bulk of the material if the results are to be meaningful. The process by which a representative fraction is acquired is termed *sampling*.**

**In sampling, a sample population is reduced in size to an amount of homogeneous material that can be conveniently handled in the laboratory and whose composition is representative of the population.**

## Obtaining the Sample—Is It Solid, Liquid, or Gas?

**Many professional societies have specified definite instructions for sampling given materials [e.g., the American Society for Testing and Materials (www.astm.org), the Association of Official Analytical Chemists International (www.aoac.org), and the American Public Health Association (www.apha.org)].**

WE CAN'T ANALYZE THE WHOLE THING

# Obtaining a representative sample

A *representative sample* is one that truly reflects the composition of the material to be analyzed within the context of a defined analytical problem.

The process by which a *representative fraction is acquired is termed* ***sampling.***

**gross sample** ⇨ **laboratory sample** ⇨ **analysis sample**

The *gross sample consists of* several portions of the material to be tested. The *laboratory sample* is a small portion of this, taken after homogenization. The *analysis sample is that actually* analyzed.

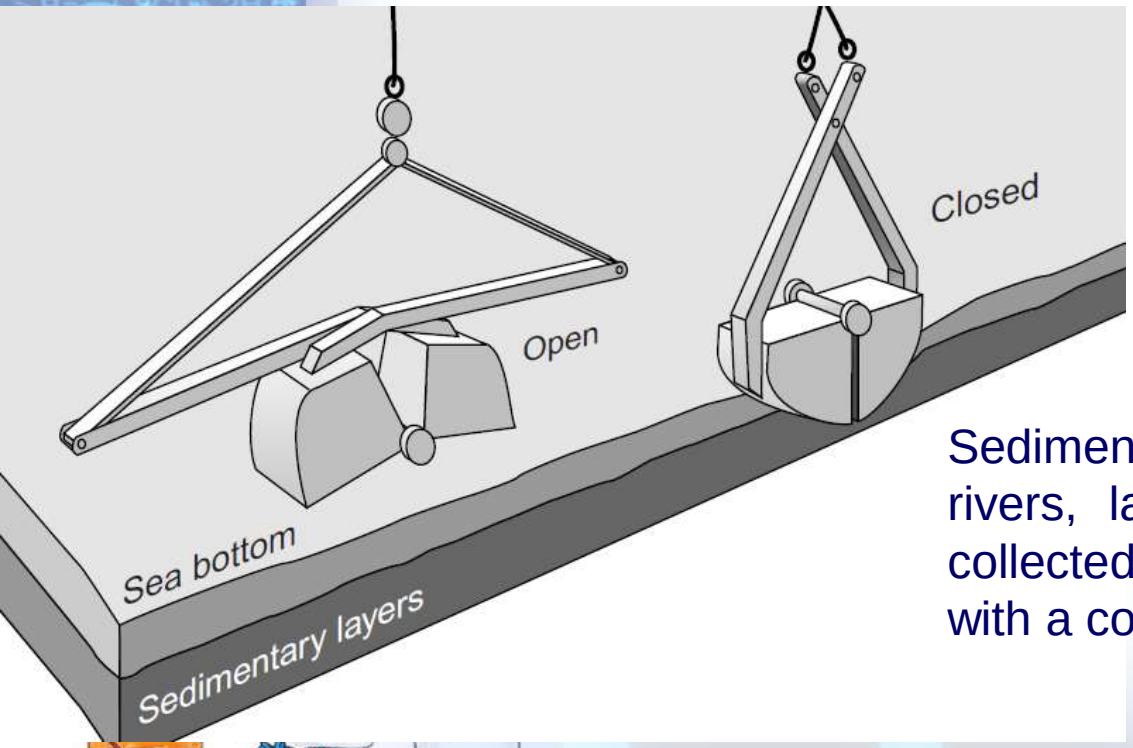
**gross sample**

Homogeneous heterogeneous

# 1. Solids

Typical examples of solid samples include large particulates, such as those found in ores; smaller particulates, such as soils and sediments; tablets, pellets, and capsules used in dispensing pharmaceutical products and animal feeds; sheet materials, such as polymers and rolled metals; and tissue samples from biological specimens.

