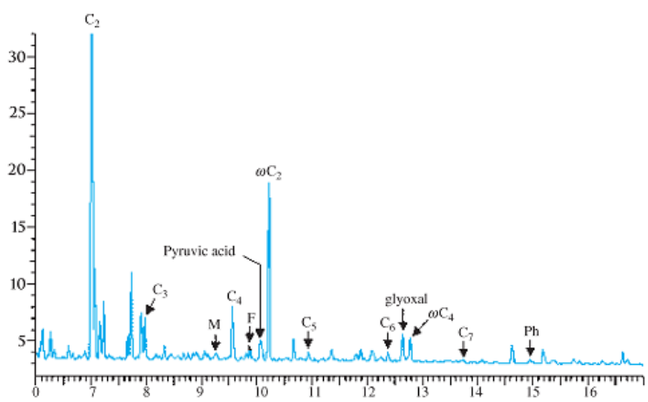
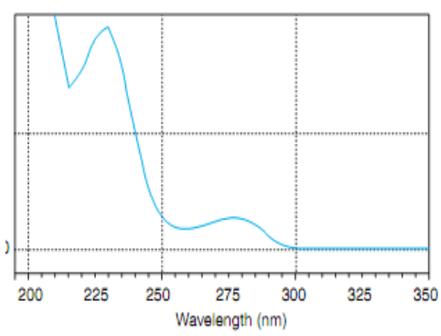
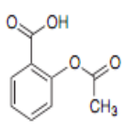
Aden University – Faculty of Science

Instrumental Chemical Analysis







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This course will address the fundamental principles and applications of instrumental analysis relevant for chemistry science students. The subject consists of a series of interrelated lectures and lab classes. The analytical techniques covered range from spectroscopy, chromatography, pH meter, as well as conductivity meter. The lecture components will address the underpinning physical principles of each analytical technique in detail. At the completion of the course, the student will have developed a firm understanding of the analytical methods employed in his or her field of study and also gained experience in carrying out analytical experiments.

1. INTRODUCTION

- Review about previous courses.

- Types of Analytical Methods.

- Instruments of Analysis.

Analytical chemistry is The Science of Chemical Measurements. It consists of a set of powerful ideas and methods that are useful in all fields of science and medicine.

Analyte: The compound or chemical species to be measured, separated, or studied.

Qualitative Analysis vs. Quantitative Analysis

— Qualitative analysis reveals the identity of the elements and compounds in a sample.

— Quantitative analysis indicates the amount of each substance in a sample.

Analytical chemistry is applied throughout the industry, medicine, and all the sciences.

Quantitative analytical measurements also play a vital role in chemistry, biochemistry, biology, geology, physics, and other sciences.

Many scientists devote much time in the laboratory gathering quantitative information about systems that are important and interesting to them.

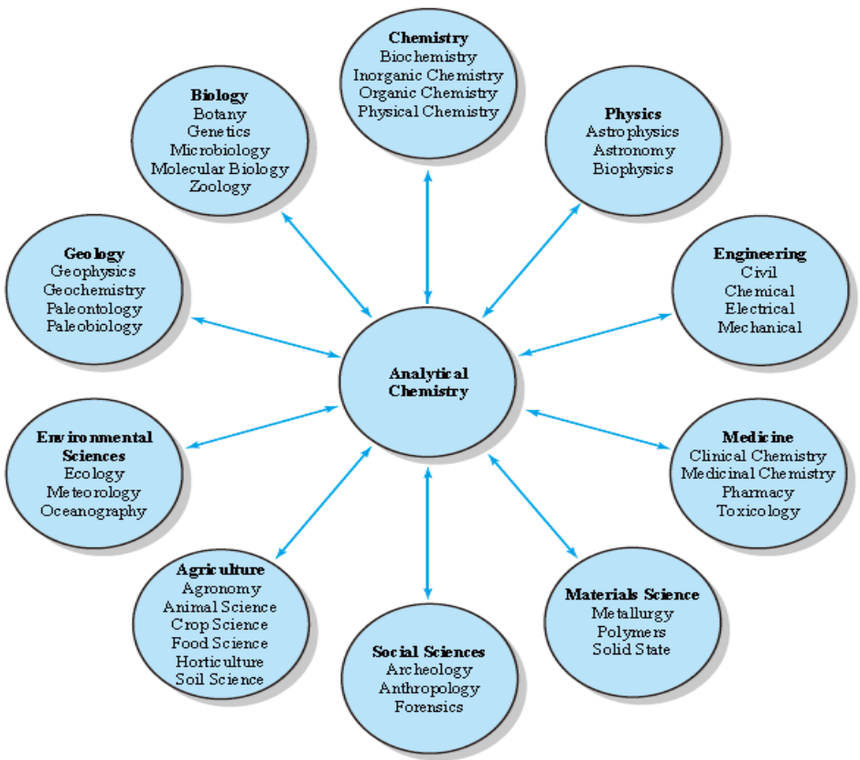


Figure 1-1 The relationship between analytical chemistry, other branches of chemistry, and the other sciences. The central location of analytical chemistry in the diagram signifies its importance and the breadth of its interactions with many other disciplines.

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.

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.

Background Terminology:

Before presenting the complete picture of an instrumental chemical analysis, it is important to distinguish the difference between an **analytical technique** and an **analytical method**.

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 spectrophotometer. 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.

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. 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**.

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.

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".

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.

It is also critical that an analyst understands the term **quality** as related to a chemical measurement. Quality is "the development, design, and supply of a product or service that is economical, useful, and always satisfactory to the customer."

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. **Quality assurance** is best considered as the proper management of the chemical analysis.

Classifying Quantitative Analytical Methods

The results of a typical quantitative analysis are computed from two measurements:

One is the mass or the volume of the sample to be analyzed.

The second is the measurement of some quantity that is proportional to the amount of analyte in the sample, such as mass, volume, intensity of light, or electrical charge.

— We classify analytical methods according to the nature of this final measurement.

1. *Gravimetric methods* determine the mass of the analyte or some compound chemically related to it.
2. *Volumetric methods* determine the volume of a solution containing sufficient reagent to react completely with the analyte.
3. *Electroanalytical methods* involve the measurement of such electrical properties as voltage, current, resistance, and quantity of electrical charge.
4. *Spectroscopic methods* are based on the measurement of the interaction between electromagnetic radiation and analyte atoms or molecules or on the production of such radiation by analytes.

— Miscellaneous methods:

1. mass-to-charge ratio
2. rate of radioactive decay
3. heat of reaction
4. rate of reaction
5. sample thermal conductivity
6. optical activity
7. refractive index

***Example methods*:**

Property	**Method Used**
Radiation emission	**Emission spectroscopy, fluorescence, phosphorescence, luminescence**
Radiation absorption	**Absorption spectroscopy spectrophotometry, photometry, nuclear magnetic resonance NMR**
Electrical potential	**Potentiometry**
Electrical charge	**Coulometry**
Electrical current	**Voltammetry - amperometry, polarography**
Electrical resistance	**Conductometry**
Thermal	**Thermal gravimetry, calorimetry**

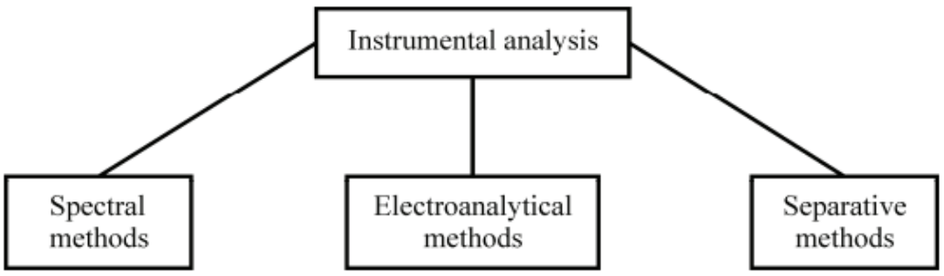


Figure. 1-2 The three major categories of instrumental methods of chemical analysis.

Flow Diagram Showing the Steps in a Quantitative Analysis

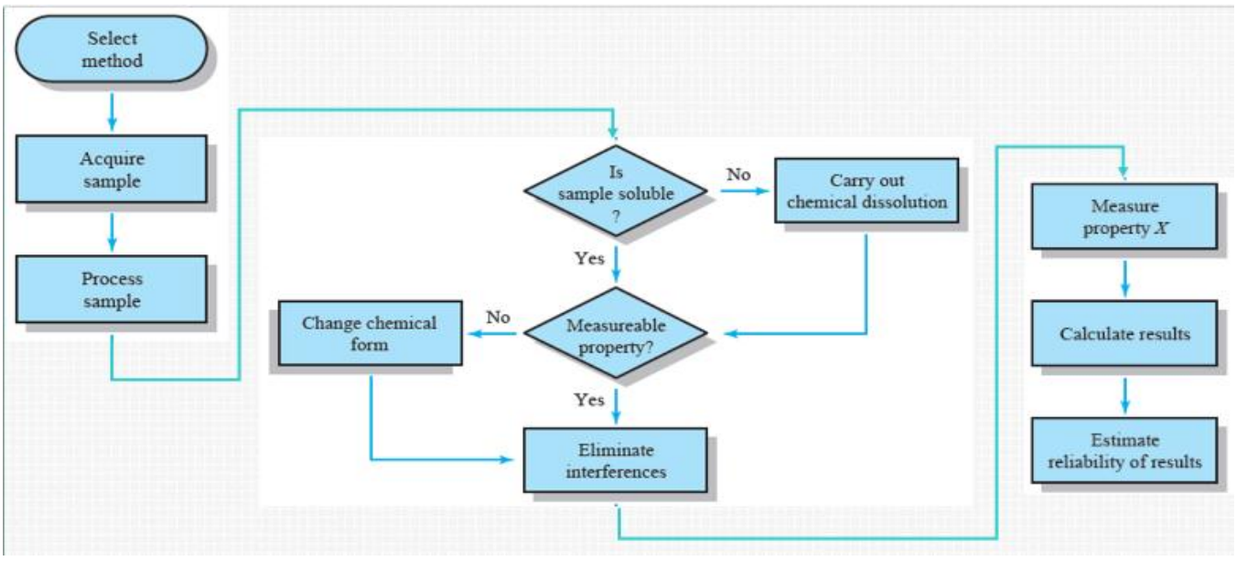


Figure 1-3 Flow diagram showing the steps in a quantitative analysis. There are a number of possible paths through the steps in a quantitative analysis. In the simplest example represented by the central vertical pathway, we select a method, acquire and process the sample, dissolve the sample in a suitable solvent, measure a property of the analyte, calculate the results, and estimate the reliability of the results. Depending on the complexity of the sample and the chosen method, various other pathways may be necessary.

Picking a Method (Choosing an Analytical Method)

- One of the first questions to be considered in the selection process is the level of accuracy required.
- A second consideration related to economic factors is the number of samples to be analyzed.
- The complexity of the sample and the number of components in the sample always influence the choice of method to some degree.

In other words, we have to ask ourselves about considered factors such as:

- What are the advantages or disadvantages of the technique versus other methods?
- How reproducible and accurate is the technique?
- How much or how little sample is required?
- How much or how little analyte can be detected?
- What types of samples can the method be used with?
- Will other components of the sample cause interference?

- Other factors: are speed, convenience, cost, availability, and skill required.

Acquiring the Sample

- *Sampling* involves obtaining a small mass of a material whose composition accurately represents the bulk of the material being sampled.
- Sampling is frequently the most difficult step in an analysis and the source of the greatest error. The final results of an analysis will never be any more reliable than the reliability of the sampling step.
- A material is *heterogeneous* if its constituent parts can be distinguished visually or with the aid of a microscope.
- An *essay* is the process of determining how much of a given sample is the material indicated by its name.
- We *analyze* samples and determine *substances.*

Processing the Sample

- Under certain circumstances, no sample processing is required prior to the measurement step.
- Under most circumstances, we must process the sample in any of a variety of different ways.
- The first step in processing the sample is often the preparation of a laboratory sample.
- Preparing a Laboratory Sample
  - A solid sample is ground to decrease particle size, mixed to ensure homogeneity, and stored for various lengths of time before analysis begins.
  - Because any loss or gain of water changes the chemical composition of solids, it is a good idea to dry samples just before starting an analysis.
  - Alternatively, the moisture content of the sample can be determined at the time of the analysis in a separate analytical procedure.
  - Liquid samples are subject to solvent evaporation.
  - If the analyte is a gas dissolved in a liquid, the analyte must be kept inside a second sealed container to prevent contamination by atmospheric gases.
  - Extraordinary measures, including sample manipulation and measurement in an inert atmosphere, may be required to preserve the integrity of the sample.
- Defining Replicate Samples
  - *Replicate samples*, or *replicates*, are portions of a material of approximately the same size that are carried through an analytical procedure at the same time and in the same way.
  - Replication improves the quality of the results and provides a measure of their reliability.
  - Quantitative measurements on replicates are usually averaged, and various statistical tests are performed on the results to establish their reliability.
- Preparing Solutions: Physical and Chemical Changes
  - Ideally, the solvent should dissolve the entire sample, including the analyte, rapidly and completely.
  - The sample may require heating with aqueous solutions of strong acids, strong bases, oxidizing agents, reducing agents, or some combination of such reagents.
  - It may be necessary to ignite the sample in air or oxygen or perform a high-temperature fusion of the sample in the presence of various fluxes.

Eliminating Interferences

- Few chemical or physical properties of importance in chemical analysis are unique to a single chemical species.

— Species other than the analyte that affect the final measurement are called *interferences*, or *interferents*.

— An *interference* is a species that causes an error in an analysis by enhancing or attenuating (making smaller) the quantity being measured.

— Techniques or reactions that work for only one analyte are said to be *specific.* Techniques or reactions that apply for only a few analytes are *selective.*

— The *matrix,* or *sample matrix,* is all of the components in the sample containing an analyte.

Remain Steps of a Typical Quantitative Analysis (Characteristics of an Analytical Methods)

— Calibration and Measurement

— Calculating Results

— Evaluating Results by Estimating Their Reliability.

Parameters used to Describe a Calibration Curve: $S = mc + S_{bl}$

S – measured signal

c – analyte concentration

S_{bl} – instrument signal for blank

Sensitivity: calibration sensitivity = slope (m) of the calibration curve.

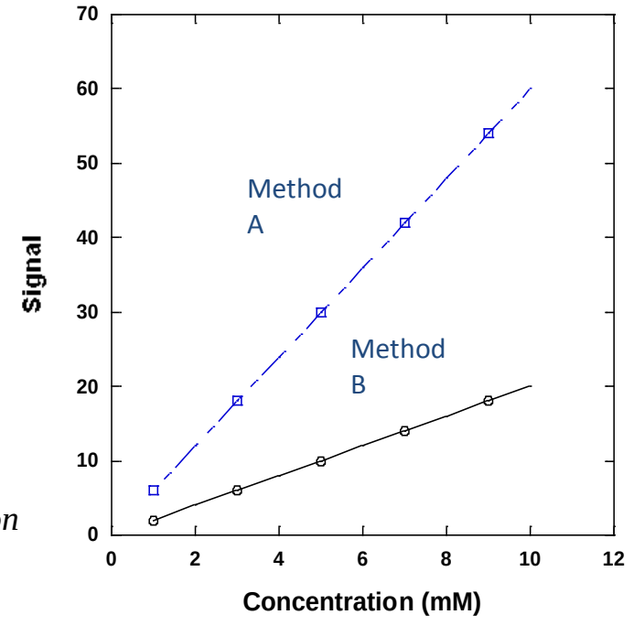
— analytical sensitivity (g) = slope (m)/standard deviation (S_s)

— ability to discriminate between small

— differences in analyte concentration.

— *Slope and reproducibility of the calibration*

— *curve.*



Selectivity: the degree to which the method is free from interference by other species.

Separative Methods

The separative methods take advantage of the physical or chemical properties of the components of a mixture to separate the components. After the separation, the components can be individually assayed either qualitatively or quantitatively.

Sometimes the separating instrument simultaneously performs the separation and the assay. In other cases, the separation is done prior to an assay by another method.

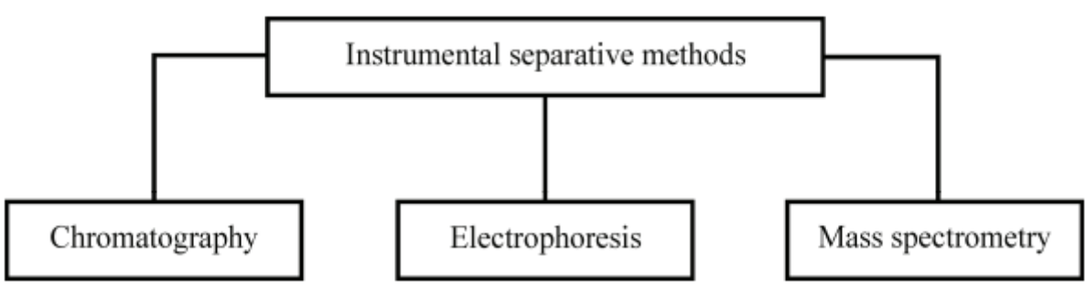


Figure 1-4: The major categories of separative methods of chemical analysis.

2. CHROMATOGRAPHY AS A SEPARATION TECHNIQUE

When two or more substances, that do not react chemically, are blended together, the result is a mixture in which each component retains its individual identity and properties. The separation of the components of a mixture is a problem frequently encountered in chemistry. The basis of the separation is the fact that each component has a different set of physical and chemical properties. The components are pure substances which are either elements or compounds. Under the same conditions of pressure and temperature, the properties of every sample of a pure substance are identical. Each sample melts at the same temperature, boils at the same temperature, has the same solubility in a given solvent, etc.

Physicochemical techniques useful for the separation of mixtures include filtration, decanting, centrifuging, sublimation, distillation/volatilization, oxidation-reduction, complex-ion formation, solvent extraction (liquid/liquid separation), precipitation and coprecipitation, electrochemistry, and *chromatography*.

Chromatography is a *physicochemical technique* for the separation of complex mixtures was discovered at the very beginning of the twentieth century by Russian–Italian botanist M. S. Tswett. When he separates plant pigments. Tswett called the new technique chromatography because the result of the analysis was 'written in color' along the length of the adsorbent column *Chroma* means “color” and *graphein* means to “write”. The components of a mixture are distributed between two phases, the *stationary* phase and the *mobile* phase. The mobile phase (**solvent** or **carrier gas**) moves through or over the surface of the fixed (stationary) phase. A solid surface on which the stationary phase is bound or coated is called a supporting medium. The stationary phase may be a solid, or a liquid supported on a solid or gel, and the mobile phase may be either a gas or a liquid. The components of the mixture have different affinities for each phase, hence some are retained longer on the stationary phase than others causing separation.

Chromatography is a technique used to *separate* and *identify* the components of a mixture. Chromatography has applications in every branch of the physical and biological sciences. The retention of a component is determined by the chemical and physical properties of the two phases and the experimental conditions (temperature and pressure). Therefore, pure reference materials must be chromatographed under the same conditions as the test samples. Following chromatography, the identity of the compounds must be substantiated by other analytical methods.

Classification

Classification of chromatography according to mobile phase:

1- Liquid chromatography: mobile phase is a liquid. (LLC, LSC).

2- Gas chromatography: mobile phase is a gas. (GSC, GLC).

Classification according to the packing of the stationary phase:

1- Thin layer chromatography (TLC): the stationary phase is a thin layer supported on glass, plastic, or aluminum plates.

2- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support.

3- Column chromatography (CC): stationary phase is packed in a glass column.

Classification according to the force of separation:

1- Adsorption chromatography.
2- Partition chromatography.
3- Ion exchange chromatography.
4- Gel filtration chromatography.
5- Affinity chromatography.

The most common chromatographic methods are:

1. Paper Chromatography
2. Thin Layer Chromatography (TLC)
3. High Performance Liquid Chromatography (HPLC)
4. Gas Liquid Chromatography (GLC).

Table 1 summarizes the common chromatographic methods.

Table 1: Classification of chromatographic methods

Method	**Symbol**	**Stationary Phase**	**Mobile Phase**	**Sorption Mechanism**	**Principal Applications**
Planar Chromatography					
Paper Chromatography	PC	Paper (cellulose)	Liquid	Partition	Analysis of mixtures
Thin Layer Chromatography	TLC	Silica, cellulose, ion exchange resin, controlled porosity solid	Liquid	Adsorption	Analysis of mixtures
Liquid Chromatography					
High performance liquid chromatography	HPLC	Solid or bonded phase	Liquid	Modified partition	Determination of nonvolatile compounds
Ion Exchange Chromatography Ion Chromatography	IEC IC	Ion exchange resin or bonded phase	Liquid	Ion Exchange	Determination of nonvolatile anions and cations.
Size Exclusion Chromatography Gel Permeation Chromatography Gel Filtration Chromatography	SEC GPC GFC	Controlled porosity solid such as silica or polymeric gel	Liquid	Exclusion	Determination of peptides, proteins and polymers
Chiral Chromatography	CC	Solid chiral selector or pre-column chiral reactions	Liquid	Selective adsorption	Separation and determination of chiral compounds
Gas Chromatography					
Gas Liquid Chromatography	GLC	Liquid phase on a wall or solid support	Gas	Partition	Determination of volatile compounds or gasses

Glossary

- ✓ **Chromatograph:** Instrument employed for a chromatography (it is equipment that enables a sophisticated Separation).
- ✓ **Chromatogram:** It is the visual output of the chromatograph.
- ✓ **Analyte (Sample):** It is the substance to be separated during chromatography.
- ✓ **Eluent:** Fluid entering a column (the solvent that will carry the analyte).

- ✓ **Eluate:** Fluid exiting the column (the mobile phase leaving the column).
- ✓ **Elution:** The process of passing the mobile phase through the column.
- ✓ **Flow rate:** How much mobile phase passed / minute (ml/min).
- ✓ **Retention time:** The characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.
- ✓ **Linear velocity:** Distance passed by mobile phase per 1 min in the column(cm/min).

Uses for Chromatography

Chromatography is used by scientists to:

- Analyze – examine a mixture, its components, and their relations to one another
- Identify – determine the identity of a mixture or components based on known components
- Purify – separate components in order to isolate one of interest for further study
- Quantify – determine the amount of the mixture and/or the components present in the sample.

Real-life examples of uses for chromatography:

J. Pharmaceutical Company
K. Hospital
L. Law Enforcement
M. Environmental Agency
N. Manufacturing Plant.

Planar Chromatography

Paper chromatography (PC) is a slightly porous paper (often filter paper) as the stationary phase, which is placed in a liquid mobile phase. The mobile phase is carried over the stationary phase by capillary action.

Thin layer chromatography (TCL) is the same method except the stationary phase is a thin layer of cellulose or silica gel coated onto a plastic or glass plate.

TLC is a useful technique because it is relatively quick and requires small quantities of material.

Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.

The stationary phase: is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.

The mobile phase: is a developing liquid which travels up the stationary phase, carrying the samples with it.

Components of the samples will separate on the stationary phase according to how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.

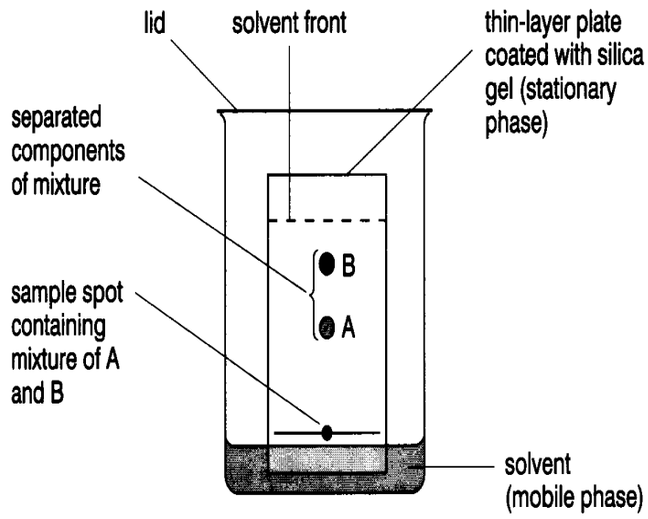
Steps:

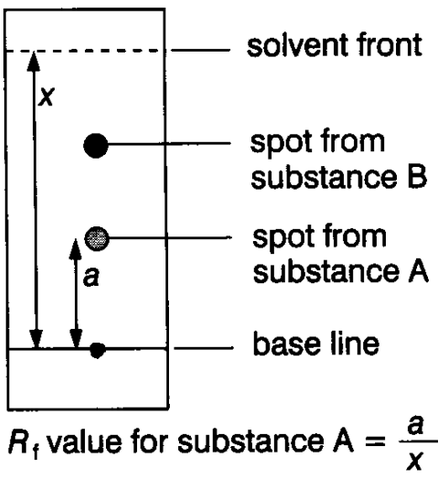
-Series of spots forms

-Compare samples in a mixture with known substances.

-Measure R_f values.

-Colored compounds & colorless compounds.





Preparing the Chamber

To a jar with a tight-fitting lid add enough of the appropriate developing liquid so that it is 0.5 to 1 cm deep in the bottom of the jar.

Close the jar tightly, and let it stand for about 30 minutes so that the atmosphere in the jar becomes saturated with solvent.

Preparing the Plates for Development

With a pencil, etch two small notches into the adsorbent about 2 cm from the bottom of the plate.

The notches should be on the edges of the plate, and each notch should be the same distance up from the bottom of the plate.

The notches must be farther from the bottom of the plate than the depth of the solvent in the jar.

Developing the Plates

After preparing the development chamber and spotting the samples, the plates are ready for development.

Be careful to handle the plates only by their edges, and try to leave the development chamber uncovered for as little time as possible.

When the plates are removed from the chamber, quickly trace the solvent front (the highest solvent level on the plate) with a pencil.

Identifying the Spots (visualization)

If the spots can be seen, outline them with a pencil.

If no spots are obvious, the most common visualization technique is to hold the plate under a UV lamp.

Many organic compounds can be seen using this technique, and many commercially made plates often contain a substance which aids in the visualization of compounds.

Visualizing Agents

Alkaloids: Dragendorff's reagent

Cardiac glycosides: Antimony trichloride

Sugar: Aniline phthalate

Amino acids: Ninhydrin.

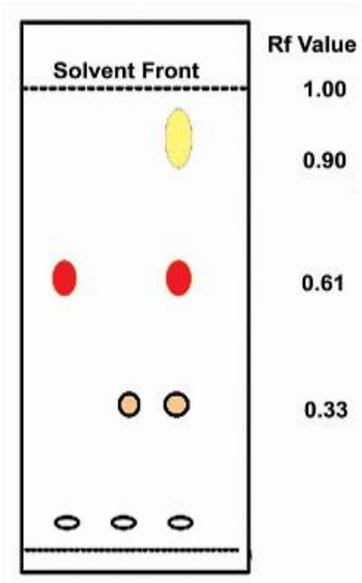
Interpreting the Data

The R_f (Retardation factor, Fraction of an analyte in the mobile phase of a chromatographic system) value for each spot should be calculated.

It is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates.

Hence, known R_f values can be compared to those of unknown substances to aid in their identifications.

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$



(Note: R_f values often depend on the temperature and the solvent used in the TLC experiment.

The most effective way to identify a compound is to spot known substances – authentic - next to unknown substances on the same plate.)

Comparing the R_f value of the sample to the standard allows identification.

In addition, the purity of a sample may be estimated from the chromatogram.

An impure sample will often develop as two or more spots, while a pure sample will show only one spot

Advanced TLC

Automated TLC is where solvent flow is forced by running the plate in a vacuum-capable chamber to dry the plate, and recording the finished chromatogram by absorption or fluorescence spectroscopy with a light source. The ability to program the solvent delivery makes it convenient to do multiple developments in which the solvent flows for a short period of time, the TLC plate is dried, and the process is repeated. This method refocuses the spots to achieve higher resolution than in a single run.

Two-dimensional TLC uses the TLC method twice to separate spots that are unresolved by only one solvent. After running a sample in one solvent, the TLC plate is removed, dried, rotated 90° and run in another solvent. Any of the spots from the first run that contain mixtures can now be separated. The finished chromatogram is a two-dimensional array of spots.

Column Chromatography

There are several chromatography methods that use a column to separate samples. Such methods are Liquid Chromatography (LC) and Gas Chromatography (GC).

Column: Tubing containing the stationary phase coated either on an inert solid support or on the column.

Liquid Chromatography

A mobile phase in this technique is a liquid solvent. Examples methods: Gel Filtration Chromatography (GFC), Size Exclusion Chromatography (SEC), Ion Exchange Chromatography (IEC), High Performance Liquid Chromatography (HPLC).

Gas Chromatography

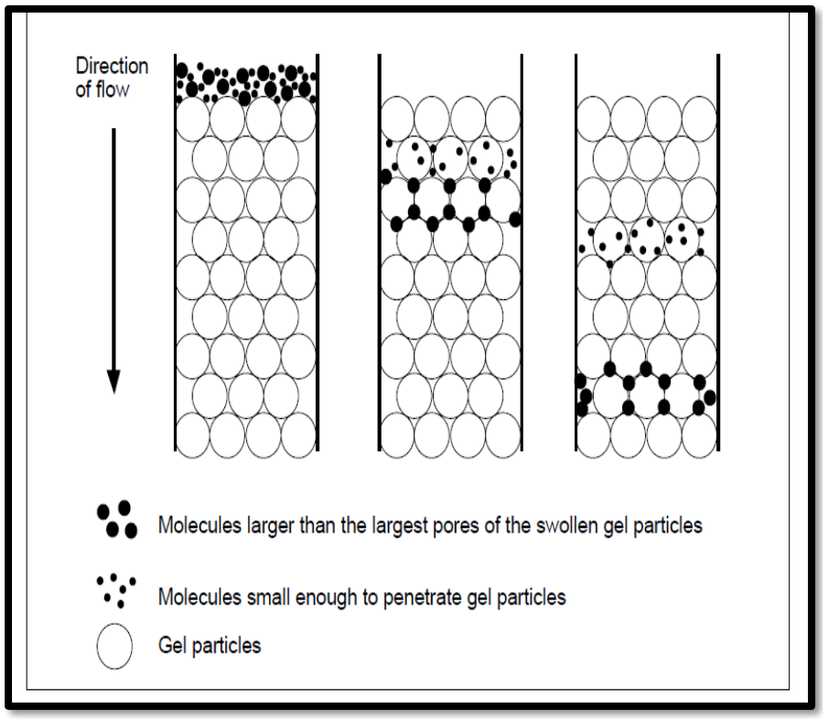
A mobile phase in this technique is a gas carrier. An example method is Gas Liquid Chromatography (GLC).

Gel Permeation Chromatography (GPC) & Gel Filtration Chromatography (GFC)

Gel permeation/filtration separates molecules according to the differences in size as they pass through the filtration medium packed in the column.

It is well suited for biomolecules that are sensitive to pH, concentration and harsh environment.

Parameters that affects gel filtration are, particle size, flow rate, packaging density, porosity of the particle and viscosity of the mobile phase.



Materials Required

Cross linked dextrans (sephadex)

Agarose (sepharose)

Polyacrylamide

Porous glass gel.

Applications

Fractionation (purification of the desired protein using suitable gel)

Molecular weight determination.

Ion Exchange Chromatography (IEC)

Ion exchange is the exchange of ions of like sign between a solution and a highly q insoluble solid body in contact with it. The solid (ion-exchanger) contains ions of its own and for the exchange to proceed sufficiently rapidly, the ion-exchanger must have an open, permeable structure so that both ions and solvent molecules can move freely in and out.

Ion exchange chromatography is used to remove ions of one type from a mixture and replace them by ions of another type.

The basic principle is reversible competitive binding.

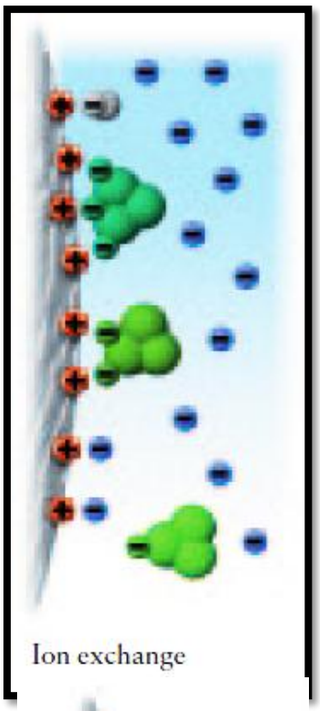
Ion Exchangers

— Cation exchangers (negative ions – stationary)

— Anion exchangers (positive ions - stationary)

Four types of polymers are commonly used. They are,

- Synthetic hydrophobic polymer resins crosslinked with divinylbenzene.
- Naturally occurring as well as synthetic polymers (cellulose)
- Synthetic hydrophilic polymers
- Silica gel.



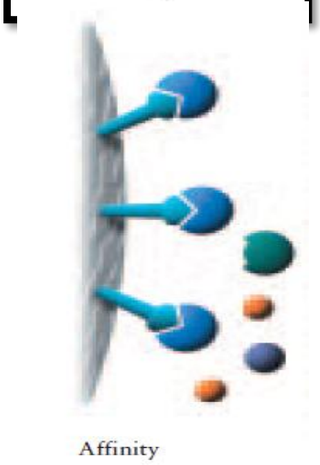
Ion exchange

Ion/Affinity Chromatography

This is a form of ion exchange chromatography for the separation of inorganic and some organic cations and anions with conductometric detection after suppressing (removing) the mobile phase electrolyte.

Affinity chromatography includes bioaffinity, dye-ligand affinity and immobilized metal ion affinity techniques.

It is based on the formation of the specific and reversible complexes between a pair of biomolecules.



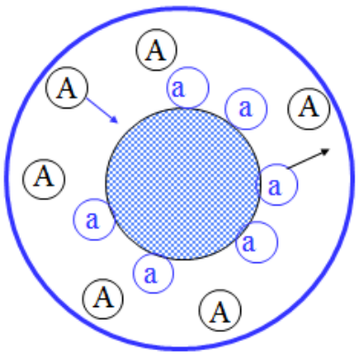
Affinity

3. GAS CHROMATOGRAPHY (GC)

Gas chromatography is a technique used for the separation of *volatile organic substances, or substances that can be made volatile* (by suitable derivatization methods or pyrolysis), from one another in a gaseous mixture at high temperatures. Thus, about 20% of chemicals available can be analyzed directly by GC. A sample containing the materials to be separated is injected into the gas chromatograph. A mobile phase (carrier gas) moves through a column that contains a wall-coated or granular solid-coated stationary phase. As the carrier gas flows through the column, the components of the sample come in contact with the stationary phase. The different components of the sample have different affinities for the stationary phase, which results in differential migration of solutes, thus leading to separation.

Principle

The organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column.
Chromatography is based on a physical equilibrium that results when a solute is transferred between the mobile and a stationary phase.

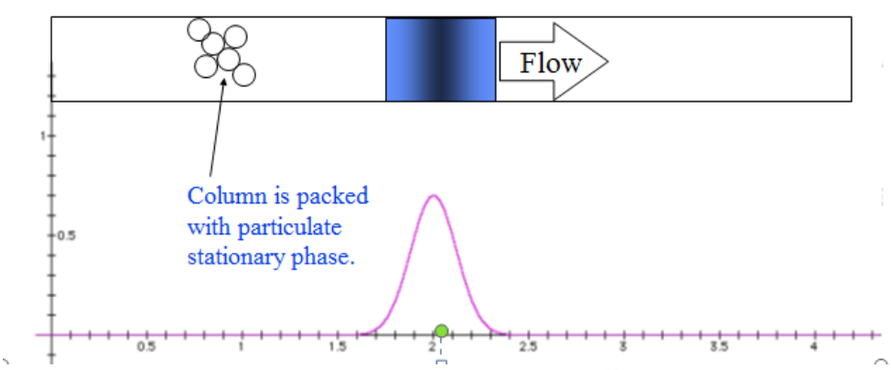


Cross Section of Equilibrium in a column.
"a" are adsorbed to the stationary phase.
"A" are traveling in the mobile phase.

$$K=\frac{C_S}{C_M}$$

K = distribution coefficient *or* partition ratio
Where C_S is the molar concentration of the solute in the stationary phase and C_M is the molar concentration in the mobile phase.

In a chromatography column, flowing gas or liquid continuously replaces the saturated mobile phase and results in the movement of A through the column.

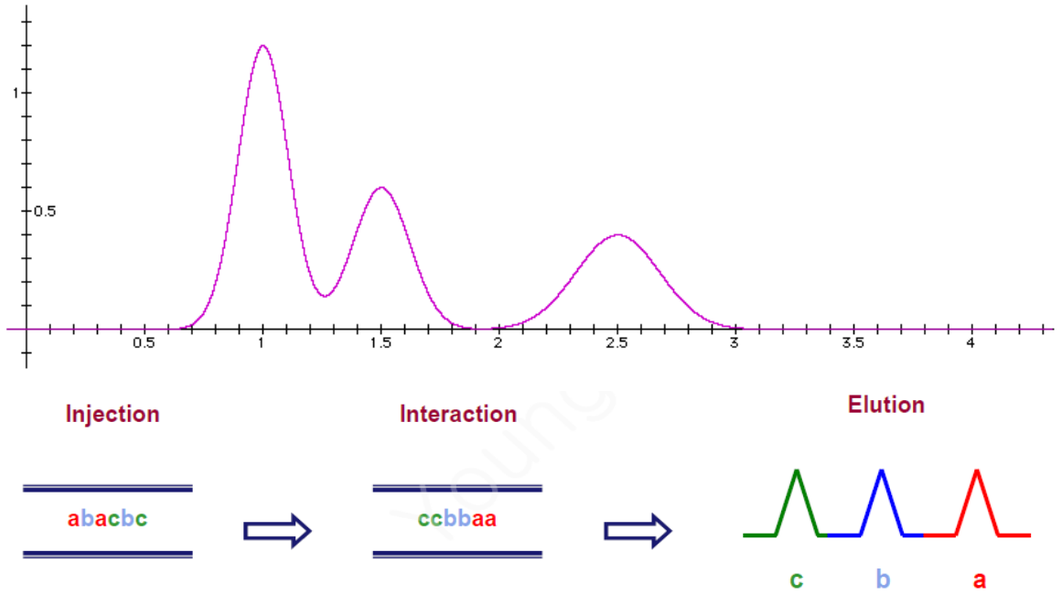


As a material travels through the column, it assumes a Gaussian concentration profile as it distributes between the stationary packing phase and the flowing mobile gas or liquid carrier phase.

In a mixture, each component has a different distribution coefficient, and thus spends a different amount of time absorbed on the solid packing phase vs being carried along with the flowing gas

More volatile materials are *carried* through the column *more rapidly* than less volatile materials, which results in a separation.

If a detector is used to determine when the components elute from the column, a series of Gaussian peaks are obtained, one for each component in the mixture that was separated by the column.

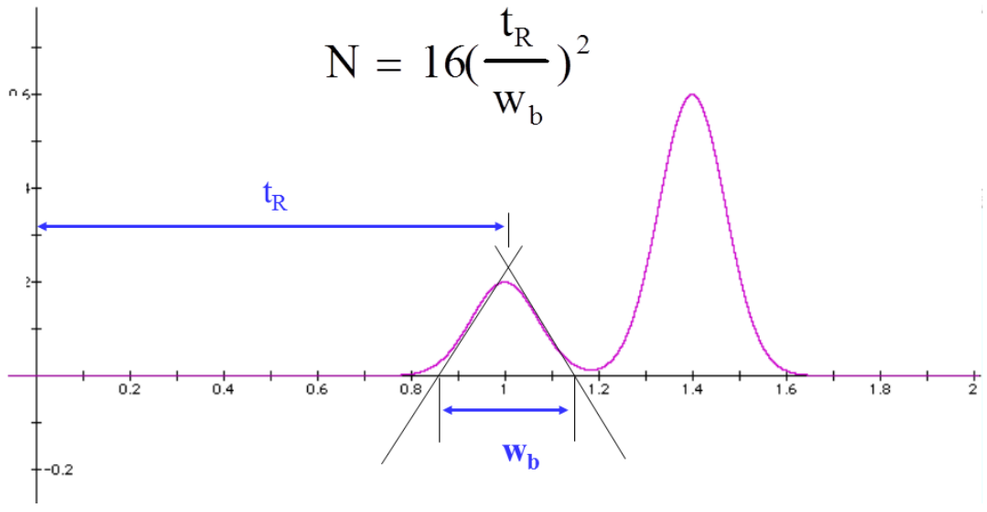


The Theoretical Plate

Theoretical plate is a term coined by Martin & Synge. It is based on a study in which they imagined that chromatographic columns were analogous to distillation columns and made up of numerous discrete but connected narrow layers or plates. Movement of the solute down the column then could be treated as a stepwise transfer.

