An Introduction to Instrumental Methods of Analysis

Instrumental methods of chemical analysis have become the principal means of obtaining information in diverse areas of science and technology. The speed, high sensitivity, low limits of detection, simultaneous detection capabilities, and automated operation of modern instruments, when compared to classical methods of analysis, have created this predominance. Professionals in all sciences base important decisions, solve problems, and advance their fields using instrumental measurements. As a consequence, all scientists are obligated to have a fundamental understanding of instruments and their applications in order to confidently and accurately address their needs.

A modern, well-educated scientist is one who is capable of solving problems with an analytical approach and who can apply modern instrumentation to problems.[1] With this knowledge, the scientist can develop analytical methods to solve problems and obtain appropriately precise, accurate and valid information. This text will present; 1) the fundamental principles of instrumental measurements, 2) applications of these principles to specific types of chemical measurements (types of samples analyzed, figures of merit, strengths and limitations), 3) examples of modern instrumentation, and 4) the use of instruments to solve real analytical problems. The text does not include information on every possible analytical technique, but instead contains the information necessary to develop a solid, fundamental understanding for a student in an upper level undergraduate class in instrumental analysis.

1-1. Background Terminology:

Before presenting the complete picture of a chemical analysis, it is important to distinguish the difference between an analytical technique and an analytical method.[2] An

analytical technique is considered to be a fundamental scientific phenomenon that has been found to be useful to provide information about the composition of a substance. Examples of analytical techniques include infrared spectrophotometry (IR) or inductively coupled plasma atomic emission spectrometry (ICP-AES). An analytical method involves the use of an analytical technique, operated within specific and appropriate measurement parameters, for solving a problem. The analysis of styrene-acrylonitrile copolymers using infrared spectrophotometry and the determination of lead in drinking water using ICP-AES are both examples of analytical methods.

It is also important to differentiate the terms procedure and protocol. A procedure represents a set of written instructions for carrying out the steps of an analytical method. Organizations such as the American Society for Testing Materials (ASTM) or the Association of Official Analytical Chemists (AOAC) publish books with standard methods for chemical analysis. These methods of analysis are standardized procedures, written with the assumption that the analyst has some prior knowledge of analytical methods and presented in the form of a general guideline of the steps to be performed. A procedure for the analysis of styrene-acrylonitrile copolymers involves the extraction of residual styrene and acrylonitrile monomers from the polymer into carbon disulfide. The remaining polymer is next dissolved and cast as a film on a sodium chloride plate. The absorbance of the carbon disulfide extract and the thin film are then measured over the range of the mid-IR frequencies using an infrared specrophotometer. The absorbances at frequencies characteristic for that of styrene and acrylonitrile are measured and compared to standards of known concentration to determine the copolymer composition.[3]

A protocol is similar to a procedure; however it contains a much more rigidly defined description of the steps of the analytical method. Generally, a protocol is used to meet the demands of a government regulatory agency or to provide information for legal purposes. A protocol developed and required by the Environmental Protection Agency (EPA) for the determination of lead in drinking water by ICP-AES includes detailed instructions for sample preparation, preservation, and storage of the water sample. It also documents the approaches for calibration, assessment of the method's performance, and other specific steps designed to assure the overall integrity of the results of the analysis.[4] The steps MUST be performed as directed without deviation for the method's results to be considered acceptable.

Not only must a scientist design an appropriate method for the analysis, but the method must also be proven acceptable for the intended purpose. The actions to prove the acceptability are termed method validation.[5] The steps required to create a valid chemical method are numerous and quite variable, depending upon the nature of the problem and the regulatory agencies that may oversee the measurements. It is beyond the scope of this text to cover validation in detail. However, additional general information related to method validation will be presented in the Figures of Merit and Calibration chapter.

Finally, the terms instrument and machine are important to clarify. Many use these terms interchangeably, but incorrectly, when describing analytical techniques. An instrument is defined as "a measuring device for determining the present value of a quantity under observation".[6] Machine should be reserved for use in describing a device used to perform work or change the direction of motion of an object. Instruments may

often contain components that are machines, but ultimately the instrument has the purpose of making a chemical measurement and should be recognized accordingly. Many practicing analytical chemists bristle when the word machine is used to describe a technique used for analysis.

1.2. Methods of Chemical Analysis:

The objective of a chemical analysis, whether the measurement is performed using classical (wet chemical) or instrumental methods, is to provide information in order to solve a problem or to make a decision.[7-8] To obtain reliable results, all scientists using instruments should consider more than the measurement, which is only one component of a chemical analysis. Instruments are important, but solid scientific procedures throughout a method of analysis are necessary in order to produce valid, trustworthy information.

A scientist's role in a method of analysis is more than understanding and making measurements. Designing a method of analysis appropriate to the problem requires experience, broad knowledge, intuition, and the problem solving skills of a detective. The analyst must deal with the nature and origin of the sample, the desired accuracy and precision, limitations in costs and time for the analysis, and the selection of appropriate techniques. Significant interactions with collaborating investigators are typically required, not only for the analyst to acquire the necessary information to solve the problem, but also to communicate the information that can realistically be provided, given the nature of the sample and measurement techniques available. Finally, the results of the analysis must be properly and accurately communicated. As a result of these varied tasks, analysts are often considered "information brokers" in that they need

to know what information is desired and how to obtain and transform data from the sample into the required information.

It is also critical that an analyst understands the term quality as related to a chemical measurement. Ishikawa described quality as "the development, design and supply of a product or service that is economical, useful, and always satisfactory to the customer."[9] When the concepts of quality are applied to a chemical measurement, the term quality assurance is more commonly used. Quality assurance in a chemical measurement involves the actions within the method of analysis that provide satisfactory measurements with appropriate confidence, high dependability, and in a cost-effective manner.[5] Quality assurance is best considered as the proper management of the chemical analysis.

In order to better understand of the role of instrumentation in a chemical analysis, it is useful to view the analytical method as a series of steps. One approach for detailing the steps is shown in Figure 1-1.[7,10] The discussion that follows will briefly highlight these steps. References providing greater detail on developing an analytical method can be found in the bibliography of this chapter.

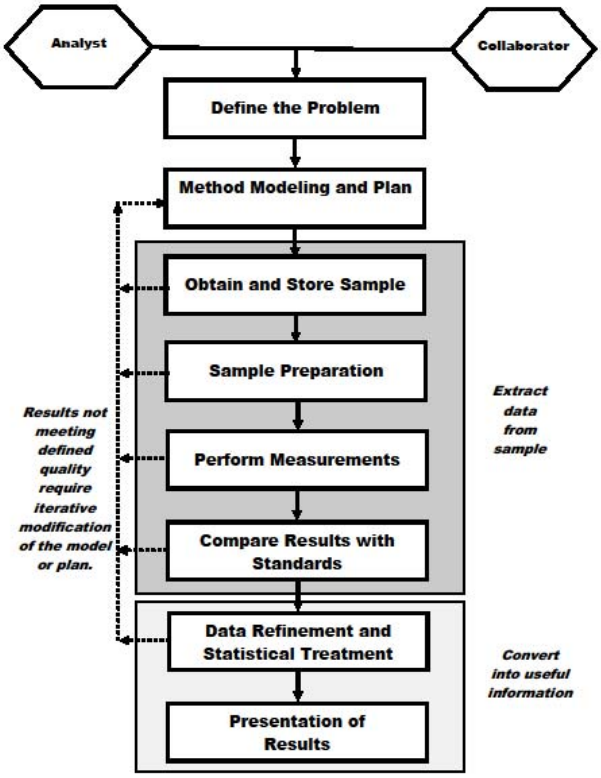

Figure 1-1. Steps in a Method of Chemical Analysis.

Define the Problem.

When presented with an analytical problem, the first important step in the development of a chemical method is to clearly define the problem. It is one of the most difficult steps to address, requiring a solid understanding of analytical techniques, problem-solving skills, experience and intuition. The analyst must address several key points to develop a method that is satisfactory.[11] These include an understanding of; 1) the intent of the measurement, 2) the necessary considerations in sampling and sample

preparation, 3) the best technique for making the measurement, 4) evaluation of the data, 5) reporting the results, and 6) the resources needed to accomplish the analysis. Table 1-1 presents a number of representative questions that the analyst and collaborators must answer in the development of an analytical method. Answers to these questions help create a clear understanding of the history of the problem and the sample involved, in order to develop satisfactory and economical solutions.