Solids are usually heterogeneous, and samples must be collected carefully if they are to be representative of the target population. As noted earlier, solids come in a variety of forms, each of which is sampled differently.

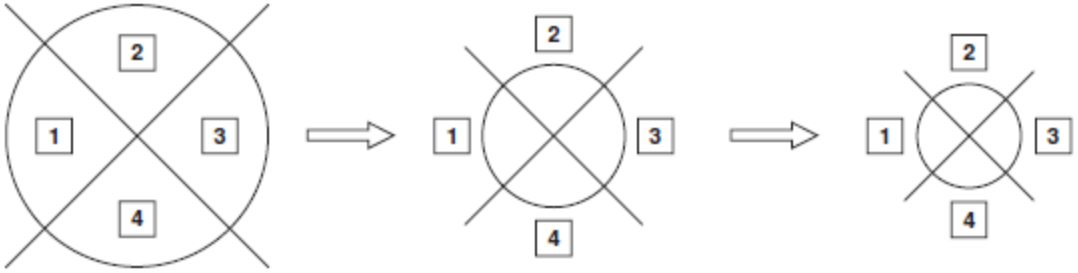



Schematic diagram of grab sampler. When the sampler reaches the sediment, the jaws of the grab sampler are closed, collecting a sample of the sediment.

Sediments from the bottom of streams, rivers, lakes, estuaries, and oceans are collected with a bottom grab sampler or with a corer.

The gross sample is the collection of individual sampling units. It must be representative of the whole in composition and in particle-size distribution.

A repeated **quartering and coning process**




1.  Form cone

2.  Pour on apex

3.  Flatten

4.  Reform cone

5.  Pour on apex

6.  Flatten

7.  Reform cone

8.  Pour on apex

9. Repeat as often as necessary

The larger the particle size, the larger the gross sample should be.

***Liquid samples*** tend to be homogeneous and representative samples are much easier to get. Liquids mix by diffusion only very slowly and must be shaken to obtain a homogeneous mixture. If the material is indeed homogeneous, a simple grab (single random) sample will suffice. For all practical purposes, this method is satisfactory for taking blood samples. The composition of some samples vary on when it is taken. This is the case for urine samples, Therefore 24-h urine sample collections are generally more representative than a single “spot sample”. The timing of sampling of biological fluids is, however, very important. The composition of blood varies considerably before and after meals, and for many analyses a sample is collected after the patient has fasted for a number of hours. Preservatives such as sodium fluoride for glucose preservation and anticoagulants may be added to blood samples when they are collected.

If liquid samples are not homogeneous, and if they are small enough, they can be shaken and sampled immediately. For example, there may be particles in the liquid that have tended to settle. Large bodies of liquids are best sampled after a transfer or, if in a pipe, after passing through a pump when they have undergone thorough mixing. Large stationary liquids can be sampled with a “thief” sampler, which is a device for obtaining aliquots at different levels. It is best to take the sample at different depths at a diagonal, rather than straight down. The separate aliquots of liquids can be analyzed individually and the results combined, or the aliquots can be combined into one gross sample and replicate analyses performed. This latter procedure is probably preferred because the analyst will then have some idea of the precision of the analysis.

**Homogeneous solutions are easily sampled by siphoning, decanting, or by using a pipet or syringe. Unfortunately, few solutions are truly homogeneous. When the material to be sampled is of manageable size, manual shaking is often sufficient to ensure homogeneity. Samples may then be collected with a pipet, a syringe, or a bottle. The majority of solutions, however, cannot be sampled in this manner. To minimize the effect of heterogeneity, the method for collecting the gross sample must be adapted to the material being sampled.**

