

5. **The sample preparation should include, if necessary, diluting or concentrating the analytes to bring their concentrations into the best range for the assay method used.** It is essential that you recognize that every assay method has an optimum concentration range. The analysis will not work if the concentration (solid, liquid, or gas) is either too high or too low. Few methods can be used to determine content with the same precision over more than about three orders of magnitude in concentration—where the high end is 1,000 times the concentration of the low end. Here again the sample preparation and the assay method are closely linked.

These five requirements often involve trade-offs, for example, a treatment that might tend to produce a better recovery might also add interferents.

In addition, there are two more themes in contemporary analysis that also influence the sample preparation. The first is the speed of contemporary instruments: They are becoming faster all the time. If, in the space of one minute, a complete assay can be carried out, the data posted to the **laboratory information management system (LIMS)** through a computerized interface, and the next sample loaded from an automatic loader of some type, then in order to utilize the instrument fully, someone has to prepare 60 samples per hour. Not long ago, when only wet chemistry assay methods were available, and all assays were done by hand—such as by manual titration—an efficient person might do ten to fifteen assays in an hour. If more than one analyte was determined in separate titrations, perhaps one sample an hour would need to be prepared. In other words, sample **throughput** has been increased by two orders of magnitude.

A second theme is the rapid increase in costs associated with purchase and disposal of solvents (and chemicals in general), not to mention the desire for less waste at all levels of chemical manipulation. The response to this trend has been to use smaller amounts of both organic solvents and acids or to find substitutes for the entire process. As an example, it has been common practice to extract organic compounds from water samples by shaking the water in a separatory funnel with an organic solvent such as ether and then using the ether phase for further processing. (For example, the ether could be evaporated to concentrate the extract.) Such ether extractions are now commonly replaced by passing the water sample through a tube filled with a solid that has a nonpolar surface. The organics adsorb on the nonpolar surface—they are **stripped** out of the sample. A small amount of organic solvent such as methanol can then be used to recover the adsorbed analyte, or the solid can be heated and the analyte vaporized from the surface and transferred directly to the assay instrument.

Because of these factors, as well as the cost of sample preparation, a great deal of research is being done to develop better methods for sample preparation. The following sections, 4.2–4.5, explain how requirements 1–5 above are fulfilled in chemical analysis.

4.2 Maximize Recovery

Central to the idea of recovery is that an analyte exists first in some matrix from which it is to be recovered. Within the requirements of the assay, in order to choose the preparation method(s) you must understand

1. The chemistry of the analytes,
2. The chemistry of the matrix, and
3. The chemistry of the interactions between the matrix and the analytes.

The chemistry to be understood here tends to be relatively complicated. The desired outcome is to recover all of the analytes in the form desired while separating them from the matrix components that cause interference. We would like to be able to state some rules about this process, but, given the range of possibilities noted in Table 4.1, generalizations are almost impossible.

An analyst's dream might be to have a solid matrix that dissolves completely in water; an assay method that requires an aqueous solution of the (soluble) analyte; and no interference from molecules or atoms of the dissolved matrix. Then, only a simple dissolution of a known amount of the sample would be required for the preparation, and there would be no problem with loss of analyte. (Occasionally you too may find yourself in such a situation, but don't hold your breath!)

Loss of Analyte

How, then, can analyte be lost? One obvious way is through adsorption, absorption, or penetration of the analyte into its storage container between the time a sample is collected and the time it is run. For instance, metal ions can *adsorb* on the surface of glass. Therefore, samples that are to be analyzed for trace metal levels require clean plastic containers (often polyethylene). However, organic materials can be *absorbed* into plastic and lost. Eventually the material can penetrate through the container wall to the outside, and clean glass containers would be better in that case. However, even for glass, the type of seal has to be considered so the analytes do not escape through the caps. Sample losses caused in these ways are unlikely to be a problem when an analysis is done on-line or *in situ*.

Analytes are also lost when they decompose, such as by air oxidation of organics; this means either oxygen has to be excluded or antioxidants added. Others materials may decompose with light, and so must be kept in the dark. A common problem with aqueous samples and biologicals is that bacteria can grow and decompose parts of samples, so bactericides or

The case study on the next page about amyloid proteins shows some other ways to avoid degradation.

Table 4.1 Analyte/Matrix Combinations

Analyte	Matrix	Examples
Solid	Solid	Molybdenum in steel; polymer in polymer; fat in fish
	Liquid	Suspended particulates in lake water; ketchup
	Gas	Smoke
Liquid	Solid	Oil in sandstone
	Liquid	Volatile organic compounds in water; mayonnaise
	Gas	Exhaled breath
Gas	Solid	Argon in basalt
	Liquid	Acid rain
	Gas	Ozone in atmosphere; exhaled breath

bacteriostats may be added to keep the sample's integrity. In addition, biological tissues contain numerous enzymes that can degrade a wide range of analytes.

To review: other than accidents and carelessness some causes for the losses are adsorption of analyte onto container surfaces, evaporation of volatile samples, losses in unanticipated chemical side reactions, and leaks in transfer systems for gases or liquids.

All these potential losses prevent **quantitative material transfer**. The quantitative part is usually expressed as a *percent recovery*. This value is simply a comparison of the amount of analyte found from the assay with the amount that is, in fact, present.

$$\% \text{ recovery} = 100 \times \frac{\text{concentration of analyte from assay}}{\text{concentration of analyte in sample}} \quad (4-1a)$$

or

$$\% \text{ recovery} = 100 \times \frac{\text{weight of analyte from assay}}{\text{weight of analyte in sample}} \quad (4-1b)$$

An example and discussion of how percent recoveries are determined will be given in Section 4.7.

Correction for Loss of Analyte

A relatively low recovery need not condemn a method outright. If the recovery is constant, we can compensate for it just as we would for any constant determinate error. For example, assume that a sample preparation method yields an 87% recovery of the analyte every time. It would only be necessary, then, to multiply the result by 1.149 to correct for the effect ($1.149 \times 0.87 = 1.00$).

Nevertheless, methods with inherently low recoveries are usually less precise. A simple comparison illustrates this point. Assume that a sample preparation procedure generally produces 99% recoveries. From the opposite viewpoint, there is a 1% loss. If the loss doubles or halves, the recovery varies from 98% to 99.5%, a relative range of about 1.5%.

In comparison, consider a sample preparation method with poor recoveries—say, 60%. With this method, 40% of the analyte is lost. Again, if the loss doubles or halves randomly, the recoveries will vary from 40% to 80%, a relative range of about 66%. Thus there is an inherently larger possible imprecision associated with low recoveries. Conclusions drawn from such analyses are, consequently, less certain.

It is best to aim for the highest analyte recovery possible, but many analytical methodologies are officially accepted for government regulation with reproducible recoveries as low as 50% for some of the analytes. As long as sample preparations are reproducible, reproducible recoveries can be obtained. Whether a treatment with a low recovery is usable can be decided by setting some maximum level of its statistical error, such as setting a maximum relative standard deviation of the recovery. The recovery error adds to the other statistical errors in the usual way:

$$\frac{\sigma_{\text{tot}}}{\text{analyte content}} = \text{RSD} = \frac{\sqrt{\sigma_{\text{sampling}}^2 + \sigma_{\text{recovery}}^2 + \sigma_{\text{assay}}^2 + \dots}}{\text{analyte content}} \quad (4-2)$$