TABLE 1-1

Representative Questions to Define the Problem

- What is the nature and background of the problem?
- What is known about the history of the sample?
- What analyte is important in the sample?
- What is the concentration range of the analyte?
- What degree of accuracy and precision is demanded?
- What other components are present in the sample (concomitants)?
- What are the physical and chemical properties of the sample, analyte, and concomitants?
- Have prior, similar efforts been documented in the literature?
- What instruments and equipment are available for the determination?
- How much time is needed to perform the work?
- How soon does the work need to be done?
- How much money is available to accomplish the work?
- How many samples must one measure?
- Are there limitations to the amount of sample that can be used?

The method chosen may provide either qualitative or quantitative information. Qualitative data may include the composition, oxidation states, structural information, or

the isotopic distributions of elements contained in a sample. Information on the polyatomic ions, functional groups, specific molecules, or all of the molecular species present in the sample may be required. Scientists should be aware that a qualitative measurement also "builds in" information semi-quantitative information on the species measured. An understanding of the instrumentation used to make the qualitative measurement also leads one to a rough approximation of the concentration of the species being measured, allowing some distinction of whether the substance is a major (>1%), minor (0.01-1%), trace (10^{-2} – 10^{-6}%), or ultra-trace (10^{-6}- 10^{-9}%) component. The problem may also require quantitative information. For quantitative methods, the analyst needs to plan tasks associated with sampling, sample preparation, and calibration more meticulously than a qualitative analysis. Often, preliminary measurements will be required to develop a quantitative method.

A careful consideration of the analyte, sample, and instrumentation are critical in the development of a valid instrumental method. A number of points are common to nearly every analytical problem. These include:

1. Properties of the sample. The sample's phase (solid, liquid, gas, dissolved, suspended), the amount available for analysis, and its homogeneity are important in method development. Each influences sampling**,** sample reduction from a gross sample to a lab-sized sample, and the sample preparation. As an example, when using infrared spectrophotometry (IR) for a measurement, the sample can often be presented to the IR for measurement as a solid, in a solution (though not aqueous), or in gaseous form. However, when using atomic spectroscopic or liquid chromatographic techniques, the sample generally must be presented as a

dilute component in a solution. With these latter techniques, if the sample is originally in the solid form, extensive decomposition or dissolution is required prior to the use of these techniques in an analytical method.

2. Analyte properties. Measurements involve instruments that rely on interactions with specific chemical or physical properties of the analyte. Knowledge of these unique analyte properties, combined with an understanding of the nature of the analyte/instrument interaction, determine the appropriate measurement technique(s). If capillary zone electrophoresis (CZE) is used to separate and quantitate a number of proteins in a biological sample, an analyst must realize that proteins need to have a net charge in order to be separated and that charge on a protein is dependent upon pH. Thus, for success in the separation, it is necessary the pH of the sample and CZE running buffer is adjusted to that necessary to keep the proteins of interest at the appropriate charge.

3. Anticipated concentration range of the analyte. The expected concentration of analyte in a sample further limits the measurement techniques that may be used in an analytical method. The analyte's expected concentration must be compared to the concentration range in which an instrument can reliably measure, referred to as the linear dynamic range. If the concentration is lower than the limit of detection (LOD) of the instrument being considered or if sample preparation steps serve to dilute the original sample below the LOD, a different technique may be required. Conversely, if the expected concentration is higher than the limit of linearity (LOL), additional dilutions might be necessary. A good analyst will

know the typical linear dynamic range of applicable instrumentation and will have an expectation for the concentration of analyte contained in a sample, either through judicious use of literature or preliminary experimentation.

4. Sample preparation. The phase of a sample, non-analyte components in the sample, properties of the analyte, and the instrumentation chosen all influence the steps needed to prepare a sample for an analytical method. The choice of decomposition or dissolution of a solid sample, dilutions made prior to the measurement, approaches taken to prevent analyte losses or contamination, and the separation of concomitants from the matrix are all important considerations. Of critical importance is the fact that when the analyte concentration decreases, the sample preparation steps become more difficult and require greater planning to obtain an accurate analytical measurement.

5. Desired precision. The desired precision of the method must also be considered when defining the problem. The overall precision achievable in an analytical method depends upon a number of contributors, including the homogeneity of the analyte in the sample, the analyte concentration, the number of steps in sample preparation, and the precision of the technique used to make the measurement.

If a method is well planned and executed, the major contributors to the overall precision are the standard deviation of the analyte in the sample, represented by s_{sample}, and the standard deviation of the technique used for the measurement, represented by $s_{technique}$. The impact of each contributing factor on the overall method of analysis can be illustrated in Equation 1. In many methods,

the sample's contribution to the method precision is generally greater than that of the contribution from the analytical technique used for measurement.

Equation 1 $$s^2_{method} = s^2_{sample} + s^2_{technique}$$

An analyst must know the contribution of each of these contributors in order to develop a solid method. As such, it may be necessary to perform preliminary experiments in the course of the method's development to determine the contribution of the sample to the overall method, using these results to further refine the sampling and preparation procedures. In some cases, it may not be feasible to improve the contribution of a sample to the overall precision of a method, limiting the precision one can achieve in the development of a method.

The precision of a technique is often estimated using the literature or measured using simple aqueous standards. It is possible to improve the precision of a technique, but it generally requires an investment of more time or money. An important point to remember when developing a method of analysis is that it is not useful to improve the precision of a technique any further than 1/3 the precision of the sample.[12] It will increase the time and cost with little gain in the method's precision.

Precision is also required to establish a number of important performance characteristics of a technique or the method, often termed figures of merit (FOM), that help validate an analytical method. Information on the importance of precision to instrument performance will be discussed in more detail in the Figures of Merit and Calibration chapter. Information on precision of the

instrumental techniques covered in this text can be found in the chapter corresponding to that technique.

6. Desired accuracy. The most common method for measuring accuracy is to determine the closeness of the measured result to a "true" value. In most real samples, a "true" value is not known, thus other measures are often used to assess the accuracy of a method. A statistical term that provides one measure to help define the accuracy of an analytical method is the confidence limit. The confidence limit defines a range of values within which the "true" value of analyte concentration is expected to lie, with a known degree of statistical confidence.[13] Equation 2 describes the confidence limit of a series of measurements, where $\bar{x}$ is the mean, *t* is the Student t-value, *s* the standard deviation, and *n* the number of experimental measurements.

Equation 2: $Confidence\ Limit = \bar{x} \pm ts/\sqrt{n}$

For example, if one desires an improvement in the confidence limit from ±2% to ±0.2%, there are limited ways to accomplish this improvement. One approach is to decrease the standard deviation of the method by a factor of 10. However, the standard deviation of the method (dependent upon the homogeneity of the sample and the precision of the technique) is not easily nor realistically improved by an order of magnitude. The alternative is to increase the number of samples measured. Since there is an inverse relationship between the square root of the number of samples and the confidence limit, a 10-fold improvement in the confidence limit can be accomplished by increasing the number of samples measured by a factor of 100! To achieve this improvement, an expected 100-fold

increase in the time and costs will be required. Thus, the analyst should be aware of this tradeoff and clearly communicate to his/her collaborators or customers the influence of increased accuracy and precision on the time and cost-effectiveness of the method.

7. Concomitants/Interferents. Knowledge of the other components in a sample, called concomitants, is also a critical factor prior to the development of a method of analysis. In complex samples, if a concomitant has a similar property to that of the analyte, it can also produce a signal in addition to that of the analyte, creating an interference in the measurement. The presence of interfering species often dictates the instrument selected for the measurement and influences the sample preparation, separation, and calibration approaches. As an example, in the determination of sodium ions in potato chips using a sodium ion selective electrode, potassium ions will also cause a direct interference with the signal response with the electrode, causing an overestimation of the sodium concentration. In potato chips, potassium ions are of comparable concentration to that of sodium and the interference will be substantial and not easily corrected. Flame atomic emission spectrometry (with a proper calibration approaches) would be more easily and accurately applied for sodium determinations in the presence of potassium, as the primary analytical emission wavelengths of sodium and potassium are sufficiently different to prevent the potassium from contributing directly to the sodium signal.

8. Existing methods. Finally, one should never proceed with the development of an analytical method without consulting the available literature for guidance. An

old adage states, “A day in the library will save a month in the lab.” Proper investigation of scientific, commercial, governmental and technical literature will provide valuable information that speeds the development of a method and improves the overall quality of its results. Web-based access to the literature facilitates information search and retrieval, often allowing the analyst to access needed materials directly from an office computer.

Method Modeling and Plan.