Sample containers for collecting solutions are made from glass or plastic. Containers made from Kimax or Pyrex brand borosilicate glass have the advantage of being sterilizable, easy to clean, and inert to all solutions except those that are strongly alkaline. The disadvantages of glass containers are cost, weight, and the likelihood of breakage. Plastic containers are made from a variety of polymers, including polyethylene, polypropylene, polycarbonate, polyvinyl chloride, and Teflon (polytetrafluoroethylene). Plastic containers are lightweight, durable, and, except for those manufactured from Teflon, inexpensive. In most cases glass or plastic bottles may be used, although polyethylene bottles are generally preferred because of their lower cost. Glass containers are always used when collecting samples for the analysis of pesticides, oil and grease, and organics because these species often interact with plastic surfaces. Since glass surfaces easily adsorb metal ions, plastic bottles are preferred when collecting samples for the analysis of trace metals.

## Gases

Typical examples of gaseous samples include automobile exhaust, emissions from industrial smokestacks, atmospheric gases, and compressed gases. Also included with gaseous samples are solid aerosol particulates.

The term *air sampling refers to collection of air or trapping of the air for analysis. Usually, it* refers to trapping pollutants in the air by various techniques, identifying them, and measuring their concentrations in the air. Direct air sampling methods include collection of air from the sites in Tedlar bags, canisters, or any appropriate container following repeated evacuation of the containers to flush out the existing air, or collecting the air in a canister under pressure. Also, air may be liquified under low temperature and high pressure and collected in a canister and brought to the laboratory for analysis. The most common sampling technique, however, involves passing a measured volume of air either through a tube packed with adsorbent materials or through a filter cassette or through an impinger solution, depending on the nature of the analyte. Among the adsorbent materials, activated charcoal is most commonly used to trap many types of organic pollutants in air. Other adsorbents include carbon molecular sieves, Tenax (2,6-diphenylene oxide), many types of porous polymers under various trade names, and silica gel. The sample may be collected rapidly (a grab sample) or over a long period of time, using a small orifice to slowly fill the bag. A grab sample is satisfactory in many cases.

After collecting the gross sample there is generally little need for sample preservation or preparation. The chemical composition of a gas sample is usually stable when it is collected using a solid sorbent, a filter, or by cryogenic cooling. When using a solid sorbent, gaseous compounds may be removed before analysis by thermal desorption or by extracting with a suitable solvent. Alternatively, when the sorbent is selective for a single analyte, the increase in the sorbent's mass can be used to determine the analyte's concentration in the sample.

## Preparing a Laboratory Sample

• For heterogeneous solids, the mass of the gross sample may range from hundreds of grams to kilograms or more.

• Reduction of the gross sample to a finely ground and homogeneous laboratory sample, of at most a few hundred grams, is necessary.

• this process involves a cycle of operations that includes crushing and grinding, sieving, mixing, and dividing the sample (often into halves) to reduce its mass.

| Sampling technique | Types of pollutants | Outline of the method |
|---|---|---|
| Filter cassettes | Particulate matter, dust particles, metal powder | A measured volume of air is passed through a membrane filter of appropriate pore size placed on a cassette; the particulate matter and dust collected on the filter is weighed to determine its concentration in air; the powder may be digested in nitric acid alone or in combination with another acid or oxidizing agent; the solution is diluted and analyzed for metals by atomic absorption (AA) or inductively coupled plasma (ICP) spectrophotometry |
| Adsorbent tubes: | | |
| Activated charcoal | Most organic pollutants | Air is passed through the front and back sections of an adsorbent tube; adsorbed organics are desorbed out from activated carbon by desorption with a solvent such as $CS_2$ or by heating under vacuum; desorbed organics are measured by GC, HPLC, or GC/MS |
| Direct sampling: | | |
| At ambient temperature and pressure | Many common gases, e.g., $CH_4$, $CO_2$, $N_2O$ | Air is collected from sampling sites in Tedlar bags or glass flasks after repeated evacuation and flushing of the containers with air from the site; the air is injected directly onto a GC column for separation of pollutants and their determination by thermal conductivity detection (TCD) or mass spectrometry |

# Sample storage

Once removed from its target population, a liquid sample's chemical composition may change as a result of chemical, biological, or physical processes.

