OBTAINING A REPRESENTATIVE SAMPLE—WE CAN'T ANALYZE THE WHOLE THING

A chemical analysis is usually performed on only a small portion of the material to be characterized. If the amount of material is very small and it is not needed for future use, then the entire sample may be used for analysis. The gunshot residue on a hand may be an example. More often, though, the characterized material is of value and must be altered as little as possible in sample collection. For example, sampling of a Rembrandt painting for authenticity would need to be done with utmost care for sample quantity, so as not to deface the artwork.

The material to be sampled may be solid, liquid, or gas. It may be homogeneous or heterogeneous in composition. In the former case, a simple "grab sample" taken at random will suffice for the analysis. In the latter, we may be interested in the variation throughout the sample, in which case several individual samples will be required. If the gross composition is needed, then special sampling techniques will be required to obtain a representative sample. For example, in analyzing for the average protein content of a shipment of grain, a small sample may be taken from each bag, or tenth bag for a large shipment, and combined to obtain a **gross sample**. Sampling is best done when the material is being moved, if it is large, in order to gain access. The larger the particle size, the larger should be the gross sample. The gross sample must be reduced in size to obtain a **laboratory sample** of several grams, from which a few grams to milligrams will be taken to be analyzed (**analysis sample**). The size reduction may require taking portions (e.g., two quarters) and mixing, in several steps, as well as crushing and sieving to obtain a uniform powder for analysis. Methods of sampling solids, liquids, and gases are discussed in Chapter 2. If one is interested in spatial structure, then homogenization must not be carried out, but spatially resolved sampling must be done.

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. See Chapter 2 for methods of sampling.

In the case of biological fluids, the conditions under which the sample is collected can be important, for example, whether a patient has just eaten. 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. Persons who have their blood checked for cholesterol levels are asked to fast for up to twelve hours prior to sampling. Preservatives such as sodium fluoride for glucose preservation and anticoagulants for blood samples may be added when samples are collected; these may affect a particular analysis.

Blood samples may be analyzed as whole blood, or they may be separated to yield plasma or serum according to the requirements of the particular analysis. Most commonly, the concentration of the substance external to the red cells (the extracellular concentration) will be a significant indication of physiological condition, and so serum or plasma is taken for analysis.

If whole blood is collected and allowed to stand for several minutes, the soluble protein **fibrinogen** will be converted by a complex series of chemical reactions (involving calcium ion) into the insoluble protein **fibrin**, which forms the basis of a gel, or **clot**. The red and white cells of the blood become caught in the meshes of the fibrin network and contribute to the clot, although they are not necessary for the clotting process. After the clot forms, it shrinks and squeezes out a straw-colored fluid, **serum**, which does not clot but remains fluid indefinitely. The clotting process can be prevented by adding a small amount of an **anticoagulant**, such as heparin or a citrate salt (i.e., a calcium complexor). Blood collection vials are often color-coded to provide a clear indication of the additives they contain. An aliquot of the unclotted whole blood can be taken for analysis, or the red cells can be centrifuged to the bottom, and the light pinkish-colored

Serum is the fluid separated from clotted blood. *Plasma* is the fluid separated from unclotted blood. It is the same as serum, but contains fibrinogen, the clotting protein.

plasma remaining can be analyzed. Plasma and serum are essentially identical in chemical composition, the chief difference being that fibrinogen has been removed from the latter.

Details of sampling other materials are available in reference books on specific areas of analysis. See the references at the end of the chapter for some citations.

Care must be taken not to alter or contaminate the sample.

Certain precautions should be taken in **handling and storing samples** to prevent or minimize contamination, loss, decomposition, or matrix change. In general, one must prevent contamination or alteration of the sample by (1) the container, (2) the atmosphere, (3) heat/temperature, or (4) light. Also, a chain of custody should be established and will certainly be required for any analysis that may be involved in legal proceedings. In the O.J. Simpson case, there were television news clips of people handling samples, purportedly without proper custody, placing them in the hot trunk of a car, for example. While this may not have affected the actual analyses and correctness of samples analyzed, it provided arguments for the defense to discredit analyses.

The sample may have to be protected from the atmosphere or from light. It may be an alkaline substance, for example, which will react with carbon dioxide in the air. Blood samples to be analyzed for CO_2 must be protected from the atmosphere.

The stability of the sample must be considered. To minimize degradation of glucose, for example, a preservative such as sodium fluoride is added to blood samples. The preservative must not, of course, interfere in the analysis. Proteins and enzymes tend to denature on standing and should be analyzed without delay. Trace constituents may be lost during storage by adsorption onto the container walls.