After obtaining adequate information about the problem, a model for the analysis is developed. The model is an idealized representation of the complex steps of the analytical method. It includes a specific statement of the problem, information about the sample and analyte (concentration levels of concern, potential interferences, location of the analyte in the sample, phase relationships, particle size distribution,….), collateral information that may impact the determination, accuracy and precision requirements, and mathematical relationships used to relate data to the original problem.

The development of a model may require experiments to obtain more information about the sample or to validate assumptions. It may also be necessary to perform measurements on the sample to determine its homogeneity, allowing the development of a sampling plan to accurately address the goals of the method. The results of these preliminary experiments are used to help refine the original model. One should also not forget to include peer review as a mandatory component of the development of the model.

After establishment of the model, a plan must be created to provide specific directions for each step in the method. The plan translates the model into standard

operating procedures (SOPs), which provide the actions and reviews necessary to complete the analysis with the necessary precision and accuracy.[2] Specific components of a plan are outlined in Table 1-2.

Table 1-2
Key Information in Method Planning

Project Organization
- Identity of principal investigator
- Identity of subordinate investigators (and their responsibilities)

Description of Problem/Work to Be Done
- Nature of the problem to be solved
- Specification of technique(s) used
- Analytical requirements of the measurement
- Description of communication of results

Define the Sample
- Written sampling plan
- Approach for sample reduction to lab-sized sample
- Procedures for drying sample
- Choice of size for measurement samples
- Approaches for protection against contamination
- Preservation of samples
- Documented chain of custody

Sample Preparation
- Identify the reagents used.
- Methods to avoid loss of or changes in the analyte
- Methods to control contamination
- Separations or extractions necessary
- Effect of dilution and relationship to instrument LOD

Technique Used
- Valid SOP cited
- Appropriate SOP for regulatory agencies involved
- Methods of validation stated
- Demonstrated methods of statistical control
- Specification of calibration materials, proper approach, and measurement schedule (sequence of calibration standards, samples, reference controls).

Control Samples
- Use of standard reference control materials
- Defined field blanks, reagent blanks and spiked samples

Ancillary Equipment and Reagents
- Appropriate cleaning of lab utensils and containers
- Documented calibration of balances, pipets, flasks, etc.
- Proper quality of reagents used
- Documentation of ancillary concerns

Reviews
- Collaborator Review
- From a statistical perspective
- Peer review
- Supervisory review
- Client review

Obtain and Store the Sample.

Expert knowledge in the mechanics of instrumental measurements is a critical aspect in the role of an analyst; however it is insufficient in gaining accurate and precise results. Proper consideration of the sampling and sample handling are equally important. Even with the best quality instrumental measurement, a poorly selected or improperly handled sample will give erroneous or inappropriate results.

Once the plan has been completed and reviewed, samples must be obtained from the bulk material. The analyte contained in the bulk sample should be "representative" of its concentration in the entire sample, though obtaining a truly representative sample is a virtual impossibility as it requires the entire sample to be analyzed.[14] In many lab situations that involve small amounts of sample, such as in measuring a product from a small-scale synthetic reaction, obtaining a representative sample is less complicated than in larger, more inhomogeneous samples. However, a scientist must not treat sampling considerations lightly, even in small samples, as some inhomogeneity can still exist. A well designed sampling plan requires a great deal of knowledge about the distribution of analyte in the sample.[15]

Typically, a sample that is much larger than required for the measurements is selected, often termed as the gross sample. The amount selected in a gross sample is determined by a number of different factors. With solid samples, the method's required precision, the particle size of the solid material, and the distribution of the analyte in the sample are important determining factors in the size of the gross sample. In liquids and gases, smaller gross sample sizes are generally needed, as homogeneity for liquids and gases can be improved with mixing prior to sampling. In large, complex samples where

it may be impossible to achieve homogeneity through mixing, a planned sub-sampling at varying locations is necessary to obtain a representative measurement. For example, a single sample is not adequate to determine the average dissolved oxygen content of a small lake. The solubility of oxygen varies greatly with temperature and the presence of aquatic life. Thus, a sampling plan that includes consideration of the topography, temperature variation, and depth of the lake is necessary to obtain a representative analytical result.

Samples must also be transported, stored and reduced into lab-sized volumes suitable for measurement. In each of these operations, steps must be made to prevent changes in the analyte concentration due to chemical changes, volatilization, absorption of moisture, contamination, and adsorption/desorption processes with sample container walls. Appropriate labeling, proper storage, and a documented chain of custody (keeping records of who handled the samples, where they are located, and when they were moved) should also be considered. Table 1-3 shows a number of factors important in developing a sampling SOP.[16]

Table 1-3
Sampling Considerations

- **Specific information on how to collect the sample**
- **Documentation of where the sample was collected and when it was collected**
- **Sampling equipment to be used**
- **Proof of the appropriate maintenance and calibration of sampling equipment**
- **Types of sample containers, including cleaning and proper stabilizers**
- **Proper storage of the samples**
- **Criteria for accepting or rejecting samples**
- **Methods for excluding or separating foreign objects**
- **Proper treatment for sample drying, mixing, homogenization, and handling**
- **Procedures for subsampling or compositing.**
- **Record keeping that documents all actions performed, traces the chain of custody, and any auxiliary information important.**

Sampling often represents the most difficult and error prone step in an analytical method. First, improperly selected samples can often lead to large errors in the accuracy of a quantitative measurement. Additionally, sampling strategies are normally a larger contributor to the overall precision (standard deviation) of an analytical method than that of the instrument used. Often, the sampling contribution to precision must be

experimentally evaluated and then used to modify the sampling plan to achieve the desired accuracy and precision.

Sample Preparation:

Few instrumental techniques can measure samples directly without pretreatment. A number of sub-samples must be chosen (as prescribed by the desired confidence limit of the method).[15] The samples must also be treated to make them compatible with the instrumental technique. Transformation of the sample into a form that can be measured using the selected technique is termed sample preparation. Sample selection and preparation usually represent the largest investment of time in the implementation of an analytical method.

It is important to realize that the majority of instrumental techniques require the sample to be in a liquid phase. For solid samples, several techniques are taken to transfer the analyte into the liquid phase. These include dissolution, extraction or decomposition *(sometimes termed digestion)* or some combination of these. Liquid or gas phase samples are not exempt from sample preparation. Filtration to remove particulate matter, extraction to remove an analyte from a complex matrix, or a chromatographic separation to remove interferences from the analyte are all commonly used for liquid or gaseous samples. Care must be taken in these steps to preserve the integrity of the analyte by considering the possibility of contamination, loss, or chemical and physical changes to the analyte. Table 1-4 defines a number of these common sample preparation approaches. Further detailed discussions of these techniques are found in the references at the end of the chapter.

Table 1-4
Approaches to Sample Preparation

	Description	Some Common Approaches
DISSOLUTION	**Homogeneously distributing the analyte in a solvent**	*Aqueous* – analyte is dissolved directly in water; preferred though relatively few materials are sufficiently water soluble.
		Non-Aqueous – analyte dissolved in solvent less polar than water; used more effectively with organic compounds and polymers
		Acid-Water Mixture –analyte dissolved in dilute non-oxidizing acid solution such as $HCl_{(aq)}$
		Complexing Agents-Water: Complexation of analyte with a ligand to aid solubility.
DECOMPOSITION	**Chemically converting the sample into a form that can be dissolved in a solvent.**	*Acid Decomposition* – analyte (typically inorganic) is chemically converted into soluble form using concentrated mineral acids (HCL, HNO_3, $HCLO_4$, HF, aqua regia) by oxidation or displacement.
		Fusion –an inorganic sample is mixed with an acidic or basic salt (K_2CO_3, $K_2S_2O_7$), melted at high temperature and cooled; cooled melt is dissolved
		Combustion – sealed oxidation of an organic sample in gaseous O_2 , followed by absorption of products in a solvent
		Wet Ashing – decomposition of an organic sample in a hot, oxidizing reagent; primarily for elemental analysis
		Dry Ashing – heating an organic sample in flame or furnace in air to oxidize; followed by dissolution of the remaining ash material
		Microwave Assisted – decomposition by heating a sample using microwaves, often assisted using high pressure in sealed vessel (bomb); used routinely to automate and speed all forms of wet decomposition
FILTRATION	**Removal of a substance (generally a solid) from a liquid or solution by an exclusion process.**	*Paper and Glass Fiber* – removal of solids by passing a solution (liquid or gas) through a paper or glass fibrous material
		Membrane Filters – filtration through polymeric structure with fine pores (as small as 0.3 μm)
		Hollow Fiber Membrane – use of pressure to force a solution through a membrane; ultrafiltration and reverse osmosis can use pore sizes as small as 0.025 μm in diameter