Without preservation, many solid samples are subject to changes in chemical composition due to the loss of volatile material, biodegradation, and chemical reactivity (particularly redox reactions).

The following effects during storage should be considered:

- increases in temperature leading to the loss of volatile analytes, thermal or biological degradation, or increased chemical reactivity;
- decreases in temperature that lead to the formation of deposits or the precipitation of analytes with low solubilities;
- changes in humidity that affect the moisture content of hygroscopic solids and liquids or induce hydrolysis reactions;
- UV radiation, particularly from direct sunlight, that induces photochemical reactions, photodecomposition or polymerization;
- air-induced oxidation;
- physical separation of the sample into layers of different density or changes in crystallinity.

A particular problem associated with samples having very low (**trace and ultra-trace) levels of analytes in solution is the possibility of losses by adsorption** onto the walls of the container or contamination by substances being leached from the container by the sample solvent.

**Sample preservation methods and maximum holding times for several analytes of importance in the analysis of water and wastewater.**

| Parameter | Preservation | Maximum Holding Time |
|---|---|---|
| ammonia | cool to 4 °C; $H_2SO_4$ to pH < 2 | 28 days |
| chloride | none required | 28 days |
| metals—Cr(VI) | cool to 4 °C | 24 h |
| metals—Hg | $HNO_3$ to pH < 2 | 28 days |
| metals—all others | $HNO_3$ to pH < 2 | 6 months |
| nitrate | none required | 48 h |
| organochlorine pesticides | 1 mL 10 mg/mL $HgCl_2$; or addition of extracting solvent | 7 days without extraction<br>40 days with extraction |
| pH | none required | analyze immediately |

After preserving, samples may be safely stored for later analysis. The maximum holding time between preservation and analysis depends on the analyte's stability and the effectiveness of sample preservation.

## Sample pretreatment

- drying at 100°C to 120°C to eliminate the effect of a **variable moisture content;**
- weighing before and after drying enables the water content to be calculated or it can be established by thermogravimetric analysis;
- separating the analytes into groups with common characteristics by distillation, filtration, centrifugation, solvent or solid phase extraction;
- removing or reducing the level of **matrix components that are known to cause interference with measurements of the analytes;**
- concentrating the analytes if they are below the concentration range of the analytical method to be used by evaporation, distillation, co-precipitation, ion exchange, solvent or solid phase extraction or electrolysis.

• Solid samples, or at least the analytes in a solid sample, must be brought into solution.

# SAMPLE DISSOLUTION

| Solution (≈ %w/w) | Uses and Properties |
|---|---|
| HCl (37%) | • dissolves metals more easily reduced than $H_2$ ($E^o < 0$)<br>• dissolves insoluble carbonates, sulfides, phosphates, fluorides, sulfates, and many oxides |
| $HNO_3$ (70%) | • strong oxidizing agent<br>• dissolves most common metals except Al and Cr<br>• decomposes organics and biological samples (wet ashing) |
| $H_2SO_4$ (98%) | • dissolves many metals and alloys<br>• decomposes organics by oxidation and dehydration |
| HF (50%) | • dissolves silicates forming volatile $SiF_4$ |
| $HClO_4$ (70%) | • hot, concentrated solutions are strong oxidizing agents<br>• dissolves many metals and alloys<br>• decomposes organics (*reactions with organics are often explosive, use only in specially equipped hoods with a blast shield and after prior decomposition with $HNO_3$*) |
| $HCl:HNO_3$ (3:1 v/v) | • also known as aqua regia<br>• dissolves Au and Pt |
| NaOH | • dissolves Al and amphoteric oxides of Sn, Pb, Zn, and Cr |

| Flux | Melting Temperature (°C) | Crucible | Typical Samples |
|---|---|---|---|
| $Na_2CO_3$ | 851 | Pt | silicates, oxides, phosphates, sulfides |
| $Li_2B_4O_7$ | 930 | Pt, graphite | aluminosilicates, carbonates |
| $LiBO_2$ | 845 | | |
| NaOH | 318 | Au, Ag | silicates, silicon carbide |
| KOH | 380 | | |
| $Na_2O_2$ | — | Ni | silicates, chromium steel, Pt alloys |
| $K_2S_2O_7$ | 300 | Pt, porcelain | oxides |
| $B_2O_3$ | 577 | Pt | silicates, oxides |