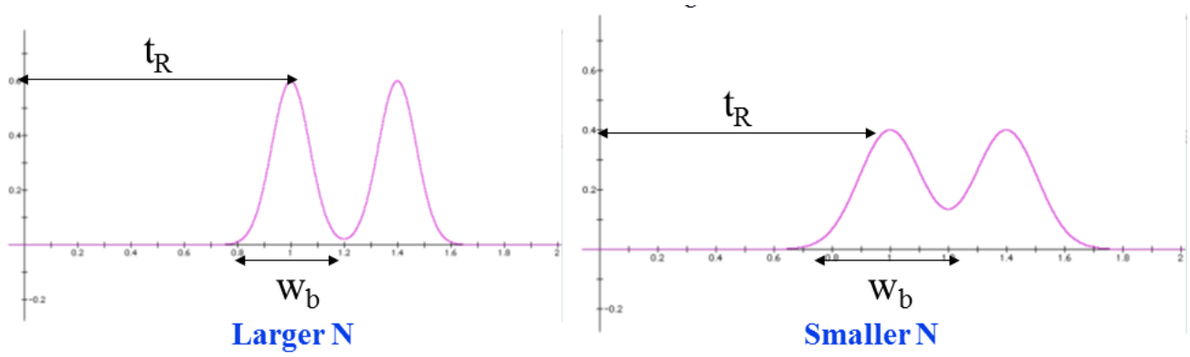
Theoretical plates (N) measure **how efficiently a column can separate a mixture into its components**. This efficiency is based on the retention time of the components and the width of the peaks.

N = Number of theoretical plates *(a measure of efficiency)*



t_R is the retention time; it is measured from the injection peak (or zero) to the intersection of the tangents.

w_b is the width of the base of the triangle; it is measured at the intersection of the tangents with the baseline.

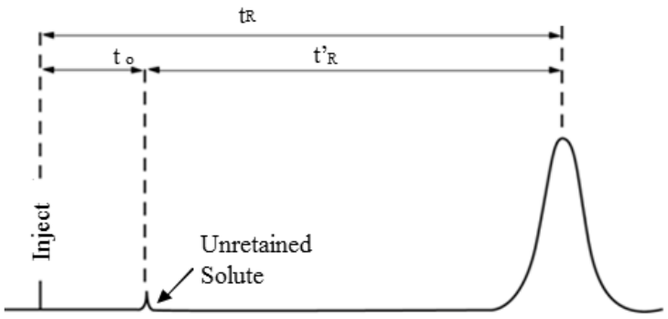


When the retention time, t_R, is held constant, the column that produces peaks with narrower bases, w_b, will be more efficient – have a greater N value.
Likewise, a column that produces wider peaks will be less efficient – have a smaller N value.
This is because a smaller denominator, w_b, will yield a larger overall number and a larger denominator will yield a smaller number.

Capacity Factor (k')

While inside the column, a retained component spends part of its time on the stationary phase and part-time in the mobile phase

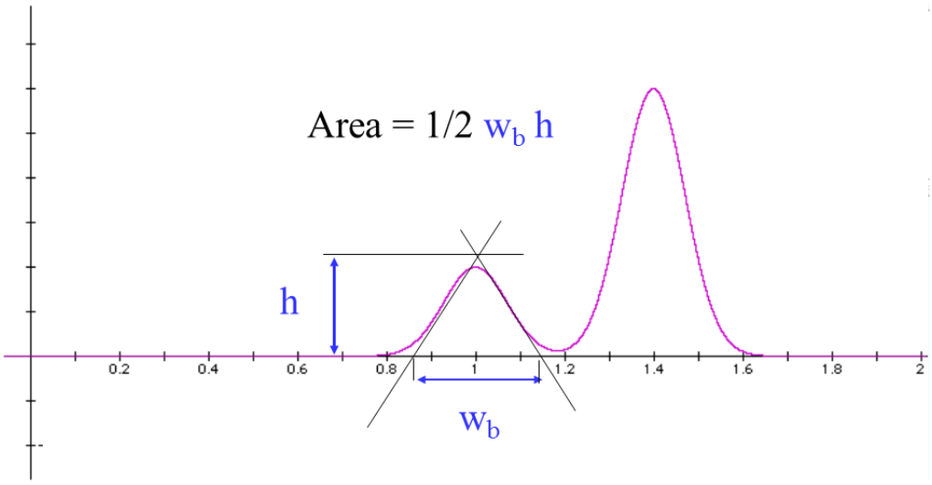
- When in the mobile phase, solutes move at the same speed as in the mobile phase
- this means that all solutes spend the same amount of time in the mobile phase (t_o)
- the amount of time the solute spends on the stationary phase is equal to t_R-t_o(adjusted retention time, t'_R)
- the ratio t'_R / t_o is the capacity of the column to retain the solute (k')



$k' = (t_r - t_0) / t_0$
$k' = (t'_r) / t_0$

Determination of the Amount of Sample Components Present

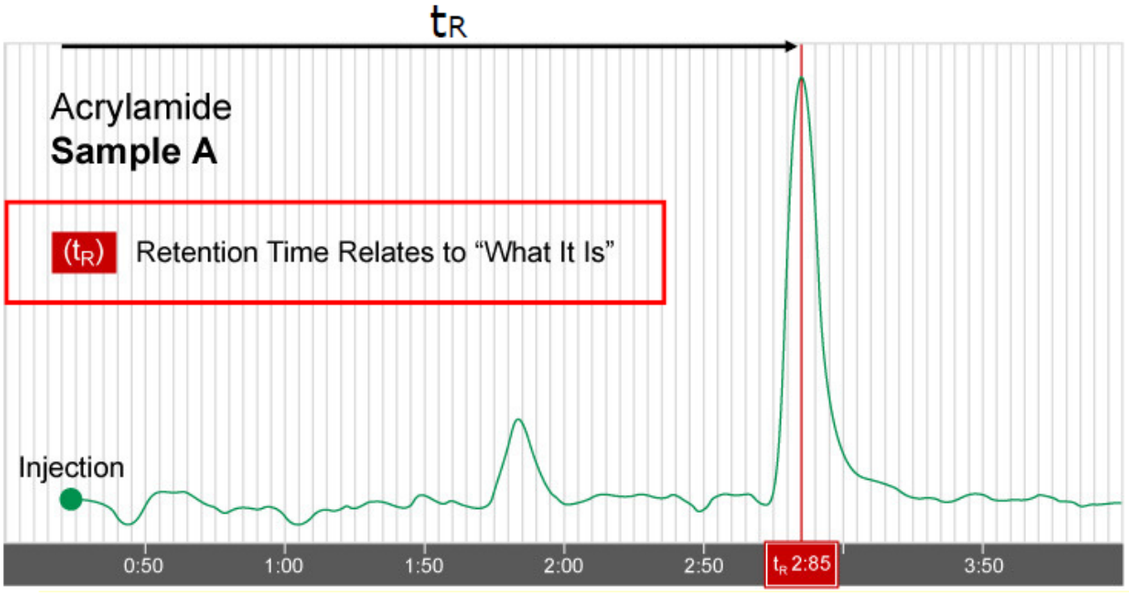
The peak height is proportional to the amount of material eluting from the column at any given time; The area under the peak is a measure of the total amount of material that has eluted from the column.
Electronic integrators are used for area measurement in commercial GCs. We will be using ALGEBRA.
The Gaussian curve can be approximated as triangular in shape, to simplify area measurement.



NOTE: the height is measured to the top of the tangents, which is above the actual curve peak.

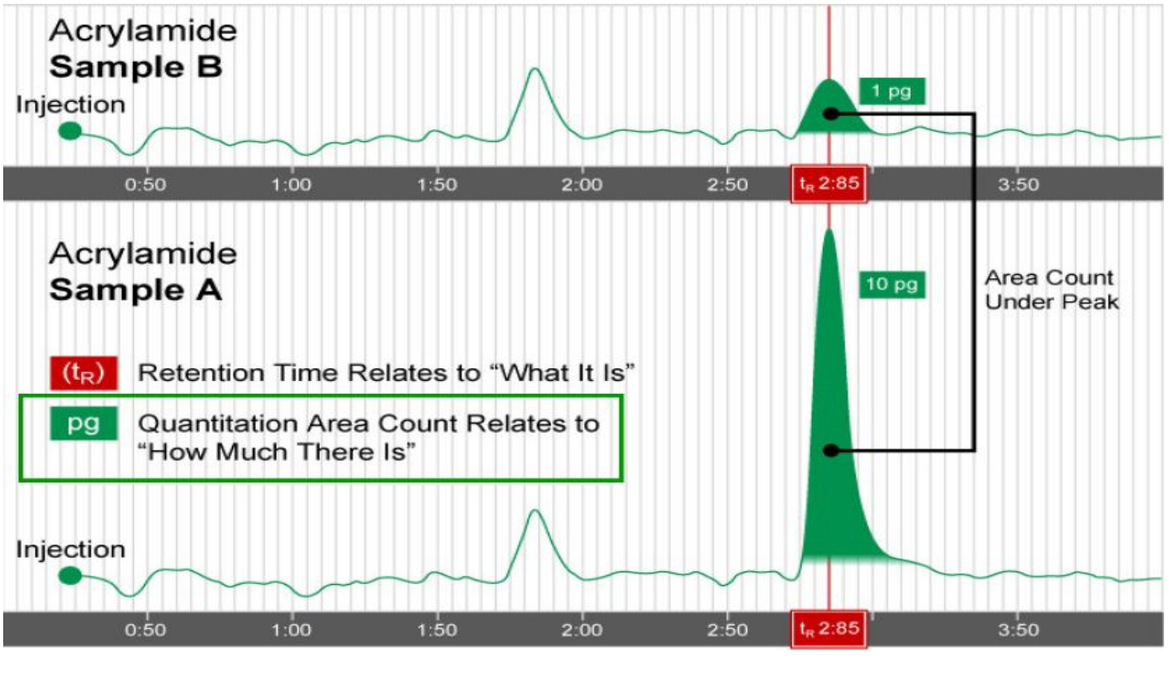
Identification and Quantitation

Compound Identification Based on Retention Time

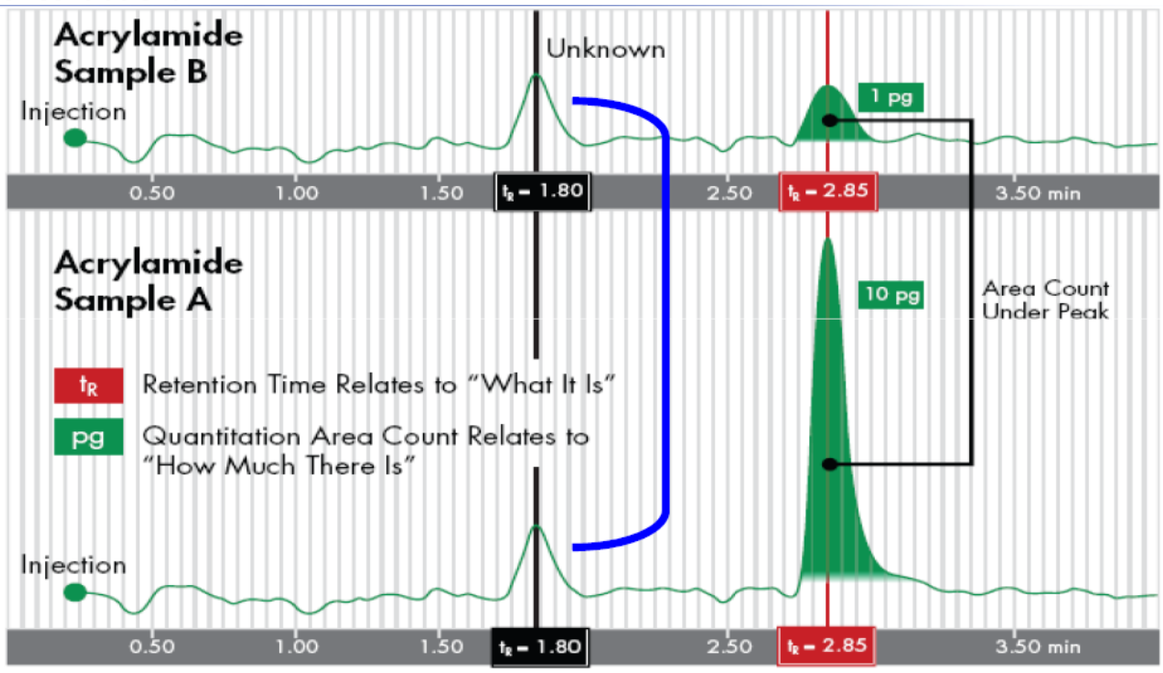


For a given mobile phase, at a given flow rate with a given column, a known pure standard of acrylamide elutes at 2.85 minutes.

Whenever a real sample is injected that contains acrylamide, you will see a peak at 2.85 minutes.



How much is present is measured by the AREA under the peak, which is related to how much was there? Both samples contain acrylamide, however, Sample B has only 1/10 the concentration.



Both samples have ~ SAME amount of this unknown compound.

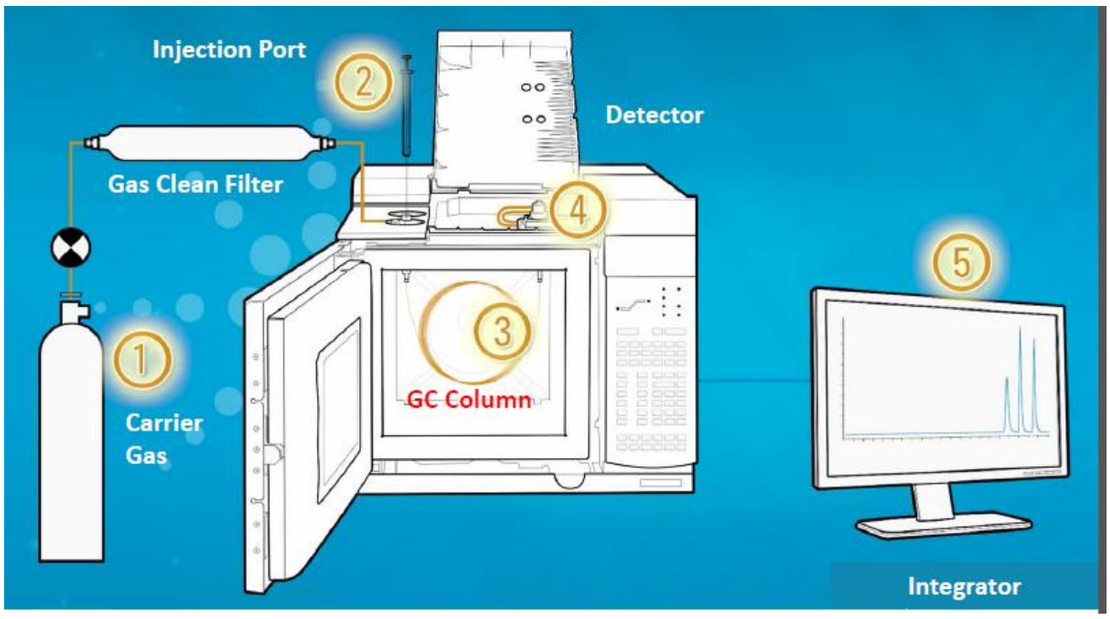
Use of GC:

- Good for volatile samples (up to about 250 °C)
- 0.1-1.0 microliter of liquid or 1-10 ml vapor
- Can detect <1 ppm with certain detectors
- Can be easily automated for injection and data analysis

The Basic Components of a GC System

GC System consists of

— gas supply: (usually N_2 or He) a flowing mobile phase.
— sample injector (an injection port): syringe / septum.
— a separation column (the stationary phase) Column: 1/8" or 1/4" x 6-50' tubing packed with small uniform size, inert support coated with thin film of nonvolatile liquid.
— an oven (the column temperature controller). the temperature of which can be varied or programmed.
— a detector: TC - thermal conductivity, FID - flame ionization detector (it should be noted that a detector will require special gas cylinders depending on the detector type utilized).
— integrator.



Types of GC:

1) Gas - Solid Chromatography (GSC)

The stationary phase, in this case, is a solid like silica or alumina. It is the affinity of solutes towards adsorption onto the stationary phase which determines, in part, the retention time. The mobile phase is, of course, a suitable carrier gas. This gas chromatographic technique is most useful for the separation and analysis of gases like CH_4, CO_2, CO, ... etc. The use of GSC in practice is considered marginal when compared to gas liquid chromatography.

2) Gas - Liquid Chromatography (GLC)

The stationary phase is a liquid with very low volatility while the mobile phase is a suitable carrier gas. GLC is the most widely used technique for the separation of volatile species. The presence of a wide variety of stationary phases with contrasting selectivities and easy column preparation adds to the assets of GLC or simply GC.

GLC Analysis Steps:

1. **Sample:** introduced by syringe.
2. **Column:** separates components, (heated in the oven).
3. **Detector**: monitors compounds emerging from the outlet.
4. **Recorder** : plots signals as a chromatogram.

A carrier gas should have the following properties:

1. Highly pure (> 99.9%)
2. Inert so that no reaction with the stationary phase or instrumental components can take place, especially at high temperatures.
3. A higher density (larger viscosity) carrier gas is preferred.
4. Compatible with the detector since some detectors require the use of a specific carrier gas.
5. A cheap and available carrier gas is an advantage.

Column Configurations and Ovens

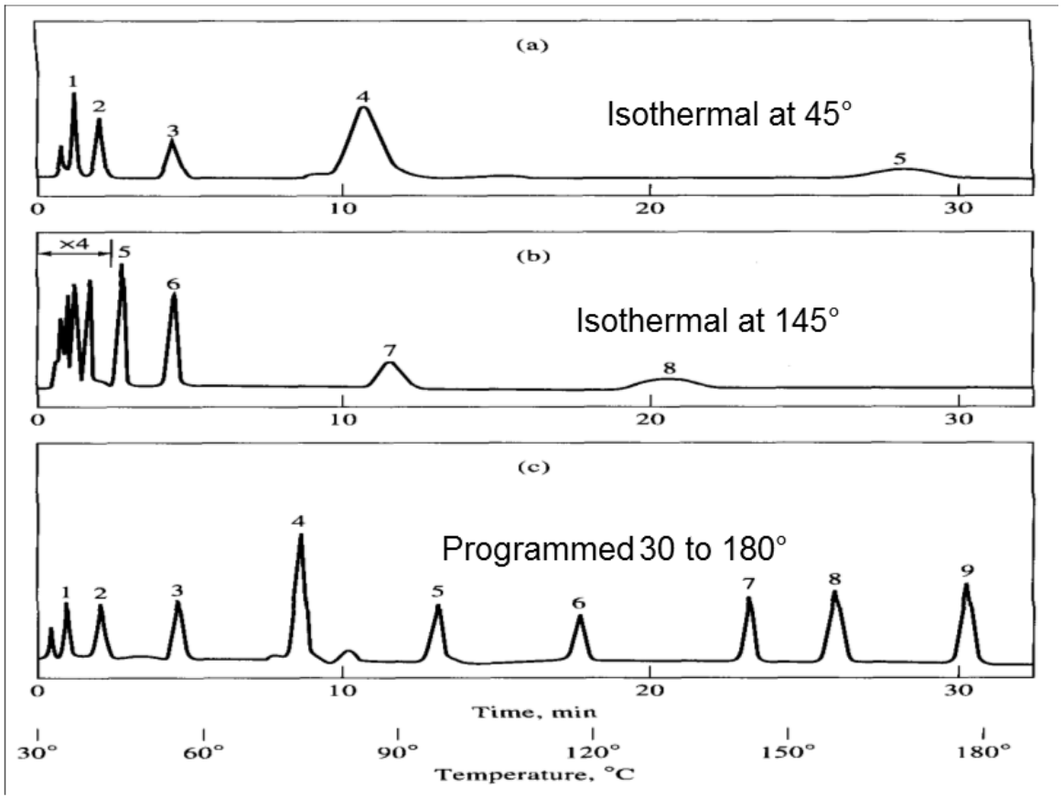
The column in chromatography is undoubtedly the heart of the technique. A column can either be a packed or open tubular. Traditionally, packed columns were the most common but fast developments in open tubular techniques and reported advantages in terms of efficiency and speed may make open tubular columns the best choice in the near future.

Packed columns are relatively short (~2 meters) while open tubular columns may be as long as 30-100 meters

Packed columns are made of stainless steel or glass while open **tubular columns** are usually made of fused silica. The temperature of the column is adjusted so that it is close to the average boiling point of the sample mixture. However, temperature programming is used very often to achieve better separations. The temperature of the column is assumed to be the same as the oven which houses the column. The oven temperature should be stable and easily changed in order to obtain reproducible results.

Temperature Programming

Gas chromatographs are usually capable of performing what is known as temperature programming gas chromatography (TPGC). The temperature of the column is changed according to a preset temperature isotherm. TPGC is a very important procedure, which is used for the attainment of excellent-looking chromatograms in the least time possible. For example, assume a chromatogram obtained using isothermal GC at 80 °C, as shown below:



Experiments:

1. **Test run of CH_2Cl_2** without sensor check for visible color, reasonable width and retention time on a column.
2. **Run of Pure Compounds**: (*1 good run of each*)
   CH_2Cl_2 (*Dichloromethane aka Methylene Chloride*)
   $CHCl_3$ (*Chloroform*)
3. **Mixture**: CH_2Cl_2:$CHCl_3$ (2:3 mix)

Collect voltage vs time data and also note visual onset and disappearance of green flame color.

Data and Calculations:

A. Graphs (3) from computer

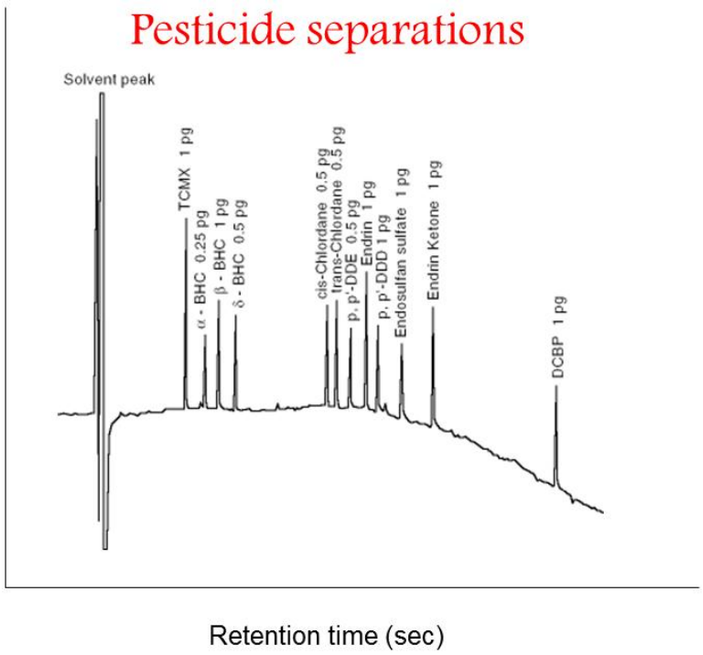
1. Elution data for pure CH_2Cl_2
2. Elution data for pure $CHCl_3$
3. Elution data for mixture

B. Calculations (handwritten, for each graph)

1. Peak Area = 1/2 (W x H)
2. Number of theoretical plates, $N = 16 (T_R / W_b)^2$

Applications

Analysis Pesticide: Analysis of pesticide residues in soil, water, and food is crucial for maintaining safe levels in the environment.
Sample: Pesticide calibration mix Detector mode: Electron capture Detector temp: 330°C Column: 25 m x 0.32 mm x 25 µm, HP-5 Column temp: 150°C to 300°C at 10°C/min Sample volume: 1 µL, 10:1 split Discharge gas: Helium, 30 mL/min Dopant gas: 5% methane in helium, 2.4 mL/min Attenuation: 1



Food Analysis: Analysis of foods is concerned with the assay of lipids, proteins, carbohydrates, preservatives, flavors, colorants and texture modifiers, and also vitamins, steroids, drugs and pesticide residues, and trace elements. Most of the components are non-volatile and although HPLC is now used routinely for much food analysis, GC is still frequently used. For example, derivatization of lipids and fatty acids to their methyl esters(FAMEs), of proteins by acid hydrolysis followed by esterification (N-propyl esters), and of carbohydrates by silylation to produce volatile samples suitable for GC analysis.

Food and Cancer: Chemicals that can cause cancer have a wide variety of molecular structures and include hydrocarbons, amines, certain drugs, some metals and even some substances occurring naturally in plants and molds. In this way, many nitrosamines have carcinogenic properties and these are produced in a number of ways such as cigarette smoke. GC can be used to identify these nitro-compounds in trace quantities.

Drugs: There are still numerous GC applications involving both quantitative and qualitative identification of the active components and possible contaminants, adulterants or characteristic features which may indicate the source of the particular sample. Forensic analysis frequently uses GC to characterize drugs of abuse, in some cases the

characteristic chromatographic fingerprint gives an indication of the source of manufacture of the sample or worldwide source of a vegetable material such as cannabis. Analytical procedures, chromatographic methods and retention data are published for over 600 drugs, poisons, and metabolites. These data are extremely useful for forensic work and in hospital pathology laboratories to assist the identification of drugs.

Environmental analysis: Environmental pollution is an age-old trademark of man and in recent years as technology has progressed, populations have increased and standards of living have improved. So the demands on the environment have increased, with all the attendant problems for the ecosystems. Combustion of fossil fuel, disposal of waste materials and products, and treatment of crops with pesticides and herbicides have all contributed to the problems. Technological developments have enabled man to study these problems and realize that even trace quantities of pollutants can have detrimental effects on health and on the stability of the environment. There is a vast amount of literature on the use of GC for studying a wide variety of these problems.

High-Performance Liquid Chromatography (HPLC)

HPLC is a column Chromatography technique in which:
- A cartridge or column is packed with a sorbent (stationary phase).
- A liquid (mobile phase) is passed through the packed column.
- A dissolved sample (in a liquid) is injected into the flow path of the mobile phase. (this is an “sample band”)
- The sample band separates into individual analyte bands as it passes through the HPLC column.
- Analytes bands are detected
- A chromatogram is generated; analyte bands are seen as “peaks”
- Peaks are quantitated.

1. Mobile Phase: Liquid
2. Stationary Phase — Separation Mechanism
   - Solid — Adsorption
   - Liquid Layer — Partition
   - Ion exchange resin — Ion exchange
   - Microporous beads — Size Exclusion
   - Chemically modified resin — Affinity

I have two separation techniques in my lab,High Performance Liquid Chromatography and Gas Chromatography. Which should I use?

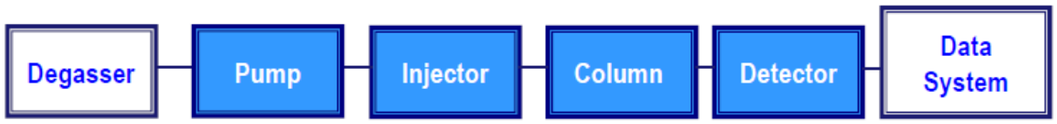
Comparison of HPLC and GC

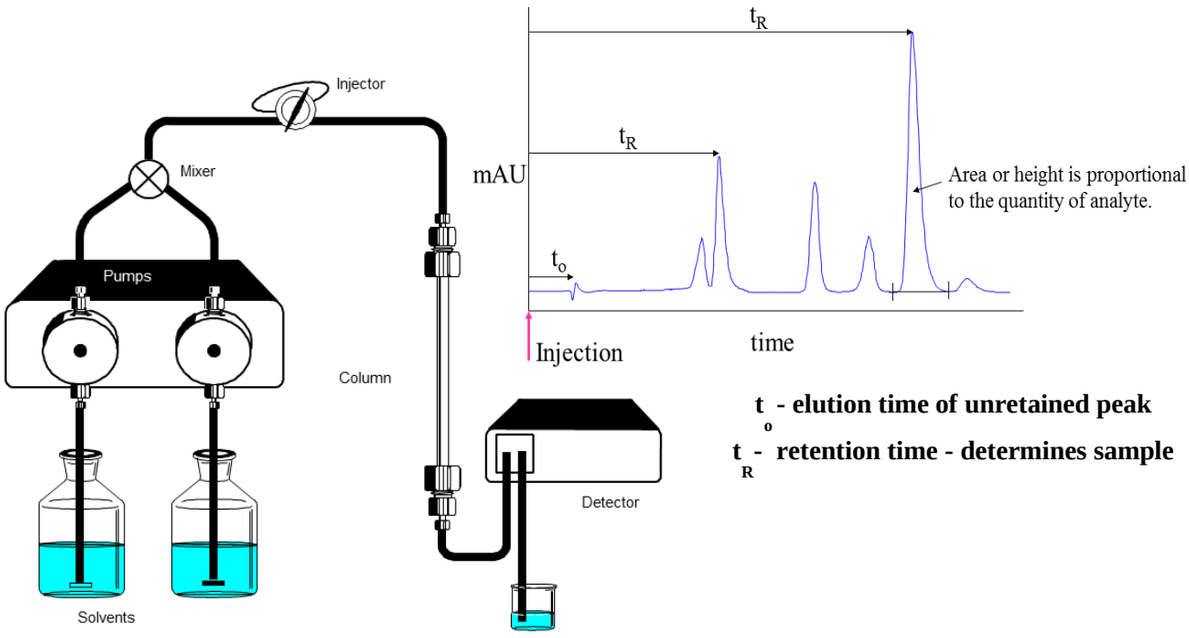
Property	HPLC	GC
Sample Volatility	-No volatility required -Sample must be soluble in mobile phase	-Sample must be volatile
Sample Polarity	-Separates both polar and nonpolar compounds -PAH ~ inorganic ions	-Samples are non-polar and polar
Sample Thermal Liability	-Analysis can take place at or below room temperature	-Sample must be able to survive high temperature injection port and column
Sample Molecular Weight	-No theoretical upper limit -In practicality, solubility is limit	-Typically < 500 amu
Sample Size	-Sample size based upon column I.D.	-Typically 1-5 uL

Sample Preparation	-Sample must be filtered -Sample should be in the same solvent as mobile phase	-Solvent must be volatile and generally lower boiling than analytes
Separation Mechanism	-Both stationary phase and mobile phase take part	- Mobile phase is a sample carrier only
Detectors	-Most common UV-Vis (Wide range of non-destructive detectors) -3-dimensional detectors - Sensitivity to pg	-Most common FID, universal to organic compounds

HPLC Instrumentation & Chromatogram

- Solvent Reservoirs , Pump , Sample Injector , Column(s) , Detector , Data System

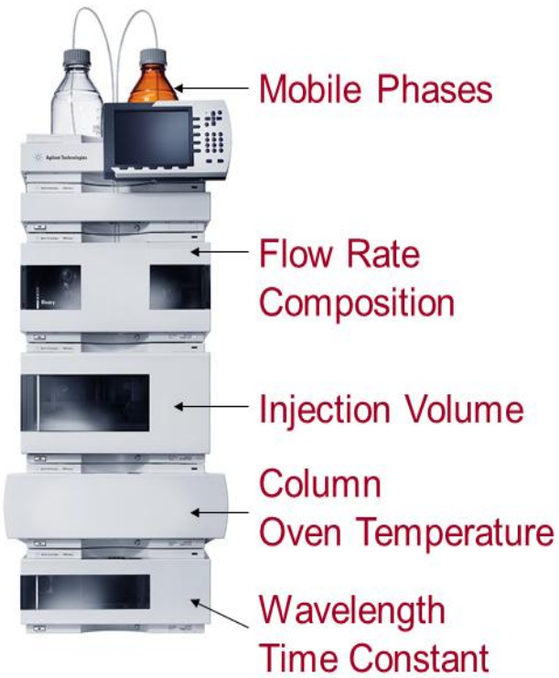


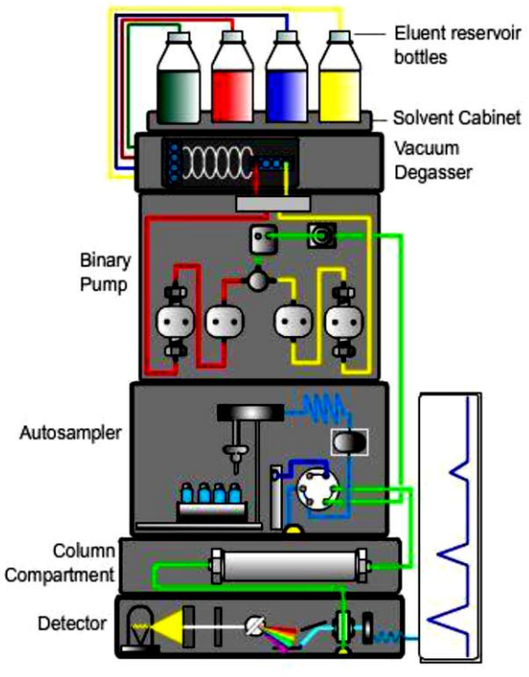


t_o - elution time of unretained peak

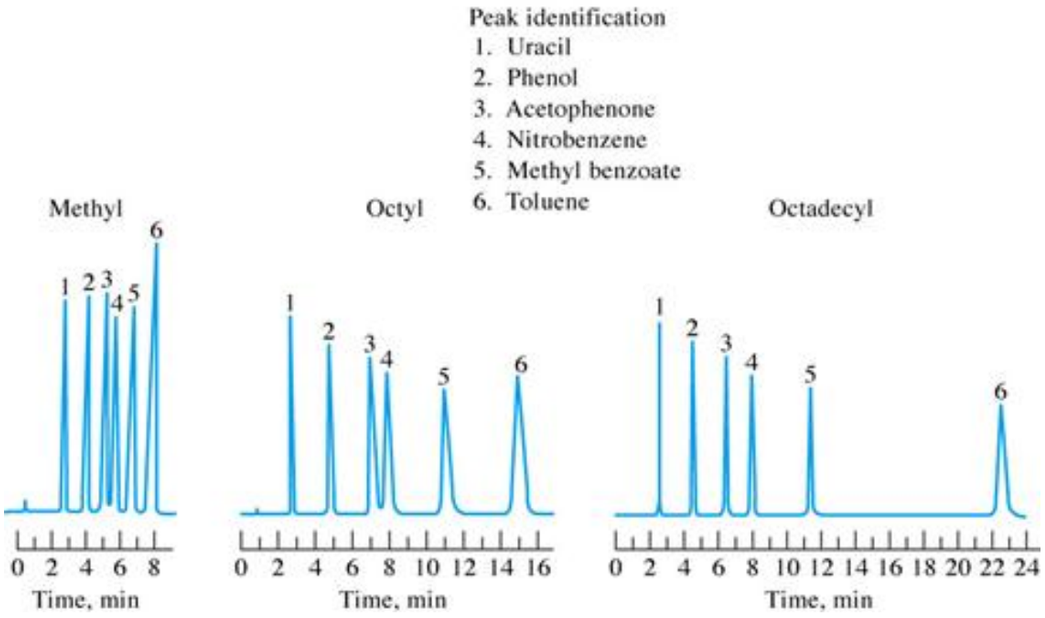
t_R - retention time - determines sample

Typical Configuration of HPLC





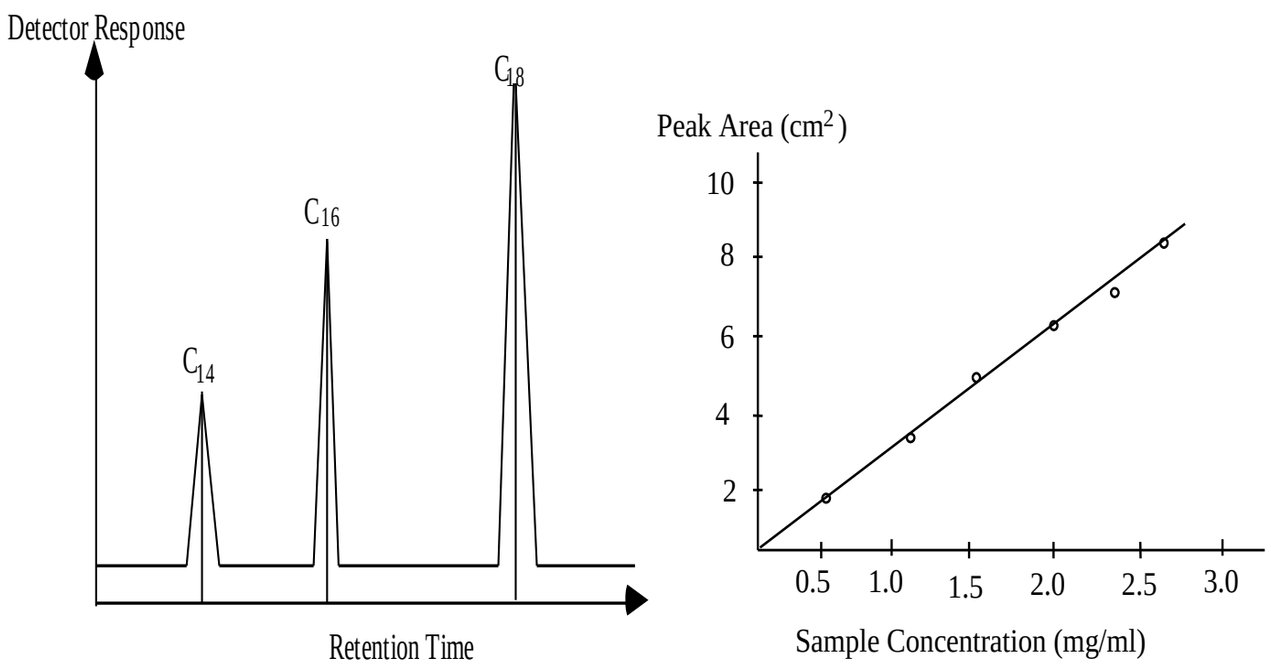
Chromatogram (An Example)



HPLC Applications

Chemical	Pharmaceuticals	Consumer Products
polystyrenes dyes phthalates	tetracyclines corticosteroids antidepressants barbiturates	lipids antioxidants sugars
Bioscience	**Environmental**	**Clinical**
proteins peptides nucleotides	polyaromatic hydrocarbons inorganic ions herbicides	amino acids vitamins homocysteine

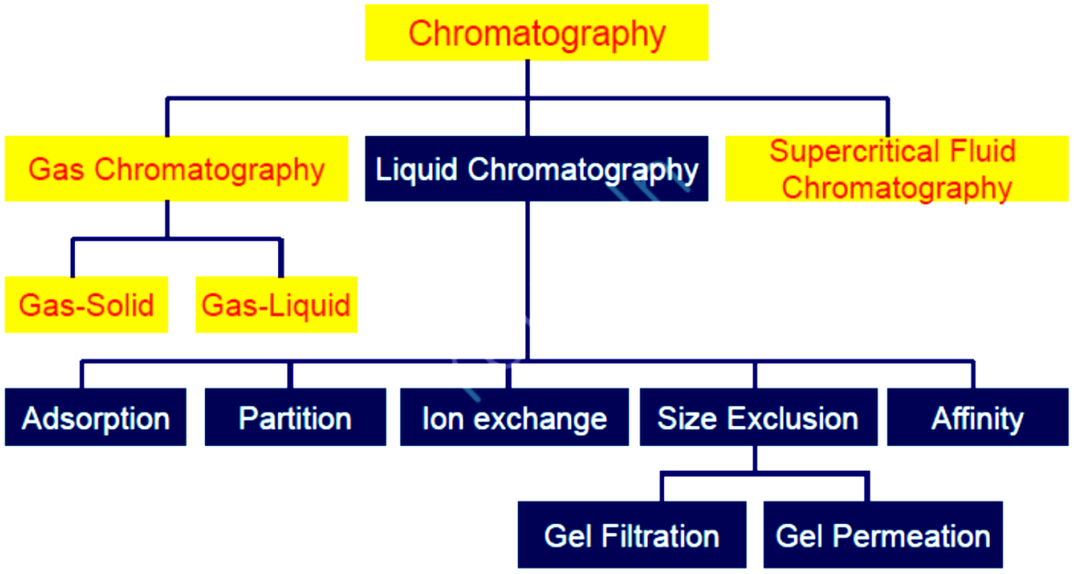
Semi- Quantitative Analysis of Fatty Acids



$$\text{The content \% of } C_{14} \text{ fatty acids} = \frac{C_{14}}{C_{14} + C_{16} + C_{18}} * 100$$

= the content % of C_{14} fatty acids

Chromatography Types Summary



4. SPECTROSCOPIC TECHNIQUES

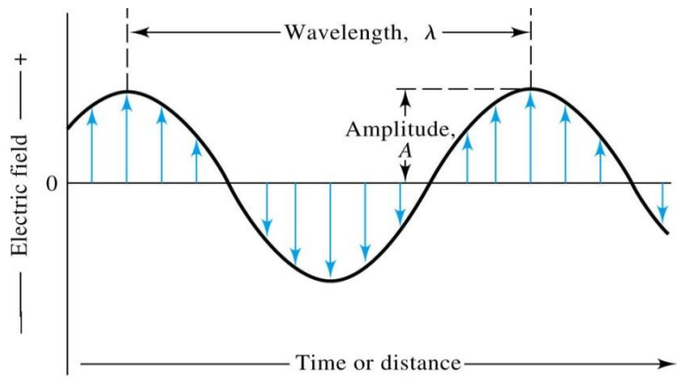
Measurements based on light and other forms of electromagnetic radiation (EMR) are widely used throughout analytical chemistry. The interactions of radiation and matter are the subject of the science called spectroscopy. Spectroscopic techniques employ *light* to interact with *matter* and thus probe certain features of a sample to learn about its consistency or structure. Spectroscopic analytical methods are based on measuring the amount of radiation produced or absorbed by *molecular* or *atomic* species of interest.
The useful interaction of matter may be represented by the excitation of atoms or molecules through their *electrons* or the induced *vibrations or rotation of functional groups* within the molecules. Practically all ranges of light found useful applications in analytical chemistry. The various spectral ranges of light require specific instrumentation for the generation or detection. The analytical signal comprises the measurement of the extent to which the incident radiant energy is absorbed by the chemical species.