Table 1-4
Approaches to Sample Preparation

	Description	Some Common Approaches
EXTRACTION	**Selective removal of an analyte from a mixture by partitioning between two immiscible phases**	*Liquid-Liquid Extraction(LLE)* – analyte distributes in two immiscible liquid phases, one aqueous and one organic; analyte solubility favored typically in organic phase
		Solid Phase Extraction (SPE) – analyte removed completely from a flowing liquid by retention on solid sorbant; analyte subsequently removed from sorbant by elution with a different solvent
		Solid Phase Microextraction (SPME) - analyte sorbed on a thin layer of sorbant (solid, liquid) coated on the outer surface of a fiber exposed to a liquid mixture; followed by redissolution or volatilization of analyte for analysis
		Supercritical Fluid Extraction (SFE) –a supercritical fluid is created using a gas (like CO_2) above the critical temperature; resulting supercritical fluid is typically used to extract organic analytes from a solid sample followed by its collection by depressurization, on a sorbant or in a solvent
		Microwave Assisted Extraction (MAE) – microwave-accelerated extraction of organic analytes from a solid sample with a liquid
		Membrane Extraction – a sample is placed in contact with a membrane that allows the sample to selectively permeate into a new gas or liquid phase
		Headspace Extraction – volatile organic compounds are allowed to diffuse from a liquid into a headspace above the liquid; analyte containing gas in the headspace is sampled.
DISTILLATION	**Removal or enrichment of a volatile substance based on differences in boiling point.**	*Batch, Fractional, Azeotropic, Vacuum Methods*: employed to remove volatile substances from a solution

Sample preparation must also compare the expected concentration of the analyte in the sample with the smallest statistically detectable concentration of the instrument

used for the measurement, the limit of detection (LOD). In some cases, care must be exercised to prevent dilution of the analyte to a concentration below that of the LOD of the method. In other cases, it may be necessary to enrich the sample concentration to make an accurate measurement, a step termed preconcentration. Suppose an analyst wished to determine the concentration of atrazine in a sample of natural water using a gas chromatographic method. If the atrazine in the sample was lower in concentration than the limit of detection possible with the GC, an approach involving preconcentration of the atrazine might be undertaken to "enrich" the atrazine concentration and allow the determination. In this approach, the analyst might pass a large volume of the aqueous sample through an adsorbant chosen to trap the herbicide. The trapped analyte would then be released into a much smaller volume (mL quantities) of solvent, increasing its effective concentration relative to the concentration in the original water sample. The result would be a sample concentration greater than the limit of detection of the intended GC method, thus allowing the sample to be measured quantitatively.

Perform Measurements.

Once the sample has been prepared, it is necessary to measure replicate samples to establish the precision of the method. The measurement depends upon the interaction of the technique with a unique chemical or physical property of the analyte. Consider the previously cited example (Section 1-1) involving the use of IR spectrophotometry to measure the residual styrene and acrylonitrile monomers remaining in a copolymer sample. The measurement relies on the fact that the aromatic carbon-carbon bonds in the styrene monomer absorb infrared radiation at approximately 1600 cm^{-1}, while the cyano group of the acrylonitrile monomer absorbs at 2275 cm^{-1}. An accurate analysis will

assure minimal, or no absorbance of the styrene monomer at the frequency of the acrylonitrile monomer (and vice versa).

Compare Results with Standards.

Reliable and convincing analytical results must involve a proper, careful comparison of the analyte's signal to that of appropriate standards of known analyte concentration as well as a calibration blank solution.[17] This is known as calibration or standardization. Calibration establishes the mathematical relationship between the analytical signal and the concentration of analyte in the calibration standards. The most common approach is to develop a "working" curve such as that illustrated in Figure 1-2, then use a statistical routine to establish a "best-fit" relationship between the measured signal and the analyte. When possible, the method of linear least squares is most desirable for representing the calibration relationship.[18] The result is a linear relationship of the general form shown in Equation 3, where S_{total} represents the total analytical signal (a combination of the analyte's signal and that of the background signal), m the slope, C is the concentration of analyte, and S_{blank} is the signal contribution of a blank sample.

Equation 3: $$S_{total} = m(C) + S_{blank}$$

Alternatively, if the background signal is subtracted from all standards and samples prior to the use of the calibration curve, S_{blank} is reduced to a small, near-zero term that is a product of the statistical curve fit routine and S_{total} becomes the net analytical signal, $S_{analyte}$. The concentration of the sample is determined by a mathematical relationship using the measured signal from the unknown sample and the values of m and S_{blank} established using linear least squares. Control samples, similar in composition to the

unknown sample, with known analyte concentration should also be measured in conjunction with calibration to help assure the accuracy of the calibration.

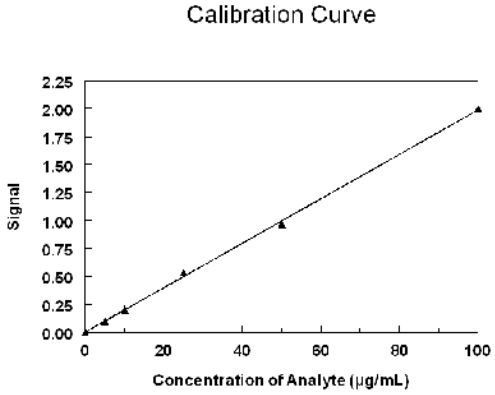

Figure 1-2. <u>Calibration Curve.</u> Calibration in an analytical method is often accomplished by a linear plot of measured signal (abscissa - y-axis) versus concentration (ordinate - x-axis). The signal, subject to random error, is plotted on the abscissa and the concentration, assumed to be subject to no random error, is plotted on the ordinate.

Alternate approaches for calibration may be necessary in cases where a linear relationship cannot be established or when sample matrix components cause differences in the signal response compared to simple standards. To achieve optimal results in these instances, the analyst may be required to dilute the sample and standard solutions, use

non-linear curve fit algorithms for calibration curves, or use more complex calibration approaches such as the method of standard additions.[19]

Adequate control of the conditions of calibration must accompany any method of analysis. Control of the temperature, the pH, or complexing properties of the sample and standards are important to retain consistency in the activity of the analyte in both sample and standard. Instrumental parameters such as the amplitude or frequency of an input signal, the sensitivity of a detector, the timing of measurement of the sample relative to the calibration, and the "drift" of measured signals must all be considered in the calibration to achieve optimal results.

Data Refinement and Statistical Treatment

Validation of a method of analysis relies on statistical methods in the treatment of results. The precision of an analytical method, a measure of the method's random error, must be presented in every analytical result. It is preferred to include information showing the independent contributions from sampling (due to inhomogeneity), sample preparation, and the measurement.[20]

Since the "true" value for the analyte is rarely available to compare the accuracy of an analytical method, the analyst must determine and report the confidence limit associated with the results as one measure of the accuracy of the method. Additional support to help demonstrate the accuracy of the method must be also performed for a valid analytical result. One or more of the following approaches must be used to detect the presence of systematic errors.

1. Analysis of a "blank" sample, prepared similarly to that of the unknown sample, but containing no analyte. The "blank" will assess analyte

contamination (through added reagents or contact with dirty containers) or non-analyte matrix interferences.

2. Analysis of the same sample with a different analytical method. Statistical differences between the two methods indicate systematic errors.
3. Comparison of results obtained by different analysts and instruments using the same analytical method on the same samples. Statistical differences will indicate the presence of systematic errors in one of the methods.
4. Use of a Certified Reference Material such as Standard Reference Materials (SRM) available from the National Institute of Standards and Technology (NIST). An SRM is a material that is similar in composition to that of the unknown sample, in which the analyte composition is accurately determined and certified by a defined, traceable method of analysis with a known degree of confidence.[21] Results outside the range of confidence of the SRM indicate the presence of systematic error. SRMs represent one of the best approaches to help validate the accuracy of an experimental result.
5. Reporting the percentage recovery of a "spiked" sample. These samples involve the addition of an exact quantity of analyte to an unknown sample in excess of the analyte already contained. Spiked samples result in analyte recoveries higher than the non-spiked unknown samples by the equivalent of the added analyte. Spiked samples generally indicate losses of analyte or the presence of interferences (physical and chemical) in the method.