# SAMPLE DISSOLUTION

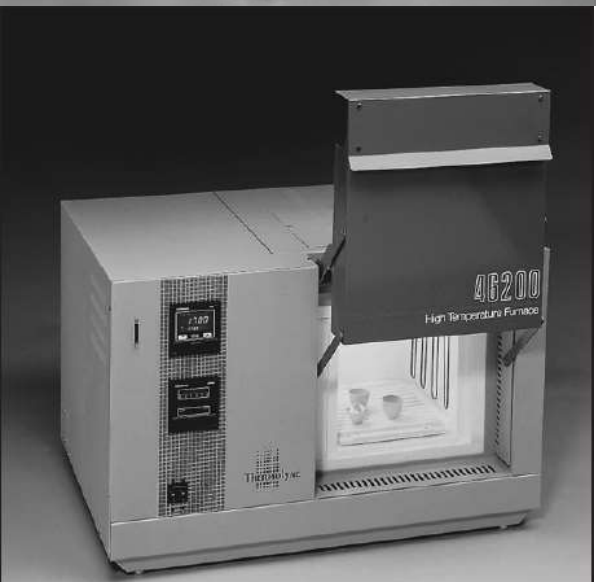
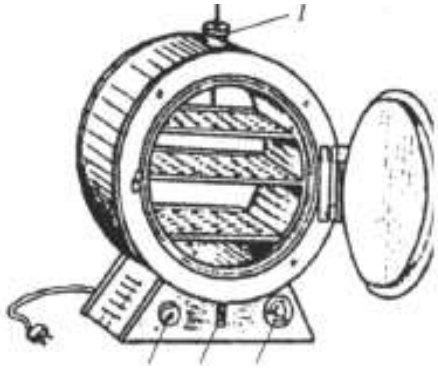



*Table 1. Some methods for sample decomposition and dissolution*

| Method of attack | Type of sample |
|---|---|
| Heated with concentrated mineral acids (HCl, $HNO_3$, aqua regia) or strong alkali, including microwave digestion | Geological, metallurgical |
| Fusion with flux ($Na_2O_2$, $Na_2CO_3$, $LiBO_2$, $KHSO_4$, KOH) | Geological, refractory materials |
| Heated with HF and $H_2SO_4$ or $HClO_4$ | Silicates where $SiO_2$ is not the analyte |
| Acid leaching with $HNO_3$ | Soils and sediments |
| Dry oxidation by heating in a furnace or wet oxidation by boiling with concentrated $H_2SO_4$ and $HNO_3$ or $HClO_4$ | Organic materials with inorganic analytes |



Muffle furnace

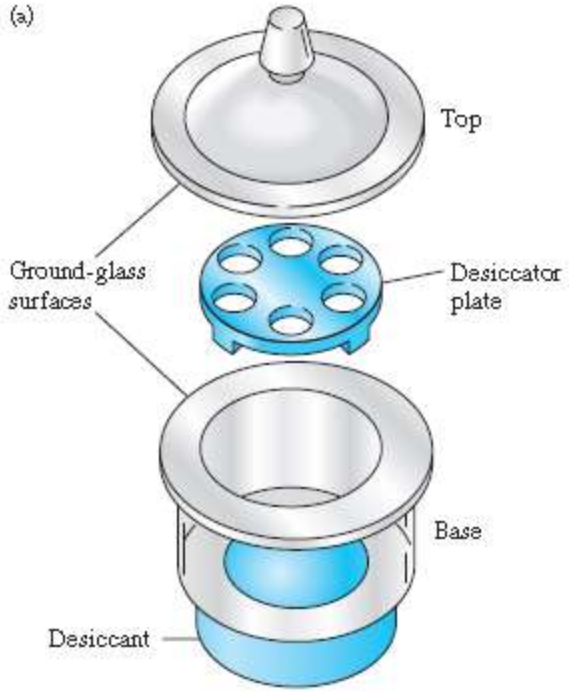
organic materials may be decomposed by dry ashing. In this method the sample is placed in a suitable crucible and heated over a flame or in a furnace. Any carbon present in the sample is oxidized to $CO_2$, and hydrogen, sulfur, and nitrogen are removed as $H_2O$, $SO_2$ and $N_2$. These gases can be trapped and weighed to determine their content in the organic material. Often the goal of dry ashing is the removal of organic material, leaving behind an inorganic residue, or ash, that can be further analyzed.