Urine samples are unstable, and calcium phosphate precipitates out, entrapping metal ions or other substances of interest. Precipitation can be prevented by keeping the urine acidic (pH 4.5), usually by adding 1 or 2 mL glacial acetic acid per 100-mL sample and stored under refrigeration. Urine, as well as whole blood, serum, plasma, and tissue samples, can also be frozen for prolonged storage. Deproteinized blood samples are more stable than untreated ones.

Corrosive gas samples will often react with the container. Sulfur dioxide, for example, is troublesome. In automobile exhaust, SO_2 is also lost by dissolving in condensed water vapor from the exhaust. In such cases, it is best to analyze the gas by an *in situ* analyzer that operates at a temperature in which condensation does not occur.

PREPARING THE SAMPLE FOR ANALYSIS—IT PROBABLY NEEDS TO BE ALTERED

The first thing you must do is measure the size of sample to be analyzed.

The first step in analyzing a sample is to measure the amount being analyzed (e.g., volume or weight of sample). This will be needed to calculate the percent composition from the amount of analyte found. The analytical sample size must be measured to the degree of precision and accuracy required for the analysis. An analytical balance sensitive to 0.1 mg is usually used for weight measurements and balances that can weigh down to 0.01 mg are becoming increasingly common. Solid samples are often analyzed on a dry basis and must be dried in an oven at 110 to 120°C for 1 to 2 h and cooled in a dessicator before weighing, if the sample is stable at the drying temperatures. Some samples may require higher temperatures and longer heating time (e.g., overnight) because of their great affinity for moisture. The amount of sample taken will depend on the concentration of the analyte and how much is needed for isolation and measurement. Determination of a major constituent may require only 100 mg of sample, while a trace constituent may require several grams. Usually **replicate samples** are taken for analysis, in order to obtain statistical data on the precision of the analysis and thus provide more reliable results.

Analyses may be nondestructive in nature, for example, in the measurement of lead in paint by X-ray fluorescence in which the sample is bombarded with an X-ray beam and the characteristic reemitted X-radiation is measured. More often, the sample must be in solution form for measurement, and solids must be dissolved. Inorganic materials may be dissolved in various acids, redox, or complexing media. Acid-resistant material may require fusion with an acidic or basic flux in the molten state to render it soluble in dilute acid or water. Fusion with sodium carbonate, for example, forms acid-soluble carbonates.

Solid samples usually must be put into solution.

Organic materials that are to be analyzed for inorganic constituents, for example, trace metals, may be destroyed by **dry ashing**. The sample is slowly combusted in a furnace at 400 to 700°C, leaving behind an inorganic residue that is soluble in dilute acid. Alternately, the organic matter may be destroyed by **wet digestion** by heating with oxidizing acids. A mixture of nitric and sulfuric acids is common. Perchloric acid digestion is used for complete oxidative digestion; this is a last resort as special extraction or fume hoods are required due to potential explosion hazards. Biological fluids may sometimes be analyzed directly. Often, however, proteins interfere and must be removed. Dry ashing and wet digestion accomplish such removal. Or proteins may be precipitated with various reagents and filtered or centrifuged away, to give a **protein-free filtrate** (PFF).

Ashing is the burning of organic matter. *Digestion* is the wet oxidation of organic matter.

If the analyte is organic in nature, these oxidizing methods cannot be used. Rather, the analyte may be extracted away from the sample or dialyzed, or the sample dissolved in an appropriate solvent. It may be possible to measure the analyte nondestructively. An example is the direct determination of protein in feeds by near-infrared spectrometry.

Once a sample is in solution, the solution conditions must be adjusted for the next stage of the analysis (separation or measurement step). For example, the pH may have to be adjusted, or a reagent added to react with and "mask" interference from other constituents. The analyte may have to be reacted with a reagent to convert it to a form suitable for measurement or separation. For example, a colored product may be formed that will be measured by spectrometry. Or the analyte will be converted to a form that can be volatilized for measurement by gas chromatography. The gravimetric analysis of iron as Fe_2O_3 requires that all the iron be present as iron(III), its usual form. A volumetric determination by reaction with dichromate ion, on the other hand, requires that all the iron be converted to iron(II) before reaction, and the reduction step will have to be included in the sample preparation.

The pH of the sample solution will usually have to be adjusted.