Validation of a method of analysis requires the presentation of a number of Figures of Merit (FOM) other than accuracy and precision to prove the method of

analysis is meeting the desired performance characteristics.[22-23] These can include performance parameters that demonstrate the quality of calibration curves by statistical assessment parameters such as the regression coefficient and standard deviation of the calibration slope and intercept. Other FOM of importance include the linear dynamic range (LDR), the sensitivity of the calibration curve, the method's limit of detection (LOD), and the selectivity of the instrument response. These terms will be discussed in the chapter titled *Figures of Merit and Calibration*.

Failure to make these assessments in the course of an analytical method will invalidate the results of the method. Failure to meet the defined standards for the confidence or FOM in an analysis will result in the need to troubleshoot the method to identify the problems and then modify the method to meet the specified goals.

Presentation of Results.

The clear, accurate presentation of results is an important requirement for a successful analytical method. Results must be considered from two different perspectives. The first is that of the analyst's organization. The results and documentation need to be presented in a technical and meaningful fashion for peer review and organizational approval. The second is that of the collaborators (often not analytical chemists) for which the analysis was performed or peers outside the institution that performed the work. Presentations often take the form of formal oral papers, poster presentations, research articles, theses or dissertations, and progress reports. In the presentation, results should be translated into a usable form, including a recommendation or conclusion related to the original problem that necessitated the work. The results should demonstrate the flow of information through the entire method of analysis and

identify the major sources of potential error in the method, including any steps that were performed to eliminate or assess them. In addition, all presentations should indicate both the accuracy and precision of the results. Documentation of all procedures is an important component of reports in order for the results to be legally acceptable. In summary, an effective analytical method must include valid results obtained by an accepted method, provided in a way that is cost effective, timely, and defensible.[11]

There is one other important point to make in relation to analytical methods. While this text focuses on instrumental methods of analysis, one should not forget classical methods of analysis. Though a large portion of current analyses involve instrumental measurements, the best solution for many problems may not always involve an instrumental technique. In many instances a classical volumetric or gravimetric technique, though more labor-intensive and less glamorous than modern instruments, will prove to be the most cost-effective, accurate and precise method. Thus, an analyst should examine both instrumental and classical techniques before choosing an appropriate technique for the problem at hand.

1-3. Classification of Instrumental Techniques.

Now that an overview of chemical analysis and a proper perspective of the role of instruments in an analytical method have been presented, a general picture of the categorization and basic functions of an instrument must be made. In this text, the classification of techniques will be made by considering the type of interaction of the instrument with a chemical or physical property of the analyte. In addition, it will be limited to the more common instrumental techniques, those of most interest to undergraduate students. Table 1-5 shows these categories organized in a way that

parallels their coverage in the text. Their acronyms, common types of chemical information that can be obtained and approximate analyte concentration ranges that can be measured are included in this table.

Table 1-5-A. Common Instrumental Techniques

	Acronym	Information Obtained[A]		Analyte Concentration[B]
		Atomic	Molecular	
Mass Spectometry				
Electron Ionization Mass Spectrometry	EI-MS	x	x	T
Chemical Ionization Mass Spectrometry	CI-MS	x	x	T
Fast Atom Bombardment Mass Spectrometry	FAB-MS	x	x	UT
Secondary Ion Mass Spectrometry	SIMS	x	x	UT
Electrospray Ionization Mass Spectrometry	ESI-MS		x	UT
Matrix Assisted Laser Desorption Ionization Mass Spectrometry	MALDI-MS		x	UT
Fourier Transform Mass Spectrometry	FT-MS		x	UT
Ion Cyclotron Resonance Mass Spectrometry	ICR-MS		x	UT
Optical Spectroscopy				
Atomic Absorption Spectrophotometry	AAS	x		UT
Graphite Furnace Atomic Absorption Spectrophotometry	GFAAS	x		UT
Inductively Coupled Plasma Atomic Emission Spectrometry	ICP-AES	x		UT
Atomic Fluorescence Spectrometry	AFS	x		UT
X-Ray Fluorescence Spectroscopy	XRF	x		UT
Ultraviolet-Visible Spectrophotometry	UV-VIS	x	x	T
Molecular Fluorescence and Phosphorescence Spectroscopy			x	UT
Infrared and Near-Infrared Spectrophotometry	IR or NIR		x	T
Fourier Transform Infrared Spectrophotometry	FTIR		x	T
Raman Spectroscopy			x	T
Nuclear Magnetic Resonance Spectroscopy	NMR		x	T

Table 1-5-B. Common Instrumental Techniques

	Acronym	Information Obtained[A]		Analyte Concentration[B]
		Atomic	Molecular	
Surface Analysis				
Scanning Tunneling Microscopy	STM	x	x	T
Atomic Force Microscopy	AFM	x	x	T
Auger Electron Spectroscopy	AES	x	x	UT
X-Ray Photoelectron Spectroscopy	XPS	x	x	UT
Electron Spectroscopy for Chemical Analysis	ESCA		x	UT
Nuclear				
Liquid Scintillation Counting		x	x	UT
Neutron Activation Analysis	NAA	x		UT
Separation Techniques				
Gas Chromatography	GC	x	x	UT
High Performance Liquid Chromatography	HPLC		x	UT
Ion Chromatography	IC	x	x	UT
Supercritical Fluid Chromatography	SFC		x	UT
Capillary Electrophoresis	CE	x	x	UT
Capillary Zone Electrophoresis	CZE	x	x	UT
High Performance Capillary Electrophoresis	HPCE		x	UT
Micellar Electrokinetic Capillary Chromatography	MECC		x	UT

Table 1-5-C. Common Instrumental Techniques

	Acronym	Information Obtained[A]		Analyte Concentration[B]
		Elemental	Molecular	
Electrochemical Techniques				
Potentiometry		x	x	T
Ion Selective Electrode Potentiometry (ISE)	ISE	x	x	T
Linear Sweep Voltammetry (LSV)	LSV	x	x	T
Cyclic Voltammetry (CV)	CV	x	x	T
Anodic Stripping Voltammetry (ASV)	ASV	x		UT
Amperometric Techniques		x	x	UT
Conductiometric Techniques		x	x	T
Hyphenated Techniques				
Gas Chromatography – Mass Spectrometry (GC-MS)	GC-MS		x	UT
Liquid Chromatography – Mass Spectrometry (LC-MS)	LC-MS		x	UT
Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	ICP-MS	x		UT
Gas Chromatography – Infrared Spectroscopy (GC-IR)	GC-IR		x	T
Mass Spectrometry – Mass Spectrometry (MS-MS)	MS-MS		x	UT

[A] Information obtained represents the chemical information most frequently gained using the technique.
[B] Analyte Concentration is the approximate lowest concentration in which the technique is applied routinely. [T = Trace level (10^{-2} to 10^{-6} %), UT = ultra-trace level (10^{-6}- 10^{-9} %)]

Mass spectrometry (MS) represents the first of these categories. MS techniques involve the creation of ions from atoms or molecules, their separation by mass-to-charge ratio, and subsequent detection. MS is a powerful technique, providing qualitative and quantitative information on the atomic or molecular composition of inorganic or organic materials. Advances in the creation of gas phase ions from macromolecules have resulted in significant applications of MS to problems in biochemistry and molecular biology.

Optical spectroscopic techniques involve the interaction of electromagnetic radiation with atoms or molecules. General subcategories of spectroscopic techniques involve techniques where matter absorbs, emits, or scatters electromagnetic radiation. In addition to quantitative data, qualitative information on the identity of atoms, molecular

functional groups, molecules, and changes in bonding environments can be obtained by optical spectroscopy.

Nuclear and *surface analytical techniques* have been grouped together in their own categories, despite some overlaps with optical spectroscopic techniques. Techniques involving nuclear processes, often termed radiochemical processes, involve the emission of particles or electromagnetic radiation from the nucleus of an element, rather than electronic phenomena common in the more traditional optical spectroscopic techniques. Some surface science techniques also are spectroscopic in nature, but differ in sampling considerations and the portion of the sample analyzed. A number of surface analytical approaches, such as atomic force microscopy (AFM) and scanning tunneling microscopy (STM), do not involve electromagnetic radiation.

Separations represent an important category of instrumental techniques. Most samples are complex mixtures of atoms, ions and molecules. Thus, the individual sample components must be separated prior to measurement. The two most common separation techniques are chromatography and electrophoresis. Chromatographic techniques involve partitioning of components in a sample between a flowing "mobile" phase and an immobile "stationary" phase. Separation occurs due to differing intermolecular interactions of the sample components in the two phases which results in different velocities of components through a column containing the stationary phase.