# DRYING THE SAMPLE

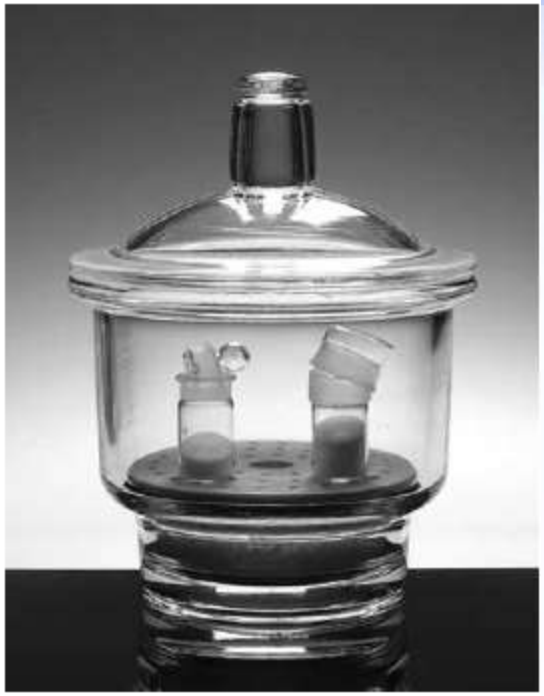
| Drying agent | Most useful for |
|---|---|
| $Al_2O_3$ | Hydrocarbons |
| $Ba(ClO_4)_2$ | Inert-gas streams |
| BaO | Basic gases, hydrocarbons, aldehydes, alcohols |
| $CaC_2$ | Ethers |
| $CaCl_2$ | Inert organics |
| $CaH_2$ | Hydrocarbons, ethers, amines, esters, higher alcohols |
| CaO | Ethers, esters, alcohols, amines |
| $CaSO_4$ | Most organic substances |
| KOH | Amines |
| $Mg(ClO_4)_2$ | Gas streams |
| $MgSO_4$ | Most organic compounds |
| Molecular sieve 4X | Molecules with effective diameter >4 Å |
| $P_2O_5$ | Gas streams; not suitable for alcohols, amines, or ketones |
| Silica gel | Most organic amines |
| $H_2SO_4$ | Air and inert-gas streams |


Desiccator

(a)


Top
Ground-glass surfaces
Desiccator plate
Base
Desiccant

(b)



An analysis requires a sample, and how we acquire the sample is critical. To be useful, the samples we collect must accurately represent their target population. Just as important, our sampling plan must provide a sufficient number of samples of appropriate size so that the variance due to sampling does not limit the precision of our analysis. A complete sampling plan requires several considerations, including the type of sampling; whether to collect grab samples, composite samples, or in situ samples; whether the population is homogeneous or heterogeneous; the appropriate size for each sample; and, the number of samples to collect.

Removing a sample from its population may induce a change in its composition due to a chemical or physical process. For this reason, samples are collected in inert containers and are often preserved at the time of collection.

When the analytical method's selectivity is insufficient, it may be necessary to separate the analyte from potential interferents. Such separations can take advantage of physical properties, such as size, mass or density, or chemical properties. Important examples of chemical separations include masking, distillation, and extractions.