Properties of ElectroMagnetic Radiation (Light)
Two different views of light: Wave Model & Particle Model.

1- Wave Model:

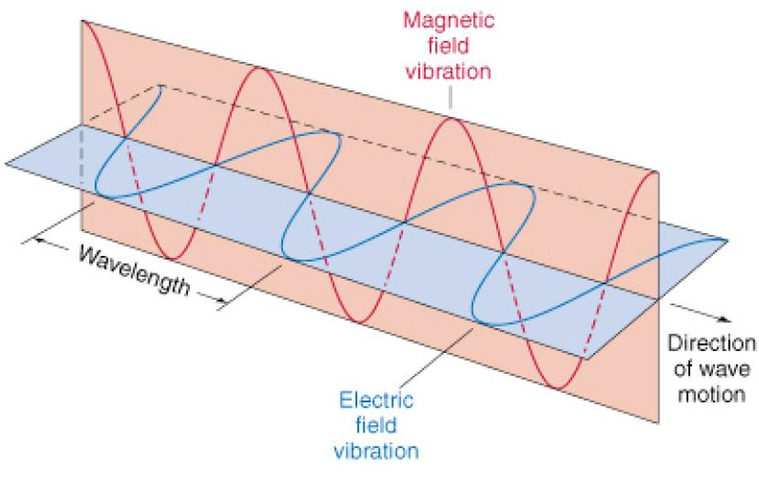
Represented by a sinusoidal wave traveling in space with an oscillating electric field and perpendicular magnetic field. (electric field is what is considered or used in most spectroscopic methods – except NMR)

- Description of wave model
  1) amplitude (A) – height of wave's electric vector



2) wavelength (l) – distance (nm, cm, m) from peak to peak

a) wave number ($\bar{\upsilon}$) = 1/ʎ (cm^{-1})



3) frequency (n) – number of cycles or oscillations per second

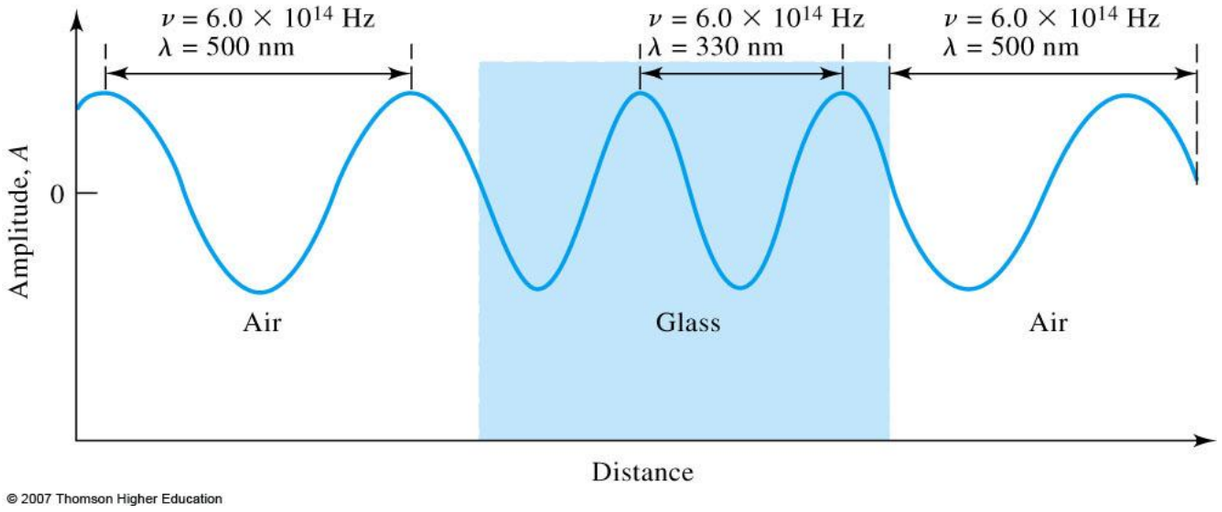
a) hertz (Hz) or s^{-1}.

4) velocity of propagation (v_i) – rate of travel through space, dependent on composition of medium

a) $v_i = \nu\lambda_i$

b) maximum velocity (c) – speed of light in a vacuum (3.00 $x10^8$ m/s)

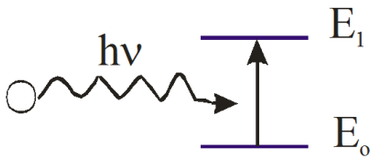
c) slower in other media (~ 0.03% slower in air)



2- Particle Model:

Light is viewed as discrete particles of energy called photons

a) like other particles, light can be scattered, counted (quantized) , etc



Energy required of photon to give this transition:
$\Delta E = E_1 - E_o$

- Energy of wave/particle:

$E = h\nu = hc/\lambda = hc\bar{\upsilon}$

h = Plank's constant (6.63×10^{-34} J·s)

ν = frequency, λ = wavelength, $\bar{\upsilon}$ =wave number

note:

energy is proportional to frequency and wave number (↑ ν ⇔↑ E)
energy is inversely proportional to wavelength (↑ λ ⇔↓ E)

Wide Range of Types of Electromagnetic Radiation in nature.

1. Only a small fraction (350-780 nm is visible light).
2. The complete variety of electromagnetic radiation is used throughout spectroscopy.
3. Different energies allow monitoring of different types of interactions with matter.

Spectrochemical Methods

Spectroscopy:A branch of science that studies the interaction between EM radiation and matter.

Spectrometry and Spectrometric methods :Measurement of the intensity of radiation with a photoelectric transducer or other types of electronic device.

We can classify spectroscopic methods according to the region of the electromagnetic spectrum used or produced in the measurement. The g-ray, X-ray, ultraviolet (UV), visible, infrared (IR), microwave, and radio-frequency (RF) regions have been used. Indeed, current usage extends the meaning of spectroscopy yet further to include techniques such as acoustic, mass, and electron spectroscopy in which electromagnetic radiation is not a part of the measurement. (see Figure).

Spectroscopy has played a vital role in the development of modern atomic theory. In addition, spectrochemical methods have provided perhaps the most widely used tools for the elucidation of molecular structure as well as the quantitative and qualitative determination of both inorganic and organic compounds.

In this part of course, we discuss the basic principles that are necessary to understand measurements made with electromagnetic radiation, particularly those dealing with the absorption of UV, visible, and IR radiation. The nature of electromagnetic radiation and its interactions with matter are stressed.

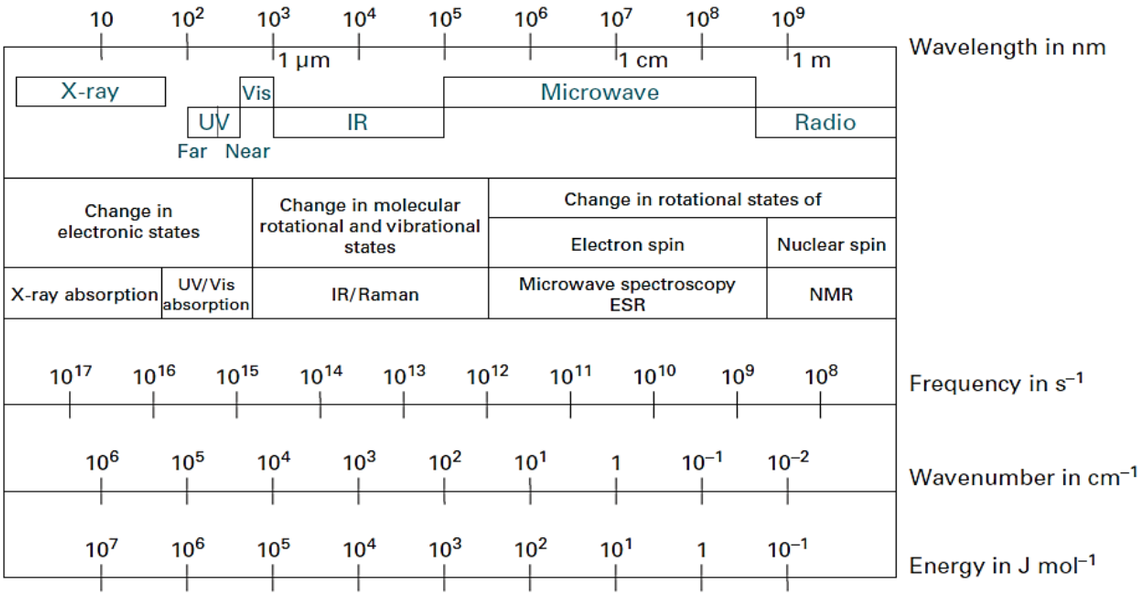


***Figure1**: The electromagnetic spectrum and its usage for spectroscopic methods.*

Molecular Analysis- Ultraviolet & Visible Light Spectroscopy

These regions of the electromagnetic spectrum and their associated techniques are probably the most widely used for analytical work and research into biological problems.

The electronic transitions in molecules can be classified according to the participating molecular orbitals (See Fig. 2). From the four possible transitions (n→p*, π→π*, n→σ*, σ→σ*), only two can be elicited with light from the UV/Vis spectrum for some biological molecules: n→π* and π→π*. The n→σ* and σ→σ* transitions are energetically not within the range of UV/Vis spectroscopy and require higher energies.

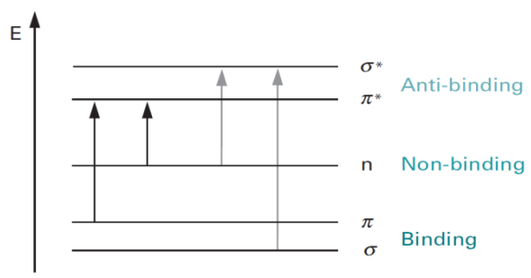


***Figure2**: Energy scheme for molecular orbitals (not to scale). Arrows indicate possible electronic transitions.*

The length of the arrows indicates the energy required to be put into the system in order to enable the transition. Black arrows depict transitions possible with energies from the UV/Vis spectrum for some biological molecules. The transitions shown by grey arrows require higher energies (e.g. X-rays).

Molecular (sub-)structures responsible for interaction with electromagnetic radiation are called chromophores. In proteins, there are three types of chromophores relevant for UV/Vis spectroscopy:

• peptide bonds (amide bond);
• certain amino acid side chains (mainly tryptophan and tyrosine); and
• certain prosthetic groups and coenzymes (e.g. porphyrin groups such as in haem).

The presence of several conjugated double bonds in organic molecules results in an extended π-system of electrons which lowers the energy of the π* orbital through electron delocalization. In many cases, such systems possess π→π* transitions in the UV/Vis range of the electromagnetic spectrum. Such molecules are very useful tools in colorimetric applications (see Table 1).

Table 1 Common colorimetric and UV absorption assays

Substance	Reagent	Wavelength (nm)
Amino acids	(a) Ninhydrin (b) Cupric salts	570 (proline : 420) 620
Protein	(a) Folin (phosphomolybdate, phosphotungstate, cupric salt)	660
	(b) Biuret (reacts with peptide bonds) (c) BCA reagent (bicinchoninic acid) (d) Coomassie Brilliant Blue (e) Direct	540 562 595 Tyr, Trp: 278, peptide bond : 190
Carbohydrate	(a) Phenol, H_2SO_4 (b) Anthrone (anthrone, H_2SO_4)	Glucose: 490, xylose: 480 620 or 625
Glucose	Glucose oxidase, peroxidase, o-dianisidine, phosphate buffer	420
DNA	(a) Diphenylamine (b) Direct	595 260
RNA	Bial (orcinol, ethanol, $FeCl_3$, HCl)	665
Cholesterol	Cholesterol oxidase, peroxidase, 4-aminoantipyrine, phenol	500

Principles

Quantification of light absorption

The chance for a photon to be absorbed by matter is given by an extinction coefficient which itself is dependent on the wavelength λ of the photon. If light with the intensity I_0 passes through a sample with appropriate transparency and the path length (thickness) *l*, the intensity I drops along the pathway in an exponential manner.

Biochemical samples usually comprise aqueous solutions, where the substance of interest is present at a molar concentration c. Algebraic transformation of the exponential correlation into an expression based on the decadic logarithm yields the law of Beer–Lambert.

Beer - Lambert Law

If we measure the intensity of the beam of light entering our sample (I_o) and compare it with the intensity of the beam of light exiting our sample (I) we can take the ratio I/I_o to get an indication of what fraction of the light entering the sample was found exiting the sample (see Fig.3). This ratio is called the Transmittance ($T=\frac{I_1}{I_0}$).

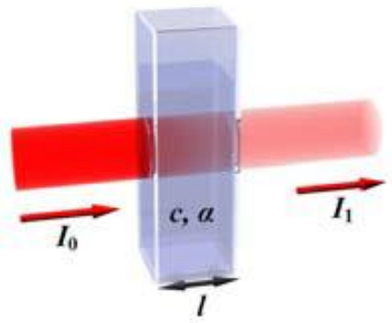


Figure3: *Transmittance in a sample solution (in cuvette)*

We can represent Beer - Lambert Law as

$$\log = \frac{I_0}{I_1} = \log \frac{1}{T} = -\log T = \varepsilon \times c \times l = A$$

where [l]=path light length (1cm), [c]=molar concentration (1mol.dm^{-3}), and ε is the *molar absorption coefficient* (also *molar extinction coefficient*) (α=2.303 x c x ε) [ε]=1dm^{3}mol^{-1}cm^{-1}. A is the absorbance of the sample, which is displayed on the spectrophotometer.

We can convert this ratio into a percentage by multiplying by 100 to get Percent Transmittance (%T); $\%T = \frac{I_1}{I_0} x100$.

The Beer–Lambert law is valid for low concentrations only. Higher concentrations might lead to association of molecules and therefore cause deviations from the ideal behavior. Absorbance and extinction coefficients are additive parameters, which complicates determination of concentrations in samples with more than one absorbing species. Note that in dispersive samples or suspensions scattering effects increase the absorbance, since the scattered light is not reaching the detector for readout.

The absorbance recorded by the spectrophotometer is thus overestimated and needs to be corrected.

The two main properties of an *absorbance peak* are:

- Absorption wavelength λ_{max}
- Absorption intensity A_{max}

The Problem

Unfortunately, in order to determine concentration of a species in solution, you need to know two key pieces of information:

1) At what wavelength does the chemical species of interest absorb electromagnetic radiation, and

2) What is the value of the molar absorptivity; ε ?

For investigating compounds in any sample, one should be first scanning the best wavelength that gives the highest absorbance. We need a spectrometer to produce a variety of wavelengths because different compounds absorb best at different wavelengths. For example, *p*-nitrophenol (acid form) has the maximum absorbance at approximately 320 nm and *p*-nitrophenolate (basic form) absorbs best at 400nm, as shown in Figure 4 below

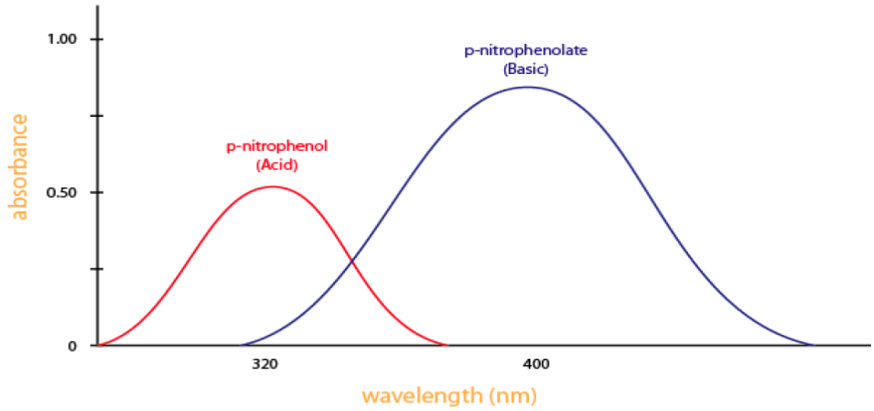


***Figure 4**: Absorbance of two different compounds*

Another example of absorption spectrum is for aromatic aldehyde below

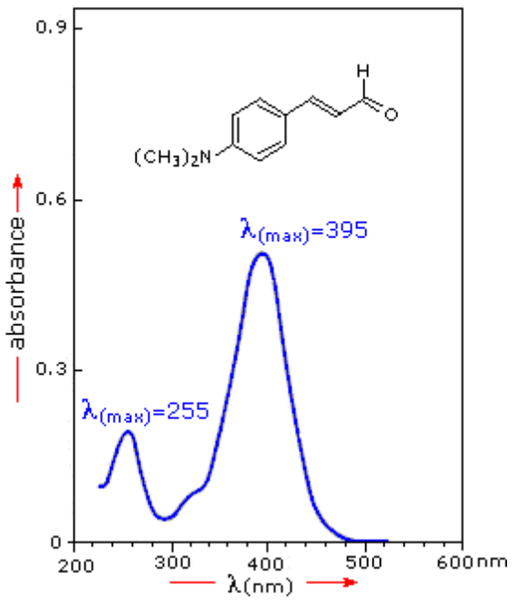


***Figure 5**: Aromatic aldehyde spectrum*

For the spectrum above, a (1.42 x 10^{-5} M) solution the aldehyde in 95% ethanol was placed in a 1 cm cuvette for measurement.
We can see here that are two maximum wavelengths (λ_{max}) for the tested aldehyde.
Using the Beer-Lambert Law formula, ε = 36,600 for the 395 nm peak, and 14,000 for the 255 nm peak.

A third example is UV-visible spectrum of 4-nitroanaline:

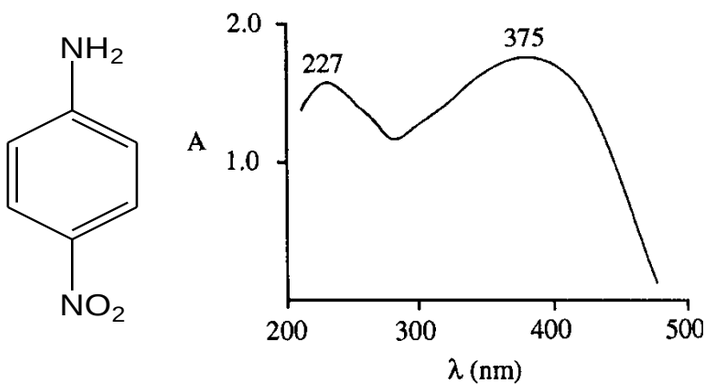


***Figure 6**: UV-visible spectrum of 4-nitroanaline; Molecular mass = 138, Solvent: Ethanol ,Concentration: 15.4 mg L^{-1} ,Pathlength: 1 cm.*

Q1. Determine the absorption maxima (λ_{max}) and absorption intensities (A) from the spectrum.

Ans. λ_{max} = 227 nm, A_{227} = 1.55 λ_{max} = 375 nm, A_{375} = 1.75

Q2. Calculate the concentration of the compound:

Ans. $(1.54 \times 10^{-2}\ g\ L^{-1})/(138\ g/mol) = 1.12 \times 10^{-4}\ mol\ L^{-1}$

Q3. Determine the molar absorptivity coefficients (ε) from the Beer-Lambert Law: $\varepsilon = A/c\ell$

Ans. $\varepsilon_{227} = 1.55/(1.0\ cm \times 1.12 \times 10^{-4}\ mol\ L^{-1}) = 13{,}900\ mol^{-1}\ L\ cm^{-1}$

$\varepsilon_{375} = 1.75/(1.0\ cm \times 1.12 \times 10^{-4}\ mol\ L^{-1}) = 15{,}700\ mol^{-1}\ L\ cm^{-1}$

Molar absorptivities are very large for strongly absorbing chromophores (ε >10,000) and very small if the absorption is weak (e = 10 to 100). The magnitude of e reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed when it strikes the chromophore. A general equation stating this relationship may be written as follows:

$$— = 0.87 \times 10^{20} P \times a$$

where P is the transition probability (0 to 1) ,a is the chromophore area in cm^2

The transition probability depends on a number of factors including whether the transition is an "allowed" transition or a "forbidden" transition.

Typical analytical application: Calibration curve

The usual procedure for (colorimetric) assays is to prepare a set of standards and produce a plot of concentration versus absorbance called **calibration curve** (Fig. 7). This should be **linear** as long as the Beer–Lambert law applies. Absorbances of unknowns are then measured and their concentration interpolated from the linear region of the plot. It is important that one never extrapolates beyond the region for which an instrument has been calibrated as this potentially introduces enormous errors.

To obtain good spectra, the maximum absorbance should be approximately 0.5 which corresponds to concentrations of about 50 mM (assuming ε = 10 000 $dm^3\ mol^{-1}\ cm^{-1}$).

Beer - Lambert law also works for mixtures: For a given ʌ,

$A_{net} = A_1 + A_2 + A_3 + \ldots$

$A_{net} = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \varepsilon_3 bc_3 + \ldots$

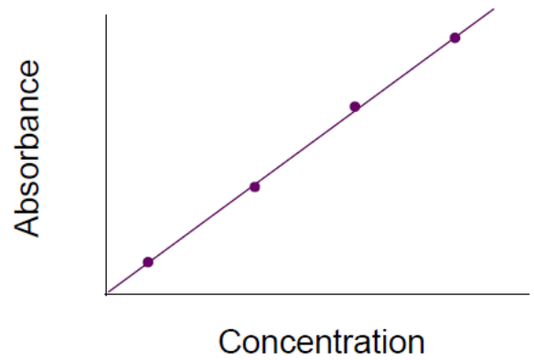


***Figure 7**: A general calibration curve*

Deviations from the Beer–Lambert law (Limitations to the Applicability of Beer's Law)

A Beer–Lambert law relationship is a linear for the most part. However, under certain circumstances the Beer relationship gives *a non-linear relationship*.

These deviations from the Beer-Lambert law can be classified into three categories:

Real Deviations - These are fundamental deviations due to the limitations of the law itself.

Chemical Deviations- These are deviations observed due to specific chemical species changes (associated with concentration changes) of the sample which is being analyzed.
Instrument Deviations- These are deviations which occur due to how the absorbance measurements are made.

According to the Beer–Lambert law, absorbance is linearly proportional to the concentration of **chromophores**. This might not be the case anymore in samples with high absorbance. Every spectrophotometer has a certain amount of stray light, which is light received at the detector but not anticipated in the spectral band isolated by the monochromator.
In order to obtain reasonable signal-to-noise ratios, the intensity of light at the chosen wavelength (I_λ) should be 10 times higher than the intensity of the stray light (I_{stray}).
If the stray light gains in intensity, the effects measured at the detector have nothing or little to do with chromophore concentration. Secondly, molecular events might lead to deviations from the Beer–Lambert law. For instance, chromophores might dimerize at high concentrations and, as a result, might possess different spectroscopic parameters.

Factors affecting UV/Vis absorption in Biochemical samples
Biochemical samples are usually buffered aqueous solutions, which has two major advantages. Firstly, proteins and peptides are comfortable in water as a solvent, which is also the 'native' solvent. Secondly, in the wavelength interval of UV/Vis (700–200 nm) the water spectrum does not show any absorption bands and thus acts as a silent component of the sample.
The absorption spectrum of a chromophore is only partly determined by its chemical structure. The environment also affects the observed spectrum, which mainly can be described by three parameters:
• protonation/deprotonation (pH, RedOx);
• solvent polarity (dielectric constant of the solvent); and
• orientation effects.
Vice versa, the immediate environment of chromophores can be probed by assessing their absorption, which makes chromophores ideal reporter molecules for environmental factors. Four effects, two each for wavelength and absorption changes, have to be considered:
• a wavelength shift to *higher values* is called **red shift or bathochromic effect**;
• similarly, a shift to *lower wavelengths* is called **blue shift or hypsochromic effect**;
• an increase in absorption is called **hyperchromicity** ('more color'),
• while a decrease in absorption is called **hypochromicity** ('less color').
Protonation/deprotonation arises either from changes in *pH* or *oxidation/reduction* reactions, which makes chromophores pH- and RedOx-sensitive reporters. As a rule of thumb, λ_{max} and ε increase, i.e. the sample displays a *batho*- and *hyperchromic* shift, if a titratable group becomes charged.
Furthermore, *solvent polarity* affects the difference between the ground and excited states. Generally, when shifting to a less polar environment one observes a batho- and hyperchromic effect. Conversely, a solvent with higher polarity elicits a hypso- and hypochromic effect.
Lastly, *orientation effects*, such as an increase in order of nucleic acids from single-stranded to double-stranded DNA, lead to different absorption behavior. A sample of free nucleotides exhibits a higher absorption than a sample with identical amounts of nucleotides but is assembled into a single-stranded polynucleotide. Accordingly, double-stranded polynucleotides exhibit an even smaller absorption than two single-stranded polynucleotides. This phenomenon is called the hypochromicity of polynucleotides. The increased exposure (and thus stronger absorption) of the individual nucleotides in the less ordered states provides a simplified explanation for this behavior.

Absorption or light scattering – optical density

In some applications, for example, measurement of turbidity of cell cultures (determination of biomass concentration), it is not the absorption but the scattering of light that is actually measured with a spectrophotometer. Extremely turbid samples like bacterial cultures do not absorb the incoming light. Instead, the light is *scattered* and thus, the spectrometer will record an apparent absorbance (sometimes also called attenuance). In this case, the observed parameter is called **optical density (OD)**.

Instruments specifically designed to measure turbid samples are *nephelometers* or *Klett meters*; however, most biochemical laboratories use the general UV/Vis spectrometer for determination of optical densities of cell cultures.

Instrumentation

UV/Vis spectrophotometers are single-beam (where only one channel contains the sample) or dual-beam spectrometers where the first channel contains the sample and the second channel holds the control (buffer) for correction.

Alternatively, one can record the control spectrum first and use this as internal reference for the sample spectrum. The latter approach has become very popular as many spectrometers in the laboratories are computer-controlled, and baseline correction can be carried out using the software by simply subtracting the control from the sample spectrum.

The light source is a tungsten filament bulb for the visible part of the spectrum, and a deuterium bulb for the UV region. Since the emitted light consists of many different wavelengths, a **monochromator**, consisting of either a prism or a rotating metal grid of high precision called **grating**, is placed between the light source and the sample.

Wavelength selection can also be achieved by using colored filters as monochromators that absorb all but a certain limited range of wavelengths. This limited range is called the **bandwidth** of the filter. Filter-based wavelength selection is used in colorimetry, a method with moderate accuracy, but best suited for specific colorimetric assays where only certain wavelengths are of interest. If wavelengths are selected by prisms or gratings, the technique is called **spectrophotometry** (Fig. 8a,b).

Since **borosilicate glass** and **normal plastics absorb** UV light, such cuvettes can only be used for applications in the visible range of the spectrum (up to 350 nm). For UV measurements, **quartz cuvettes** need to be used. However, disposable plastic cuvettes have been developed that allow for measurements over the entire range of the UV/Vis spectrum.

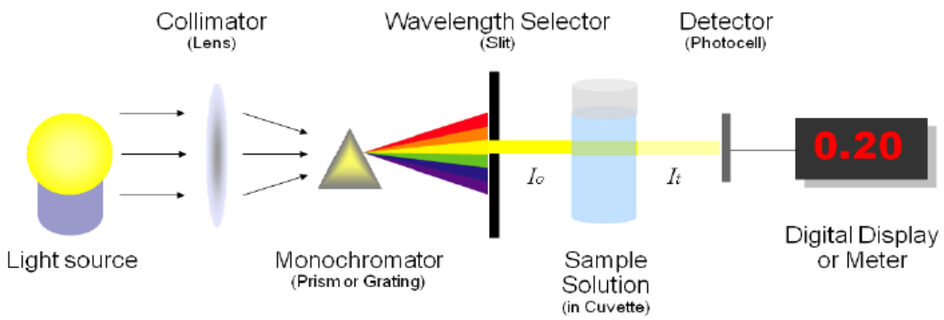


***Figure8a**:Basic structure of spectrophotometers.*

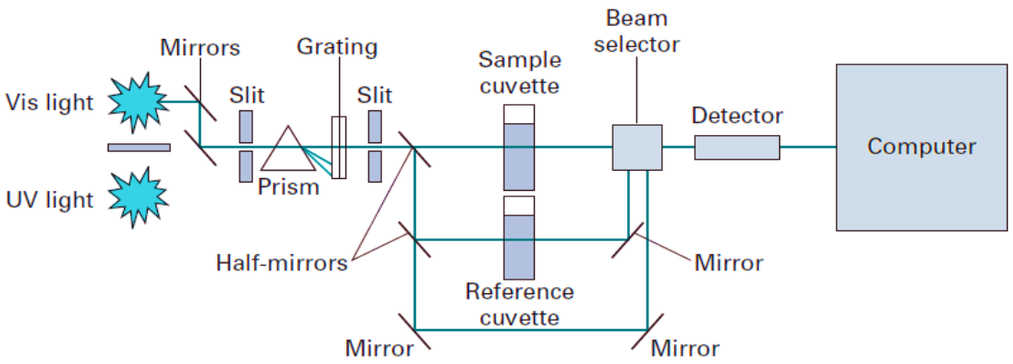


***Figure8b**: Optical arrangements in a dual-beam spectrophotometer. Either a prism or a grating constitutes the monochromator of the instrument. Optical paths are shown as green lines.*

Analytical Applications

- Metals analysis (complexes).
- Organic compound analysis.
- Nutrient analysis.
- Pharmaceutical industry.

-pH determination (use of indicator dye).

-Gas analysis (IR, e.g., CO_2).

Qualitative & Quantitative Analysis

Qualitative analysis may be performed in the UV/Vis regions to identify certain classes of compounds both in the pure state and in biological mixtures (e.g. protein-bound).

The application of UV/Vis spectroscopy to further analytical purposes is rather limited, but possible for systems where appropriate features and parameters are known.

Most commonly, this type of spectroscopy is used for quantification of biological samples either directly or via colorimetric assays. In many cases, proteins can be quantified directly using their intrinsic chromophores, tyrosine and tryptophan.

Protein spectra are acquired by scanning from 500 to 210 nm. The characteristic features in a protein spectrum are a band at 278/280 nm and another at 190 nm. The region from 500 to 300 nm provides valuable information about the presence of any prosthetic groups or coenzymes. Protein quantification by single wavelength measurements at 280 and 260nm only should be avoided, as the presence of larger aggregates (contaminations or protein aggregates) gives rise to considerable Rayleigh scatter that needs to be corrected for.

Determination of Concentrations

Question (1) The concentration of an aqueous solution of a protein is to be determined assuming:

(i) knowledge of the molar extinction coefficient ε

(ii) molar extinction coefficient ε is not known.

Question (2) What is the concentration of an aqueous solution of a DNA sample?

Answer (1)

(i) The protein concentration of a pure sample can be determined by using the Beer–Lambert law. The absorbance at 280 nm is determined from a protein spectrum, and the molar extinction coefficient at this wavelength needs to be experimentally determined or estimated:

$$\rho^* = \frac{A\ x M.wt}{\varepsilon\ X\ l}$$

where ρ* is the mass concentration in mg cm^{-3} and M.wt the molecular mass of the assayed species in g mol^{-1}.

(ii) Alternatively, an empirical formula known as the Warburg–Christian formula can be used without knowledge of the value of the molar extinction coefficient:

$$\rho^* = (1.52 \times A_{280} - 0.75 \times A_{260}) \text{ mg cm}^{-3}$$

Other commonly used applications to determine the concentration of protein in a sample make use of colorimetric assays that are based on chemicals (folin, biuret, bicinchoninic acid or Coomassie Brilliant Blue) binding to protein groups. Concentration determination in these cases requires a calibration curve measured with a protein standard, usually bovine serum albumin.

Answer (2) As we have seen above, the genetic bases have absorption bands in the UV/Vis region. Thus, the concentration of a DNA sample can be determined spectroscopically. Assuming that a pair of nucleotides has a molecular mass of M= 660 g mol^{-1}, the absorbance A of a solution with double-stranded DNA at 260 nm can be converted to mass concentration ρ* by:

$$\rho^* = 50\ \mu\text{g cm}^{-3} \times A_{260}$$

The ratio A260/A280 is an indicator for the purity of the DNA solution and should be in the range 1.8–2.0.

Quantitative Analysis for Mixture

For two components (M and N) system

$$A_\lambda^{total} = A_\lambda^M + A_\lambda^N = \varepsilon_\lambda^M l[M] + \varepsilon_\lambda^N l[N] = l\left(\varepsilon_\lambda^M [M] + \varepsilon_\lambda^N [N]\right)$$

Measurements under two wavelengths

$$A_{\lambda_1} = l\left(\varepsilon_{\lambda_1}^M [M] + \varepsilon_{\lambda_1}^N [N]\right)$$

$$A_{\lambda_2} = l\left(\varepsilon_{\lambda_2}^M [M] + \varepsilon_{\lambda_2}^N [N]\right)$$

SO

$$[M] = \frac{1}{l}\left(\frac{\varepsilon_{\lambda_2}^N A_1 - \varepsilon_{\lambda_1}^N A_2}{\varepsilon_{\lambda_1}^M \varepsilon_{\lambda_2}^N - \varepsilon_{\lambda_2}^M \varepsilon_{\lambda_1}^N}\right)$$

$$[N] = \frac{1}{l}\left(\frac{\varepsilon_{\lambda_1}^M A_2 - \varepsilon_{\lambda_2}^M A_1}{\varepsilon_{\lambda_1}^M \varepsilon_{\lambda_2}^N - \varepsilon_{\lambda_2}^M \varepsilon_{\lambda_1}^N}\right)$$

Worked example: Solutions containing the amino acids tryptophan (trp) and tyrosine (tyr) can be analyzed under alkaline conditions (0.1M KOH) from their different UV spectra. The extinction coefficients under these conditions at 240 nm and 280 nm are:

240 nm- ε_{trp} =11300, ε_{tyr} =1960

280 nm - ε_{trp} =1500, ε_{tyr} =5380

A 10-mg sample of the protein glucagon is hydrolyzed to its constituent amino acids and diluted to 100 mL in 0.1 M KOH. The absorbance of this solution (1 cm path) was 0.717 at 240 nm and 0.239 at 280 nm. Estimate the content of tryptophan and tyrosine in mol (g protein)$^{-1}$.

$$[trp] = \frac{(5380 \times 0.717) - (1960 \times 0.239)}{(11300 \times 5380) - (1500 \times 1960)} = 5.85 \times 10^{-5} M$$

$$[tyr] = \frac{(11300 \times 0.239) - (1500 \times 0.717)}{(11300 \times 5380) - (1500 \times 1960)} = 2.81 \times 10^{-5} M$$

Problems:

Problem1: Guanosine has a maximum absorbance of 275 nm. ε at 275 = 8400 M^{-1} cm^{-1} and the path length is 1 cm. Using a spectrophotometer, you find the that A== 0.70. What is the concentration of guanosine?
*Clue:*To solve this problem, you must use Beer's Law.(Ans. 8.33x10 mol/L)

Problem2:There is a substance in a solution (4 g/liter). The length of cuvette is 2 cm and only 50% of the certain light beam is transmitted. What is the absorption coefficient?
Clue: use first $-log = \frac{I_1}{I_0} = logT = A$.(Ans. ε= 0.0376).
Problem3:In problem 2 above, how much is the beam of light is transmitted when 8 g/liter ?
Clue:find transmittance. first. (Ans. I_1 = 0.2503 = 25%).

Problem4: In problem 2 above, what is the molar absorption coefficient if the molecular weight is 100?
Clue: multiply the absorption coefficient by the molecular weight.(Ans. ε=3.76 L . mol^{-1} cm^{-1}).

Problem5: The absorption coefficient of a glycogen-iodine complex is 0.20 at light of 450 nm. What is the concentration when the transmission is 40 % in a cuvette of 2 cm?(Ans. C= 0.9948).

5. MOLECULES SPECTROSCOPY:fLUORESCENCE SPECTROSCOPY

Emission of Radiation: Excitation needs energy such particle bombardment (e-), electrical currents (V), fluorescence, and heat.
Because excitation state is unstable state, the electrons lose energy by process called emission.
$X^* \rightarrow X + h\nu$
where X* is an excited atom or (molecule).
Atoms and molecules have different spectra (Figure.1): *line spectra* (individual atoms, well separated, in a gas phas) and *band spectra* (small molecules and radicals).

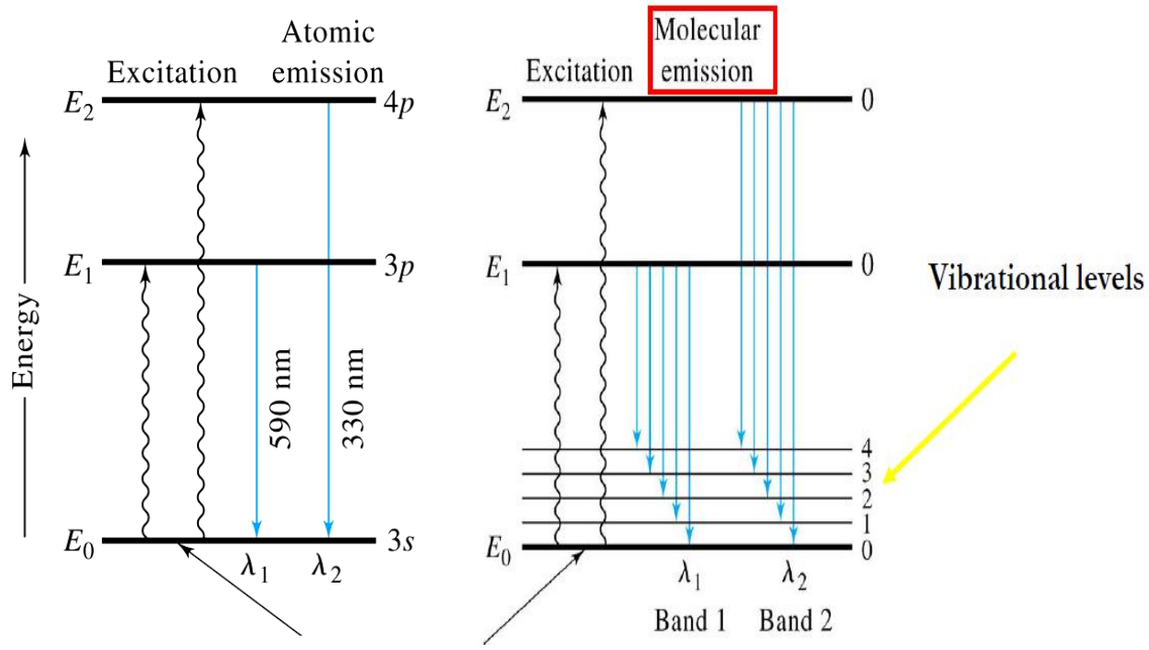
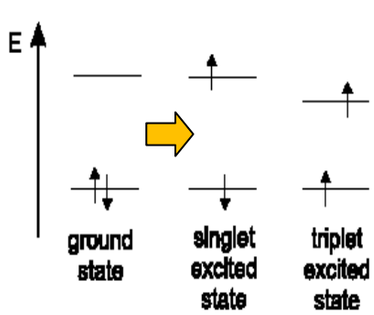


Figure 1. (a) line spectra (b) band spectra

Luminescence (light from nonthermal sources)
Luminescence is *an emission* of photons from electronically excited states at low temperature.
There are two types of luminescence:

1- Relaxation from singlet excited state and
2- Relaxation from triplet excited state.



A ground state level has two electrons per orbital; electrons have opposite spin and are paired.
-*Singlet excited state (S):* Electron in higher energy orbital has the opposite spin orientation relative to electron in the lower orbital
-*Triplet excited state (T):* The excited valence electron may spontaneously reverse its spin (spin flip). This process is called intersystem crossing. Electrons in both orbitals now have same spin orientation.

Types of Emission
There are two types of emission used in a luminescence analysis: *fluorescence* and *phosphorescence*.
Fluorescence: It happens when electrons return from excited single tstate to ground state; does not require change in spin orientation (more common of relaxation).