Electrophoretic techniques involve separation of charged molecules by their migration in an electrical field. Both approaches to separations are applied qualitatively and quantitatively to the determination of ions and molecules present in a mixture. Electrophoretic techniques are traditionally associated with separations in biochemical

systems. Instruments used in separations require the integration of a detection technique to measure the separated components. Numerous detection methods are available, often employing common instrumental techniques, such as mass spectrometry or spectroscopy. The choice of an appropriate chromatographic detector is governed by the specific requirements of the analysis.

Electrochemical techniques comprise another broad, general classification. These methods generally depend upon some approach to monitoring the process of electron transfer to or from an analyte. Potentiometric techniques involve measurements that are made based on the ability of electrons to be transferred in an electrochemical system. Electrolytic techniques, such as those categorized as voltammetric techniques, actually force the electron transfer to occur. Electrochemical techniques are used to derive both qualitative and quantitative information about elements, ions, and compounds in a wide variety of situations.

Two or more instrumental techniques are often used in tandem to gain advantages that neither can provide singly. These are often referred to as hyphenated techniques, some of which are listed in Table 1-5. Often, these techniques couple a separation technique with a mass spectrometer. One common hyphenated technique couples gas chromatography with a mass spectrometer (GC-MS). This instrument allows for the separation of volatile compounds from a complex mixture followed immediately by sequential mass specific detection (and structural elucidation) of each of the separated compounds. Hyphenated techniques are certainly not limited to combinations similar to GC-MS. The presentation of major hyphenated techniques will be integrated throughout the text in chapters appropriate to their use, rather than discussed in a specific chapter.

1-4. Basic Function of Instrumentation

The role of a chemical instrument is to obtain information about a sample. This process involves converting the information contained in the chemical or physical properties of analytes, into meaningful data. Several transformations may be necessary to accomplish the measurement; the number needed depends upon a variety of factors including the instrument, the quality of data needed and the quantity of data required. (23)

The flow of information in an instrumental measurement may be divided into four steps, as illustrated in Figure 1-3a.[24] The measurement begins with a signal generator, the portion of the instrument that creates a signal as a result of direct interaction of energy with the analyte. The energy involved is often electromagnetic radiation, thermal heating, or electricity. The resulting signal is directed to an input transducer, a device that transforms the signal from the non-electrical domain (the desired physical or chemical characteristic ---- chemical composition, light intensity, pressure, chemical activity) into the electrical domain (encoded as an electrical quantity such as voltage, current or resistance). The electrical signal is then transformed into a more usable form by signal modifiers. This involves operations such as amplification, attenuation or filtering. Finally, the modified electrical signal is converted by an output transducer to information in a format which can be recorded and interpreted by the analyst.

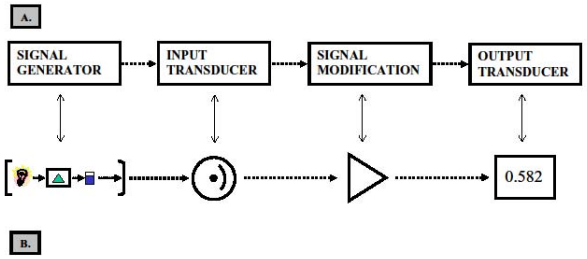

Figure 1-3. (A) General Flow of Information in an Instrument and (B) Flow in a Simple Spectrophotometer. The Signal Generator consists of a light source, monochromator to isolate a "single" wavelength and cuvette containing liquid sample. The transmitted light is focused to a phototube detector which converts light power into an analog current. Signal modification occurs by current-to-voltage and other mathematical modifications. The modified signal is converted into sample absorption for signal readout.

Figure 1-3b illustrates an example of this process using a simple spectrophotometer to determine the concentration of copper in a solution. Copper, when complexed with the reagent dithiozone, forms a red-violet colored complex that absorbs visible light at 525 nm. The concentration of copper can be determined by creating a ratio of the power of light transmitted through an unknown sample of the copper complex with the power of light transmitted through a blank sample that contains no complex, resulting in a signal called the transmittance (T).

The actual measurement of the transmittance of the copper-dithiozone complex is accomplished as follows. A lamp generates visible light which is introduced into a monochromator, a device that isolates 525 nm radiation, then passes it through a cuvette containing a solution of the copper complex. These components constitute the signal

generator portion of the spectrophotometer. After passing the cuvette, the transmitted light is focused on a photodetector, the input transducer. The photodetector accomplishes a domain conversion changing the radiant light power (energy per time per detector surface area) into an electrical current. Signal modifiers within the instrument then serve to convert the current into a voltage which is amplified and mathematically ratioed with a previously measured reference signal to obtain a voltage, corresponding to the transmittance of the sample. The transmittance, as an analog voltage is converted into data for the analyst (in the form of a digital display, computer storage, needle deflection, or chart recorder tracing).

Signal Generators.

A major objective of this text is the presentation of the fundamental processes involved in obtaining a signal form the sample. Signal generators serve to convert information in the non-electrical domain (chemical or physical property) of the analyte into an electrical signal. There are two general ways to generate a signal within most instruments: 1) Modification of an applied external signal by the analyte, as illustrated in the previous example involving the measurement of copper by absorbance spectrophotometry, and 2) the creation of a controlled environment which allows the analyte to produce a signal, as in the application of elevated thermal energy to a population of gaseous atoms, causing the atoms to emit light energy as the signal. The manner in which the signal generator functions is unique to each instrumental technique.

Input Transducers.

To preface an explanation of the input transducer, it is useful to briefly describe the analog electrical domain. The analog domain consists of electrical signals that are

usually represented by the magnitude of a voltage, current, charge or power. Data in the analog domain are represented by the amplitude of the signal at any instant in time and are generally considered to be continuous.[24-25]

The majority of instruments use detectors as input transducers to convert the signal generated by interaction with the analyte into a continuous analog signal. This analog signal is either monitored continuously or sampled at specific time intervals. In the previous example involving a spectrophotometric measurement, the phototube detector converts the power of light incident upon the detector into a continuous electrical current whose magnitude is proportional to the power of the incident light.

In some instances, the magnitude of the signal reaching the detector may not be of interest. Instead, data relating to analog signal fluctuation are important. Analog signals in which time relationships are of interest are known as time-domain signals. For example, the measurement of temperature using the frequency of the sinusoidal signal produced by a crystal oscillator detector is a common time-domain instrumental measurement. Another example of time-domain data is the signal produced by a gamma radiation detector in response to random events over time. A number of input transducers are shown in Table 1-6. Specific details about the variety and function of input transducers will be highlighted throughout the text.

Table 1-6
Transducers and Modifiers

A. INPUT TRANSDUCERS

Physical Quantity Measured	Input Transducer	Analog Electrical Output
Concentration of Electroactive Species	Potentiostat	Current
Solution Ion Activity	Ion Selective Electrode	Voltage
Light Photon Power	Phototube	Current
	Photodiode	Current
	Photomultiplier Tube	Current
	Charge Coupled Device (CCD)	Total Charge
Temperature	Thermistor	Resistance
	Thermocouple	Voltage

B. ELECTRICAL SIGNAL MODIFICATIONS

Amplification	Filtering
Analog-to-Digital Conversion	Digital-to-Analog Conversion
Integration	Differentiation
Logarithmic Conversion	Antilogarithmic Conversion
Comparison	Rectification
Attenuation	Summation
Counting	Voltage-to-Frequency Conversion
Current-to-Voltage Conversion	Voltage-to-Current Conversion

C. OUTPUT TRANSDUCERS

Analog Meters	Digital Meters
Strip Chart Recorders	X-Y Recorders
Video Displays	Oscilloscopes (Analog or Digital)
Computer Disk Drives	Printers

Signal Modifications.

Once an analog signal has been created by the input transducer, circuit components in the instrument modify and convert the data into a usable form. The modifications can take many forms depending upon the type of analog signal that is provided by the input transducer and the signal necessary for presention to the output transducer. Common signal modifications include current-to-voltage conversion (i-V), amplification, or mathematical operations such as summation, integration, differentiation, or logarithmic conversions. The electrical components responsible can range from a simple resistor to an operational amplifier to more complex digital circuits.