## Standardization and calibration

- Calibration determines the relationship between the analytical response and the analyte concentration, which is usually determined by the use of ***chemical standards prepared from purified reagents.***
- To reduce interferences from other constituents in the sample matrix, called concomitants, standards are added to the analyte solution (internal standard methods or standard addition methods) or matrix matching or modification is done.
- Almost all analytical methods require calibration with chemical standards.
- Gravimetric methods and some coulometric methods are absolute methods that do not rely on calibration with chemical standards.

### Comparison with Standards

Two types of comparison methods are:

- direct comparison techniques
- titration procedures.

### *Direct Comparison*

- Some analytical procedures involve comparing a property of the analyte with standards such that the property being tested matches or nearly matches that of the standard. This is called null comparison or isomation methods.
-Some modern instruments use a variation of this procedure to determine if an analyte concentration exceeds or is less than some threshold level. Such a comparator can be used to determine whether the threshold has been exceeded.

***External Standard Calibration***

- A series of standard solutions is prepared separately from the sample.
- The standards are used to establish the instrument calibration function, which is obtained from analysis of the instrument response as a function of the known analyte concentration.
- The calibration function can be obtained graphically or in mathematical form.
- Generally, a plot of instrument response versus known analyte concentrations is used to produce a calibration curve, sometimes called a working curve.

**Minimizing Errors in Analytical Procedures**

The overall accuracy and precision of an analysis is not limited to the measurement step and might instead be limited by factors such as sampling, sample preparation, and calibration.

***Separations***

Sample cleanup by separation methods is an important way to minimize errors from possible interferences in the sample matrix.

Techniques such as filtration, precipitation, dialysis, solvent extraction, volatilization, ion exchange, and chromatography can be used.

In most cases, separations may be the only way to eliminate an interfering specimen.

***Saturation, Matrix Modification, and Masking***

* The saturation method involves adding the interfering species to all the samples, standards, and blanks so that the interference effect becomes independent of the original concentration of the interfering species in the sample.
* A matrix modifier is a species, not itself an interfering species, added to samples, standards, and blanks in sufficient amounts to make the analytical response independent of the concentration of the interfering species.
•Sometimes, a masking agent is added that reacts selectively with the interfering species to form a complex that does not interfere.

***Dilution and Matrix Matching***

* The dilution method can sometimes be used if the interfering species produces no significant effect below a certain concentration level.
* The matrix-matching method attempts to duplicate the sample matrix by adding the major matrix constituents to the standard and blank solutions.
* Errors in procedures can be minimized by saturating with interfering species, by adding matrix modifiers or masking agents, by diluting the sample, or by matching the matrix of the sample

## Internal Standard Methods

* An internal standard is a reference species, chemically and physically similar to the analyte, that is added to samples, standards, and blanks.
* The ratio of the response of the analyte to that of the internal standard is plotted versus the concentration of analyte.
* In the internal standard method, a known amount of a reference species is added to all the samples, standards, and blanks.
* The response signal is then not the analyte signal itself but the ratio of the analyte signal to the reference species signal.

## *Validation*

Validation determines the suitability of an analysis for providing the sought-for information and can apply to samples, to methodologies, and to data.

Validation is often done by the analyst, but it can also be done by supervisory personnel. There are several different ways to validate analytical methods. The most common methods include: analysis of standard reference materials when available, analysis by a different analytical method, analysis of “spiked” samples, and analysis of synthetic samples approximating the chemical composition of the test samples.

Individual analysts and laboratories often must periodically demonstrate the validity of the methods and techniques used.

Data validation is the final step before release of the results. This process starts with validating the samples and methods used. Then, the data are reported with statistically valid limits of uncertainty after a thorough check has been made to eliminate blunders in sampling and sample handling, mistakes in performing the analysis, errors in identifying samples, and mistakes in the calculations used.

## *Reporting Analytical Results*

Analytical results should be reported as the mean value and the standard deviation.