Phosphorescence: It happens when electrons return from a triplet excited state to a ground state; electron requires change in spin orientation.
Note: Emissive rates of fluorescence are several orders of magnitude faster than that of phosphorescence.
The Jablonski diagram (Figure 2) offers a convenient representation of the excited state structure and the relevant transitions.

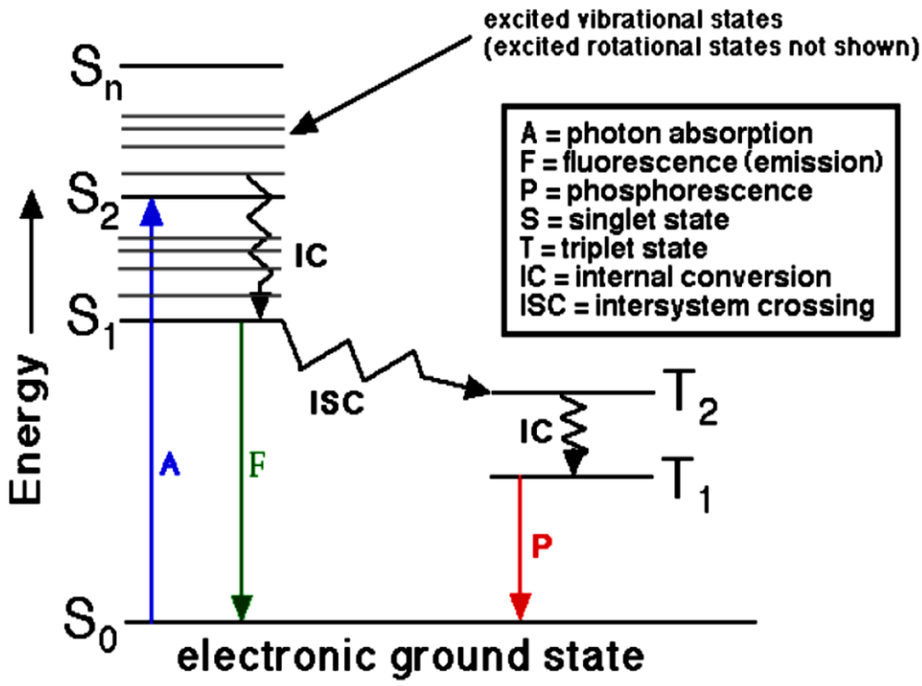


Figure 2. Energy level diagram (Jablonski diagram)

Like a *chromophore* (the chemical group that gives color to a molecule), luminescence compounds have polyatomic fluorescent molecules *(fluorophores*). Photonic processes involve transitions between electronic and vibrational states of fluorophores. Figure 3 below represents examples of fluorophores.

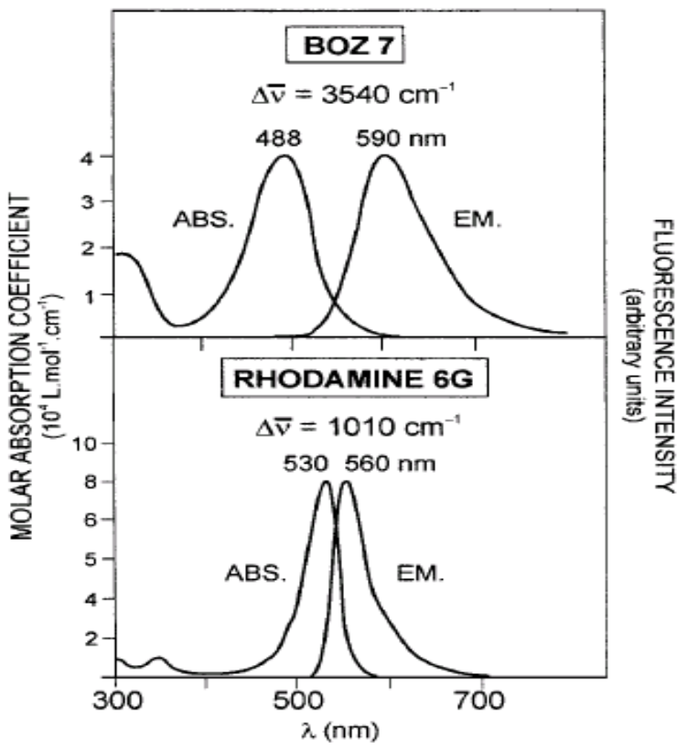


Figure 3. Wavelengths and wavenumbers of Boz 7 and Rhodamine 6G fluorophores in fluorescent dyes

Instrumentation for Fluorescence Spectrophotometry

Fluorescence is a highly sensitive method (can measure analyte concentration of 10^{-8} M).
Important to minimize interference from:
• Background fluorescence from solvents
• Light leaks in the instrument
• Stray light scattered by turbid solutions
Instruments do not yield ideal spectra:
• Non-uniform spectral output of light source
• Wavelength dependent efficiency of detector and optical elemens.

A fluorescence instrument has major components (Figure. 4):
Illumination source:Broadband (Xe lamp) or Monochromatic (LED, laser).
Light delivery to sample: Lenses/mirrors or Optical fibers.
Wavelength separation (potentially for both excitation and emission): Filters, Monochromator and Spectrograph.
Detector: PMT, CCD camera.

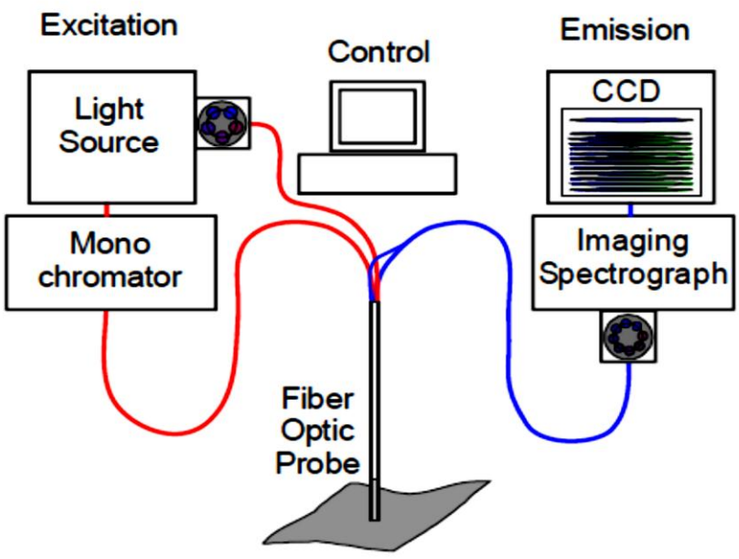


Figure 4. Major components for fluorescence instrument

Quenching
A number of processes can lead to a reduction in fluorescence intensity, i.e., quenching. These processes can occur during the excited state lifetime – for example collisional quenching, energy transfer, charge transfer reactions or photochemistry – or they may occur due to formation of complexes in the ground state.
We shall focus our attention on the two quenching processes usually encountered – namely collisional (dynamic) quenching and static (complex formation) quenching.
Collisional quenching occurs when the excited fluorophore experiences contact with an atom or molecule that can facilitate non-radiative transitions to the ground state. Common quenchers include O_2, I^-, Cs^+ and acrylamide.
In some cases, the fluorophore can form a stable complex with another molecule. If this *ground-state* is non-fluorescent then we say that the fluorophore has been *statically quenched*.

Applications:
Fluorescence spectophotometry is widely used in many areas of biology and medicine. A basic understanding of fluorescence principles, fluorophores properties, instruments

and techniques is a prerequisite to the study of a wide range of biological systems. Fluorescence techniques can be use in:

1- Analysis of proteins and peptides
Table 1. Intrinsic fluorescence of proteins and peptides.

	Lifetime ns	*Absorption*		*Fluorescence*	
		Wavelength nm	Absorptivity	Wavelength nm	Quantum
Tryptophan	2.6	280	5,600	348	0.20
Tyrosine	3.6	274	1,400	303	0.14
Phenylalanine	6.4	257	200	282	0.04

2- Fluorescence lifetime measurements
a) Autofluorescence lifetimes measured from colon tissue in vivo (taking place in a living organism).
b) Autofluorescence lifetimes used to distinguish adenomatous from non-adenomatous polyps in vivo.

3- Detection of lung carcinoma in situ using the LIFE imaging system.

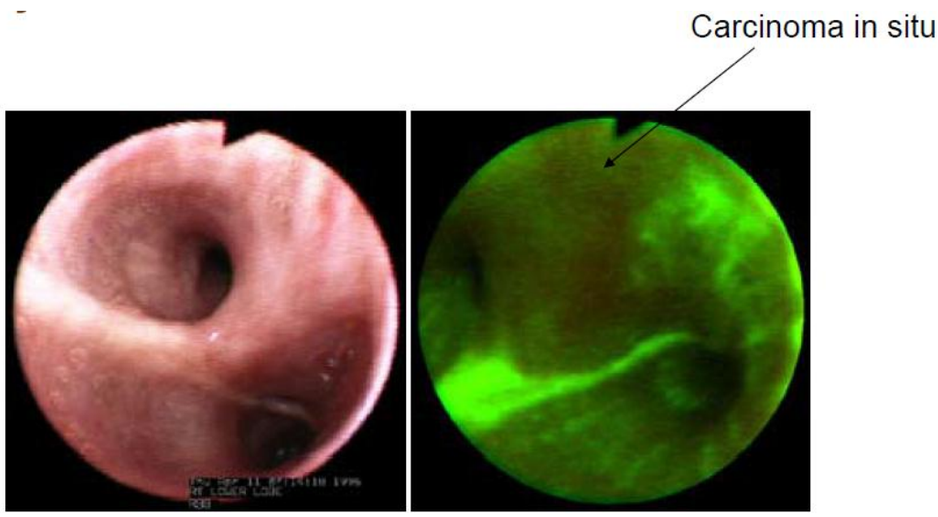


Figure 5. White light bronchoscopy vs. Autofluorescence ratio image

Autofluorescence enhances ability to localize small neoplastic lesions.

4- Polarization measurement
Fluorescence polarization measures the rotational diffusion rate of macromolecules. Rotational diffusion contains information related to the shape of the proteins. Diffusional restrictions of molecules in biological macrostructures, such as cellular membrane or the cytoskeleton can also be quantified based on polarization measurement.

Molecules Spectrocopy:IR Spectroscopy

Infrared Spectroscopy (IR) – Triggering molecular vibrations through irradiation with infrared light. Provides mostly information about the presence or absence of certain *functional groups*.

Upon irradiation with infrared light, certain bonds respond by vibrating faster. This response can be detected and translated into a visual representation called a spectrum.

Infrared radiation is largely thermal energy.

It induces stronger **molecular vibrations** in covalent bonds, which can be viewed as springs holding together two masses, or atoms.

Specific bonds respond to (absorb) **specific** frequencies.

Covalent bonds can vibrate in several modes, including **stretching, rocking, scissoring, wagging, and twisting**.

The most useful bands in an infrared spectrum correspond to stretching frequencies, and those will be the ones we'll focus on.

Region of infrared that is most useful lies between 2.5-16 μm (4000-625 cm^{-1}) depends on transitions between vibrational energy states: stretching and bending.

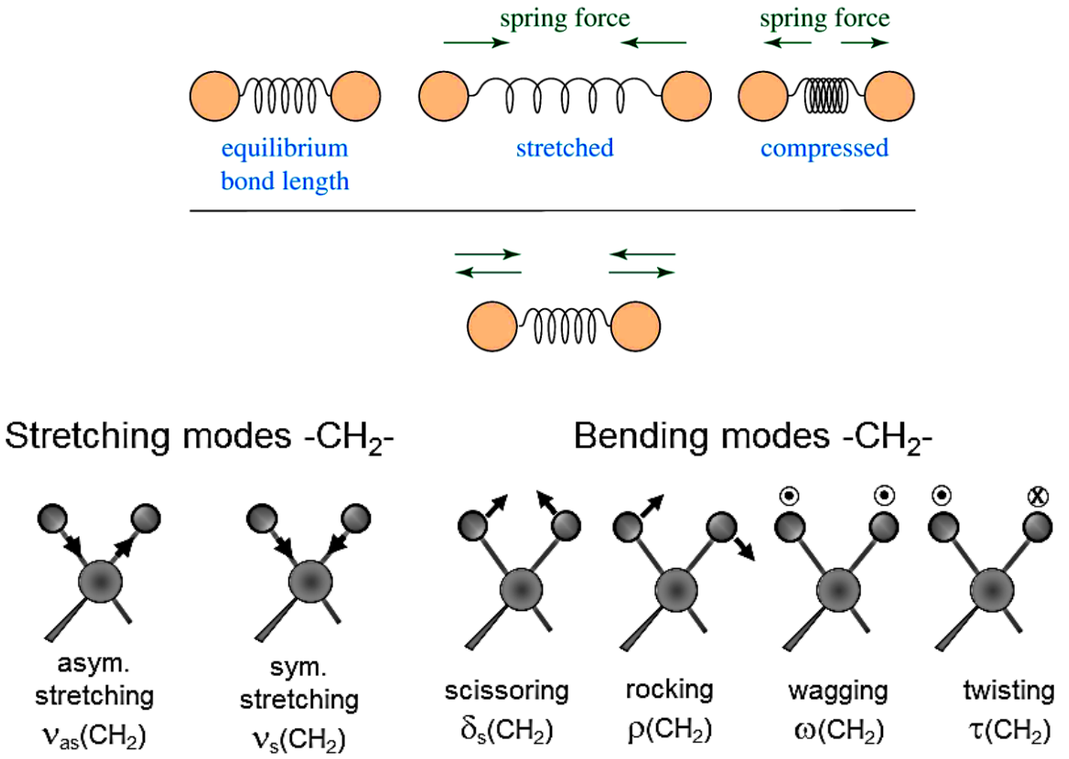
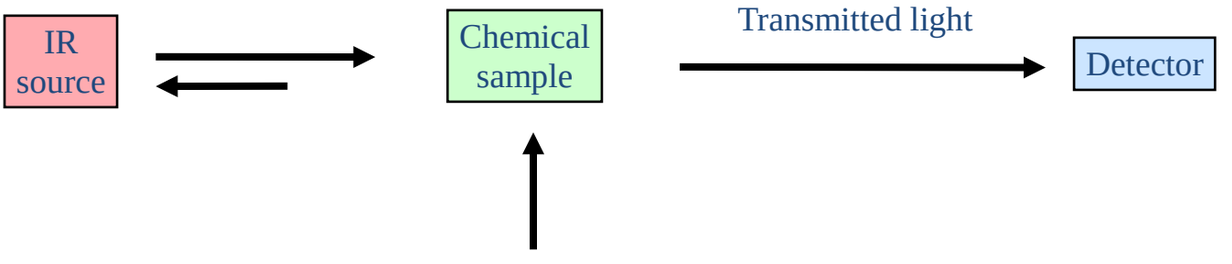


Figure 6. Covalent bonds vibrational modes

Instrumentation for IR Spectroscopy

When a chemical sample is exposed to the action of **IR LIGHT**, it can **absorb** some frequencies and **transmit** the rest. Some of the light can also be reflected back to the source.



From all the frequencies it receives, the chemical sample can **absorb** (retain) **specific frequencies** and allow the rest to pass through it (transmitted light).

The detector detects the transmitted frequencies, and by doing so also reveals the values of the absorbed frequencies. Since all bonds in an organic molecule interact with infrared radiation, IR spectra provide a considerable amount of structural data.

An Infrared Spectrum in Transmisson or Absorption Mode

The IR spectrum is basically a plot of transmitted (or absorbed) frequencies vs. intensity of the transmission (or absorption) (Figure 7). Frequencies appear in the *x*-axis in units of inverse centimeters (wavenumbers), and intensities are plotted on the *y*-axis in percentage units.

A transmission mode **is the most commonly used representation** and the one found in most chemistry and spectroscopy books.

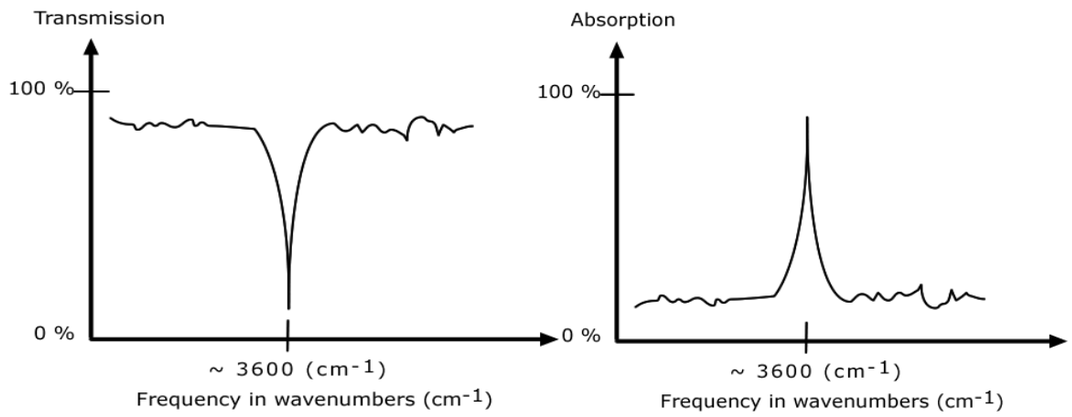


Figure 7. Transmisson and absorption modes in IR Spectrum

Classifiaction of IR Bands

IR bands can be classified as **strong** (s), **medium** (m), or **weak** (w) (Figure 8), depending on their relative intensities in the infrared spectrum. A strong band covers most of the *y*-axis. A medium band falls to about half of the *y*-axis, and a weak band falls to about one third or less of the *y*-axis.

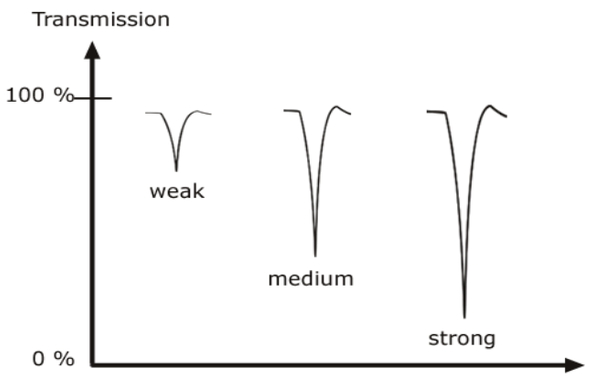


Figure 8. Strength of IR bands

Infrared Active Bonds

Not all covalent bonds display bands in the IR spectrum. *Only polar bonds do so. These are referred to as IR active*.

The intensity of the bands depends on the magnitude of the dipole moment associated with the bond in question:

- Strongly polar bonds such as carbonyl groups (C=O) produce strong bands.
- Medium polarity bonds and asymmetric bonds produce medium bands.
- Weakly polar bond and symmetric bonds produce weak or non observable bands.

IR Band Shape

Infrared band shapes come in various forms. Two of the most common are **narrow** and **broad**. Narrow bands are thin and pointed, like a dagger. Broad bands are wide and smoother.

Figure 9 shows a typical example of a broad band that is displayed by O-H bonds, such as those found in alcohols and carboxylic acids, as shown below.

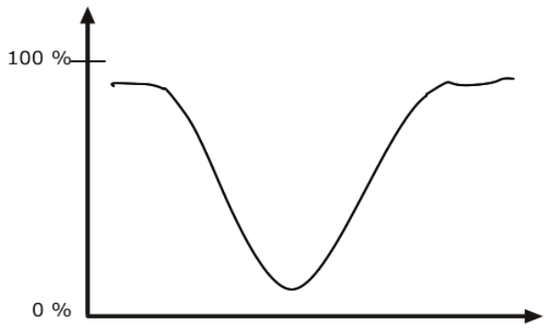


Figure 9. A broad band of O-H bonds

Information Obtained From IR Spectra

IR is most useful in providing information about the presence or absence of specific **functional groups**.

IR can provide a **molecular fingerprint** that can be used when comparing samples. If two pure samples display the same IR spectrum it can be argued that they are the same compound.

IR **does not** provide detailed information or proof of molecular formula or structure. It provides information on molecular fragments, specifically functional groups.

Therefore it is very limited in scope, and must be used in conjunction with other techniques to provide a more complete picture of the molecular structure.

IR Absorption Range

The typical IR absorption range for covalent bonds is **600 - 4000 cm^{-1}** (Figure 10). The graph shows the regions of the spectrum where the following types of bonds normally absorb. For example a sharp band around 2200-2400 cm^{-1} would indicate the possible presence of a C-N or a C-C triple bond.

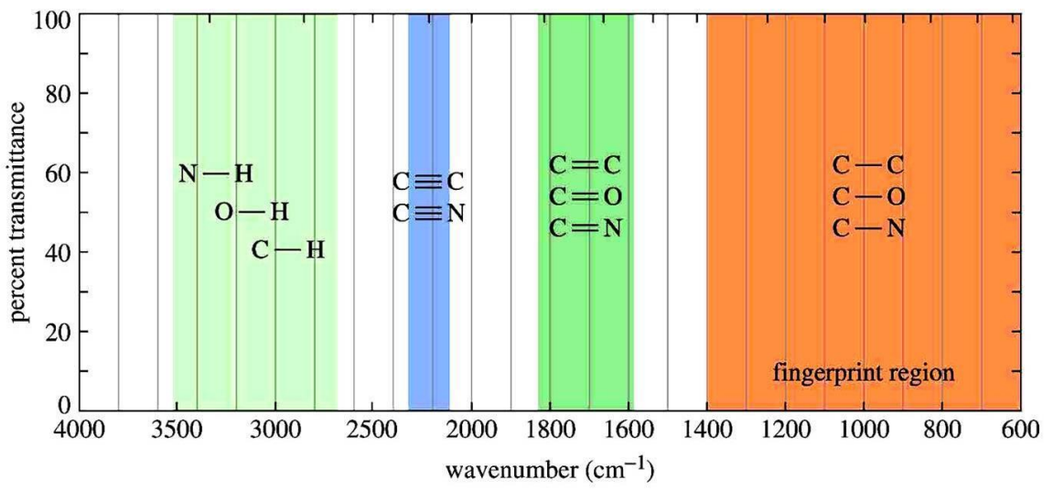


Figure 10. IR absorption range for some covalent bonds

The Fingerprint Region

Although the entire IR spectrum can be used as a fingerprint for the purposes of comparing molecules, the **600 - 1400 cm^{-1}** range is called the **fingerprint region** (Figure

11). This is normally a complex area showing many bands, frequently overlapping each other. This complexity limits its use to that of a fingerprint, and should be ignored by beginners when analyzing the spectrum. As a student, you should focus your analysis on the rest of the spectrum, that is the region to the left of 1400 cm^{-1}.

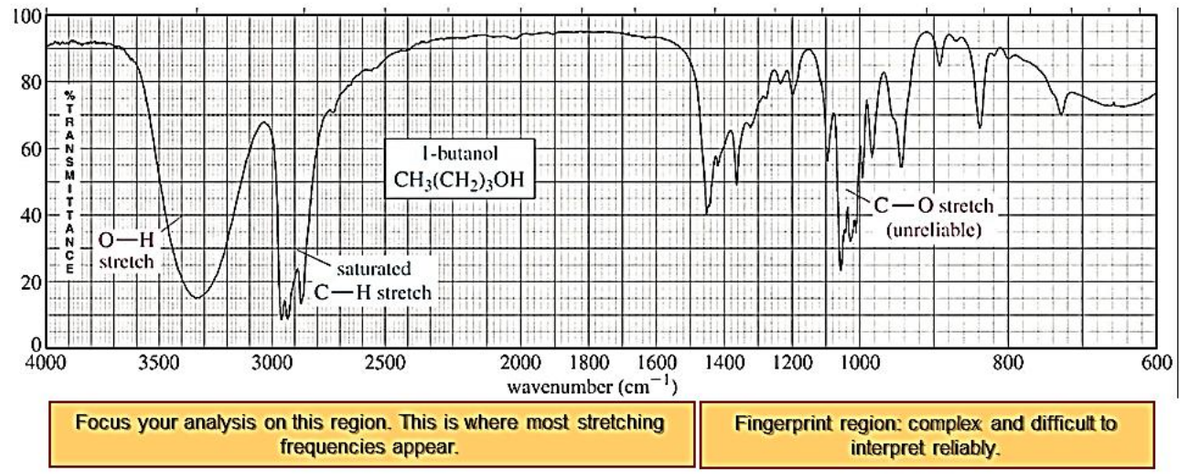


Figure 11. The fingerprint region in IR spectrum

Functional Groups and IR Tables

IR spectrophotometer identifies various functional groups such as alkenes, alcohols, ketones, carboxylic acids, etc. Basic knowledge of the structures and polarities of these groups is assumed. If you need a refresher please turn to your organic chemistry textbook. A table relating IR frequencies to specific covalent bonds can be found on sevral sources such as textbook and websites…etc.

Examples of IR Spectra

1- Alkanes

Alkanes have no functional groups. Their IR spectrum displays only C-C and C-H bond vibrations. Of these the most useful are the **C-H bands**, which appear around **3000 cm^{-1}** (Figure 12). Since most organic molecules have such bonds, most organic molecules will display those bands in their spectrum.

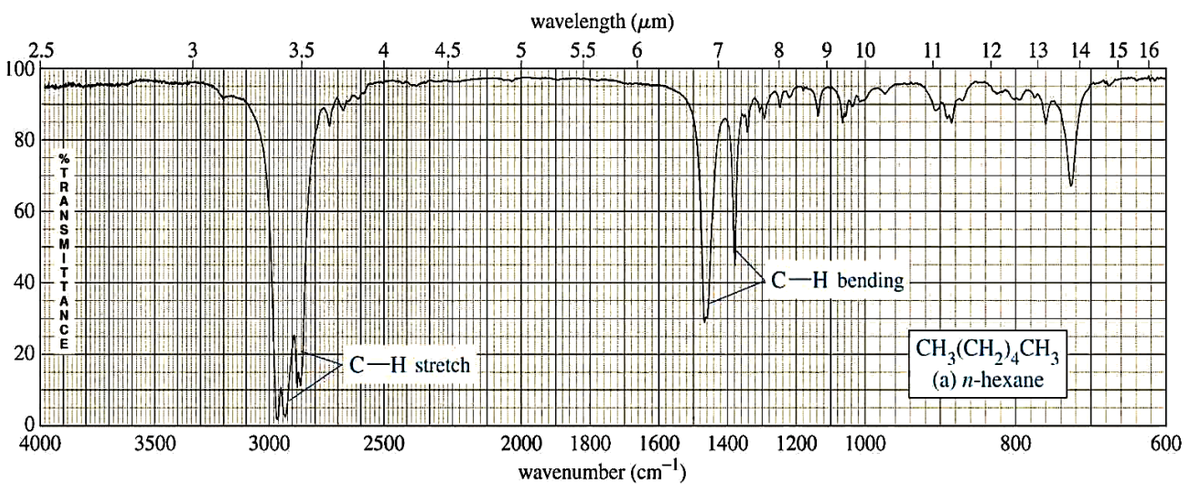


Figure 12. n-hexane IR spectrum

2- Alkynes

The most prominent band in alkynes corresponds to the **carbon-carbon triple bond**. It shows as a sharp, weak band at about **2100 cm^{-1}** (Figure 13 a,b). The reason it's weak is because the triple bond is not very polar. In some cases, such as in highly symmetrical alkynes, it may not show at all due to the low polarity of the triple bond associated with those alkynes.

Terminal alkynes, that is to say those where the triple bond is at the end of a carbon chain, have C-H bonds involving the *sp* carbon (the carbon that forms part of the triple bond). Therefore they may also show a sharp, weak band at about **3300 cm^{-1}** corresponding to the C-H stretch.

Internal alkynes, that is those where the triple bond is in the middle of a carbon chain, do not have C-H bonds to the *sp* carbon and therefore lack the aforementioned band.

The following slide shows a comparison between an unsymmetrical terminal alkyne (1-octyne) and a symmetrical internal alkyne (4-octyne).

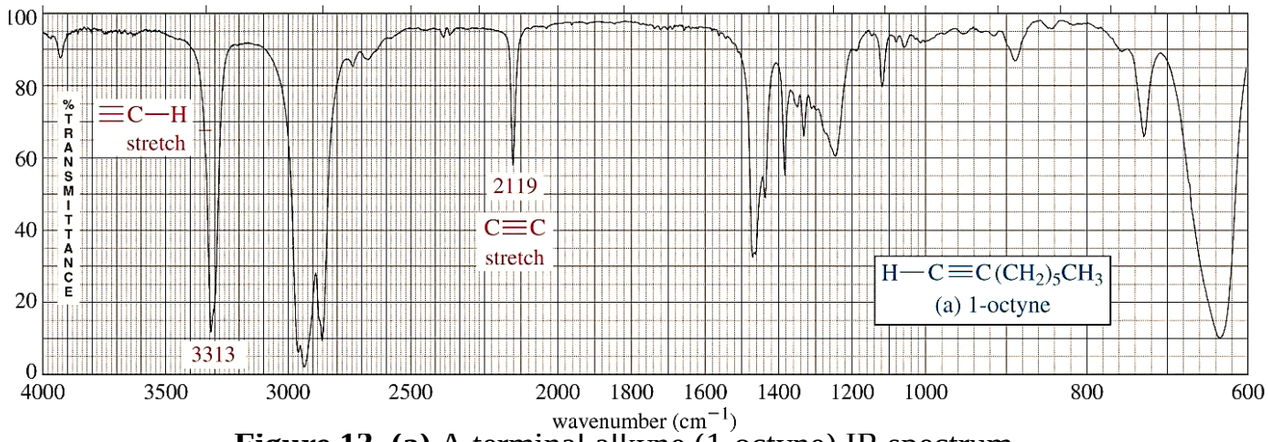


Figure 13. (a) A terminal alkyne (1-octyne) IR spectrum

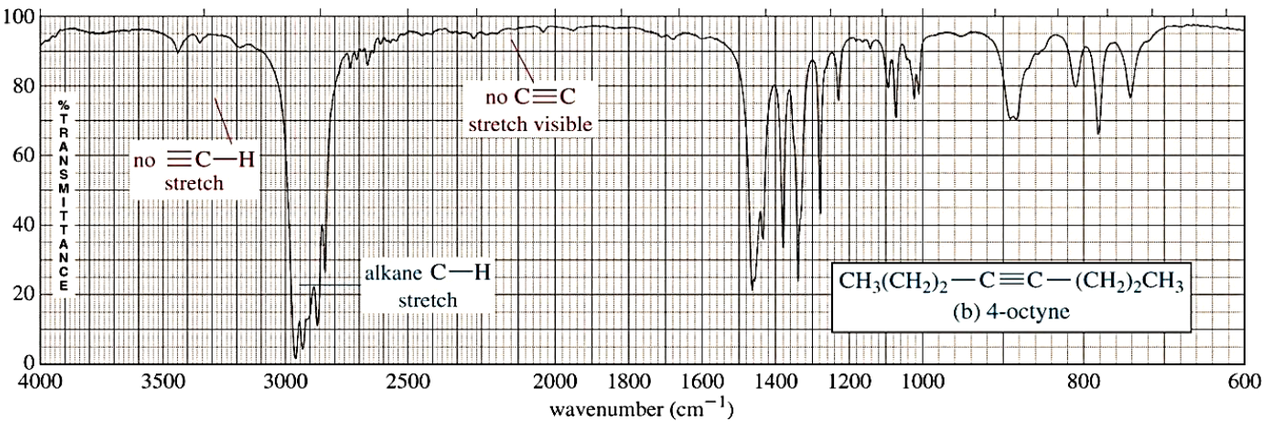


Figure 13. (b) An internal alkyne (4-octyne) IR spectrum

3- Alcohols

The most prominent band in alcohols is due to the **O-H bond**, and it appears as a strong, broad band covering the range of about **3000 - 3700 cm^{-1}**. The sheer size and broad shape of the band dominate the IR spectrum and make it hard to miss.

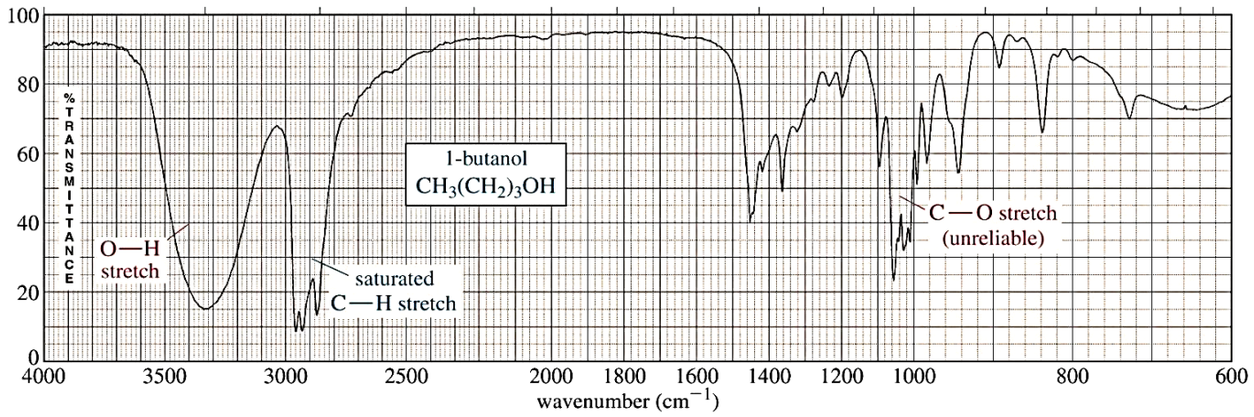


Figure 14. 1-Butanol IR spectrum

4- Carboxylic Acids

A carboxylic acid functional group combines the features of alcohols and ketones because it has both the **O-H bond** and the **C=O bond**. Therefore carboxylic acids show a

very strong and broad band covering a wide range between **2800** and **3500 cm^{-1}** for the O-H stretch. At the same time they also show the stake-shaped band in the middle of the spectrum around **1710 cm^{-1}** corresponding to the C=O stretch.

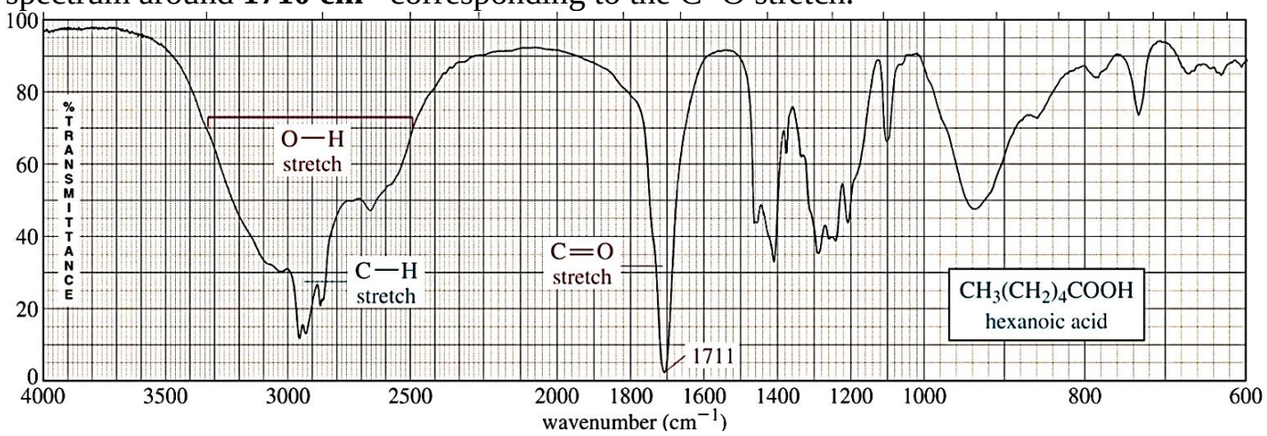
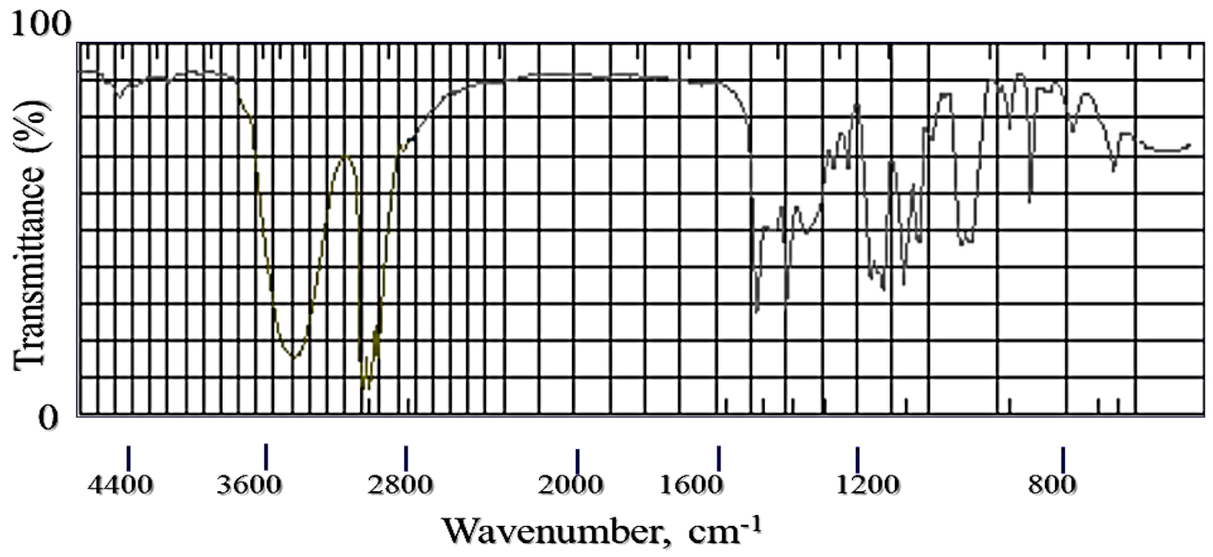


Figure 15. Hexanoic acid IR spectrum

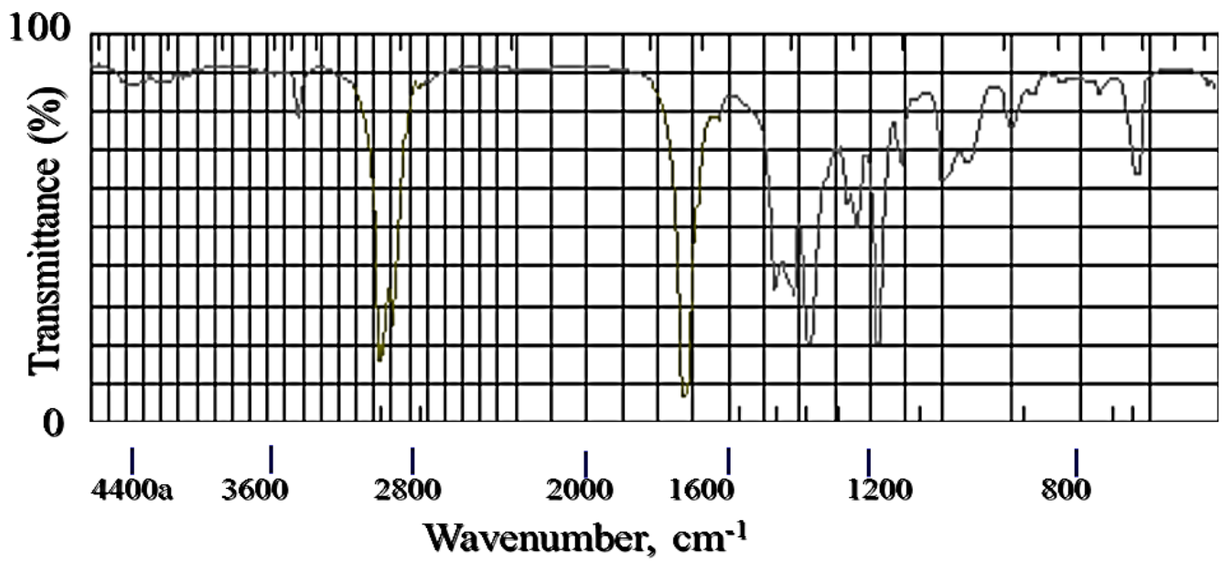
Problems:

In help textbooks with a table relating IR frequencies, Identity the compounds below and explain how you deduced this.

(1)



(2)



6. ELEMENTAL ANALYSIS TECHNIQUES: THE SPECTROSCOPY TECHNIQUES

Optical spectroscopic methods are based on six phenomena (1) absorption, (2) fluorescence. (3) phosphorescence, (4) scattering, (5) emission, and (6) chemiluminescence. As in molecular spectroscopy, atomic spectroscopy is divided broadly into absorption and emission spectroscopy. The notable differences are that atomic spectrometry is always carried out in the gas phase. The measurement conditions require elevated temperatures; with the exception of Hg, Cd and the inert gases, elements are not present as a monoatomic gas at room temperature. Also, as the name implies, we measure atoms; atomic spectroscopy is then a form of elemental analysis.