The conversions of analog domain signals into the digital domain and vice versa are important steps in the manipulation, processing and storage of data in computers. Data in the digital domain are represented by binary signals, characterized by a HI/LO transition; typically measured as a voltage, where the HI signal has a higher positive voltage than a threshold level that differentiates the LO signal. Transistor-Transistor Logic (TTL) circuits typically represent a voltage of about +2.4 V or higher as a logic 1 (HI) and a voltage of less than about +0.5V as a logic 0 (LO). Numbers or characters in the digital domain are represented as a sequence of HI/LO pulses in a logic channel, termed a serial digital signal or as a group of logic levels in a group of parallel channels, known as a parallel digital signal. Each logic level signal defined by the HI/LO characters is termed a *bit*. Modern 64 bit processors allow conversion of analog signals into digital values with high bit resolution and virtually error-free storage, processing and transmission of information. The Introduction to Electronics and Domain Conversions chapters will provide more detailed information on electronic signal modification and its importance in an instrumental measurement.

Output Transducers.

Finally, in the process of creating a chemical measurement, the data must be converted into a form useful to the analyst. The output transducer accomplishes this conversion. Common output transducers and their displays are shown in Table 1-6. Output data are most often transferred to an electronic storage device, such as a data file on a hard disk drive. In some cases, results may be displayed directly as the concentration of analyte, after proper calibration tasks are performed by the computer.

Output transducers are limited in number and the details of their operation are beyond the scope of this text. Further reading on the subject is found in the references cited in the bibliography in this chapter.

1-5 Organization of the Information in This Text

Following this chapter, the text will be organized into eight specific sections; each covering a category of information of general importance to all instrumental measurements or a group of techniques that rely on similar signal-analyte interactions. Section II of this text covers issues related to signals, signal modifications, calibration, method validation and figures of merit that are common to instruments. The remaining sections cover specific categories of instrumental techniques, and will follow an organization that parallels Table 1-5. Most of these sections will begin with a chapter presenting the fundamental theories associated with the category, followed by chapters that address specific techniques within the broad classification. The major hyphenated techniques will be integrated into appropriate chapters where one of the techniques is a component of the hyphenated instrument. Two additional chapters are also presented covering aspects of instrumental techniques that are important in modern chemistry but difficult to categorize elsewhere. The first involves applications of analytical instruments for process analysis, where the instrument operates in real-time as a component of an industrial production processes. The latter chapter describes some of the developments in instrumentation that are being driven by new technologies, particularly the need to develop smaller, more portable instrumentation.

All chapters addressing specific instrumental techniques will be organized as follows.

1. The General Theory of the Technique. An overview of the fundamental chemical/physical principles important to the technique and the types of chemical information obtainable will be presented. The flow of data through the instrument will be presented, with the components of the instrument responsible for data transformations.
2. Examples of Common Instrumentation. Specific information that describes some common approaches to modern instruments will be presented. Diagrams of these instruments will be included along with specific explanations of components that create, isolate, modify and detect the analytical signal. Interpretation of the results obtained with these instruments will be discussed.
3. Advantages and Limitations. The capabilities and limitations of the techniques will be presented. Major emphasis on quantitative methods of analysis will include sample requirements, applicable concentration ranges, limits of detection, expected precision and accuracy, and considerations related to selectivity and specificity.
4. Application of Techniques. Examples of the application of these techniques to modern chemical analysis will be presented. The applications presented have been selected to illustrate common or interesting approaches involving the techniques described in the chapter.
5. Problems. Illustrated questions and problems involving an understanding of the techniques described in the chapter are included in each chapter. These contain problems illustrating both qualitative and quantitative treatment of data relevant to the method.

6. Recommended Literature and Literature Cited. Specific references are cited within the text in each chapter. In addition, a bibliography is included containing books or papers that present greater detail on the techniques or more examples of their applications.

1-8. Future of Modern Instrumentation

As predicted in 1985, today's instruments are smaller, more highly automated, faster, and offer much significantly improved performance characteristics compared to their predecessors.[26] Future advances will continue these trends in a number of areas. These include:

1. Improved Performance. As is traditional in analytical chemistry, a better understanding of the science of measurements leads to improvements in the sensitivity, selectivity, application of instrumentation to complex sample matrices, and innovative configurations of instruments. Many of these developments are driven by the needs of unique problems, such as the study the dynamics of chemical reactions or the mapping of small inhomogeneities in the surface composition of a polymer. Most certainly, probe microscopy instruments that image three-dimensional micro- and nano-scale surfaces and subsurfaces will continue to evolve.
2. Miniaturized Instruments. One of the major trends in modern instrumental development is to create smaller, robust and low cost instruments. Developments in light sources (diode lasers), polymeric materials, optics, microelectrical mechanical systems (MEMS), electronics, and microfabrication technologies have impacted instrument design. Laboratory instruments occupy a much smaller

footprint than their predecessors. Small, portable instruments and sensors with few or no moving parts allow the instrument to come to the sample in locations such as deep, ocean environments, rather than bringing the sample to the lab for analysis. Micro total analysis systems (μ-TAS) or "lab on a chip" instruments are also being applied to many interesting analytical problems.[26-29] In μ-TAS systems an entire instrument, including electronics and power source fit into a space often the size of a cell phone. The "working end" of the instrument is created on a postage stamp sized polymeric plate, where the sample, reagent solutions, a separation device that employs chromatographic or capillary electrophoretic principles, and a detector are all located. M-TAS systems are finding a wide range of applications in biochemical analyses.

3. Remote and Process Analysis. Monitoring chemical processes in harsh environments, at high temperatures, and for different manufacturing processes is an expanding area in instrumental analysis. For example, the pharmaceutical industry uses instruments that can monitor mixing, drying, and tableting processes in real time, allowing for better quality control.[30-31] Continual development of more powerful methods for these applications can be expected.
4. Computers. Computers are now integral components of almost every instrument, where they control measurement parameters as well as the collection, processing, storage and display of data. Parallel and multiplexing data allows simultaneous, real-time collection of information on multiple analytes. Systems which integrate the preparation and sample introduction with measurements are now common and will become more intelligent in the future, increasing productivity and reducing

operator intervention. Expert systems used for data interpretation and advances in wireless communication will also impact future instrumental methods.

5. Bioanalytical Methods. The life sciences, including medical, clinical, agricultural, toxicological, and environmental analyses are experiencing the greatest expansion of instrumental methods. Interest in tracking or identifying small molecules, peptides, proteins or other molecules of biological importance is growing rapidly and providing new challenges for the analytical community.[32-38] The application of combinatorial chemical syntheses requires the development of new, unique analytical tools. The fields of proteomics, genomics, and metabolomics depend heavily on mass spectrometry, capillary electrophoresis, and "lab on a chip" techniques to help solve problems.

Although many of the instruments that will be designed and used in the future may be smaller and more automated, it is useful to remember that the fundamental principles on which they are based still involve those presented in this text. Thus, a solid understanding of the basic instrumental measurement processes provides a good foundation needed to adapt to these rapid changes.

1-8. Literature of Instrumentation/Analytical Chemistry

The scientific and technical literature is important to the analyst. A literature review can save time, avoid unnecessary lab experiments, and reduce costs. Thus, it is important for the analyst or research chemist to keep abreast of new developments in instrument design and applications to analytical problems.

The literature of analytical chemistry is similar to that of other branches of chemistry. It is organized into three major categories, by that nature of the information presented. These categories are: primary, secondary and tertiary sources.

Primary literature sources present recent, original material. They include journals, other periodicals, government publications, patents, dissertations, and manufacturers' technical information. The information presented in many of these sources may be grouped by a general technique of analysis, but it is often presented in a less organized manner. Journals, for example, will present information on the basis of the timing of acceptance of the paper after a peer-review process. Typically, to find literature in these sources one must use a computerized search by topic, keywords, or authors.

A number of periodicals of general interest to the field of analytical chemistry exist. These include *Analytical Chemistry*, *The Analyst (London)*, *Talanta*, *Journal of the Association of Official Analytical Chemists*, and *Zeitschrift für Analytische Chemie*. Numerous journals cover more specific fields within analytical chemistry. For example, developments in the field of gas chromatography may be found in the *Journal of Chromatography*, *Journal of High Resolution Chromatography*, *Chromatography Newsletter*, *Journal of Chromatographic Science* and *Chromatographia*.