The solvents and reagents used for dissolution and preparation of the solution should be of high purity (reagent grade). Even so, they may contain trace impurities of the analyte. Hence, it is important to prepare and analyze replicate **blanks**, particularly for trace analyses. A blank theoretically consists of all chemicals in the unknown and used in an analysis in the same amounts (including water), run through the entire analytical procedure. The blank result is subtracted from the analytical sample result to arrive at a net analyte concentration in the sample solution. If the blank is appreciable, it may invalidate the analysis. Oftentimes, it is impossible to make a perfect blank for an analysis.

Always run a blank!

PERFORMING NECESSARY CHEMICAL SEPARATIONS

In order to eliminate interferences, to provide suitable selectivity in the measurement, or to preconcentrate the analyte for more sensitive or accurate measurement, the analyst must often perform one or more separation steps. It is preferable to separate the analyte away from the sample matrix, in order to minimize losses of the analyte. Separation steps may include precipitation, extraction into an immiscible solvent, chromatography, dialysis, and distillation.

PERFORMING THE MEASUREMENT—YOU DECIDE THE METHOD

The method employed for the actual quantitative measurement of the analyte will depend on a number of factors, not the least important being the amount of analyte present and the accuracy and precision required. Many available techniques possess varying degrees of selectivity, sensitivity, accuracy and precision, cost, and rapidity. Analytical chemistry research often deals with the optimization of one or more of these parameters, as they relate to a particular analysis or analysis technique. **Gravimetric analysis** usually involves the selective separation of the analyte by precipitation, followed by the very nonselective measurement of mass (of the precipitate). In **volumetric**, or **titrimetric**, **analysis**, the analyte reacts with a measured volume of reagent of known concentration, in a process called **titration**. A change in some physical or chemical property signals the completion of the reaction. Gravimetric and volumetric analyses can provide results accurate and precise to a few parts per thousand (tenth of 1 percent) or better. However, they require relatively large (millimole or milligram) quantities of analyte and are only suited for the measurement of major constituents, although microtitrations may be performed. Volumetric analysis is more rapid than gravimetric analysis and is therefore preferred when applicable.

Instruments are more selective and sensitive than volumetric and gravimetric methods. But they may be less precise.

Instrumental techniques are used for many analyses and constitute the discipline of **instrumental analysis**. They are based on the measurement of a physical property of the sample, for example, an electrical property or the absorption of electromagnetic radiation. Examples are spectrophotometry (ultraviolet, visible, or infrared), fluorimetry, atomic spectroscopy (absorption, emission), mass spectrometry, nuclear magnetic resonance spectrometry (NMR), X-ray spectroscopy (absorption, fluorescence), electroanalytical chemistry (potentiometric, voltammetric, electrolytic), chromatography (gas, liquid), and radiochemistry. Instrumental techniques are generally more sensitive and selective than the classical techniques but are less precise, on the order of 1 to 5% or so. These techniques are usually much more expensive, especially in terms of initial capital investment. But depending on the numbers of analyses, they may be less expensive when one factors in personnel costs. They are usually more rapid, may be automated, and may be capable of measuring more than one analyte at a time. Chromatography techniques are particularly powerful for analyzing complex mixtures. They integrate the separation and measurement steps. Constituents are separated as they are pushed through (eluted from) a column of appropriate material that interacts with the analytes to varying degrees, and these are sensed with an appropriate detector as they emerge from the column, to give a transient peak signal, proportional to the amount of each.

Table 1.1 compares various analytical methods to be described in this text with respect to sensitivity, precision, selectivity, speed, and cost. The numbers given may be exceeded in specific applications, and the methods may be applied to other uses, but these are representative of typical applications. The lower concentrations determined by titrimetry require the use of an instrumental technique for measuring the completion of the titration. The selection of a technique, when more than one is applicable, will depend, of course, on the availability of equipment, and personal experience, and preference of the analyst. As examples, you might use spectrophotometry to determine the concentration of nitrate in river water at the sub parts-per-million level, by first reducing to nitrite and then using a diazotization reaction to produce a color. Fluoride in toothpaste may be determined potentiometrically using a fluoride ion-selective electrode. A complex mixture of hydrocarbons in gasoline can be separated using gas chromatography and determined by flame ionization detection. Glucose in blood can be determined kinetically by the rate of the enzymatic reaction between glucose and oxygen, catalyzed by the enzyme glucose oxidase, with measurement of the rate of oxygen depletion or the rate of hydrogen peroxide production. The purity of a silver