Atomic and/or molecular spectroscopy is a powerful tool to analyze chemical composition or structure of a substance, which may be a pure compound or a simple mixture or solution of two or more different phases of a crystalline or amorphous material. The chemistry and industry people sometimes also talk of minerals, ores, and pollutants, but these comprise the same crystalline or amorphous structures. They are characterized using the same instrumental techniques.

Atomic absorption (AA) is a spectrophotometric analysis technique which allows metal determination. Quantification is based on light absorption by metal atoms at their ground state. It is specially indicated for the determination of alkaline elements, alkaline-earth and heavy metals present in any type of sample, previously dissolved. Attainable levels range from pph to ppb. The service is requested by firms, research departments in universities, research public organisms and individuals. The technique of atomic absorption is complemented by the techniques of elemental analysis with inductively coupled plasma linked to mass spectrometry and optic plasma.

Atomic Absorption Spectroscopy (AAS) is typically carried out using a hot flame for atom formation **(flame AAS)**, or an electrically heated mini-furnace, most commonly consisting of graphite **[electrothermal AA, graphite furnace AA (GFAA)]**, and rather uniquely for Hg (and occasionally Cd), it is possible to do this without heating **[cold vapor AA (CVAA)]**. Similar to molecular luminescence, atomic luminescence can be produced by excitation of atoms by photons; this represents the technique of **atomic fluorescence spectrometry (AFS)**. Also, unlike molecules that thermally decompose long before they can be thermally excited sufficiently to emit light, atoms can be heated to high enough temperatures to emit their characteristic radiation. While atoms undergo true thermoluminescence, this term is not used to describe the relevant measurement techniques, rather they are always described with reference to the specific means of exciting the atoms. Examples are the techniques of **flame emission spectrometry** (in practical use today, the desired wavelengths are selected using interference filters—hence, the technique is more accurately called **flame photometry**), arc/spark source emission spectrometry, direct current plasma (DCP) emission spectrometry, and **induction coupled plasma** (also called **inductively coupled plasma**) **atomic** (or **optical**) **emission spectrometry (ICP-AES** or **ICP-OES)**. The ICP is sufficiently energetic to strip off one (or more) outer shell electron(s) to generate positive ions from the analyte atoms; they can be analyzed by a mass spectrometer after being sorted by their m/z values this technique is hence called **induction coupled plasma mass spectrometry (ICP-MS)**.

Atomic Spectroscopy (deals with atoms)

Atomic spectroscopy measures *absorbance* or *emission* of the atomic vapor (and e.g. Fe^{2+} and Fe^{3+} will not be distinguished).

AAS is based on the principle that a *ground state atom* with **outer shell electrons** is capable of absorbing light of the same *characteristic wavelength* as it would emit if excited to a higher energy level.

Spectra of atoms consist of 50 sharp (inherent linewidth of 0.003 nm-0.00001 nm) lines for small metals to over 5000 sharp lines for larger metals per atom. Each element has a characteristic spectrum. Due to the sharpness of lines, there is little overlap/little interference between the spectral lines of different elements.

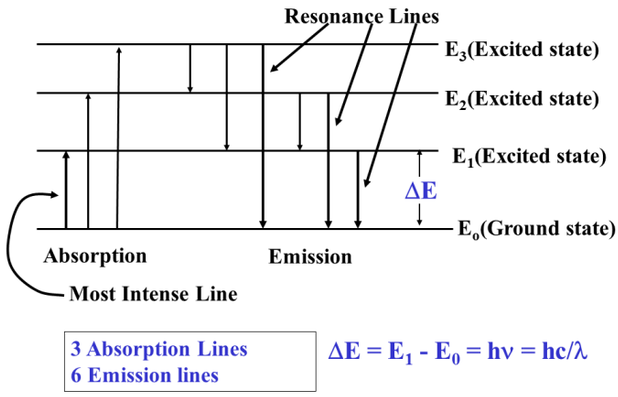


Figure .1 Atomic absorption and emission lines

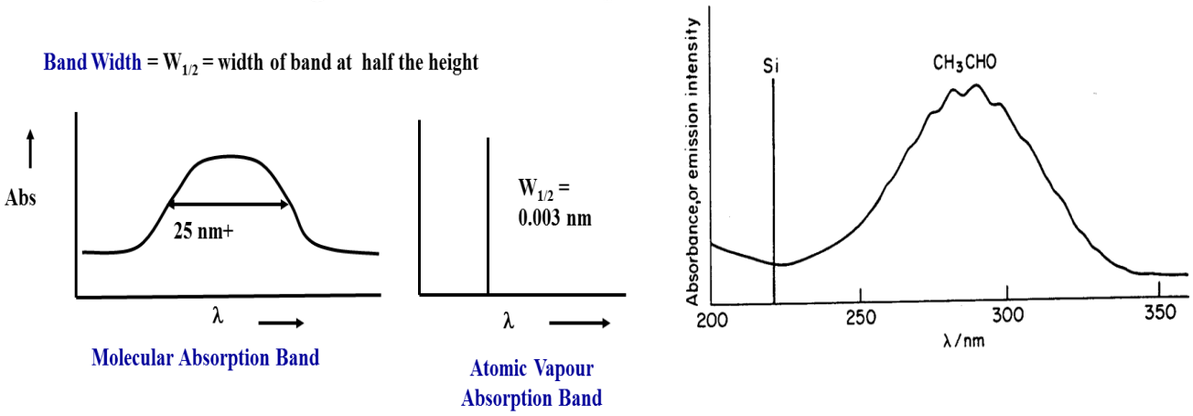


Figure .2 Comparison of the atomic emission spectrum (i.e. silicon) with the molecular absorption spectrum (i.e. ethanal).

AAS is widely used for metal analysis in biological fluids and tissues, in environmental samples such as air and water, and in occupational health and safety areas. In the clinical laboratory, *alkali* and *alkaline earth* metals were measured by *flame photometry*.

Sample Preparation

Sample preparation in flame AAS can often be kept to a minimum. As long as chemical or spectral interferences are absent, essentially all that is required is to obtain the sample in the form of a diluted solution, filtered to be free from particles. Atomic spectrometry measures the elements; the specific chemical form of the element is immaterial; it will be dissociated to the free elemental atomic vapor in the measurement process. Thus, several elements can be determined in blood, urine, cerebrospinal fluid, and various biological fluids by direct aspiration of the diluted sample. GFAA allows the analysis of not only dilute solutions, but liquid samples containing suspended solids and even solids. Specialized atomizers have been developed that facilitate the accurate analysis of solids and are used if solids are frequently analyzed.

In preparing standards, the analyte *matrix* must be matched *as closely as possible*.

Methylcyclopentadienyl manganese tricarbonyl (MMT) is added to make high-octane number gasoline. If gasoline is to be analyzed for Mn, an appropriate hydrocarbon solvent matrix must be used for standards, not water.

Direct (or Multiple External Standards) Calibration Curve Procedure

1.Prepare a series of standard solutions (analyte solutions with known concentrations).
2.Plot [analyte] vs. Analytical Signal.

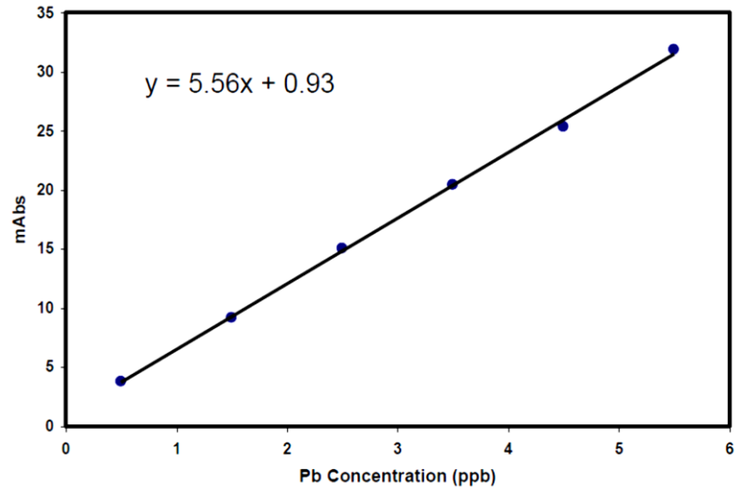
3.Use signal for unknown to find [analyte].

Example 1. A lead (Pb) in Blood is determined by GFAAS and gave the results below:

[Pb] **(ppb)**	Signal (mAbs)
0.50	3.76
1.50	9.16
2.50	15.03
3.50	20.42
4.50	25.33
5.50	31.87

Draw a calibration curve for the previous data.

Solution:



Results of linear regression can be done using the equation: S = mC + b
where m = 5.56 mAbs/ppb, b = 0.93 mAbs

Internal Standard and Standard Addition Calibration Procedure

In atomic spectrometric methods, signals can frequently vary with time due to factors like fluctuations in gas flow rates and aspiration rates. Precision can be improved by the technique of **internal standards**. For the measurement of sodium and potassium in serum, the accuracy can be significantly improved by adding *a fixed concentration* of lithium to *all standards* and *samples* and interpreting the data in terms of the ratios of the K/Li and Na/Li signals. If the aspiration rate, for example, fluctuates, each signal is affected to the same extent and the ratio, at a given K or Na concentration, remains constant. Ideally, the internal standard element should be chemically similar to the analyte element, and their wavelengths should not be too different.

In **standard addition**, known quantities of analyte are added to the unknown.

-From the increase in signal, we deduce how much analyte was in the original unknown.

-This method requires a linear response to analyte.

-Standard addition is especially appropriate when the sample composition is unknown or complex and affects the analytical signal.

-The matrix is everything in the unknown, other than analyte.

-A **matrix effect** is a change in the analytical signal caused by anything in the sample other than analyte.

As mentioned above, the technique of **standard addition** can be utilized to minimize matrix-induced errors.

One principal difference between atomic spectrometry (and most other analytical techniques) with potentiometry is the linear dependence of the analytical response on

concentration rather than on the logarithm of the concentration as is encountered in potentiometry.

A linear dependence generally simplifies the interpretation of the data.

Consider the blank-corrected absorbance A_s arising from a sample of unknown concentration (C_{unk}). We take V_s mL of a sample and **spike** it with V_{std} mL volume of a standard of known concentration C_{std} ($V_{std} << V_s$ to minimally affect matrix composition. C_{std} is so chosen that the change in concentration of the spiked sample from the original sample concentration is of the same order as the anticipated sample concentration). We measure the blank corrected absorbance of the **spiked sample** to be A_{spk}, representing C_{spk}, which is the sum of the sample plus spiked concentrations.

Assuming a linear analytical response,

$$A_s = kC_{unk} \text{ and}$$
$$A_{spk} = kC_{spk}$$

Where

$$C_{spk} = \frac{A_s\, V_{std}\, C_{std}}{A_{spk}(V_s + V_{std}) - A_s V_s}$$

In practice, at least two standard addition readings resulting in two different final concentrations should be taken in addition to the original sample to ensure that one is still operating within the linear range. Further, as blanks can often be significant, it is important to perform blank corrections. Multiple standard additions are often carried out for high accuracy.

Spike recovery

If C_s is the concentration of analyte in a sample, then the spike recovery is defined as

$$\%\ \text{recovery} = \frac{C_{spk} - C_{Unspk}}{C_{added}} = 100\%$$

Example 2. A serum sample is analyzed for potassium by flame emission spectrometry using the method of standard additions. Two 0.500-mL aliquots are added to 5.00-mL portions of water. To one portion is added 10.0 µL of 0.0500 M KCl solution. The net emission signals in arbitrary units are 32.1 and 58.6. What is the concentration of potassium in the serum?

Solution:

The amount of standard added is

$$0.0100\ \text{mL} \times 0.0500\ M = 5.00 \times 10^{-4}\ \text{mmol}$$

This produces a signal of

$$58.6 - 32.1 = 26.5\ \text{arbitrary units}$$

The millimoles of potassium in the sample, then, is

$$5.00 \times 10^{-4}\ \text{mmol} \times \frac{32.1\ \text{units}}{26.5\ \text{units}} = 6.06 \times 10^{-4}\ \text{mmol}$$

This is contained in 0.500mL serum, so the concentration of potassium in the serum is

$$\frac{6.06 \times 10^{-4}\text{mmol}}{0.500\ \text{mL}} = 1.21\text{x}10^{-3}\ \text{mmol/mL serum}$$

Graphical Procedure for Standard Addition

There are two common methods to perform standard addition:

1- If the analysis does not consume solution, we begin with an unknown solution and measure the analytical signal. Then, we add a small volume of concentrated standard and measure the signal again.

2- We add several more small volumes of standard and measure the signal after each addition.

Standard should be concentrated so that only small volumes are added and the sample matrix is not appreciably altered.

Added standards should increase the analytical signal by a factor of 1.5 to 3.

Example 3.

The quantitative analysis of cadmium in water- standard addition experiment

Add 0.279 M Cd^{2+} (as a standard solution) to 50.0 ml water (sample)

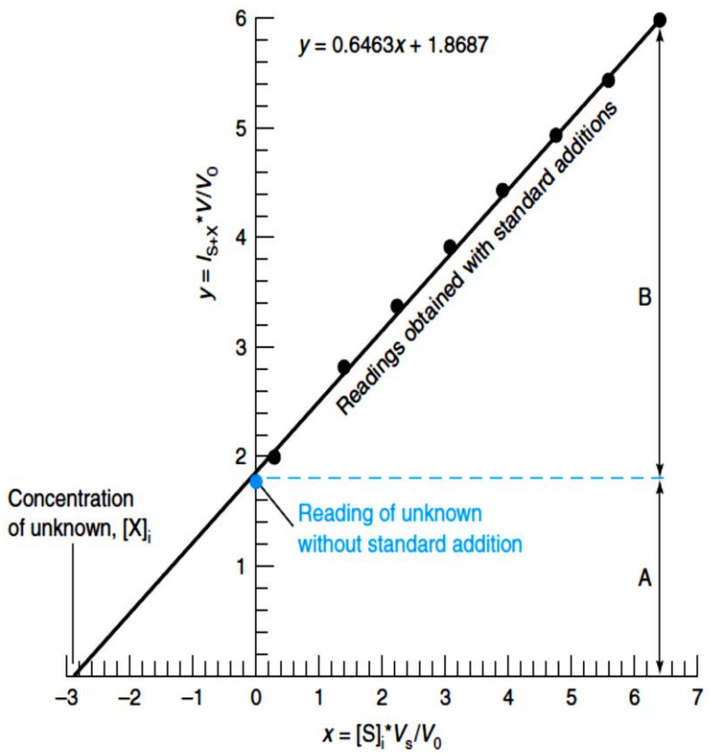
	V_s =			
V_o (ml) =	mL Cd^{2+}	$I_{(S+X)}$ =	x-axis function	y-axis function
50	solution added	signal (µA)	S_i*Vs/Vo	$I_{(s+x)}$*V/Vo
$[S]_i$ (mM) =	0.000	1.78	0.000	1.780
279	0.050	2.00	0.279	2.002
	0.250	2.81	1.395	2.824
	0.400	3.35	2.232	3.377
	0.550	3.88	3.069	3.923
	0.700	4.37	3.906	4.431
	0.850	4.86	4.743	4.943
	1.000	5.33	5.580	5.437
	1.150	5.82	6.417	5.954

$$I_{S+X}\left(\frac{V}{V_o}\right) = I_X + \frac{I_X}{[X]_i}[S]_i\left(\frac{V_s}{V_o}\right)$$

Function to plot on y-axis

Function to plot on x-axis

The right side of Equation is 0 when $[S]_i(V_S/V_o) = -[X]_i$



Instrumentation

In this part, we will briefly describe four techniques, and point out the important criteria by which to judge their applicability to your own analytical problems. Table 1 below shows a checklist of common analytical requirements and may help in the assessment of the techniques.

Table 1: Checklist of analytical requirements

•	How many samples/week?

• What are the sample types? (Steels, rocks, effluents, soils, etc)
• What method of dissolution may be employed?
• How many and what elements need to be determined?
• Is Chlorine important (far UV option for some ICP-OES spectrometers)?
• What are the concentration ranges?
• What sample volume is typically available?
• What other options/accessories are being considered? Why?
• How important is isotope information to you?
• How much money is available to purchase or lease?
• What is the cost of ownership and running costs for the techniques to fulfill the requirements?
• What level of skill operator is available to you?

ICP-MS offers initially, albeit at a higher cost, the advantages of ICP-OES and the detection limit advantages of graphite furnace-atomic absorption spectrometry (GF-AAS). Basically, the sample introduction system and plasma of ICP-OES and ICP-MS look similar. In ICP-OES, the optical spectrum with a typical range of 165-800 nm is viewed and measured, either sequentially or simultaneously. The simultaneous ICP-OES can be faster for a large number of elements, but more expensive, than sequential ICP-OES. This greatly depends on the number of elements, and the concentrations required. More recently several ICP-OES spectrometers are able to reach to 120 nm (3), thus enabling the determination of Cl at the primary wavelength of 134.664 nm with sub-ppm detection limits.

(1) Flame Atomic Absorption Spectrometry (FAAS)

In flame AA, a cloud of ground state atoms is formed by aspirating a solution of the sample into a flame of a temperature sufficient to convert the element to its *atomic state*.
The degree of absorption of characteristic radiation produced by a suitable source will be proportional to the population of ground state atoms in the flame, and hence to the *concentration of the element* in the *analyte*.
Either an air/acetylene or a nitrous oxide/acetylene flame is used to evaporate the solvent and dissociate the sample into its component atoms. When light from a hollow cathode lamp (selected based on the element to be determined) passes through the cloud of atoms, the atoms of interest absorb the light from the lamp. This is measured by a detector, and used to calculate the concentration of that element in the original sample.
The use of a flame limits the excitation temperature reached by a sample to a maximum of approximately 2600 °C (with the N_2O/acetylene flame). For many elements this is not a problem. Compounds of the alkali metals, for example, and many of the heavy metals such as lead or cadmium and transition metals like manganese or nickel are all atomized with good efficiency. The flame atomic absorption spectrophotometer allows detection of metals in any type of industrial sample provided it admits solubilization. Detection levels in this case are within the ppm range (mg/Kg).
However, there are a number of refractory elements like **V**, **Zr**, **Mo** and **B** which do not perform well with a flame source. This is because the maximum temperature reached, even with the N_2O/acetylene flame, is insufficient to break down compounds of these elements. As a result, flame AAS sensitivity for these elements is not as good as other elemental analysis techniques.

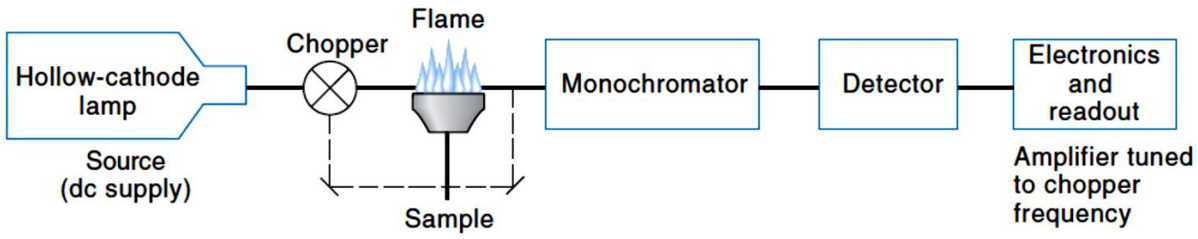


Figure 3. Schematic diagram of a flame atomic absorption spectrometry

(2) Graphite Furnace- Atomic Absorption Spectrometry (GF-AAS)

This technique is essentially the same as flame AA, except *the flame is replaced by a small, electrically heated graphite tube, or cuvette*, which is heated to *a temperature up to 3000 °C* to generate the *cloud of atoms*. The higher atom density and longer residence time in the tube improve furnace AAS detection limits by a factor of up to 1000x compared to flame AAS, down to the sub-ppb range.

Atomic absorption spectrophotometry with graphite furnace allows introduction of small samples (less than 100 µl) or direct introduction of liquid organic samples. Usually, biological samples of clinical origin are analyzed(blood, serum, urine, hepatic biopsies, etc.). Because of its high sensibility (ppb levels), this technique is used for metal detection in high purity products, such as drugs, food (fish and meat) and industrial products, as well as in clean waters (determination of the presence of Cu, As, Pb, etc.).

However, because of the temperature limitation and the use of graphite cuvettes, refractory element performance is still somewhat limited.

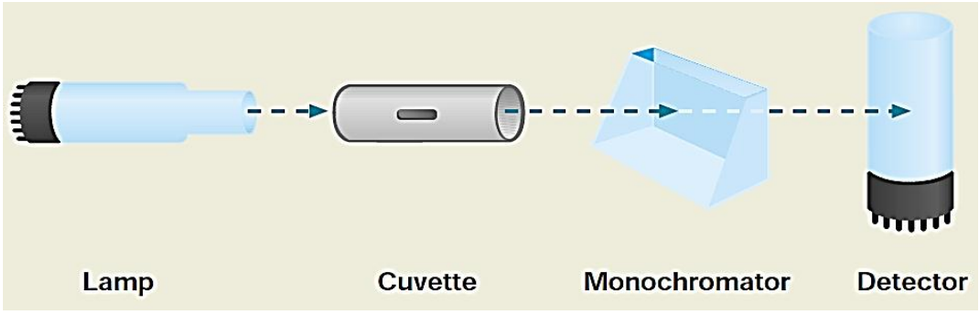


Figure 4. Schematic diagram of a graphite furnace atomic absorption spectrometry

(3) Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, or ICP)

ICP-AES, often referred to simply as ICP, is a multi-element analysis technique that uses an inductively coupled plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. A detector measures the intensity of the emitted light, and calculates the concentration of that particular element in the sample.

When undergoing ICP analysis, the sample experiences temperatures as high as 10,000 °C, where even the most refractory elements are atomized with high efficiency.

As a result, detection limits for these elements can be orders of magnitude lower with ICP than with FAAS techniques, typically at the 1-10 parts-per-billion level.

ICP instruments come in two “flavors,” radial and axial. In the traditional radial configuration, the plasma source is viewed from the side, across the narrow emitting central channel of the plasma. Many newer systems view the emitting channel horizontally along its length; this is known as the axial method. Axial viewing increases the path length and reduces the plasma background signal, resulting in detection limits as much as 5-10x lower than with the radial configuration.

Simultaneous ICP instruments can screen for up to 60 elements in a single sample run of less than one minute, with no compromise of precision or detection limits.

Sequential ICPs can provide analytical results for about five elements per minute.

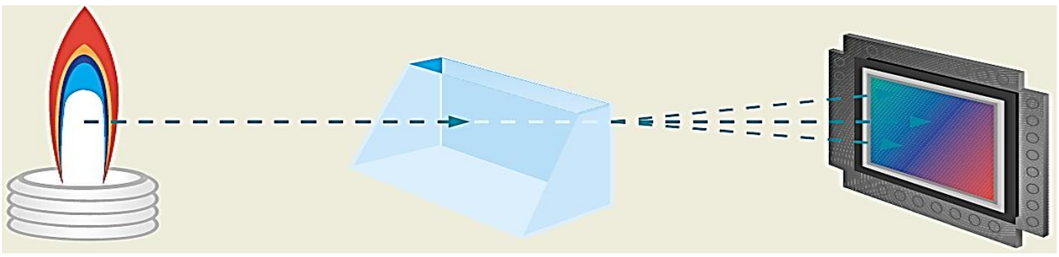


Figure 5. Schematic diagram of an inductively coupled plasma atomic emission spectrometry

(4) Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is a multi-element technique that also uses an ICP plasma source to dissociate the sample into its constituent atoms or ions. However, in this case, the ions themselves are detected, rather than the light that they emit. The ions are extracted from the plasma and passed into the mass spectrometer, where they are separated based on their atomic mass-to-charge ratio by a quadrupole or magnetic sector analyzer.

The high number of ions produced, combined with very low backgrounds, provides the best detection limits available for most elements, normally in the parts-pertrillion range. It is important to remember that detection limits can be no better than lab cleanliness allows.

Quadrupole mass spectrometers are most common in ICP-MS, yet magnetic sector instrumentation fulfills exacting requirements that demand the ultimate detectability and isotope ratio measurement.

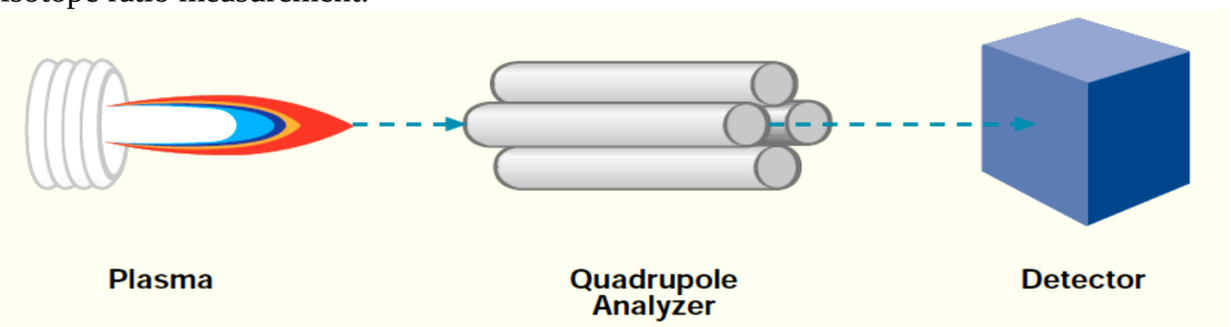


Figure 6. Schematic diagram of an inductively coupled plasma mass spectrometry

The following table summarizes strengths and limitations for te previous techniques.

Table 2. Strengths and limitations for the four mentioned techniques

Strengths	Limitations
(1)Flame Atomic Absorption Spectrometry (FAAS)	
• Easy to use **• Very fast** **• Lowest capital cost** **• Relatively few interferences** **• Very compact instrument** **• Good performance** **• Robust interface**	• Moderate detection limits • Element limitations • 1-10 elements per determination • No screening ability
(2) Graphite Furnace Atomic Absorption Spectrometry (GFAAS)	
•Very good detection limits **• Small sample size** **• Moderate price** **• Very compact instrument** **• Few spectral interferences**	•Slower analysis time • Chemical interferences • Element limitations • 1-6 elements per determination • No screening ability • Limited dynamic range
(3) Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, or ICP)	
• Easy to use **• Multi-element**	• Moderate to low detection limits (but often much better than FAAS)

• High productivity **• Very economical for many samples and/or elements** **• Few chemical interferences** **• Robust interface** **• Excellent screening abilities** **• High total dissolved solids** **• Solid and organic samples**	• Spectral interferences possible • Some element limitations
(4) Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	
• Excellent detection limits **• Multi-element** **• High productivity** **• Very economical for many samples and/or elements** **• Wide dynamic range** **• Isotopic measurements** **• Fast semiquantitative screening** **• Hybrid techniques** **LA-ICP-MS (solids)#** **LC-ICP-MS (speciation)#** **• Easily interpreted spectra** ***#LA = laser ablation LC = liquid chromatography***	• Some method development skill required • Higher initial capital cost • Some spectral interferences, but well defined • Limited to <0.2% dissolved solids

Table 3 summarizes the characteristics of mentioned atomic spectroscopy techniques.

Table 3. Comparison table of the atomic spectroscopy techniques

	Flame AAS	**GFAAS**	**ICP-AES**	**ICP-MS**
Detection limits	Very good for some elements	Excellent for some elements	Very good for most elements	Excellent for most elements
Sample throughput	10-15 secs per element	3-4 mins per element	1-60 elements/ minute	All elements in <1 minute
Dynamic range	10^3	10^2	10^6	10^8
Precision	0.1-1.0%	0.5-5%	0.1-2%	0.5-2%
short term	2-beam 1-2%	1-10%	1-5%	2-4%
long term	1-beam < 10%	(tube lifetime)	--	--
Interferences				
Spectral	Very few	Very few	Many	Few
Chemical (matrix)	Many	Very many	Very few	Some
Physical (matrix)	Some	Very few	Very few	Some
Dissolved solids in solution	0.5-5%	> 20% (slurries)	0-20%	0.1-0.4%
Elements applicable to	68+	50+	73	82
Sample volumes required	Large	Very small	Medium	Very small to medium
Semiquantitative analysis	No	No	Yes	Yes
Isotopic analysis	No	No	No	Yes
Ease of use	Very easy	Moderately easy	Easy	Moderately easy
Method development	Easy	Difficult	Moderately	Difficult

			easy	
Unattended operation	No	Yes	Yes	Yes
Capital costs	Low	Medium to high	High	Very high
Running costs	Low	Medium	High	High
Cost per elemental analysis				
High volume – few elements	Low	High	Medium	Medium
High volume –many elements	Medium	High	Low-Medium	Low-Medium

Problems:

1. A standard containing 1.0 ppm of NO_3-N was treated with colorizing agents to give an absorbance of 0.20 at 507 nm. A sample treated in the same manner, gave an absorbance of 0.15 at the same wavelength. Determine the concentration of nitrate-nitrogen in this sample. (*Ans.* 0.75 ppm NO_3-N).

2. An unknown was found to contain 10.0 µg of analyte per liter. A spike of 5.0 µg/L was added to a replicate portion of unknown. Analysis of the spiked sample gave a concentration of 14.6 µg/L. Find the percent recovery of the spike. The acceptable recovery limit is ±4%. (*Ans.* 92%, recovery limit range is 96 to 104%)

3. A 5.00-mL sample of blood was treated with trichloroacetic acid to precipitate proteins. After centrifugation, the resulting solution was brought to pH 3 and extracted with two 5-mL portions of methyl isobutyl ketone containing the lead-complexing agent APCD. The extract was aspirated directly into an air/acetylene flame and yielded an absorbance of 0.502 at 283.3 nm using AAS. Five-milliliter aliquots of standard solutions containing 0.400 and 0.600 ppm of lead were treated in the same way and yielded absorbances of 0.396 and 0.599. Find the concentration of lead in the sample in ppm assuming that Beer's law is followed. (*Ans.* 0.504 ppm Pb).

4. *STANDARD ADDITION (with dilution)* problem- An unknown sample of Cu^{2+} gave an absorbance of 0.262 in an atomic absorption analysis. Then 1.00 mL of solution containing 100.0 ppm Cu^{2+} was mixed with 95.0 mL of unknown and the mixture was diluted to 100.00 mL in a volumetric flask. The absorbance of the new solution was 0.500.
   a) Denoting the initial unknown concentration as $[Cu^{2+}]_i$, write an expression for the final concentration $[Cu^{2+}]_f$ after dilution in units of concentration of ppm. (*Ans.* Use Dilution law).
   b) In a similar manner, write the final concentration of added standard, designated as $[S]_f$. (*Ans.* 1 ppm $[S]_f$).
   c) Find $[Cu^{2+}]_i$ in the unknown. (*Ans.* 0.504 ppm $[Cu^{2+}]_i$).

5. *INTERNAL STANDARD (with dilution)* problem- A solution containing 3.46 mM X (analyte) and 1.72 mM S (standard) gave peak areas of 3473 and 10,222, respectively, in a chromatographic analysis. Then 1.00 mL of 8.4 7 mM S was added to 5.00 mL of unknown X and the mixture was diluted to 10.0 mL. This solution gave peak areas of 5428 and 4431 for X and S, respectively.
   a) Calculate the response factor for the analyte. (*Ans.* Use the equation $F = A_X/[X]/(A_S/[S])$, 0.168_3).
   b) Find the concentration of S (mM) in the 10.0 mL of mixed solution. (*Ans.*$[S]_f$ =0.847 mM).
   c) Find the concentration of X (mM) in the 10.0 mL of mixed solution. (*Ans.* $[X]_f$ = 6.61_5 mM).
   d) Find the concentration of X in the original unknown solution. (*Ans.* $[X]_i$ =12.3_3 mM).

6. Strontium was being determined by flame emission spectrophotometry. A set of strontium standards was prepared and to each of the standards and the samples a fixed amount of scandium (as an **internal standard**) was also added. The emission intensities for each of the solutions was measured:

	Sr Conc (mg/L)	Sr Emission	Sc Emission
Standards	0.00	0.0	19.4
	1.00	16.6	21.5
	2.00	37.8	24.7
	3.00	43.2	18.6
	4.00	68.7	22.3
	5.00	95.2	24.6
Samples	A	45.6	20.2
	B	102.7	21.6

Determine the strontium concentration (mg/L) in each of the samples. (*Ans.* A = 2.92 and B = 6.15 mg/L).

7. ELECTROANALYTICAL CHEMISTRY (EECTROANALYSIS)

Electrochemistry (Figure 1) is the study of the relations between chemical reactions and electricity. or:

- It is the study of the interconversion of chemical energy and electrical energy.

-The study of redox reactions.

-Electrochemical processes involve the transfer of electrons from one substance to another.

It encompasses a group of quantitative analytical methods that are based upon the electrical properties of a solution of the analyte when it is made part of an electrochemical cell.

Analysts always ask questions such as ‘what is it?’, ‘how much of it is present?’, and sometimes, ‘how fast does it change?’. Electrochemistry is an ideal analytical tool for answering each of these questions - sometimes simultaneously.

Electroanalytical chemistry deals with the relationship between electricity and chemistry using analytical calculations that based on the measurement of electrical quantities (current, potential, charge, or resistance) and their relationship to chemical parameters. It is the use of electrochemical techniques to characterize a sample.

Advantages

Electroanalytical methods have certain advantages over other analytical methods.

-- Electroanalytical techniques are capable of producing exceptionally low detection limits (low concentrations of analytes are determined without difficulty) and

-- an abundance of characterization information including chemical kinetics information; stoichiometry and rate of interfacial charge transfer, rate of mass transfer, extent of adsorption or chemisorption, and rates and equilibrium constants for chemical reactions.

-- Electroanalytical techniques are often specific for a particular oxidation state of an element, whereas other techniques are only capable of revealing total concentrations of an element.

-- Electroanalytical techniques require relatively inexpensive/low-cost equipment than spectroscopy instruments.

-- Electroanalytical techniques provide information about activities rather than concentrations. Ordinarily, it’s the activities of ions that are significant in “ how things work”.

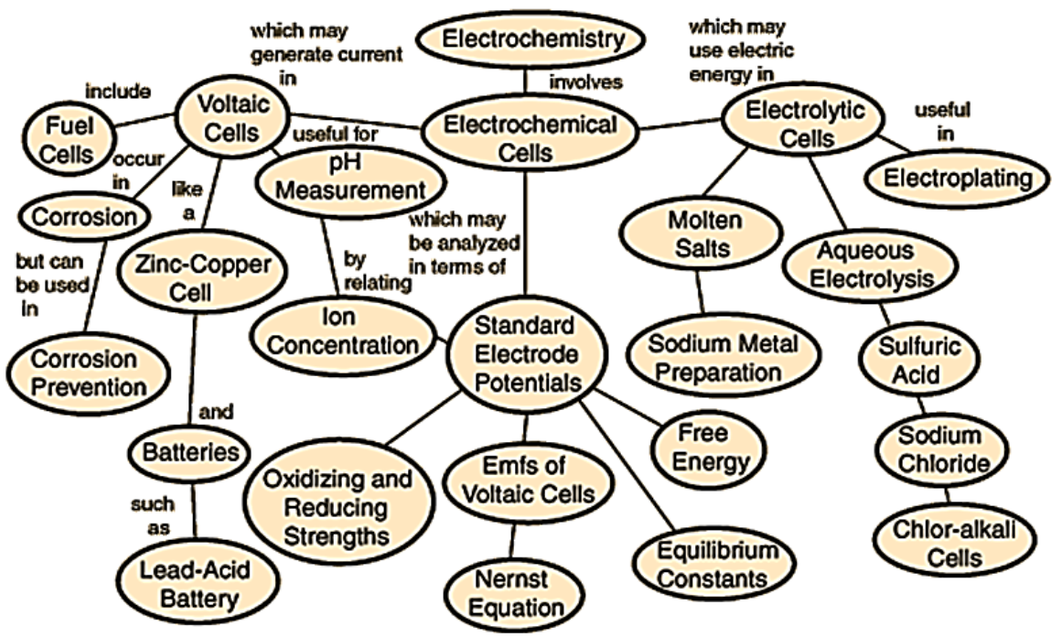


Figure 1 Electrochemistry and its relations between chemical reactions and electricity in electrochemical cells

Electroanalysis (Figure 2) is the science of carrying out analytical chemistry by the use of electrochemistry.
The electrochemist will say that a measurement that accompanies compositional changes is **dynamic** in nature, while a measurement that is performed without compositional changes occurring is **static** or **at equilibrium.**

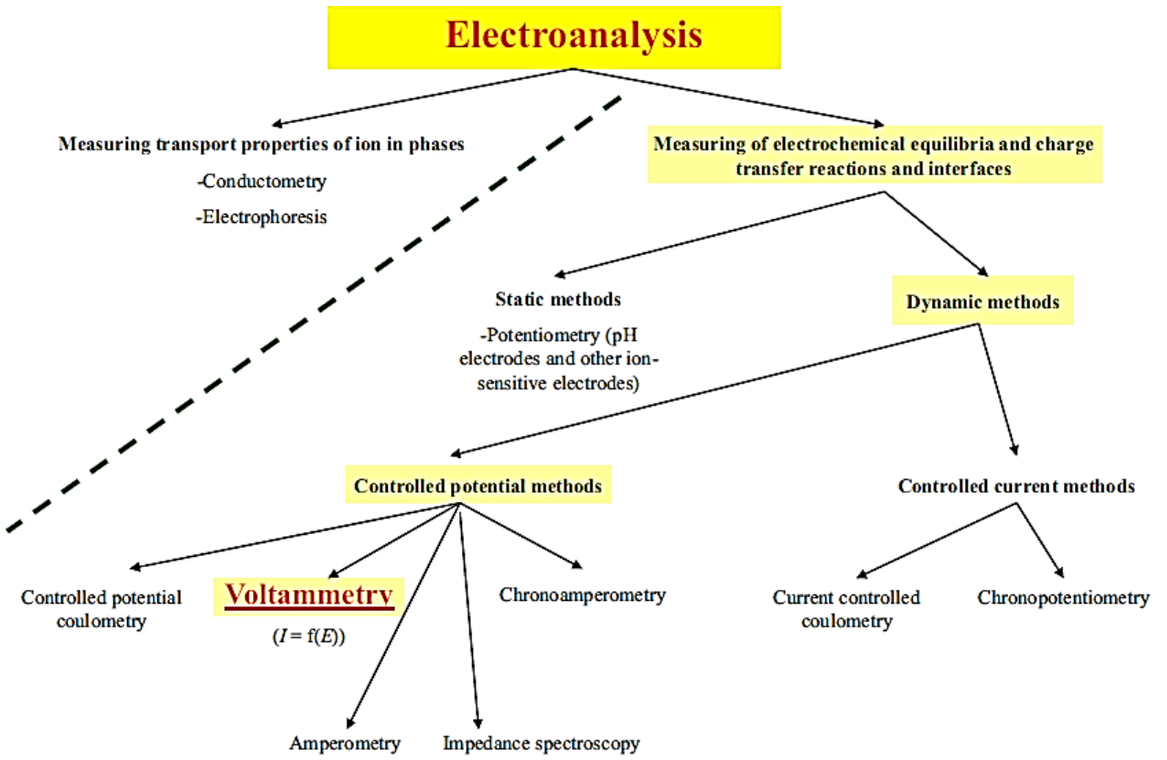


Figure 2 A possible subdivision of electroanalysis. ‘‘Dynamic techniques’’ are those in which the electrochemical equilibrium is shifted with the help of an excitation (potential changes or current changes), whereas in the ‘‘static techniques’’ the electrochemical equilibrium at the electrode is not affected. Sometimes, dynamic techniques are described as active, and the static techniques as passive techniques

Electroanalytical methods are a class of techniques in analytical chemistry which study an analyte by measuring the potential (volts) and/or current (amperes) in an electrochemical cell containing the analyte.
Figure 3 shows two main types: *Potentiometric and Potentiostatic.*
- The type of technique reflects the type of electrical signal used for quantitation.
- Techniques require at least two electrodes and an electrolyte (containing solution).
Electrodes can be working (indicator) electrode, reference electrode, and counter electrode.

Measurement of potential. Analysts employing electrochemistry usually measure potentials with a **voltmeter,** or any device capable of replicating the behavior of an accurate voltmeter. Typically, the potential is measured under conditions of **equilibrium.** While there are many definitions of ‘equilibrium’ in the broad topic of electrochemistry, the simplest, when measuring a potential, is to say that the measurement was performed at zero current.
Measurement of ***current.*** In order to measure a current, we must use an **ammeter**, or any device capable of acting as an ammeter.

(1) Potentiometric Technique
- Based on a **static** (zero-current) situation.
- Based on measurement of the potential established across a membrane.

- Used for direct monitoring of ionic species (Ca^{2+}, Cl^-, K^+, H^+).

(2) Potentiostatic Technique

- Controlled-potential technique.
- Based on **dynamic** (non-zero-current) situation.
- Deals with the study of charge transfer processes at the electrode-solution interface.
- Chemical species are forced to gain or lose electrons.