Secondary sources typically contain information that has been previously published, but collected and presented in a more usable or organized format. Review articles in scientific journals, bibliographies, tabular compilations, treatises, monographs, and textbooks all represent examples of secondary sources. These secondary sources assemble information from a wide variety of sources (primary) in a cohesive, organized

and usable form. Because of the time in collecting and assembling this information, the secondary sources usually represent information that lags behind the current developments in science. The journal *Analytical Chemistry* publishes one of the more useful comprehensive reviews annually. In even number years, developments in fundamental analytical techniques are covered and in odd number years the review articles present summaries of the applications of analytical techniques to areas such as the environment, food, coatings, and biochemical interests. These reviews are accompanied by extensive bibliographies of primary sources. Another secondary source of interest is also published in *Analytical Chemistry*. A magazine-type section of the journal, called the "A" pages, provides reviews of trends in instrumental techniques or methods in a brief format. A number of different journals publish similar types review articles, including a periodical devoted to reviewing analytical methods titled *Critical Reviews of Analytical Chemistry*.

Many other secondary sources are also available to provide information of importance. A number of trade periodicals such as *American Laboratory*, *Spectroscopy*, *LC/GC*, and *Research and Development* provide articles that review instrumental techniques and applications. Treatises, such as the *Treatise on Analytical Chemistry*, edited by Kolthoff and Elving or *Wilson and Wilson's Comprehensive Analytical Chemistry* present a concise, comprehensive treatment of modern analytical chemistry. Finally, a large number of monographs (an entire book devoted to a single topic) are available from a number of different publishers. Similar to the review articles found in journals, the information presented in monographs is typically well organized but several years older than the most current advancements in the field. Unlike review articles,

monographs present a more detailed and completely explained discussion of important advances in their respective topics.

Tertiary sources are publications that are designed to help the analyst use the primary and secondary sources and to keep posted on developments in laboratory equipment, chemicals and instrumentation. *Chemical Abstracts* and *Scientific Citation Index* are important sources that abstract journal publications. Lab guides, published annually by *Analytical Chemistry*, *American Laboratory*, and others provide information of manufacturers of laboratory equipment. Additionally, sources of procedures and protocols for chemical analysis are published by private or governmental organizations such as the Association of Official Analytical Chemists (AOAC), American Society for Testing and Materials (ASTM), Environmental Protection Agency (EPA) and Food and Drug Administration (FDA). Many of these lab guides, procedures and protocols can also be freely accessed electronically on the World Wide Web.

The majority of scientific literature searches are now performed using computer searching tools to access the tertiary abstract sources. A number of on-line search engines are available for searching the chemical literature, including the Scientific and Technical Information Network (STN) which accesses technical literature in several international databases and SciFinder, which provides access to the Chemical Abstracts Service database. An analyst must have a general understanding of computer search procedures to obtain the specific, desired information. Modern searching software has made this task much easier, but it is still important to carefully define the search or important information may be overlooked.

A second important development that has made accessing the literature of analytical chemistry is that many journal publishers now provide access to current and past journal articles on-line, either by personal or organizational subscriptions. A researcher can now perform a search, link directly with the publishers of the journal articles they wish to retrieve, and obtain electronic copies of these publications without leaving their office.

Another important tool in obtaining information is the World Wide Web, which provides access to technical information in a variety of areas. Instrument manufacturers create web sites with a significant quantity of information on the principles of their instrumentation and applications to analytical methods. Academic scientists often post information on research being conducted and on material presented in their classes. Government and regulatory agencies often post standard procedures and protocols for chemical analyses. When finding and using web information from these sites, a degree of skepticism should be used, as much of the information is not a part of the standard scientific peer review system that is a vital part of publishing the results of scientific research. A recent project designed to collect, catalog and link to peer-reviewed database of web information on the analytical sciences is the Analytical Sciences Digital Library (ASDL), tied with the National Science Foundation's National Sciences Digital Library project.[39] However, because of the fluid nature sites of the World Wide Web, citations to web information in this text will be consciously avoided due to issues with the rapid changes that occur with web addresses. Instead, literature cited in this text will be material that is accessible in scientific journals, monographs, and texts.

Chapter Summary.

Instrumental methods of analysis have become the predominant approach for performing chemical measurements. It is critical for scientists who rely on these powerful tools to understand the fundamentals and applications of analytical instruments. However, expertise with chemical instruments is not entirely sufficient in intelligently solving problems. The accurate and appropriate instrumental measurement of an incorrect or irrelevant sample obtained from a poorly designed method will not solve the intended problem. In other words, the instrument and analyst are only as good as the sample presented for analysis. To provide results in a method that are valid, an analyst must understand the role an instrument plays in a method, making certain the appropriate sample is presented to the instrument and also applying a technique that is adequate for the desired answer.

A variety of instrumental techniques are available for the application to chemical problems. They include mass spectrometric, optical spectroscopic, nuclear, surface, electrochemical and separation methods. They are commonly used individually and in manners where two or more techniques are combined beneficially, creating hyphenated techniques. In general, all of these instrumental techniques function by converting information in the non-electrical domain into the electrical domain, where the information can be transformed into meaningful information, then converted into a form that is meaningful to the analyst. The manner in which each instrument accomplishes this varies, driven by the nature of the interaction of the probe with the chemical property of the analyst, but generally the overall pathway of the flow of information is similar from instrument to instrument.

The intent of this text is to present information covering many of the major instrumental techniques, allowing a student to more fully understand the theory of each technique, examples of common instruments using this approach for measurement, advantages and limitations of the techniques, and a few applications to specific methods of analysis. It is not intended to provide a comprehensive, encyclopedic coverage of techniques, but rather to provide information that all who use instruments should retain in their toolbox of knowledge. Should a more advanced understanding be needed, recommended sources are provided in each chapter.

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Problems

1. What is the difference between an analytical technique and an analytical method? Give at least two examples of each.
2. What differences might one expect when using a procedure for analysis versus that of a protocol?
3. Describe the term, method validation.
4. Describe what is meant by the term quality assurance.
5. Define what is meant by a quantitative analysis. How might a qualitative analysis differ from this? What types of information might be sought in the two types of analyses?
6. Outline and briefly describe the major steps in a chemical method.
7. Asking questions to help fully understand a problem that requires a chemical method of analysis is an important part of developing a method. When considering any general chemical analysis, describe five questions you would consider to be universally important to ask and have answered prior to the development of a method. Justify your responses.
8. Four specific terms are applied to describe a range of concentration of a component in a sample. What are these terms and what approximate range of concentrations would be expected to correspond to each?
9. Accuracy is important in an analytical method. In what ways can the accuracy of an analytical method be measured?
10. What is the typical measure of precision in an analytical method? Be specific.
11. Define the confidence limit of a chemical method, including those factors that affect it.
12. What specific changes or manipulations in a chemical method can improve the confidence limit?
13. There is a significant tradeoff made when making an analytical method more accurate. What is this tradeoff?
14. What is the difference between a model and a plan?
15. Name at least four different important considerations in the collection of a sample for analysis and in its reduction from a gross sample to a laboratory sample.
16. Briefly describe what is meant by the term
    a. dissolution.
    b. extraction
    c. decomposition
    d. filtration
    e. chromatography
    f. spectroscopy
    g. electrochemistry
17. What is the purpose of a blank in a chemical measurement?
18. How are “spiked” samples and standard reference materials used in a chemical analysis?

19. Calibration with most techniques is accomplished by use of a working curve. Describe the typical working curve, the relationships that they define, and how they are used to extract quantitative information about a sample.
20. What is meant by a figure of merit (FOM)? Give two examples of FOMs.
21. Using a diagram and brief explanation, describe the flow of information within a typical instrument used for a chemical measurement.
22. What is the purpose of a device called a transducer?
23. Briefly describe each of the following terms, giving an example of each.
    a. signal generator
    b. input transducer
    c. signal modifier
    d. output transducer
24. What is the difference between an electrical and non-electrical domain?
25. What are the three types of electrical domains? Explain how they are different.
26. There are two predominant ways in which a signal generator in an analytical instrument functions. Describe each of these approaches.
27. How does an analog signal differ from a digital signal?

Figure Captions

Figure 1-1. Steps in a Method of Chemical Analysis.

Figure 1-2. Calibration Curve. Calibration in an analytical method is often accomplished by a linear plot of measured signal (abscissa - y-axis) versus concentration (ordinate - x-axis). The signal, subject to random error, is plotted on the abscissa and the concentration, assumed to be subject to no random error, is plotted on the ordinate.

Figure 1-3. (A) General Flow of Information in an Instrument and (B) Flow in a Simple Spectrophotometer. The Signal Generator consists of a light source, monochromator to isolate a "single" wavelength and cuvette containing liquid sample. The transmitted light is focused to a phototube detector which converts light power into an analog current. Signal modification occurs by current-to-voltage and other mathematical modifications. The modified signal is converted into sample absorption for signal readout.