Methods such as **potentiometry**, **coulometry**, **voltammetry**, and **polarography** are classified according to the variable being measured.

- One variable (current, voltage, charge) is measured and the others are controlled.

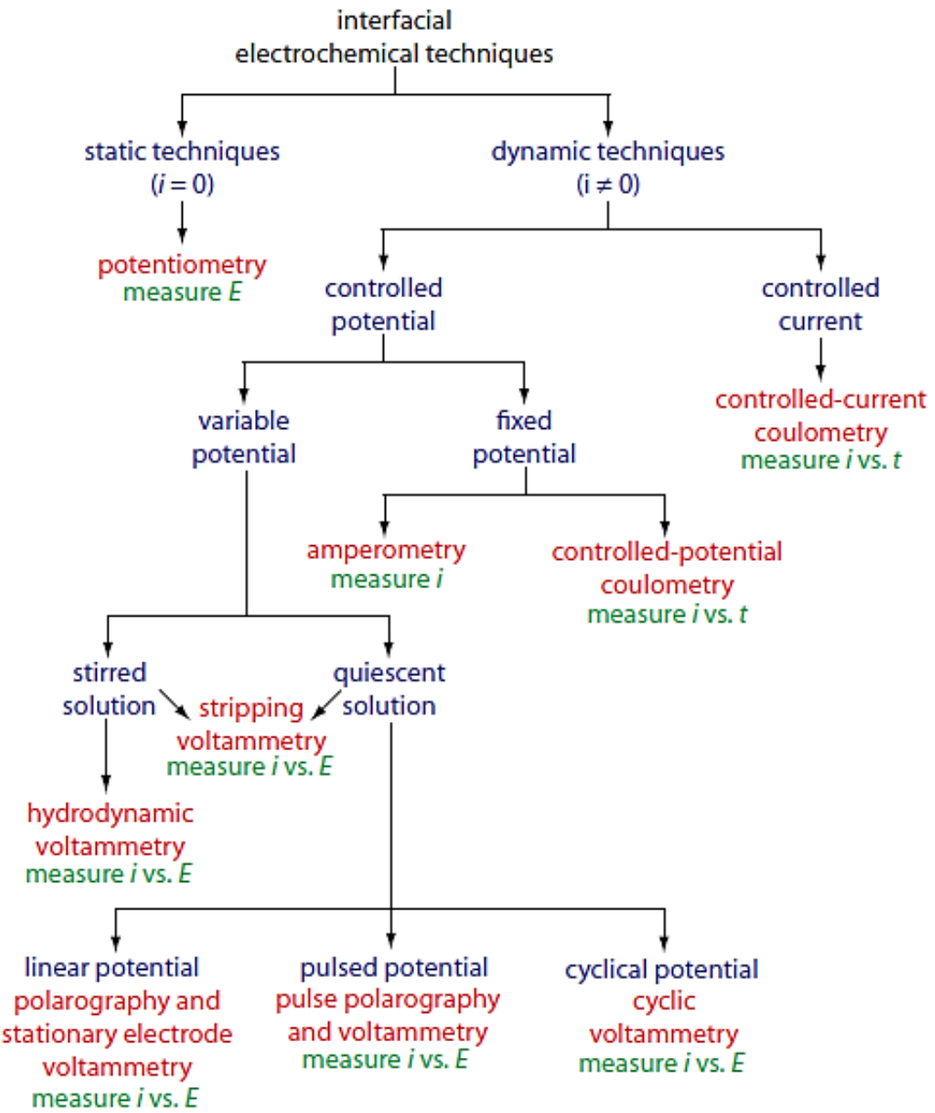


Figure 3 Family tree highlighting a number of interfacial electrochemical techniques. The specific techniques are shown in red, the experimental conditions are shown in blue, and the analytical signals are shown in green.

Some Important Concepts

1) Charge (q)

Charge (q) of an electron = - 1.602 x 10^{-19} C

Charge (q) of a proton = + 1.602 x 10^{-19} C

C = coulombs: Charge of one mole of electrons

= (1.602 x 10^{-19} C)(6.022 x 10^{23}/mol) = 96,485 C/mol

= Faraday constant (F)

The charge (q) transferred in a redox reaction is given by **q = n x F**.

2) Current (I)

- The quantity of charge flowing past a point in an electric circuit per second

I = q/time

Units: Ampere (A) (or 'amp', for short).= coulomb per second (C/s); 1A = 1C/s.
- Amperometric experiments measure current. The ***current I*** is the rate at which charge is passed, while the **current density** is symbolized as i . Current density is defined as the current per unit electrode area A, so we can write the following:

i= *I*/A

3) Voltage or Potential Difference (E)

- The amount of energy required to move charged electrons between two points
- Work done by or on electrons when they move from one point to another:

w = E x q or **E = w/q**

Units: volts (V or J/C); 1V = 1J/C

4) Ohm's Law

I= E/R

R = resistance

Units: Ω (ohm) or V/A.

5) Electrode

- Conducts electrons into or out of a redox reaction system.
- The electrode surface serves as a junction between an ionic conductor and an electronic conductor.

Examples are platinum wire, carbon (glassy or graphite), Gold, Silver…etc.

6) Electroactive Species

- Species that undergo an oxidation or a reduction during reaction.
- Species may be complex, solvated, molecule, or ion.
- Species may be in aqueous or nonaqueous solution.

7) Measurements

Electrochemical Measurements- Occur at the electrode – solution interface.

Chemical Measurements - Involve homogeneous bulk solutions.

8) Electrochemical Cell

Electrode Potential

- A measure of the ability of the half-cell to do work (the driving cell for the half-cell reaction).

Anode

- Electrode where oxidation occurs

$M^o \rightarrow M^{n+} + ne^-$

- Metal loses electrons and dissolves (enters solution)

$Cd(s) \rightarrow Cd^{2+} + 2e^-$

$Ag(s) \rightarrow Ag^+ + e^-$

Cathode

- Electrode where reduction occurs

$M^{n+} + ne^- \rightarrow M^o$

- Positively charged metal ion gains electrons
- Neutral atoms are deposited on the electrode
- The process is called electrodeposition

$Cd^{2+} + 2e^- \rightarrow Cd(s)$

$Ag^+ + e^- \rightarrow Ag(s)$

Electrolysis

- Voltage is applied to drive a redox reaction that would not otherwise occur.

Examples are production of aluminum metal from Al^{3+} , production of Cl_2 from Cl^-…etc.

Electrochemical Cells can be

Electrolytic Cell

-Nonspontaneous reaction.
- Requires electrical energy to occur.

- Consumes electricity from an external source.

Galvanic Cell or (Voltaic Cell)

- Spontaneous reaction.
- Produces electrical energy.
- Can be reversed electrolytically for reversible cells.
- A spontaneous redox reaction generates electricity.
- One reagent is oxidized and the other is reduced.
- The two reagents must be separated (cannot be in contact).
- Electrons flow through a wire (external circuit).

An example is rechargeable batteries.

Conditions for Non-reversibility

- If one or more of the species decomposes.
- If a gas is produced and escapes.

Galvanic Cell-Oxidation Half-Reaction

- Loss of electrons
- Occurs at anode (negative electrode)
- The left half-cell by convention

Galvanic Cell -Reduction Half-Reaction

- Gain of electrons
- Occurs at cathode (positive electrode)
- The right half-cell by convention

Galvanic Cell -Salt Bridge

- Connects the two half-cells (anode and cathode).
- Filled with gel containing saturated aqueous salt solution (KCl).
- Ions migrate through to maintain electroneutrality (charge balance).
- Prevents charge buildup that may cease the reaction process.
- Figure 4 shows the overall reaction for :

$$Cu^{2+}(aq) + Zn(s) \rightarrow Cu(s) + Zn^{2+}(aq)$$

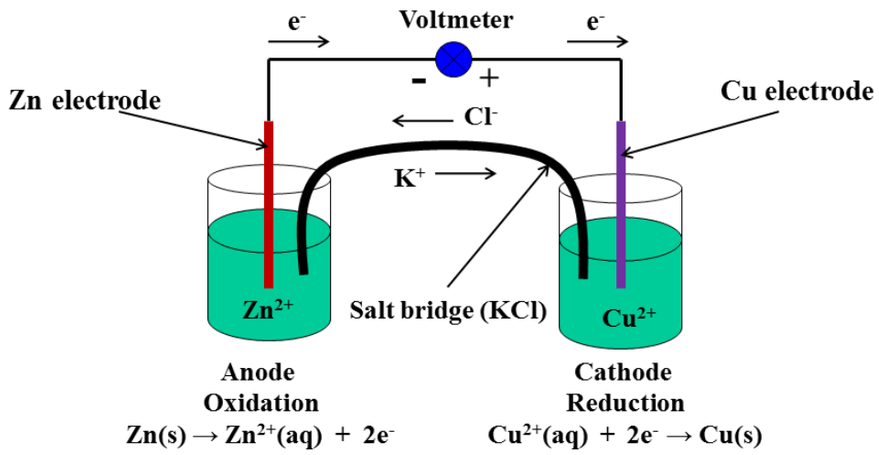


Figure 4 A galvanic cell for the reaction between Zn and Cu

9) Line Notation

Phase boundary: represented by one vertical line (|).

Salt bridge: represented by two vertical lines (‖).

$Fe(s) \mid FeCl_2(aq) \| CuSO_4(aq) \mid Cu(s)$

10) Standard Potentials

Standard Reduction Potential (E^o) (Table1) is used to predict the voltage when different cells are connected.

- Potential of a cell as cathode compared to standard hydrogen electrode
- Species are solids or liquids

- Activities = 1
- Concentrations can be used for simplicity; concentration = 1 M, pressures = 1 bar.

Standard Hydrogen Electrode (SHE) is a reference electrode half-cell.
- Used to measure E^o for half-reactions (half-cells), Table 1.
- Connected to negative terminal (anode).

Assigned E^o = 0.000 under standard state conditions (T = 25 ^oC, concentration = 1M, pressure = 1 bar, pure solid or liquid).

SHE consists of
- Platinized Pt electrode immersed in a solution of 1M HCl.
- H_2 gas (1 bar) is bubbled over the Pt electrode

$2H^+(aq, 1\ M) + 2e^- \leftrightarrow 2H_2\ (g, 1\ bar)$.

Table 1. Standard half-reaction potential at 25 ^oC

	Half Reaction		E^o (V)
Oxidizing agents	$F_2 + 2e^- \leftrightarrow 2F^-$	Reducing agents	2.890
	$MnO_4^- + 5e^- \leftrightarrow Mn^{2+}$		1.507
	$Ce^{4+} + e^- \leftrightarrow Ce^{3+}$ (in HCl)		1.280
	$O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$		1.229
Increasing oxidizing power ↑	$Ag^+ + e^- \leftrightarrow Ag(s)$	↓ Increasing reducing power	0.799
	$Cu^{2+} + 2e^- \leftrightarrow Cu(s)$		0.339
	$2H^+ + 2e^- \leftrightarrow H_2(g)$		0.000
	$Cd^{2+} + 2e^- \leftrightarrow Cd(s)$		-0.402
	$Fe^{2+} + 2e^- \leftrightarrow Fe(s)$		-0.440
	$Zn^{2+} + 2e^- \leftrightarrow Zn(s)$		-0.763
	$Al^{3+} + 3e^- \leftrightarrow Al(s)$		-1.659
	$K^+ + e^- \leftrightarrow K(s)$		-2.936
	$Li^+ + e^- \leftrightarrow Li(s)$		-3.040

11) Cell Potential

In practice, we have to measure the difference or separation in energy between two (or more) electrodes. This separation is termed the ***emf***, following from the somewhat archaic expression 'electromotive force'. In other texts, the alternative name is E_{cell} sometimes given to the *emf*; some texts (rather confusingly) call it just *E*.
- The potential for a cell containing a specified concentration of reagent other than 1 M.

Standard Cell Potential

$E^o_{cell} = E^o_{cathode} - E^o_{anode}$

Cell Potential

The potential of the cell is given by the equation;

$E_{cell} = E_{cathode} - E_{anode}$ or $E_{cell} = E_{ind} - E_{ref} + E_j$
- E_{cell} is positive for spontaneous reactions.
- Half-reaction is more favorable for more positive E^o.

Junction Potential
- Is produced when there is a difference in concentration or types of ions of the two half-cells.
- Is created at the junction of the salt bridge and the solution.
- Is a source of error.
- Minimized in KCl salt bridge due to similar mobilities of K^+ and Cl^-.

12) Nernst Equation

Nernst equation gives relationship between the potential of an electrochemical cell and the concentration of reactants and products.

$$O + ne^- \leftrightarrow R$$

$$E = E^O + \frac{2.3RT}{nF}\log\left(\frac{[O]}{[R]}\right)$$

were, E = electrode potential.
E^o = standard potential for the redox reaction.
R = gas constant = 8.314 J/K-mol.
T = absolute temperature in Kelvin.
F = Faraday's constant = 96,485 C/mol.
n = number of electrons transferred.
For the half-reaction
$aA + ne^- \leftrightarrow bB$
The half-cell potential (at 25 °C), E, is given by

$$E = E^O - \frac{RT}{nF}\ln\left(\frac{[B]^b}{[A]^a}\right) \text{ or } E = E^O - \frac{2.3RT}{nF}\log\left(\frac{[B]^b}{[A]^a}\right) \text{ converted to } E = E^O - \frac{0.05916}{n}\log\left(\frac{[B]^b}{[A]^a}\right)$$

For the overall reaction
$aA + bB \leftrightarrow cC + dD$
The potential at 25 °C is given by

$$E = E^O - \frac{0.05916}{n}\log\left(\frac{[C]^c[D]^d}{[A]^a[B]^b}\right)$$

Potentiometry (Potentiometric Methods)
In potentiometry, we measure the potential of an electrochemical cell under static conditions. Because no current—or only a negligible current—flows through the electrochemical cell, its composition remains unchanged. For this reason, potentiometry is a useful quantitative method. The first quantitative potentiometric applications appeared soon after the formulation, in 1889, of the Nernst equation, which relates an electrochemical cell's potential to the concentration of electroactive species in the cell. Potentiometry initially was restricted to redox equilibria at metallic electrodes, limiting its application to a few ions.

Reference Electrodes
An ideal reference electrode.has a fixed potential over time and temperature, long-term stability, ability to return to the initial potential after exposure to small currents (reversible), and obeys the Nernst equation.
• One of the half-cells provides a known reference potential, and the potential of the other half-cell indicates the analyte's concentration.
By convention, $E_{cell} = E_{ind} - E_{ref}$
The ideal reference electrode (Figures 5-7) must provide a stable potential so that any change in E_{cell} is attributed to the indicator electrode, and, therefore, to a change in the analyte's concentration.

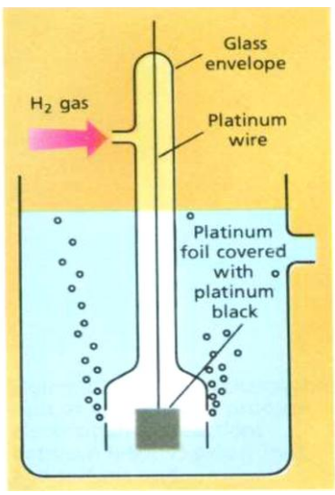


Figure 5 Standard Hydrogen Electrode-SHE (or Normal Hydrogen Electrode NHE) as a reference Electrode, H_2 gas 1.000 atm, E^0 = 0.00000 V

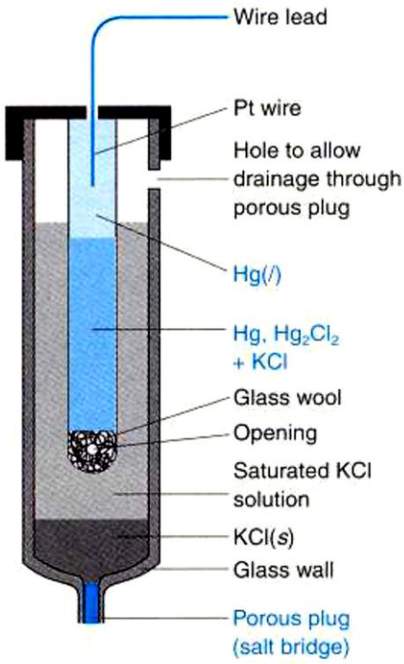


Figure 6 Saturated Calomel, Hg_2Cl_2 Electrode a- S.C.E s as a reference Electrode, E^0= +0.241 V. A small hole connects the two tubes, and an asbestos fiber serves as a salt bridge to the solution in which the SCE is immersed. The stopper in the outer tube may be removed when additional saturated KCl is needed

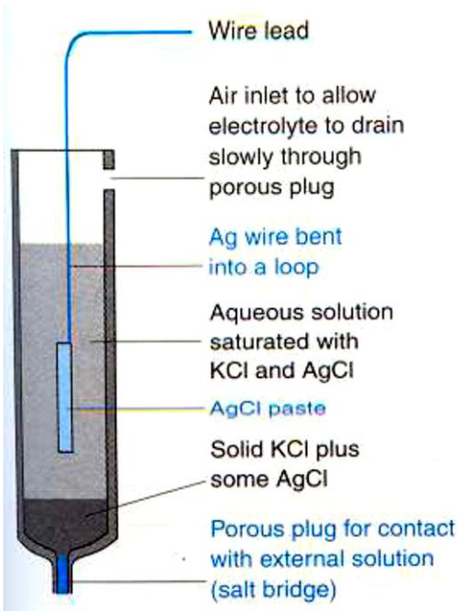


Figure 7 Silver/Silver Chloride Reference Electrode, If the KCl solution is saturated then E^0 (saturated KCl) = 0.197 V

Indicator Electrodes

The potential of the indicator electrode in a potentiometric electrochemical cell is proportional to the concentration of analyte.

• Two classes of indicator electrodes are used in potentiometry: metallic electrodes, and membrane ion-selective electrodes.

Potential measuring device

The indicator electrode produces a voltage that is proportional to the concentration of the $M^{\pm}$ concentration, and the measurement is made by a pH meter (Figure 8).
•The indicator electrode is attached to control electronics which convert the voltage to a pH (in case of a pH electrode) reading and displays it on a meter.

Figure 8 A pH-potential measuring device

Membrane Ion-Selective Electrodes
The discovery, that a thin glass membrane develops a potential, called a membrane potential, when opposite sides of this membrane are in contact with solutions of different pH led to the eventual development of a whole new class of indicator electrodes called **ion selective electrodes (ISEs).**

Using Ion-Selective Electrodes
Advantages
• Linear response to log A over a wide range.
• Don't consume unknown.
• Don't contaminate unknown.
• Have short response time.
• Color and turbidity do not hinder the electrode.

Disadvantages
• Respond to the activity (not concentration).
• Only responds to uncomplexed analyte ions.
• Precision is rarely better than 1%.
• Certain ions interfere with or poison particular electrodes which leads to sluggish, drifting response.
• Some are fragile and have limited shelf life.

Types of Ion-selective Electrodes
There are four classes of ion-selective electrodes:
1. *Glass membranes*
– These are selective to H^+ and certain monovalent cations.

2. *Solid-state electrodes*
– These are made of inorganic salt crystals.
– The inorganic salt is made such to have vacancies in its lattice structure.
– The vacancies allow the ion (needed to fill the vacancy) to migrate through the salt.

3. *Liquid-based electrodes*
– A mobile carrier transports the selected ion across a membrane impregnated with a liquid solution of the carrier.

4. *Molecular Electrodes*
1. These contain a conventional electrode surrounded by a membrane that isolates (or generates) the analyte to which the electrode responds.
2. For example, a CO_2 electrode responds the change in pH due to the presence of the CO_2.

Practical Applications of Potentiometry with Ion-Selective Electrodes
ISE are unique in determining:
– Free ions (good for toxicity).
– Determination of anions.
– Monitoring toxic gases e.g., SO_2, H_2S, NH_3, CN.
– Accessible to automated continues .
Scope of Applications:
• Water analysis:
– Surface, Sea, ground, potable, and wastewater.
• Atmospheric analysis:
• Gases are absorbed in solutions aerosol is deposited on filters.
• Sediment dust and soil are tedious to prepare.
• Analysis of foodstuffs.
• Clinical analysis.

Combined Glass Electrodes for measuring pH (Figure 8)
These consists of a thin glass bulb at the bottom that is selective to H^+.
• Two reference electrodes (usually Ag/AgCl) measure the potential difference across the membrane.

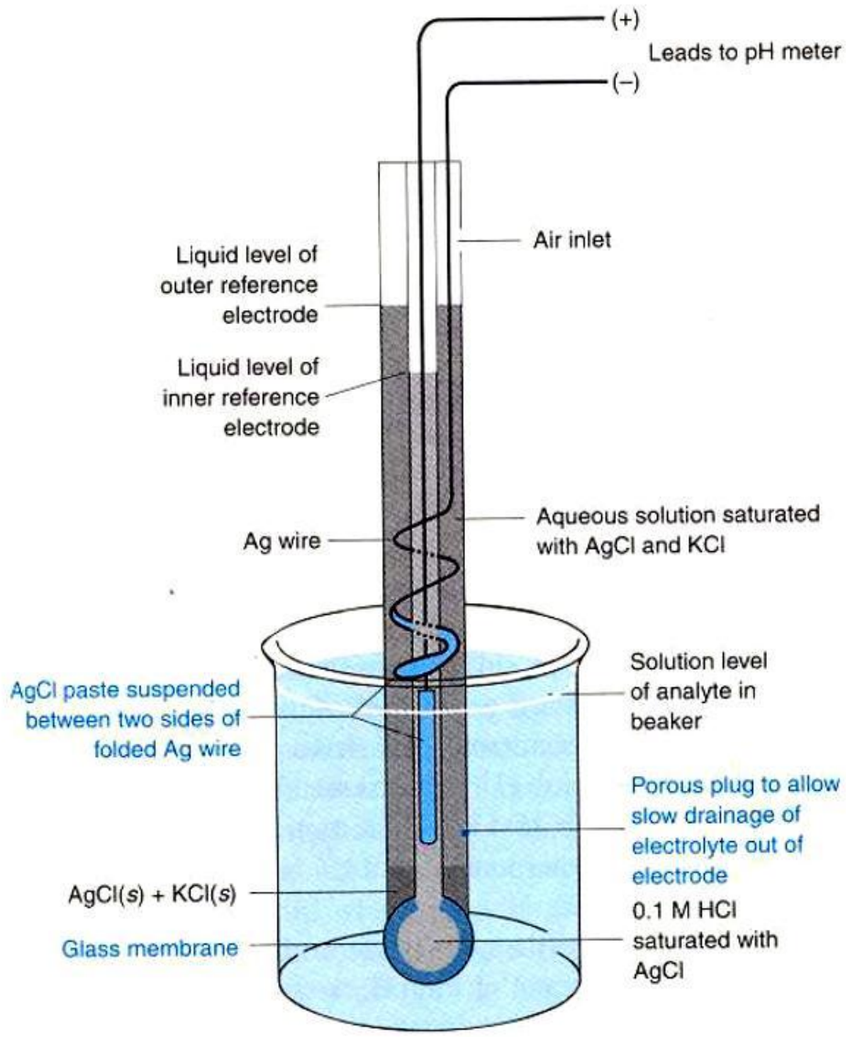
Membrane Potentials
• ISE, such as the glass pH electrode, function by using a membrane that reacts selectively with a single ion.
$E_{cell} = K + (0.0592/z) \log [A]$ sample
• This equation applies to all types of ISE's.
• For the glass electrode:
$E_{cell} = K + (0.0592/1) \log [H^+]$ sample
$E_{cell} = K - (0.0592pH)$.

Example 1
A glass electrode- SCE pair is calibrated at 25°C with a pH standard buffer, the measured voltage being 0.814 V. What voltage would be measured in a 1.00 X 10^{-3} M acetic acid solution (pH =3.88)? Assume $a_{H^+} = [H^+]$.

Solution

$$\therefore 3.88 = 4.01 + \frac{0.814 - E_{cell\ unkn}}{0.0592}$$

$E_{\text{cell unkn}} = +0.822$ V



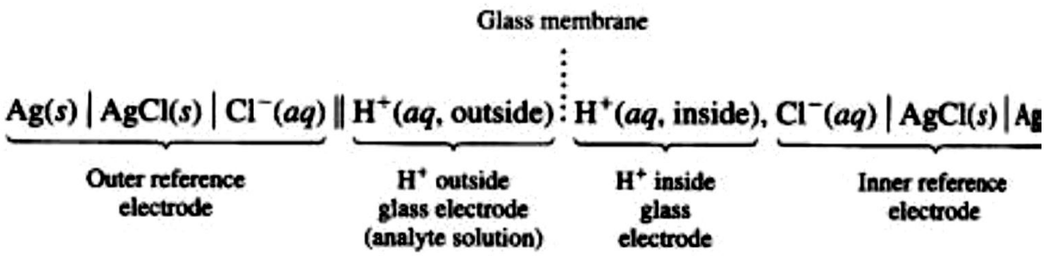


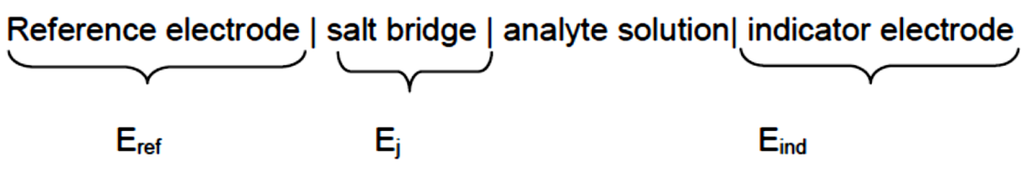
Figure 8 A typical combined glass electrodes for measuring pH

Potentiometric Methods (Figures 9)

a) Direct Potentiometry

Measure the electrode response in an unknown solution and read the concentration directly from the calibration graph (either manually or using special computer graphics and calculations) or from the meter display on a self-calibrating ion meter.

A typical cell for potentiometric analysis consists of a reference electrode, an indicator electrode and a salt bridge. This cell can be represented as



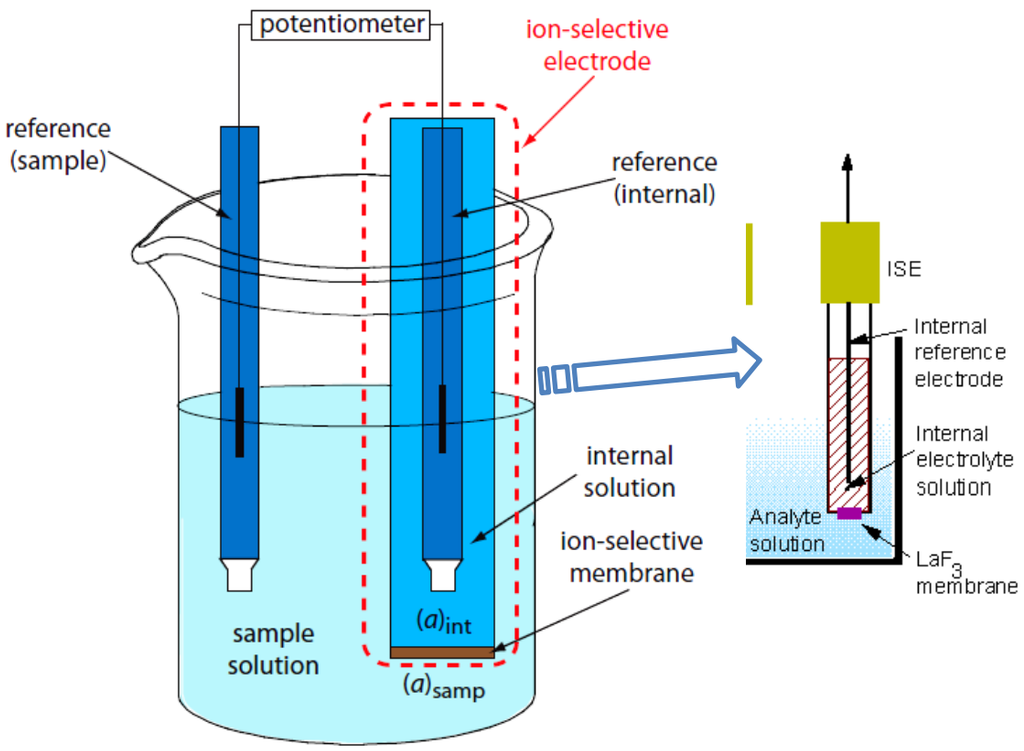


Figure 9 Schematic diagram showing a typical potentiometric cell with an ion-selective electrode. The ion-selective membrane electrode's membrane separates the sample, which contains the analyte at an activity of (a_A)samp, from an internal solution containing the analyte with an activity of (a_A)int.

b) Potentiometric Titrations

Potentiometry is generally valuable as a technique for detecting the end-point of titrations where there is often a drastic change in the concentrations of the reactants and thus a big shift in the electrode potential (Figure 11).

• These endpoint determinations can often be made more precisely than other ISE methods because they depend on the accuracy of the volumetric measurements rather than the measurement of the electrode potential.

• For example, when a chloride ion solution is titrated against the silver nitrate reagent (see figure 12) there is a gradual decrease in the Cl ion concentration as more $AgNO_3$ is added until the endpoint when all the Cl^- disappears from solution (precipitate).

• The progress of this titration can be monitored using a chloride electrode.

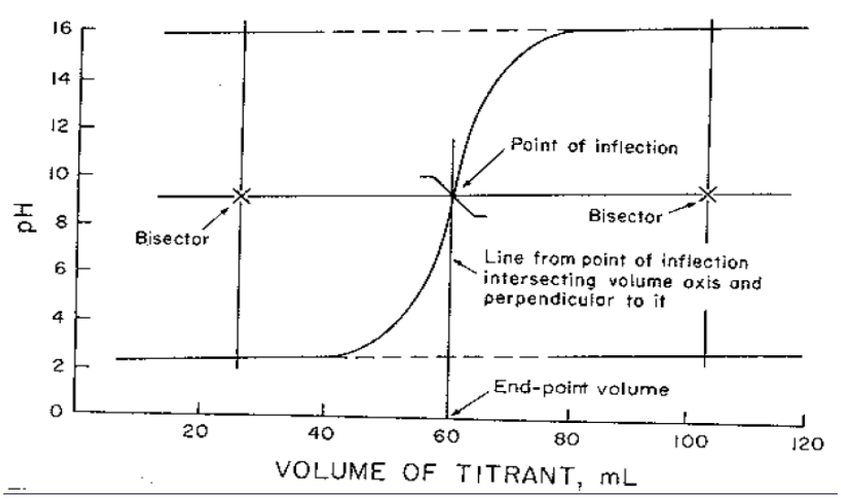


Figure 11 Potentiometric titration

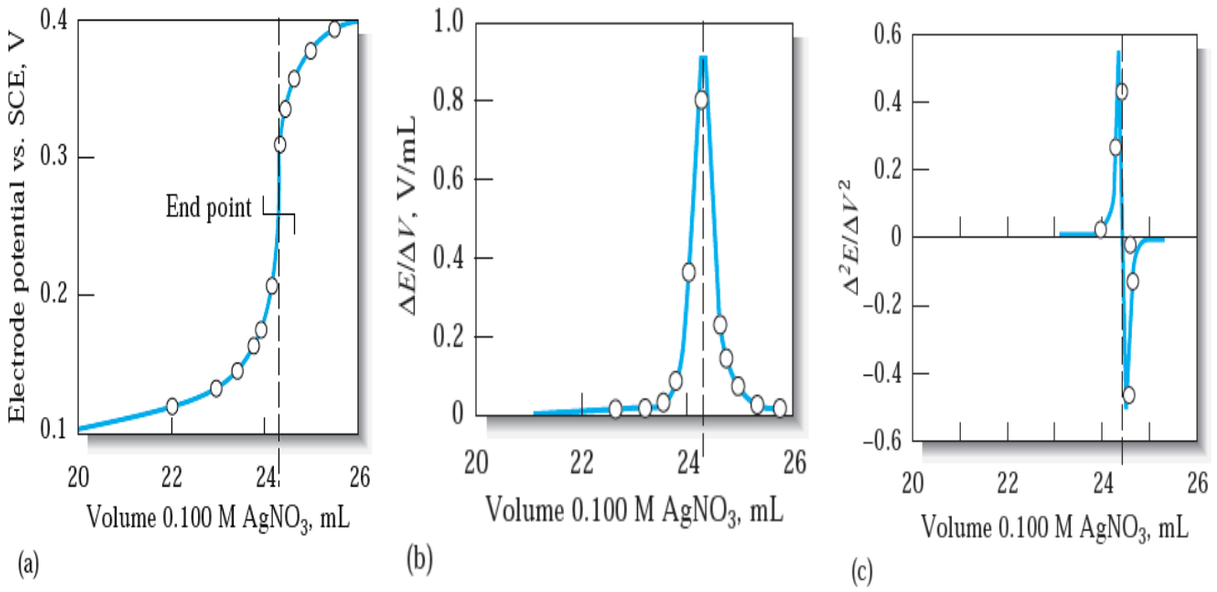


Figure 12 Titration of 2.433 mmol of chloride ion with 0.1000 M silver nitrate. (a) Titration curve. (b) First-derivative curve. (c) Second-derivative curve.

Example 2 Response of an Ion-Selective Electrode

When an F^- electrode was immersed in standard F- solutions (maintained at a constant ionic strength of 0.1M with $NaNO_3$), the following potentials (versus SCE) were observed:

[F-] (M)	*E* (mV)	
1.00 x 10^{-5}	100.0	– 58.5 mV
1.00 x 10^{-4}	41.5	
1.00 x10^{-3}	-17.0	– 58.5 mV

Since the ionic strength was held constant. the response should depend upon the logarithm of the F- *concentration.*
1-What potential is expected if [F^-] = 5.00x 10^{-5}M?
2-What concentration of F^- will give a potential of 0.0 V?

Solution
1- We seek to fit the calibration data with an equation:

$$E = m \log [F^-] + b$$

Plotting *E* versus log [F^-] gives a straight line with a slope of –58.8 mV and a y-intercept of –192.5 mV. Setting [F^-] = 5.00 x 10^{-5} M, gives

E =(– 58.5) log [5.00 x 10-5] – 192.5 = 59.1 mV.

2- If *E*= 0.0 mV, we can solve for the concentration of [F^-]:
0.0 = (– 58.5) log [F^-] – 192.5 = [F^-] = 5.1 x10^{-4}M.

8. CONDUCTOMETRIC METHOD (CONDUCTOMETRY)

The analytical chemistry method by changes in the composition of solution so it can be detected.
It is another method of end-point detection.

Measured Equivalent Conductance Λ

It is defined as
(1) the ability of the medium to carry the electric current.
(2) the migration of **positively** charged species towards the **cathode** and **negatively** charged ones through the **anode**.
(3) the conductance of one gram equivalent of solute contained between electrodes spaced one centimeter apart.
Conductance (Λ) of a solution can be represented by the expression:

$$\Lambda = B \sum C_i \lambda_i Z_i$$

where,
B = constant characteristic of geometry and size of conductance cell
λ = equivalent ionic conductance
C = molar concentration of the individual ion
Z = ionic charge of the individual ion

Conductivity is the measurement of the electrolytes in a solution. It is defined as the conductance in a given volume of sample. Conductance is the ability of the solution to conduct electric current.
Conductivity (κ) = Conductance x Probes cell constant (K). Or
Conductivity = Electrical Current/Voltage x Distance/Area

$$G = \frac{1}{R}$$ ohm^{-1}(mho or, Seimen)

From Ohm's law $R = \frac{E}{i}$, E is the potential difference, i is the current intensity.

Conductance (G) is the reciprocal of the electrical resistance. G = 1/R (mhos or, Siemens)
Conductivity(specific conductance), κ = G*l/A where l/A is the cell constant whose unit is cm^{-1}. An example of conductivity related to concentration at different temperatures is shown in Table 1.

The cell constant, K, is equal to the distance in cm between the probe's electrodes divided by the surface area of the electrodes in cm^2. For solutions with low conductivities, the electrodes can be placed closer together or made larger so that the cell constant is less than one. This has the effect of raising the conductance to produce a value more easily interpreted by the meter. The reverse also applies, in high conductivity solutions, the electrodes are placed farther apart or made smaller to reduce the conductance of the sample. By using the appropriate probe, K =0.1 for low conductivity solutions, K =1 for normal solutions, and K =10 for high conductivity solutions, accurate measurements across the full range of conductivity values can be made.
All substances in aqueous/nonaqueous medium possess some conductive properties. Generally, organic compounds (such as benzene, alcohols, and petroleum products) have very low conductivities, while metals have very high conductivities. Measuring the conductivity of highly flammable liquids is very risky.

Equivalent ionic conductance Λ_0

The equivalent conductivities of electrolytes all diminish with concentration (or more accurately, with the square root of the concentration), but they do so in several distinct ways that are distinguished by their behaviors at very small concentrations. This led to the classification of electrolytes as weak, intermediate, and strong (Figure 1).

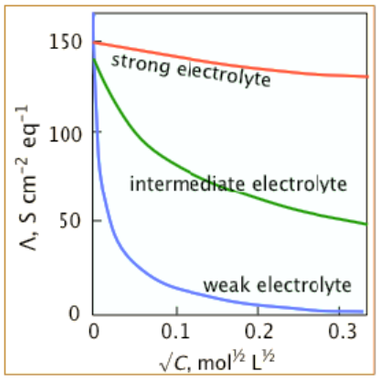


Figure 1 Classification of electrolytes

You will notice that plots of conductivities vs. $\sqrt{c}$ start at c=0. It is of course impossible to measure the conductance of an electrolyte at vanishingly small concentrations (not to mention zero!), but for strong and intermediate electrolytes, one can extrapolate a series of observations to zero. The resulting values are known as *limiting equivalent conductances* or sometimes as "equivalent conductances at infinite dilution", designated by Λ_0.

The specific conductance of electrolytic solutions depends on the concentration of the ionic species present (Table 2).

At infinite dilution, interactions become nil; the overall conductance of the solution consists of the sum of the individual equivalent ionic conductance

$\Lambda_0 = \lambda_+^o + \lambda_-^o$

The gram-equivalent weight is equal to the gram-formula (or atomic) weight divided by the charge on the ion. Hence, the number of gram equivalents is the number of gram-formula weights (moles) multiplied by the charge, and the normality is the molarity multiplied by the charge.

Molar conductivity or equivalent conductance of a solution with concentration of M molar:

$$\Lambda_M = \frac{1000 * k}{c} \ (S.cm^2 .mol^{-1})$$

Table1 Conductivity of KCl solutions

Concentration/M	**Conductivity (κ) / S cm^{-1}**				
	19°C	20°C	22°C	24°C	25°C
1.000	0.1001	0.1021	0.1059	0.1098	0.1118
0.1000	0.01143	0.01167	0.01215	0.01264	0.01288
0.01000	0.001251	0.001278	0.001332	0.001386	0.00141

The conductance of the solution depends on:

1. Temperature:

It is increased by increase of temperature.

The effect of temperature on conductivity readings depends on the solution being measured. The effect is greatest in low ionic strength (low conductivity) solutions. A general rule to follow is there will be about 2% change (increase)/degree C. This rule can be followed for most aqueous solutions, however, if you require a high degree of accuracy, you should consult a chart for the particular solution you are measuring.

When not working with water, we should find correct temperature coefficient by comparing with water. Water correction factor is set at a default value of 1.91% per degree C. Check the conductivity of the sample at 25°C, then using the same sample, find

the conductivity at another temperature to see what the percent change is. This will give you the temperature correction factor.

Table2 Equivalent ionic conductance at 25^0 C

Cations	λ_+^0	**Anions**	λ_-^0
H_3O^+	349,8	OH^-	198
Li^+	38,7	Cl^-	76,3
Na^+	50,1	Br^-	78,4
K^+	73,5	I^-	76,8
NH_4^+	73,4	NO_3^-	71,4
Ag^+	61,9	ClO_4^-	68
$1/2Mg^{2+}$	53,1	$C_2H_3O_2^-$	40,9
$1/2Ca^{2+}$	59,5	$1/2SO_4^{2-}$	79,8
$1/2Ba^{2+}$	63,6	$1/2CO_3^{2-}$	70
$1/2Pb^{2+}$	73	$1/2C_2O^{2-}$	24
$1/3Fe^{3+}$	68	$1/4Fe(CN)^{4-}$	110,5

2. Nature of ions
Such as size, molecular weight, number of charges, the ion carries and other factors.
3. The concentration of ions:
As the number of ions increases the conductance of the solution increases.
4. The size of the electrodes

$$G = k\frac{A}{l} \qquad k = G\frac{l}{A}$$

where, l/A is cell constant, κ is the specific conductance or conductivity in $ohm^{-1}cm^{-1}$ or seimen/cm.

Using Conductivity Probes/Meters
1) Cleaning
Cells can be cleaned with mild liquid detergent and/or dilute nitric acid (1% wt) by dipping or filling the cell with solution and agitating for 2 to 3 minutes. Dilute HCl (hydrochloric acid) or H_2SO_4 (sulfuric acid) may also be used. When stronger cleaning is needed, try concentrated HCl mixed into 50% isopropanol (rubbing alcohol). Rinse the cell several times with distilled or deionized water and recalibrate before use.
2) Storing
Rinse probe in distilled/deionized water when you are finished using it. You can store your electrode either wet or dry. If it is stored dry you will need to recondition the electrode before use.
3) Calibration
In case the dissolved solids are not the same as those in the calibration solution, a meter for TDS can be calibrated by making your own standard will yield the most accurate results. This is done by formulating a mixture of salts in relative proportions to those that simulate the solution being tested, then dissolving the mixture into distilled water. This should be done according to the following formula:
1 mg salt mixture/litre of distilled water = 1 ppm TDS,

or,
X ppm TDS = X mg of salts + one liter of distilled water.
Remember that "X mg of salts" is the number of milligrams of the mixture of salts which simulate your test solution, not X milligrams of each salt in the mixture. An appropriate value for "X" is determined by the following rule:
Choose a ppm value for a calibration solution which is as close as possible to the expected ppm values of the test solutions. If the ppm content of the test solution is expected to vary a great deal, it is best to choose a ppm value for the calibration solution in the upper 1/3 of the expected TDS measurement range.

Some Applications of Electrolytic Conduction
From the chemist's standpoint, the most important examples of conduction are in connection with electrochemical cells, electrolysis, and batteries.
Determination of Equilibrium Constants
Owing to their high sensitivity, conductivity measurements are well adapted to the measurement of equilibrium constants for processes that involve very small ion concentrations. These include
K_s values for sparingly soluble solids
Autoprotolysis constants for solvents (such as K_w)
Acid dissociation constants for weak acids.
As long as the ion concentrations are so low, their values can be taken as activities, and limiting equivalent conductivities Λ_0 can be used directly.
Example 1: Use the appropriate limiting molar ionic conductivities to estimate the autoprotolysis constant K of water at 25° C.
Use the reaction equation $2H_2O \rightarrow H_3O^+ + OH^-$.
Solution:
The data we need are $\lambda_{H+} = 349.6$ and $\lambda_{OH-} = 199.1$ S cm^2 mol^{-1} .
The conductivity of water is $\kappa = [H^+]\ \lambda_{H+} + [OH^-]\ \lambda_{OH-}$ whose units work out to (mol cm^{-3}) (S cm^2 mol^{-1}). In order to express the ionic concentrations in molarities, we multiply the cm term by (1 L / 1000 cm^{-3}), yielding

$$1000\ \kappa = [H^+]\ \lambda_{H+} + [OH^-]\ \lambda_{OH-}$$ with units S cm^{-1} L^{-1} .

Recalling that in pure water, $[H^+] = [OH^-] = K^{1/2}$, we obtain
$1000\ \kappa = (K_w^{1/2})(\lambda_{H+} + \lambda_{OH-}) = (K_w^{1/2})(548.7$ S cm^2 mol^{-1}).
Substituting Kohlrausch's water conductivity value of 0.043×10^{-6} S cm^{-1}) for κ gives
$K_w = (1000 \times 0.043 \times 10^{-6}$ S cm^{-1} / 548.7 S cm^2 mol^{-1})$^2 = 0.614 \times 10^{-14}$ mol^2 cm^{-6} (i.e., mol^2 L^{-2}).
The accepted value for the autoprotolysis constant of water at 25° C is $K_w = 1.008 \times 10^{-14}$ mol^2 L^{-2} .

Example 2: *Solubility Products*
A saturated solution of silver chloride AgCl has a conductance 2.28 x 10^{-6} S cm^{-1} at 25°C. The conductance of the water used to make up this solution is 0.116 x 10^{-6} S cm^{-1} . The limiting ionic conductivities of the two ions are $\lambda_{Ag+} = 61.9$ and $\lambda_{Cl-} = 76.3$ S cm^2 mol^{-1} . Use this information to estimate the molar solubility of AgCl.
***Solution*:**
The limiting molar conductivity of the solution is
$\Lambda_0 = \lambda_{Ag+} + \lambda_{Cl-} = 138.2$ S cm^{-1} .
The conductance due to the dissociated salt alone is the difference
(2.28 – 0.116) = 2.16 x 10^{-6} S cm^{-1} .
Substituting into the expression $\Lambda = 1000\kappa/c$ yields
c= 1000 x 2.16 x 10^{-6} S cm^{-1} / 138.2 S cm^{-1} mol^{-1} = 1.56 x 10^{-5} mol/1000 cm^3 or 1.56 x 10^{-5} mol L^{-1} .

Conductance Ratio: Weak Electrolytes

One of the early uses of limiting conductances was to determine the degree of dissociation of weak electrolytes. Arrhenius suggested that, at any given concentration, the measured equivalent conductance (when compared to the limiting equivalent conductance where all ions are dissociated) should be a measure of the degree of dissociation, α. This can be expressed as $\alpha = \Lambda / \Lambda_0$

The ionization constant K_a of acetic acid is expressed as

$$K_a = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]} = \frac{\alpha[CH_3COOH]\ x\ \alpha[CH_3COOH]}{[CH_3COOH](1-\alpha)} = \frac{\alpha^2[CH_3COOH]}{(1-\alpha)}$$

Using the data from Table 2, the limiting equivalent conductance of acetic acid is $\Lambda_{0CH3COOH} = \boldsymbol{\lambda}^0_{H+} + \boldsymbol{\lambda}^0_{CH3COO-} =$ 349.8 + 40.9 = 390.7 cm^2/(eq-ohm).

Example 3: The equivalent conductance of a 0.0125 *N* acetic-acid solution was determined at 25 °C to be 14.4. Calculate both the degree of dissociation and the ionization constant.

***Solution*:**

$$\alpha = \Lambda / \Lambda_0 = 14.4/390.7 = 0.0369$$

$$K_a = \frac{\alpha^2 C_a}{(1-\alpha)} = \frac{(0.0369)^2\ x\ 0.0125}{0.9631} = 1.77\ x\ 10^{-5}$$

Conductivity and TDS

Salts, minerals, and even dissolved gases contribute uniformly to the conductivity of a solution. This means that the conductivity can be used as an indicator of the amount of dissolved materials in a solution. A **Total Dissolved Salt (TDS)** can be used fairly accurately when determining the concentration of a single salt, such as NaCl, but errors can arise when trying to compare two different types of solutions. It is necessary to calibrate the meter using the same dissolved materials that are in the test solution.

Difference between conductivity and salinity

The probe is the same for conductivity and **salinity**, but for salinity readings a correction factor is applied to the conductivity value. The correction factor takes the conductivity reading and converts it to **ppm** of NaCl.

Conductometric Titrations

A chemical reaction in which there is a significant change in the number or mobilities of ionic species can be followed by monitoring the change in conductance. The equivalence point may be located graphically by plotting the change in conductance as a function of the volume of titrant added.

Conductometry can be used as an endpoint signaling tool in titrations when molar conductivity of the solution changes significantly in the chemical reaction. For example the

$$A^+ + B^- + C^+ + D^- \rightarrow AD + B^- + C^+$$

reaction will result decrease of conductance. Acid-base titrations are good candidates for using conductometry to signal the end point of titrations.

Consider, for example, the titration of the strong acid HCl by the strong base NaOH. In ionic terms, the process can be represented as

$$H^+ + Cl^- + Na^+ + OH^- \rightarrow H_2O + Na^+ + Cl^-$$

At the endpoint, only two ionic species remain, compared to the four during the initial stages of the titration, so the conductivity will be at a minimum.

In order to reduce the influence of errors in the conductometric titration to a minimum, the angle between the two branches of the titration curve should be as small as possible (see Figure 2). If the angle is very obtuse, a small error in the conductance data can cause a large deviation. The following approximate rules will be found useful.

- The smaller the conductivity of the ion which replaces the reacting ion, the more accurate will be the result. Thus it is preferable to titrate a silver salt with lithium chloride rather than with HCl. Generally, cations should be titrated with lithium salts and anions with acetates as these ions have low conductivity.
- The larger the conductivity of the anion of the reagent which reacts with the cation to be determined, or vice versa, the more acute is the angle of titration curve.
- The titration of a slightly ionized salt does not give good results, since the conductivity increases continuously from the commencement. Hence, the salt present in the cell should be virtually completely dissociated; for a similar reason; the added reagent should also be as strong electrolyte.
- Throughout a titration the volume of the solution is always increasing, unless the conductance is corrected for this effect, non-linear titration curves result. The correction can be accomplished by multiplying the observed conductance either by total volume ($V+V'$) or by the factor ($V+ V'$)/V, where V is the initial volume of solution and V´ is the total volume of the reagent added. The correction presupposes that the conductivity is a linear function of dilution, this is true only to a first approximation.
- In the interest of keeping V small, the reagent for the conductometric titration is ordinarily several times more concentrated than the solution being titrated (at least 10-20 times). A micro burette may then be used for the volumetric measurement.

The main advantages to the conductometric titration are its applicability to very dilute, and colored solutions and to system that involve relative incomplete reactions. For example, which neither a potentiometric, nor indicator method can be used for the neutralization titration of phenol ($K_a = 10^{-10}$) a conductometric endpoint can be successfully applied.

Application: Acid-base titration, especially at trace levels. Relative precision is better than 1% at all levels. There are also few disadvantages with this technique. As you know the conductance is a non-specific property, concentration of another electrolyte can be troublesome.

The electrical conductance of a solution is a measure of its currents carrying capacity and therefore determined by the total ionic strength. It is a non-specific property and for this reason, direct conductance measurements are of little use unless the solution contains only the electrolyte to be determined or the concentrations of other ionic species in the solution are known. Conductometric titrations, in which the species in the solution are converted to non-ionic for by neutralization, precipitation, etc. are of more value.

Advantages of Conductometric Titration

- No need of indicator
- Colored or dilute solutions or turbid suspensions can be used for titrations.
- Temperature is maintained constant throughout the titration.
- Endpoint can be determined accurately and errors are minimized as the endpoint is being determined graphically.

Some Typical Conductometric Titration Curves

1. **Strong Acid with a Strong Base, e.g. HCl with NaOH:** Before NaOH is added, the conductance is high due to the presence of highly mobile hydrogen ions. When the base is added, the conductance falls due to the replacement of hydrogen ions by the added cation as H^+ ions react with OH^- ions to form undissociated water. This decrease in the conductance continues till the equivalence point. At the equivalence point, the solution contains only NaCl.

After the equivalence point, the conductance increases due to the large conductivity of OH^- ions (Figure 2).

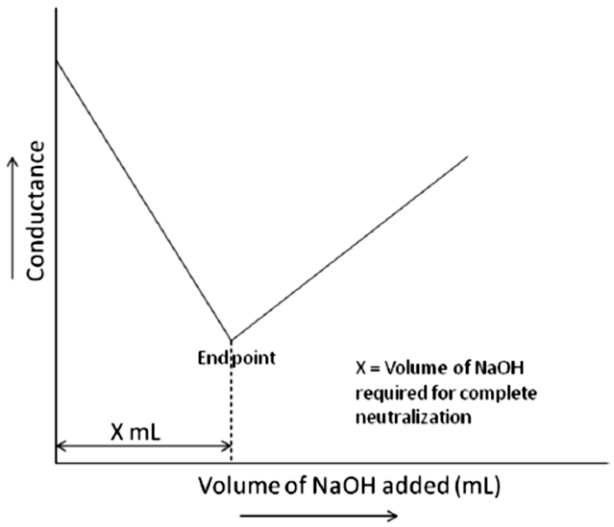


Figure 2 Conductometric titration of a strong acid (HCl) vs. a strong base (NaOH)

2. **Weak Acid with a Strong Base, e.g. acetic acid with NaOH:** Initially the conductance is low due to the feeble ionization of acetic acid. With the addition of base, there is decrease in conductance not only due to the replacement of H^+ by Na^+ but also suppresses the dissociation of acetic acid due to common ion acetate. But very soon, the conductance increases on adding NaOH as NaOH neutralizes the un-dissociated CH_3COOH to CH_3COONa which is the strong electrolyte. This increase in conductance continues to raise up to the equivalence point. The graph near the equivalence point is curved due the hydrolysis of salt CH_3COONa. Beyond the equivalence point, conductance increases more rapidly with the addition of NaOH due to the highly conducting OH^- ions (Figure 3).

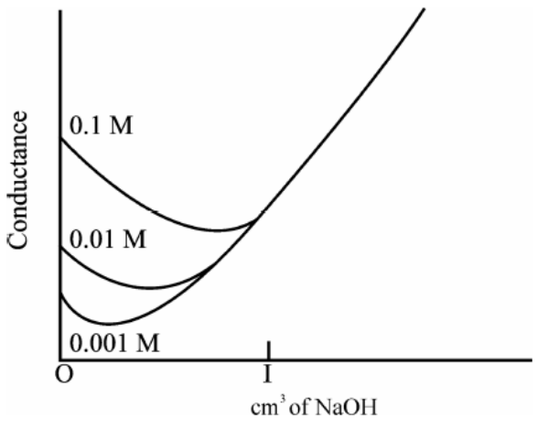


Figure 3 Conductometric titration of a weak acid (acetic acid) vs. a strong base (NaOH)

3. **Strong Acid with a Weak Base, e.g. sulphuric acid with dilute ammonia**: Initially the conductance is high and then it decreases due to the replacement of H^+. But after the endpoint has been reached the graph becomes almost horizontal, since the excess aqueous ammonia is not appreciably ionized in the presence of ammonium sulfate (Figure 4).

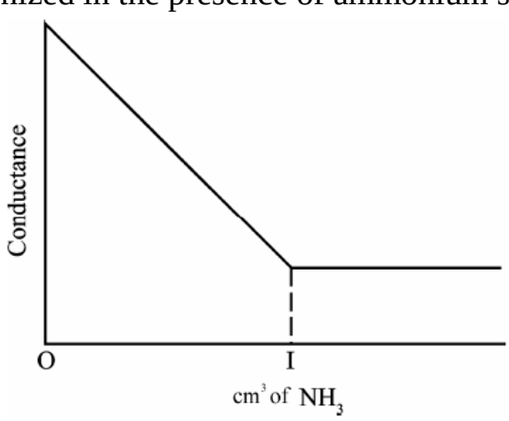


Figure 4 Conductometric titration of a strong acid (H_2SO_4) vs. a weak base (NH_4OH)

4. **Weak Acid with a Weak Base**: The nature of curve before the equivalence point is similar to the curve obtained by titrating weak acid against strong base.
After the equivalence point, conductance virtually remains the same as the weak base which is being added is feebly ionized and, therefore, is not much conducting (Figure 5).

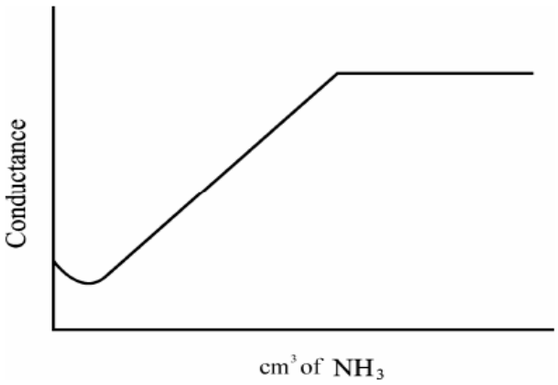


Figure 5 Conductometric titration of a weak acid (acetic acid) vs. a weak base (NH_4OH)

5. **Mixture of a Strong Acid and a Weak Acid vs. a Strong Base or a Weak Base**: In this curve there are two break points. The first break point corresponds to the neutralization of strong acid. When the strong acid has been completely neutralized only then the weak acid starts neutralizing. The second break point corresponds to the neutralization of weak acid and after that the conductance increases due to the excess of OH^- ions in case of a strong base as the titrant.
However, when the titrant is a weak base, it remains almost constant after the endpoint similar to Figure 5 (Figure 6).

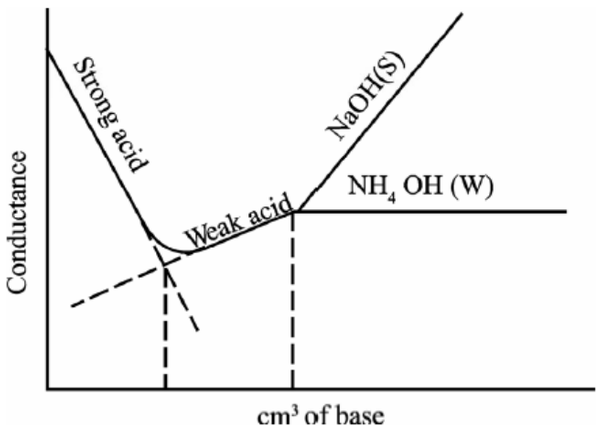


Figure 6 Conductometric titration of a mixture of a strong acid (HCl) and a weak acid (CH_3COOH) vs. a strong base (NaOH) or a weak base (NH_4OH)

6. **Displacement (or Replacement) Titrations**: When a salt of a weak acid is titrated with a strong acid, the anion of the weak acid is replaced by that of the strong acid and weak acid itself is liberated in the undissociated form. Similarly, in the addition of a strong base to the salt of a weak base, the cation of the weak base is replaced by that of the stronger one and the weak base itself is generated in the undissociated form. If for example, M-HCl is added to 0.1 M solution of sodium acetate, the curve shown in Figure 7 is obtained, the acetate ion is replaced by the chloride ion after the endpoint. The initial increase in conductivity is due to the fact that the conductivity of the chloride ion is slightly greater than that of acetate ion. Until the replacement is nearly complete, the

solution contains enough sodium acetate to suppress the ionization of the liberated acetic acid, so resulting a negligible increase in the conductivity of the solution. However, near the equivalent point, the acetic acid is sufficiently ionized to affect the conductivity and a rounded portion of the curve is obtained.

Beyond the equivalence point, when an excess of HCl is present (ionization of acetic acid is very much suppressed) therefore, the conductivity arises rapidly.

Care must be taken that to titrate a 0.1 M-salt of a weak acid, the dissociation constant should not be more than 5×10^{-4}, for a 0.01 M -salt solution, $K_a < 5 \times10^{-5}$ and for a 0.001 M-salt solution, $K_a < 5 \times10^{-6}$, i.e., the ionization constant of the displace acid or base divided by the original concentration of the salt must not exceed above 5×10^{-3}. Figure 6. Also includes the titration of 0.01 M- ammonium chloride solution versus 0.1 M - sodium hydroxide solution. The decrease in conductivity during the displacement is caused by the displacement of ammonium ion of greater conductivity by sodium ion of smaller conductivity.

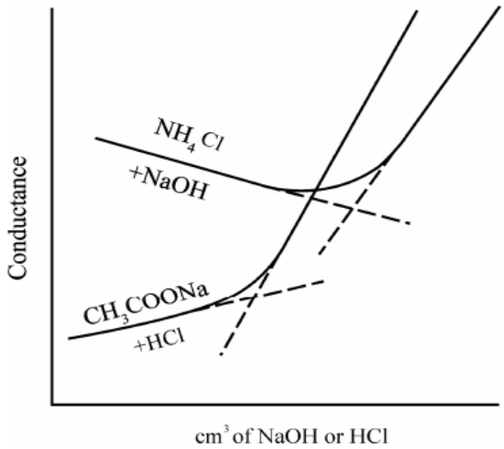


Figure 7 Conductometric titration of a salt of weak acid (sodium acetate) vs. strong acid (HCl); salt of a weak base (NH_4Cl) vs. a strong base (NaOH)

7. **Precipitation Titration and Complex Formation Titration**: A reaction may be made the basis of a conductometric precipitation titration provided the reaction product is sparingly soluble or is a stable complex. The solubility of the precipitate (or the dissociation of the complex) should be less than 5% that means the solubility product, when divided by the concentration of the titrant, should not be greater than about $5 \times I0^{-6}$. For example, the concentration of $AgNO_3$ with CI^- titrant should be at least 0.3×10^{-4} N, since AgCl has a K_{sp} of $1.7 \times I0^{-10}$. A few typical precipitation-titration curves are shown in Figure 8.

The addition of ethanol is sometimes recommended to reduce the solubility in the precipitations. An experimental example is a titration of ammonium sulfate in aqueous-ethanol solution with barium acetate.

Errors in locating true endpoints during precipitation reactions can be caused by several factors: contamination of the electrodes by the adhering precipitate, occlusion of ions by the precipitate, and incomplete or slow precipitation reaction.

Complexometric reactions require that stable complexes be formed. A typical example is the formation of the cyanide complex of Hg^{2+} according to the reaction

$$Hg^{2+} + 2NO_3^- + 2K^+ + 2CN^- \rightarrow Hg(CN)_2 + 2K^+ + 2NO_3^-$$

A number of metal ions, M^{2+}, can be titrated conductometrically with the disodium salt of ethylenediaminetetraacetic acid, Na_2H_2EDTA, in buffered solutions.

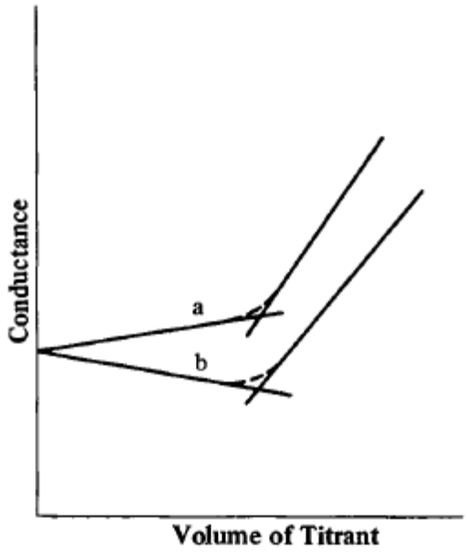


Figure 8 Precipitation reactions: Titration of $AgNO_3$ with (a) KCl and (b) LiCl titrant.

Solved Problems

Problem 1. Specific conductivity of 0,1 M NaOH aqueous solution amounts to 0,0221 $S \cdot cm^{-1}$. After addition of the same volume of 0,1 M HCl aqueous solution, the specific conductivity falls down to 0,0056 $S \cdot cm^{-1}$, while after addition of the second equal aliquot of the same HCl solution, its value increases to 0,0170 $S \cdot cm^{-1}$. Calculate: a) molar conductivity of NaOH, Λ(NaOH); b) molar conductivity of NaCl, Λ(NaCl); c) molar conductivity of HCl, Λ(HCl); d) sum of molar conductivities of H^+ and OH^- ions.

Solution:

This problem is one dealing with conductometric titrations. Namely, we must notice that after addition of first aliquot (the same volume as the original volume of NaOH solution) of HCl, which may be considered a titrant in this case, we neutralized totally the base. Hence, after the first aliquot the solution is 0,05 M NaCl (neutralized and diluted twice). After the second aliquot we have: ⅓·0,1 M NaCl (⅔·0,05, as we added another aliquot and the volume is now ⅓ of the original volume and ⅔ of that after addition of the first aliquot) and ⅓·0,1 M HCl (the extra acid has not reacted, as there was no base, and was just diluted thrice).

The decrease in conductivity after first addition of HCl is due to the neutralization (removal) of very mobile (so conductive) OH^- ions, which are replaced by less conductive Cl^- ions, while also added (high conductivity) H+ ions react with OH– ions, forming water (one can neglect here tiny concentrations of both OH^- ions and H^+ ions in a neutral aqueous solution (both at 10^{-7} M at 298K). After the second aliquot conductivity increases as extra HCl does not react, hence, we actually just add both highly mobile H+ ions and less mobile Cl^- ions.

We must realize that what we can calculate of the basis of the data available is not quite exact, as molar conductivities depend on the concentration and we should assume they do not if we want to solve the problem. Hence: From measurements of solution I (original) we have:

$$\Lambda(NaOH) = \Lambda_{NaOH} = \frac{1000\kappa_I}{c} = \frac{1000 \cdot 0{,}0221}{0{,}1} = 221[S \cdot cm^2 \cdot mol^{-1}].$$

You should remember that the formula used is correct only when specific conductivity is expressed in $[S \cdot cm^{-1}]$ and concentration in $[mol \cdot dm^{-3}]$, because only then the coefficient 1000 should be used. It has its dimensions, too, which are $[cm^3 \cdot dm^{-3}]$. Check it by yourself!

From measurements of solution II (after the addition of the first aliquot) we have:

$$\Lambda(NaCl) = \Lambda_{NaCl} = \frac{1000\kappa_{II}}{c} = \frac{1000 \cdot 0{,}0056}{0{,}05} = 112[S \cdot cm^2 \cdot mol^{-1}]$$

From measurements of solution II (after the addition of the first aliquot) we have:

$\kappa_{III} = \frac{c_{NaCl} \cdot \Lambda_{NaCl} + c_{HCl} \cdot \Lambda_{HCl}}{1000} = \frac{\frac{1}{3} \cdot 0{,}1 \cdot 112 + \frac{1}{3} \cdot 0{,}1 \cdot \Lambda_{HCl}}{1000} = 0{,}0170[S \cdot cm^{-1}]$, hence, we can find Λ(HCl):

$$\Lambda_{HCl} = \frac{0{,}0170 \cdot 1000}{\frac{1}{3} \cdot 0{,}1} - 112 = 510 - 112 = 398[S \cdot cm^2 \cdot mol^{-1}]$$

Finally, one can find the required sum of molar conductivities of H^+ and OH^- ions, $\lambda(H^+) + \lambda(OH^-)$:

$$\lambda_{H^+} + \lambda_{OH^-} = \Lambda_{NaOH} - \Lambda_{NaCl} + \Lambda_{HCl} = 221 - 112 + 398 = 507[S \cdot cm^2 \cdot mol^{-1}]$$

The explanation of the last calculation is as follows (Kohlrausch's law):

$$\lambda_{H^+} + \lambda_{OH^-} = \Lambda_{NaOH} - \Lambda_{NaCl} + \Lambda_{HCl} = (\lambda_{Na^+} + \lambda_{OH^-}) - (\lambda_{Na^+} + \lambda_{Cl^-}) + (\lambda_{H^+} + \lambda_{Cl^-}) =$$
$$= \lambda_{Na^+} + \lambda_{OH^-} - \lambda_{Na^+} - \lambda_{Cl^-} + \lambda_{H^+} + \lambda_{Cl^-} = \lambda_{OH^-} + \lambda_{H^+}$$

Problem 2. You have two aqueous HCl solutions, denoted as I and II. Specific conductivity of solution I, $\kappa_I = 4{,}25 \cdot 10^{-2}$ S·cm^{-1}, while that of solution II, $\kappa_{II} = 8{,}5 \cdot 10^{-3}$ S·cm^{-1}. You want to obtain an HCl solution of such conductivity, that a resistance of a conductometric cell (cell constant k=0,51 cm^{-1}) would amount to 20,0Ω. Calculate volumes of solutions I and II which should be mixed yielding 180 cm^3 of the desired solution. Additional data: $\lambda(Cl^-)$=75 S·cm^2·mol^{-1}, $\Lambda(HNO_3)$=410 S·cm^2·mol^{-1}, t(NO_3^- in HNO_3)=0,146.

Solution:

The problem is about mixing two solutions of the same substance of apparently different concentrations to obtain a third solution of the same substance. The concentration of the third solution must be between those of the two-component solutions, according to the rule: $C_{III} \cdot V_{III} = C_I \cdot V_I + C_{II} \cdot V_{II}$, where $V_{III} = V_{II} + V_I$ (the latter may be assumed for majority, though not all, solutions). Hence, we must find the concentrations of the three solutions, using available conductivity (specific and molar) data.

Let's calculate concentrations of solutions I and II first (we have their respective specific conductivities). If we want to get concentrations we must find molar conductivity of HCl, Λ(HCl), first.

$\Lambda_{HCl} = \lambda_{H^+} + \lambda_{Cl^-}$. We know the latter value, while the first must be calculated from the given molar conductivity of the nitric(V) acid and the transference number of NO_3^- ions. Remember, the transference number is a fraction of total charge transferred by one type of charge carriers (cations or anions). In solutions there are cations and anions, moving in opposite directions, while when passing any given cross-section of the solution they transfer charge q. This charge is a sum: $q = q_- + q_-$, i.e., that of charges transferred by each type of ions. Hence, a transference numbers are given by the following equations:

$$t_+ = \frac{q_+}{q} = \frac{q_+}{q_+ + q_-}; \quad t_- = \frac{q_-}{q} = \frac{q_-}{q_+ + q_-}$$

This is true when only a single dissociating compound is present in the solution (one source of both the cations and the anions in question). Under this assumption, the following must also be true: $t_+ + t_- = 1$.

One can prove that:

$$t_+ = \frac{\lambda_+}{\Lambda} = \frac{\lambda_+}{\lambda_+ + \lambda_-}; \quad t_- = \frac{\lambda_-}{\Lambda} = \frac{\lambda_-}{\lambda_+ + \lambda_-}$$

, where Λ is the molar conductivity of the salt. This gives our solution for the missing λ_{H+} Namely:

$$t_{H^+} = \frac{\lambda_{H^+}}{\Lambda_{HNO_3}} = 1 - t_{NO_3^-},\ yielding:\ \lambda_{H^+} = \left(1 - t_{NO_3^-}\right)\Lambda_{HNO_3} = (1 - 0{,}146)\cdot 410 = 350{,}14[S\cdot cm^2\cdot mol^{-1}]$$

which we can round to 350[S·cm^2·mol^{-1}]. Finally:

$$\Lambda_{HCl} = \lambda_{H^+} + \lambda_{Cl^-} = 350 + 75 = 425[S\cdot cm^2\cdot mol^{-1}]\ \ and:$$

$$\Lambda_{HCl} = \frac{1000\kappa_I}{c_I} = \frac{1000\cdot 0{,}0425}{c_I} \Rightarrow c_I = \frac{1000\cdot 0{,}0425}{\Lambda_{HCl}} = \frac{1000\cdot 0{,}0425}{425} = 0{,}1[mol\cdot dm^{-3}]$$

$$c_{II} = \frac{1000\cdot\kappa_{II}}{\Lambda_{HCl}} = \frac{1000\cdot 0{,}0085}{425} = 0{,}02[mol\cdot dm^{-3}]$$

We still miss, however, the target concentration (C_{III}). To get it, we must find the specific conductivity first:

$$\kappa_{III} = \frac{k}{R} = \frac{0{,}51}{20{,}0} = 0{,}0255[S\cdot cm^{-1}],\ and\ subsequently:\ c_{III} = \frac{1000\cdot\kappa_{III}}{\Lambda_{HCl}} = \frac{1000\cdot 0{,}0255}{425} = 0{,}06[mol\cdot dm^{-3}].$$

Therefore: 0, 06.180 01 V_I +0,02.(180-V_I) → V_i = 90 [cm^3]; VII = 180-90 =90[cm^3].
Remark: There was no need to calculate it, an observation that the desired concentration is equal to just the arithmetic mean of the two-component solutions would lead us to the conclusion that the same volumes of both solutions must be taken.
Another remark is that our results are approximations only, as also here we must have assumed Λ_{HCl} as concentration-independent (the same in all the three solutions), which is not quite exact.

Problem 3. A conductometric cell is characterized by its constant, k=1,30 cm^{-1}. Once filled with a solution of NaOH of 0,2 mol·dm^{-3} concentration, its resistance amounted to 31,71Ω. After adding to certain volume of this solution equal volume of H2SO4 solution of 0,1 mol·dm–3 concentration, resistance of the cell was equal to 141,30Ω. Adding second equal aliquot of the same H_2SO_4 solution resulted in 55,71Ω result of its resistance measurement. Calculate molar conductivities of the following species Λ(NaOH), Λ(½H_2SO_4), and Λ(½Na_2SO_4). What assumptions must be made to solve this problem?

Solution:
This problem quite similar to the first one, a bit more complex, though, as here we do not deal with a simple 1:1 electrolyte. In such a case we must carefully consider (and indicate it properly) what kind of chemical species we have in mind. For example 1 mole of SO_4^{2-} carry charge equal to 2F, while 1 mole of ½SO_4^{2-} that of 1F only.
Therefore, we must clearly describe the composition of each solution. Solution I contains NaOH at 0,2 mol·dm^{-3} concentration:

Solution II contains Na_2SO_4 at 0,05 mol×dm^{-3} concentration. Check it! We added equal volumes of NaOH and H_2SO_4, while molar concentration of the latter was twice smaller than the former (remember that 1 mole of H_2SO_4 reacts with 2 moles of NaOH). The base is exactly neutralized by the acid added and altogether diluted twice (equal volumes were added, so total volume was doubled). Hence, one can write:

$$c_{Na_2SO_4,II} = 0{,}05[mol\cdot dm^{-3}];\ c_{Na^+,II} = 0{,}1[mol\cdot dm^{-3}];\ c_{SO_4^{2-},II} = 0{,}05[mol\cdot dm^{-3}];\ c_{\frac{1}{2}SO_4^{2-},II} = 0{,}1[mol\cdot dm^{-3}];$$

$$c_{\frac{1}{2}Na_2SO_4,II} = 0{,}1[mol\cdot dm^{-3}]$$

Hence:

$$\Lambda_{\frac{1}{2}Na_2SO_4} = \frac{1000\kappa_{II}}{c_{\frac{1}{2}Na_2SO_4,II}} = \frac{1000\ k/R_{II}}{c_{\frac{1}{2}Na_2SO_4,II}} = \frac{1000 \cdot 1{,}30/141{,}30}{0{,}1} = 92[S \cdot cm^2 \cdot mol^{-1}]$$

Remember that $\Lambda_{Na2SO4} = 2\Lambda_{1/2Na2SO4} = 184[S \cdot cm^2 \cdot mol^{-1}]$

Solution II contains Na_2SO_4 at 0,05 mol·dm^{-3} concentration. Check it! We added equal volumes of NaOH and H_2SO_4, while molar concentration of the latter was twice smaller than the former (remember that 1 mole of H_2SO_4 reacts with 2 moles of NaOH). The base is exactly neutralized by the acid added and altogether diluted twice (equal volumes were added, so total volume was doubled). Hence, one can write:

$$c_{Na_2SO_4,III} = \tfrac{2}{3} \cdot 0{,}05[mol \cdot dm^{-3}];\quad c_{\frac{1}{2}Na_2SO_4,III} = \tfrac{2}{3} \cdot 0{,}1[mol \cdot dm^{-3}];\quad c_{Na^+,III} = \tfrac{2}{3} \cdot 0{,}1[mol \cdot dm^{-3}];$$

$$c_{H_2SO_4,III} = \tfrac{1}{3} \cdot 0{,}1[mol \cdot dm^{-3}];\quad c_{\frac{1}{2}H_2SO_4,III} = \tfrac{2}{3} \cdot 0{,}1[mol \cdot dm^{-3}];\quad c_{H^+,III} = \tfrac{2}{3} \cdot 0{,}1[mol \cdot dm^{-3}];$$

$$c_{SO_4^{2-},III} = \tfrac{2}{3} \cdot 0{,}05 + \tfrac{1}{3} \cdot 0{,}1 = \tfrac{2}{3} \cdot 0{,}1[mol \cdot dm^{-3}];\quad c_{\frac{1}{2}SO_4^{2-},III} = \tfrac{2}{3} \cdot 0{,}1 + \tfrac{2}{3} \cdot 0{,}1 = \tfrac{4}{3} \cdot 0{,}1[mol \cdot dm^{-3}]$$

and subsequently:

$$\kappa_{III} = \frac{k}{R_{III}} = \frac{c_{\frac{1}{2}Na_2SO_4} \cdot \Lambda_{\frac{1}{2}Na_2SO_4} + c_{\frac{1}{2}H_2SO_4} \cdot \Lambda_{\frac{1}{2}H_2SO_4}}{1000} = \frac{\tfrac{2}{3} \cdot 0{,}1 \cdot 92 + \tfrac{2}{3} \cdot 0{,}1 \cdot \Lambda_{\frac{1}{2}H_2SO_4}}{1000} = \frac{1{,}30}{55{,}71} = 0{,}023335[S \cdot cm^{-1}]$$

$$\Lambda_{\frac{1}{2}H_2SO_4} = \frac{0{,}023335 \cdot 1000}{\tfrac{2}{3} \cdot 0{,}1} - 92 = 350 - 92 = 258[S \cdot cm^2 \cdot mol^{-1}]$$

The assumption here is again the one about invariability of Λ with concentration, which is not quite obeyed. It also explains the fact that in problem 1 we got Λ_{NaOH}=221[S·cm^2·mol^{-1}], while in this problem it amounts to 205[S·cm^2·mol^{-1}].

9. KINETIC METHODS OF ANALYSIS

Introduction

The basic types of reactions used for determinative purposes encompass the traditional four in equilibrium-based measurements: precipitation (ion exchange), acid-base (proton exchange), redox (electron exchange), and complexation (ligand exchange). These four basic types, or cases that can be reduced to them, are also found in kinetic-based measurements with some distinguishable trends. The influence of concentration on the position of a chemical equilibrium is described in quantitative terms by means of an equilibrium-constant expression. Such expressions are important because they permit the chemist to predict the direction and completeness of a chemical reaction. However, the size of one equilibrium constant tells us nothing about the rate (the kinetic) of the reaction. There are many ways to categorize analytical techniques, several of which we introduced in earlier this course. Techniques classified by the signal are proportional to the absolute amount of analyte or by the relative amount of analyte. For example, precipitation gravimetry is a total analysis technique because the precipitate's mass is proportional to the absolute amount, or moles, of analyte. UV/Vis absorption spectroscopy, on the other hand, is a concentration technique because absorbance is proportional to the relative amount, or concentration, of analyte.

An additional way to classify analytical techniques is by whether the analyte's concentration is determined under a state of equilibrium or by the kinetics of a chemical reaction or a physical process.

A large equilibrium constant does not imply that a reaction is fast. In fact, we sometimes encounter reactions that have highly favorable equilibrium constants but are of slight analytical use because their rates are low. Commonly used kinetic methods based on chemistry of reaction employed have been selected.

Kinetic Methods Versus Equilibrium Methods

In an **equilibrium method** the analytical signal is determined by an equilibrium reaction that involves the analyte or by a steady-state process that maintains the analyte's concentration. When we determine the concentration of iron in water by measuring the absorbance of the orange-red $Fe(phen)_3^{2+}$ complex, the signal depends upon the concentration of $Fe(phen)_3^{2+}$, which, in turn, is determined by the complex's formation constant. In the flame atomic absorption determination of Cu and Zn in tissue samples, the concentration of each metal in the flame remains constant because each step in the process of atomizing the sample is in *a steady-state*. In a **kinetic method** the analytical signal is determined by the rate of a reaction that involves the analyte or by *a nonsteady-state process*. As a result, the analyte's concentration changes during the time in which we monitor the signal.

Kinetic methods of analysis are based on the fact that for most reactions the rate of the reaction and the analytical signal increase with an increase of the analyte concentration. In **kinetic method**s, measurement of the analytical signal is made under dynamic conditions in which the concentrations of reactants and products are changing as a function of time.

Generally, in analytical chemistry many methods of analysis are based on the equilibrium state of the selected reaction. In contrast to kinetic methods, **equilibrium or thermodynamic methods** are performed on systems that have come to equilibrium or steady state, so that the analytical signal should be stable during measurements. Kinetic and equilibrium parts of the selected chemical reaction are illustrated in the figure 1.

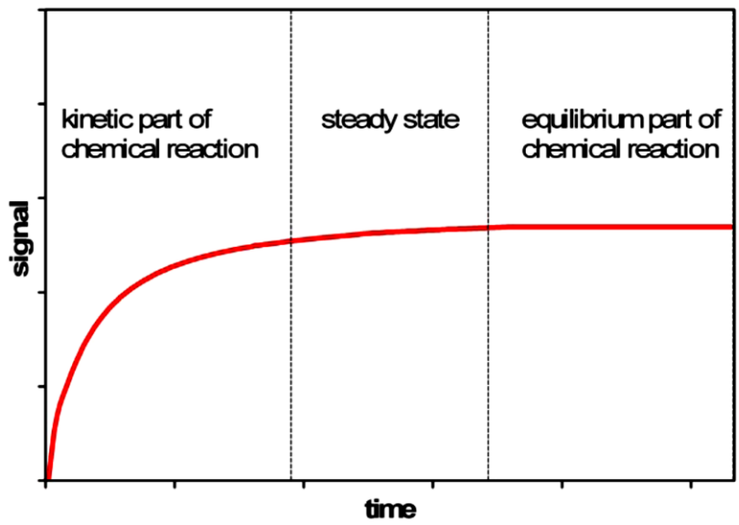


Figure 1. Kinetic, steady state and equilibrium parts of the selected chemical reaction

In many cases we can choose to complete an analysis using either an equilibrium method or a kinetic method by changing when we measure the analytical signal.

The **advantages** of kinetic methods include *sensitivity*, *simple instrumentation* and *simplicity*. The most important advantage of the kinetic method of the analysis is the ability to use chemical reaction that is slow to reach equilibrium. By using kinetic methods determination of a single species in a mixture may be possible when species have sufficient differences of reaction rates. There are three techniques that rely on measurements made while the analytical system is under kinetic control: (1) chemical kinetic techniques, in which we measure the rate of a chemical reaction; (2) radiochemical techniques, in which we measure the decay of a radioactive element; (3) and flow injection analysis, in which we inject the analyte into a continuously flowing carrier stream, where its mixes with and reacts with reagents in the stream under conditions controlled by the kinetic processes of convection and diffusion.

Chemical Kinetics

The earliest analytical methods based on chemical kinetics— defines as the study of the rate at which a chemical process occurs—took advantage of the **catalytic activity of enzymes**. In a typical method used in the late nineteenth century, an enzyme was added to a solution that contained a suitable substrate and their reaction was monitored for a fixed time. The enzyme's activity was determined by the change in the substrate's concentration. Enzymes also were used for the quantitative analysis of hydrogen peroxide and carbohydrates. The development of chemical kinetic methods continued in the first half of the twentieth century with the introduction of nonenzymatic catalysts and noncatalytic reactions.

Despite the diversity of chemical kinetic methods, by 1960 they no longer were in common use. The principal limitation to their broader acceptance was a susceptibility to significant errors from uncontrolled or poorly controlled variables—temperature and pH are two such examples—and the presence of interferents that activate or inhibit catalytic reactions. By the 1980s, improvements in instrumentation and data analysis methods compensated for these limitations, ensuring the further development of chemical kinetic methods of analysis.

Classifying Chemical Kinetic Methods

Kinetic methods were classified into two categories,(1) catalyzed reactions (2) and unanalyzed reactions. Figure 2 provides one useful scheme for *recent* classifying **chemical kinetic methods** of analysis. Methods are divided into two broad categories: (1) direct-computation methods (2) and curve-fitting methods. In a **direct-computation method** we calculate the analyte's initial concentration, $[A]_0$, using the appropriate rate

law. For example, if the reaction is first-order in analyte, we can use calculations to determine $[A]_0$ given values for *k*, t, and $[A]_t$. With a **curve-fitting method**, we use regression to find the best fit between the data—for example, $[A]_t$ as a function of time—and the known mathematical model for the rate law.

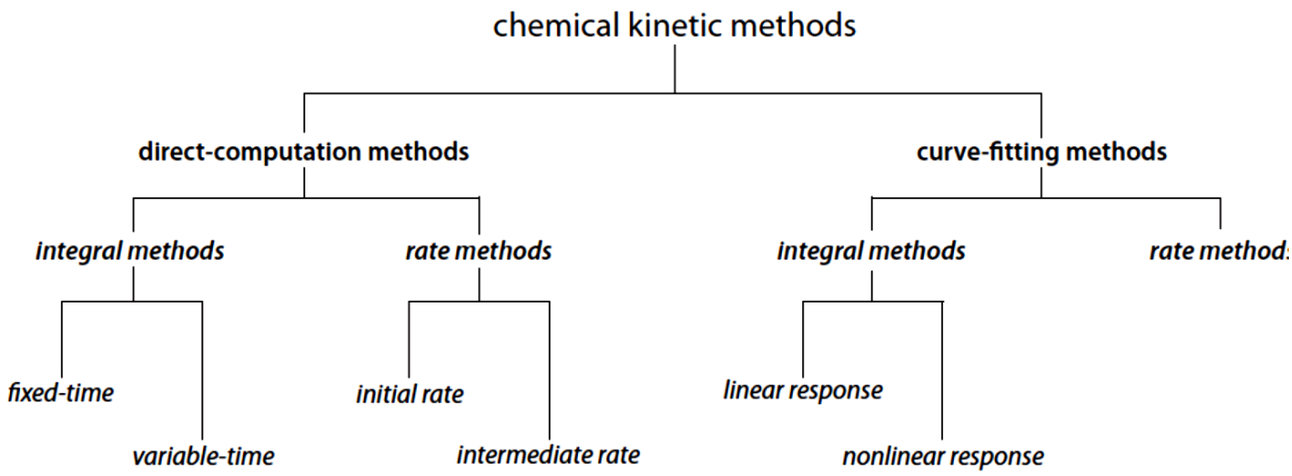


Figure 2. Classification of chemical kinetic methods of analysis

Classification of **kinetic methods** will be discussed later under **types of kinetic methods** part.

Principle of kinetic method

The rate of the reaction is either accelerated or retarded by a catalyst. This results in the alteration of activation energy or the kinetic order of a reaction without changing the point of equilibrium. For example

$$C + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X \qquad (1)$$

$$X + R \xrightarrow{k_2} P + Z + C \qquad (2)$$

$$S + R \xrightarrow{k_3} P + Z \qquad (3)$$

In the above

C = catalyst

X = the intermediate

S = substrate

R =reagent molecule that reacts with X to yield the product P with the elimination of Z.

The overall reaction rate K is the sum of the rates of the catalyzed reaction (1) and (2) and the uncatalyzed reaction (3), i.e.,

$$\frac{dx}{dt}(\text{Total}) = \frac{dx}{dt}(\text{Cat.}) + \frac{dx}{dt}(\text{Uncat.})$$

$$= K_{\alpha c}[C]_o([R]_o - X) + K_3[S]_o([R]_o - X) \qquad (4)$$

K is the overall constant and α_c is the product concentration or more complex function of all the concentrations of the components participating in the reaction except $[C]_0$ which is

the original concentration of the catalyst. $[R]_0$ is the initial concentration of the species and changes in its concentration in the course of the reaction and ($[R]_0$ - X) is the observed concentration of [R] at any time ' t' after the reaction has started. $[S]_0$ is the initial concentration of the substrate. Experimentally the concentration $[S]_0$ is often kept in large excess such that α_c and $[S]_0$ can be considered to be constant throughout the experiment. If ($[R]_0 - x$) ≈[R], equation (4) reduces to the differential equation

$$\frac{dx}{dt}\,(\text{Total}) = F[C]_o + F' \qquad (5)$$

where F is the rate of the uncatalyzed reaction (3). The equivalent integrated form of the

$$\ln([R]_1/[R]_2) = (F[C]_o + F')(t_2 - t_1) \qquad (6)$$

equation is obtained by integrating the expression (4)

One of the last two kinetic expressions is useful for the determination of catalyst concentration.

Equation (5) is simpler to interpret than equation (6) and the factor F is generally much larger than 1. It corresponds to an amplification factor and this is the one of the unique features of the catalytic methods. Catalytic methods use chemical methods signal amplification. Most kinetic methods involve the measurement of initial rate or two-point fixed time approaches. They are *dependent* on *experimental conditions* such as *temperature, pH, inhibitors and solvents*, etc.

The procedures employed in kinetic methods of analysis are variable time procedure and fixed time procedure.

1. Variable time procedure

The relationship between the rate of the chemical reaction and the amount of the reactant or the catalyst is first established by following the course of the reaction with time. The amount of the reactant or the catalyst is then determined by making use of the relationship.

2. Fixed time procedure

Any physical property, for example, absorbance of a system under investigation which varies proportionally with the concentration of the reactant or the catalyst is first identified. The mathematical relationship between this property and the concentration of the catalyst at any fixed interval of time is later established. This fact is employed for the determination of the concentration of the reactant or the catalyst.

Kinetics—The Basics

Kinetics is the description of **reaction rates**. The **order** of a reaction defines the dependence of reaction rates on the concentrations of reacting species. Order is determined empirically and is not necessarily related to the stoichiometry of the reaction. Rather, it is governed by the **mechanism** of the reaction, that is, by the number of species that must collide for the reaction to occur.

Reaction Rate

The rate of the chemical reaction—how quickly the concentrations of reactants and products change during the reaction—must be fast enough that we can complete the analysis in a reasonable time, but also slow enough that the reaction does not reach equilibrium while the reagents are mixing. As a practical limit, it is not easy to study a

reaction that reaches equilibrium within several seconds without the aid of special equipment for rapidly mixing the reactants.

Rate Law

The second requirement is that we must know the reaction's rate law—the mathematical equation that describes how the concentrations of reagents affect the rate—for the period in which we are making measurements.

The rate of a chemical reaction is expressed as a change in concentration of some species with time. Therefore, the dimensions of the rate must be those of concentration divided by time (moles=liter sec, moles=liter min, etc.). A reaction that can be written as

$$A \rightarrow P \qquad (7)$$

has a rate that can be expressed either in terms of the disappearance of A or the appearance of B. Because the concentration of A is decreasing as A is consumed, the rate is expressed as - d[A]= dt. Because the concentration of P is increasing with time, the rate is expressed as +d[P]= dt. The mathematical equation relating concentrations and time is called the rate equation or the rate law. The relationships between the concentrations of A and P with time are represented graphically in Figure 3 for a first-order reaction in which $[A]_o$ is 1.00 M and k = 0.050 min^{-1}.

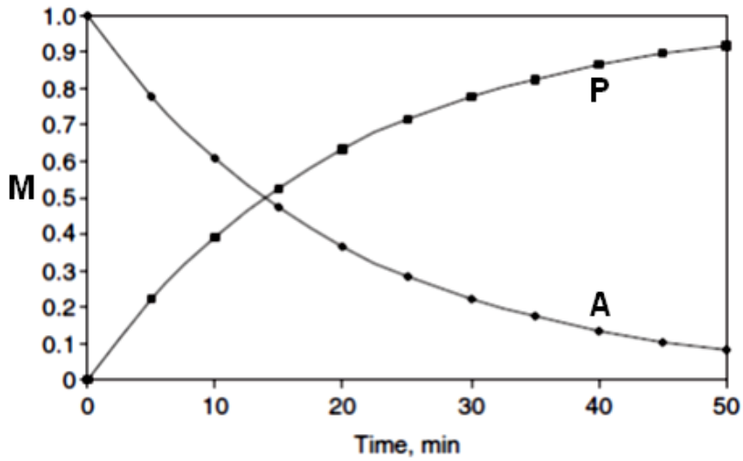


Figure 3. Change in concentration of A and P for the reaction A → P.

If we consider a reaction that can be shown as

$$aA + bB \rightarrow cC + dD \qquad (8)$$

the rate law will usually be represented in terms of a constant times some function of the concentrations of A and B, and it can usually be written in the form

$$\text{Rate} = k[A]^x[B]^y \qquad (9)$$

where x and y are the exponents on the concentrations of A and B, respectively. In this rate law, k is called the rate constant and the exponents x and y are called the order of the reaction with respect to A and B, respectively. The exponents x and y may or may not be the same as the balancing coefficients a and b in Eq. (8).

The overall order of the reaction is the sum of the exponents x and y. Thus, we speak of a second-order reaction, a third-order reaction, etc., when the sum of the exponents in the rate law is 2, 3, etc., respectively. These exponents can usually be established by studying the reaction using different initial concentrations of A and B. When this is done, it is possible to determine if doubling the concentration of A doubles the rate of the reaction. If it does, then the reaction must be **first-order in A**, and the value of x is 1. However, if doubling the concentration of A quadruples the rate, it is clear that [A] must have an exponent of 2, and the reaction is **second-order in A**. One very important point to remember is that *there is no necessary correlation between the balancing coefficients in the chemical equation and the exponents in the rate law.* They may be the same, but one can not assume that they will be without studying the rate of the reaction.

If a reaction takes place in a series of steps, a study of the rate of the reaction gives information about the slowest step of the reaction.

Classification of Reaction Rates

Reactions are roughly classified as fast reactions – and the rest (Table1). The borderline is indistinct, but the general consensus is that a 'fast' reaction is one which is over in one second or less. Reactions slower than this lie in the conventional range of rates, and any of the techniques described previously can be adapted to give rate measurements. Fast reactions require special techniques. A very rough general classification of rates can also be given in terms of the time taken for reaction to appear to be virtually complete, or in terms of half-lives.

Table 1. Relationship between types of reaction and half-life

Type of reaction	Time span for apparent completion	Half-life
very fast rate	microseconds or less	10^{-12} to 10^{-6} second
fast rate	seconds	10^{-6} to 1 second
moderate rate	minutes or hours	1 to 10^{3} second
slow rate	weeks	10^{3} to 10^{6} second
very slow rate	weeks or years	$>10^{6}$ second

First-Order Reactions

Reactions in which the rate of the reaction is directly proportional to the concentration of a single substance are known as first-order reactions. Consider the reaction in Equation 7. Substance A might be a compound that is decomposing to one or more products. The rate of the reaction is equal to the rate of disappearance of A, and it is proportional to the concentration of A:

$$-\frac{dA}{dt} = k(A) \qquad (10)$$

This is a **rate expression**, or **rate law**. The minus sign is placed in front of the term on the left side of the equation to indicate that A is decreasing in concentration as a function of time. The constant *k* is the **specific rate constant** at the specified temperature and has the dimensions of reciprocal time, for example, s^{-1}. The **order of a reaction** is the sum of the exponents to which the concentration terms in its rate expression are raised. Thus, this is a first-order reaction and its rate depends only on the concentration of A.

Equation 10 is known as the **differential form** of the first-order rate law. The **integrated form** of the equation is

$$\log[A] = \log[A]_0 - \frac{kt}{2.303} \qquad (11)$$

where $[A]_0$ is the initial concentration of A*(t* = 0*)* and [A] is its concentration at time *t* after the reaction is started. This equation gives the amount of A that has reacted after a given time interval. It is a straight-line equation, and if *t* is plotted versus log[A] (which can be measured at different times), a straight line with slope −*k*/2.303 and intercept $\log[A]_0$ is obtained. Thus, the rate constant can be determined.

Note that, from Equation 10, the rate of the reaction (*not* the rate constant) decreases as the reaction proceeds because the concentration of A decreases. Since [A] decreases logarithmically with time (see Equation 11), it follows that the rate of the reaction will decrease exponentially with time. The time required for one-half of a substance to react is

called the **half-life** of the reaction, $t_{1/2}$. The ratio of [A]/[A]$_0$ at this time is 1/2 . By inserting this in Equation 11 and solving for $t_{1/2}$, we see that for a first-order reaction

$$t_{1/2} = \frac{0.693}{k} \qquad (12)$$

After the reaction is half complete, then one-half of the remaining reacting substances will react in the same time $t_{1/2}$, and so on (see Figure 4).
This is the exponential decrease we mentioned. Theoretically, it would take an infinitely long time for the reaction to go to completion, but for all practical purposes, it is complete (99.9%) after 10 half-lives. It is important to note that the half-life, and hence, the time for the reaction to go to completion is independent of the concentration for first-order reactions.
Radioactive decay is an important example of a first-order reaction.

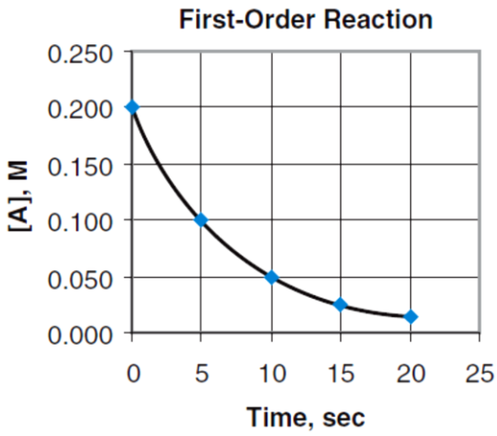


Figure 4. The reaction is half-complete every 5 seconds. Shown here are four half-reactions. The rate of reaction slows down with time.

Second-Order Reactions
Suppose we have the following reaction:

$$A + B \rightarrow P \qquad (13)$$

The rate of the reaction is equal to the rate of disappearance of either A or B. If it is empirically found to be

$$-\frac{dA}{dt} = -\frac{dB}{dt} = k[A][B] \qquad (14)$$

then the reaction is first order with respect to [A] and to [B] and second order overall (the sum of the exponents of the concentration terms is 2). The specific reaction rate constant has the dimensions of reciprocal molarity and time, for example, $M^{-1}s^{-1}$.
The integrated form of Equation 14 depends on whether the initial concentrations of A and B ([A]$_0$ and [B]$_0$) are equal. If they are equal, the equation is

$$kt = \frac{[A]_0 - [A]}{[A]_0[A]} \qquad (15)$$

If [A]$_0$ and [B]$_0$ are not equal, then

$$kt = \frac{2.303}{[B]_0 - [A]_0} \log \frac{[A]_0[B]}{[B]_0[A]} \qquad (16)$$

If the concentration of one species, say B, is very large compared with the other and its concentration remains essentially constant during the reaction, then Equation 14 reduces to that of a first-order rate law:

$$-\frac{dA}{dt} = k'[A] \tag{17}$$

where *k*' is equal to *k*[B]; the integrated form becomes

$$kt = \frac{2.303}{[B]_0}\, log\frac{[A]_0}{[A]} \tag{18}$$

Since $[B]_0$ is constant, Equation 18 is identical in form to Equation 11. This is ***a pseudo first-order reaction***.

The half-life of a second-order reaction in which $[A]_0 = [B]_0$ is given by

$$t_{1/2} = \frac{1}{k[A]_0} \tag{19}$$

Thus, unlike the half-life of a first-order reaction, the half-life here depends on the initial concentration.

A reaction between A and B is not necessarily second order. Reactions of a fraction rate order are common. A reaction such as $2A + B \rightarrow P$ may be third order (rate $\propto [A]^2[B]$), or it may be second order (rate $\propto [A][B]$), or a more complicated order (even a fractional order).

Zero Order Reaction

A reaction is of zero order when the rate of reaction is independent of the concentration of materials. The rate of reaction is a constant. When the limiting reactant is completely consumed, the reaction stops abruptly.

The zero-order rate law for the general reaction in Equation 7 is written as

$$-\frac{d[A]}{dt} = k \tag{20}$$

This means that the rate of the reaction never changes; it's always equal to the value of the rate constant.

Rearranging of previous equation gives

$$d[A] = -k.dt \tag{21}$$

which on integration of both sides

$$\int_{[A]_0}^{[A]} d[A] = -k \int_{t_0}^{t} dt \tag{22}$$

leads to

$$[A] = -kt + C \tag{23}$$

When **t = 0**, the concentration of **A** is $\mathbf{[A]_0}$. The constant of integration must be $\mathbf{[A]_0}$.

Now the integrated form of zero-order kinetics can be written as follows

$$[A] = -kt + [A]_0 \tag{24}$$

Plotting [A] versus t will give a straight line with slope $-k$ (see Figure 5) .

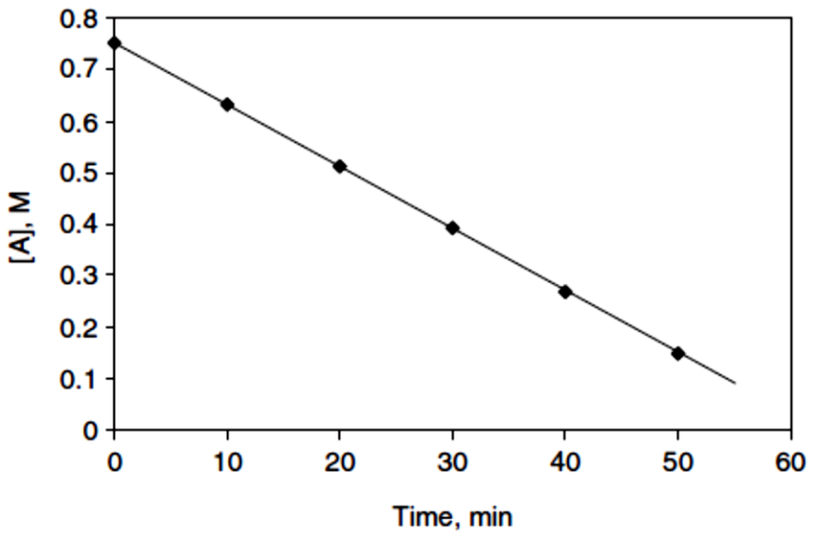


Figure 5. A zero-order rate plot for a reaction where $[A]_o$ = 0.75M and k = 0.012 mol/L.

What is meant by **a *pseudo* zero order reaction**?

The important detail is that the protonation of the ketone and enolization is slower than the step that involves the iodine. An example is the reaction between propanone and iodine.
So, if the rate-determining step of the reaction involves propanone and hydrogen ions (not the iodine). Usually, the experiment is done with a very large excess of propanone (and so its concentration is effectively unchanged during the experiment - a constant); the acid is a catalyst (and so is 'regenerated' and its concentration is unchanged - a constant).

So the rate equation that possibly starts out as: Rate = k[propanone][H^+], becomes Rate = *k*' because [propanone] and [H^+] are effectively constant throughout the experiment.

So the reaction appears (hence the '*pseudo*') to be zero order and the iodine 'disappears' at a constant rate.

If you do the experiment with the concentrations of propanone and iodine more or less equal, then the order ceases to be zero order and, in fact, appears to change as time goes on - interesting, but difficult to explain completely.

Units for Rate Constants

Since reaction rates are always expressed in terms of concentration per unit time, the units of the rate constant are determined by the overall order p of the reaction according to the relation

$$\frac{\text{concentration}}{\text{time}} = (\text{units of } k)(\text{concentration})^p \qquad (25)$$

where $p = m + n$. Rearranging leads to

$$\text{units of } k = (\text{concentration})^{1-P} \text{ X time}^{-1} \qquad (26)$$

Thus, the units for a first-order rate constant are s^{-1}, and the units for a second-order rate constant are $M^{-1}\ s^{-1}$.

Table 2 below summarizes the order of reactions

Table 2 Reaction order and its laws

Reaction Order	Differential Rate Law	Integrated Rate Law	Linear Plot	Slope of Linear Plot	Units of Rate Constant
0	$-d[A]/dt = k$	$[A] = [A]_o - kt$	[A] versus t	$-k$	$mol \cdot L^{-1} \cdot s^{-1}$
1	$-d[A]/dt = k[A]$	$[A] = [A]_o \cdot e^{-kt}$	ln [A] versus t	$-k$	s^{-1}
2	$-d[A]/dt = k[A]^2$	$1/[A] = 1/[A]_o + kt$	1 / [A] versus t	k	$L \cdot mol^{-1} \cdot s^{-1}$

Reaction Time

The time for a reaction to go to completion will depend on the rate constant *k* and, in the case of second-order reactions, the initial concentrations. A first-order reaction is essentially instantaneous if *k* is greater than $10 s^{-1}$ (99.9% complete in less than 1 s).
When *k* is less than $10^{-3} s^{-1}$, the time for 99.9% reaction exceeds 100 min. Although it is more difficult to predict the time for second-order reactions, they can generally be considered to be instantaneous if *k* is greater than about 10^3 or $10^4 M^{-1} s^{-1}$. If *k* is less than $10^{-1} M^{-1} s^{-1}$, the reaction requires hours for completion.

Example1: A reaction is first order with k = 0.0370 s^{-1}. Calculate the concentration of reactant remaining 18.2 s after initiation of the reaction if its initial concentration is 0.0100 M.

Solution:

$[A]_{18.2} = (0.0100\ M)e^{-(0.0370\ s^{-1}) \times (18.2\ s)} = 0.00510\ M.$

Example2: Calculate the time required for a first-order reaction with k = 0.0500 s^{-1} to proceed to 99.0% completion.

Solution: For 99.0% completion, $[A]_t/[A]_o = (100 - 99)/100 = 0.010$; substitution into equation

$$ln\frac{[A]}{[A]_0} = -kt$$

then gives: $\ln 0.010 = -kt = -(0.0500\ s^{-1})t$

$$t = \frac{\ln 0.010}{0.0500\ s^{-1}} = 92\ s$$

Example 3: For a pseudo-first-order reaction in which the reagent is present in 100-fold excess, find the relative error resulting from the assumption that *k*[R] is constant when the reaction is 40% complete.

Solution:

The initial concentration of the reagent can be expressed as

$[R]_0 = 100[Al_0$

At 40% reaction, 60% of A remains. Thus,

$[A]_{40\%} = 0.60[A]_0$

$[R]_{40\%} = [R]_0 - 0.40[A]_0 = 100[A]_0 - 0.40[A]_0 = 99.6[A]_0$

Assuming *pseudo*-first-order behavior. the rate at 40% reaction is

$$-\frac{d[A]_{40\%}}{dt} = k[R]_0[A]_{40\%}$$

The true rate at 40% reaction is $k(99.6[A]_0)(0.60[A]_0)$. Thus, the relative error is

$$\frac{k(100[A]_0)(0.60[A]_0) - k(99.6[A]_0)(0.60[A]_0)}{k(99.6[A]_0)(0.60[A]_0} = 0.004 \ \ (or\ 0.4\%)$$

As Example shows, the error associated with the determination of the rate of a *pseudo*-first-order reaction with a 100-fold excess of reagent is quite small. A 50-fold reagent excess leads to a 1% error, which is usually deemed acceptable in kinetic methods. Moreover, the error is even less significant at times when the reaction is less than 40% complete.

Types of Kinetic Methods

Kinetic methods are classified according to the type of relationship that exists between the measured variable and the analyte concentration.

A major advantage of kinetic methods is their immunity to errors resulting from long-term drift of the measurement system.

The Differential Method

In the differential method, concentrations are computed from reaction rates by means of a differential form of a rate expression. Rates are determined by measuring the slope of a curve relating analyte or product concentration to reaction time.

$$-\frac{d[A]}{dt} = k[A]_t = k[A]_0 e^{-kt}$$

As an alternative, the rate can be expressed in terms of the product concentration.

That is,

$$\left(\frac{d[P]}{dt}\right) = k[A]_0 e^{-kt}$$

Those equations show the dependence of the rate on *k*, t, and, most important, $[A]_0$, the initial concentration of the analyte. At any fixed time t, the factor ke^{-kt} is a constant, and the rate is directly proportional to the initial analyte concentration. Example below illustrates the use of the differential method to calculate the initial analyte concentration.

Example 4: The rate constant for a *pseudo*-first-order reaction is 0.156 s^{-1}. Find the initial concentration of the reactant if its rate of disappearance 10.00 s after the initiation of the reaction is 2.79×10^{-4} M s^{-1}.

Solution:

The proportionality constant ke^{-kt} is

$$ke^{-kt} = (0.156\ s^{-1})e^{-(0.156 s^{-1} x (10.00 s)} = 3.28\ x\ 10^{-2} s^{-1}$$

Rearranging equation and substituting numerical values, we have

$$[A]_0 = rate/ke^{-kt}$$
$$= (2.79 \times 10^{-4}\ M\ s^{-1})/3.28 \times 10^{-2}\ s^{-1}$$
$$= 8.51 \times 10^{-3} M$$

Integral Methods

In contrast to the differential method, **integral methods** take advantage of integrated forms of rate laws.

Graphical Methods

From the equation

$$\ln [A]_t = -kt + \ln [A]_0$$

Thus, a plot of the natural logarithm of experimentally measured concentrations of A (or P) as a function of time should yield a straight line with a slope of -*k* and a y-intercept of ln $[A]_0$. Use of this procedure for the determination of nitromethane is illustrated in the next example.

Example 5: The data in the first two columns of Table below were recorded for the *pseudo*-first-order decomposition of nitromethane in the presence of excess base. Find the initial concentration of nitromethane and the pseudo-first-order rate constant for the reaction.

Data for the Decomposition of Nitromethane

Time, s	$[CH_3NO_2]$, M	$\ln[CH_3NO_2]$
0.25	3.86×10^{-3}	-5.557
0.50	2.59×10^{-3}	-5.956
0.75	1.84×10^{-3}	-6.298
1.00	1.21×10^{-3}	-6.717
1.25	0.742×10^{-3}	-7.206

***Solution*:**

Computed values for the natural logarithms of nitromethane concentrations are shown in the third column of Table . The data are plotted in Figure 6.

A least-squares analysis of the data leads to an intercept *b* of

$$b = \ln[CH_3NO_2]_o = -5.129$$

which after exponentiation gives

$$[CH_3NO_2]_0 = 5.92 \text{ X } 10^{-3} \text{ M}$$

The least-squares analysis also gives the slope of the line m, which in this case is

$$m = -1.62 = -k$$

and thus,

$$k = 1.62 s^{-1}$$

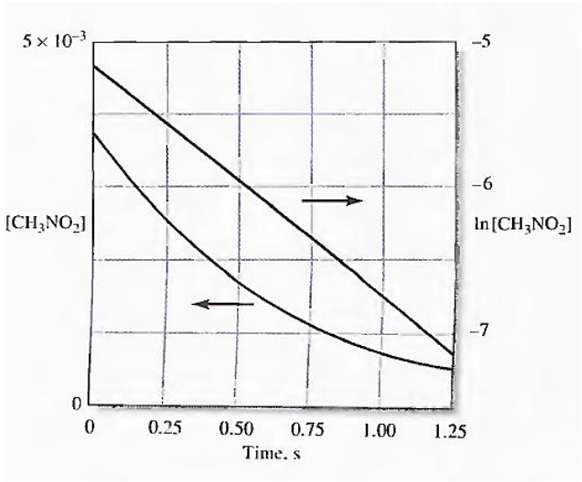


Figure 6. Plots of nitromethane concentration and the natural logarithm of nitromethane concentration as a function of time.

Fixed-Time Methods Fixed-time methods are based on equation

$$[A]_0 = \frac{[A]_t}{e^{-kt}}$$

The simplest way of employing this relationship is to perform a calibration experiment with a standard solution that has a known concentration $[A]_0$. After a carefully measured reaction time t, $[A]_t$ is determined and is used to evaluate the constant e^{-kt} by means of previous equation. Unknowns are then analyzed by measuring [A], after exactly the same reaction time, and employing the calculated value for e^{-kt} to compute the analyte concentrations.

Last equation is easily modified for the situation in which [P] is measured experimentally rather than lA]. Then, rearrangement to solve for $[A]_0$.

That is,

$$[A]_0 = \frac{[P]_t}{1 - e^{-kt}}$$

A more desirable approach to the use of previous equations is to measure [A] or [P] at two times, t_1 and t_2. For example, if the product concentration is determined, we can write

$$[P]_{t1} = [A]_0(1 - e^{-kt1})$$

$$[P]_{t2} = [A]_0(1 - e^{-kt2})$$

Subtracting the first equation from the second and rearranging yields

$$[A]_0 = \frac{[P]_{t2} - [P]_{t1}}{e^{-kt1} - e^{-kt2}} = C([P]_{t2} - [P]_{t1})$$

The reciprocal of the denominator, C, is constant for fixed t_1 and t_2.

The use of this equation has a fundamental advantage common to most kinetic methods-that is, the absolute determination of concentration or of a variable proportional to concentration is unnecessary. The difference between two concentrations is proportional to the initial concentration of the analyte.

Curve-Fitting Methods

With computers attached to instruments, fitting a mathematical model to the concentration or signal versus time curve is straight-forward. These techniques compute values of the model parameters, including the initial concentration of analyte, that 'best fit" the data. The most sophisticated of these methods use the parameters of the model to estimate the value of the equilibrium or steady-state response. These methods can provide error compensation because the equilibrium position is less sensitive to such experimental variables as temperature, pH, and reagent concentrations. Figure 7 illustrates the use of this approach to predict the equilibrium absorbance from data obtained during the kinetic regime of the response curve. The equilibrium absorbance is then related to the analyte concentration in the usual way.

The computer enables many innovative techniques for kinetic methods. Some recent error compensation methods do not require prior knowledge of the reaction order for the system employed but instead use a generalized model. Still, other methods calculate the model parameters as the data are collected instead of employing batch processing methods.

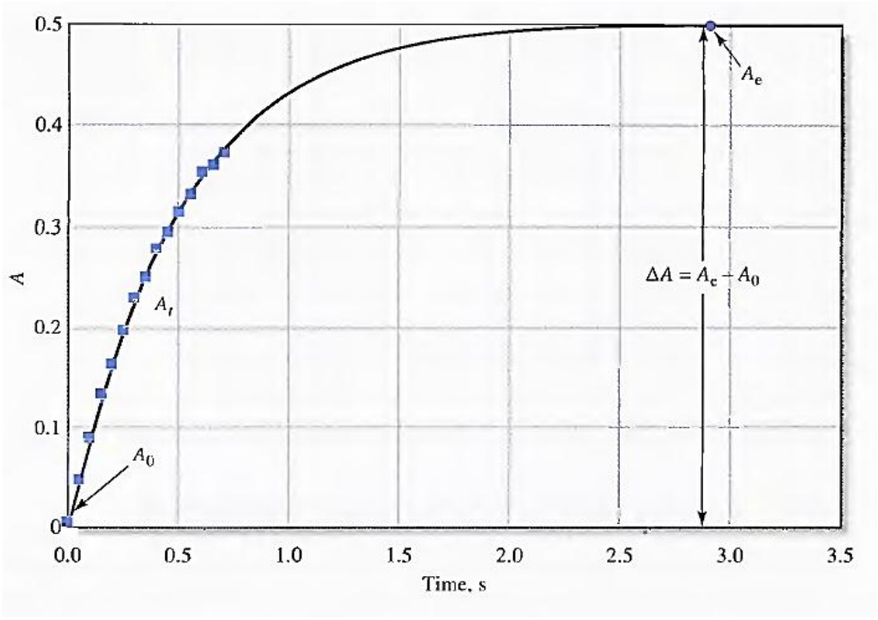


Figure 7. The predictive approach in kinetic methods. A mathematical model, shown as the blue squares, is used to fit the response, shown as the solid line, during the kinetic regime of a reaction. The model is then used to predict the equilibrium value of the signal, A_e, which is related to the analyte concentration. In the example shown, the absorbance is plotted *vs*. time, and the early-time data is used to predict A_e, the equilibrium value, shown as the blue circle.

Applications

(1) Kinetic Methods of Analysis with Potentiometric Detector

Modern work generally uses major techniques such as , potentiometer and spectroscopy, although there is a wide range of other techniques available.

A potentiometric chemical sensor or ion-selective electrode confirms to the Nernst equation (27)

$$E = E^0 + k \log a_i \qquad (27)$$

Direct potentiometric measurements are usually time-consuming experiments.
Kinetic potentiometric methods are powerful tools for analysis, since they permit sensitive and selective determination of many samples within a few minutes with no

sample pretreatment in many cases. The application of kinetic potentiometric methods offers some specific advantages over classical potentiometry, such as improved selectivity due to measurements of the evolution of the analytical signal with the reaction time. To construct calibration graphs the initial rate of the complex (product) formation reaction or change in potential during fixed time interval are used.

Kinetic method of potentiometric determination of Fe(III) with a copper(II) selective electrode based on a metal displacement reaction is described. Addition of various amounts of iron(III) to the buffered (pH 4) Cu(II)-EDTA cell solution alters the concentration of free copper(II) ion in the solution. EDTA is well-known abbreviation for ethylenediaminetetraacetic acid, a compound that forms strong 1:1 complexes with most metal ions. EDTA is a hexaprotic system, designated H_6Y^{+2} . When iron(III) is added to a buffered aqueous solution containing CuY^{-2} species same cupric ion will be displaced because

$K_{FeY^-} > K_{CuY2^-}$:

$$CuY^{2-} + Fe^{3+} = FeY^{-} + Cu^{2+}$$

The above ligand exchange between two metals is often sluggish because the reaction involves breaking a series of coordinate bonds in succession. As already noted, the rate of change in the potential, expressed as d*E*/d*t*, is directly proportional to the rate of change of the concentration of the potential determining ion, Cu^{2+} in this experiment, with time. The calculated values, $\Delta E/\Delta t$ versus log $C_{Fe(III)}$ were found to be linear for different concentrations of the Cu-EDTA complex, which was used as "kinetic substrate". The linear or analytical range for each tested concentration of Cu-EDTA was close to one decade of iron concentration.

(2) Spectroscopic Techniques

the spectrophotometric technique is the most widely used in pharmaceutical analysis, due to its inherent simplicity, economic advantage, and wide availability in most quality control laboratories. Kinetic spectrophotometric methods are becoming a great interest for the pharmaceutical analysis.

The application of these methods offers some specific advantages over classical spectrophotometry, such as improved selectivity due to the measurement of the evolution of the absorbance with the reaction time.

There are three important features of spectra which are of prime interest to the kineticist.

1. The frequency and the fine structure of the lines give the identity of the molecule; this is particularly important in detecting intermediates and minor products.
2. The intensity of the lines gives the concentration; this is useful for monitoring the concentrations of reactants and intermediates with time.
3. The line width enables kinetic features of the transition and the excited state to be determined.

Using **Beer's law**, concentrations can be found from the change in intensity of the radiation passed through the sample.

$$\log_e A = \log_e c + \text{constant} \qquad (28)$$

A plot of $\log_e$ A versus time differs from a plot of $\log_e$ c versus time only in so far as it is displaced up the y-axis by an amount equal to $\log_e$ εd (see Figure 8). The slope of the graph of loge absorbance versus time is the same as the slope of the graph of loge[reactant] versus time (Figure 8). The slope of this latter graph gives the quantity which characterizes the reaction.

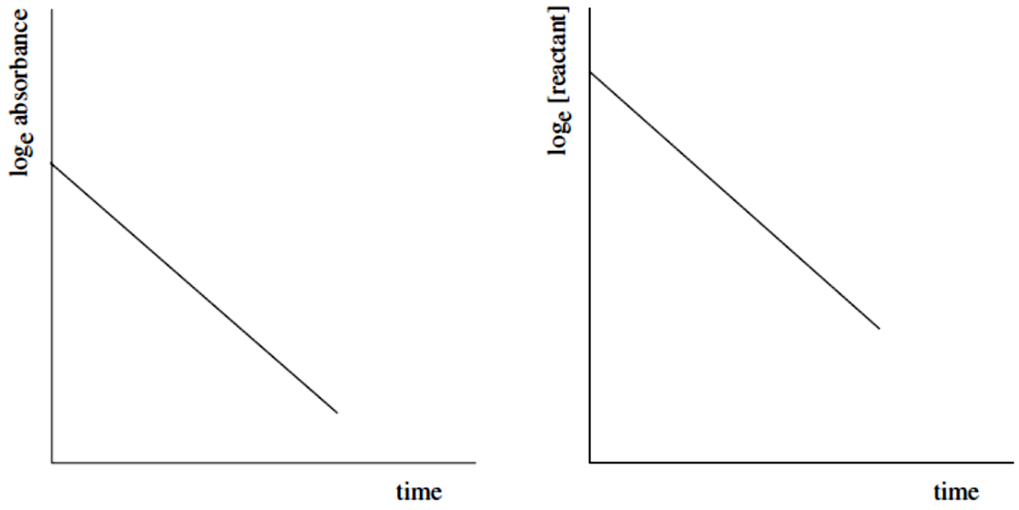


Figure 8. Graphs of $\log_e$ absorbance *vs* time, $\log_e$ [reactant] *vs* time.

In case of fluorescence spectroscopy , intensity is generally proportional to [reactant], so that

$$\text{Intensity} = f[\text{reactant}] \qquad (29)$$

where f is a constant of proportionality.

$$\log_e \text{intensity} = \log_e f + \log_e [\text{reactant}] \qquad (30)$$

and so again there is no need to convert to concentrations (see Figure 9). This can sometimes be particularly useful, e.g. when laser-induced fluorescence is being used for monitoring concentrations.

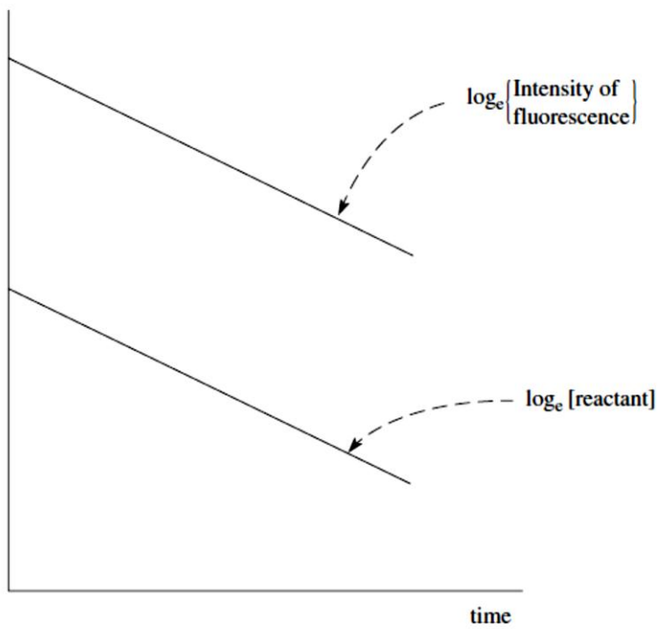


Figure 9. Graphs of $\log_e$ intensity of fluorescence versus and, $\log_e$ [reactant] versus time.

Worked Problem 1

In a 1.000 cm spectrophotometric cell, solutions of $C_6H_5CH{=}CHCCl_2$ in ethanol of known concentration give the following values of the absorbance, A.

$\frac{10^5 \times \text{conc.}}{\text{mol dm}^{-3}}$	0.446	0.812	1.335	1.711	2.105
A	0.080	0.145	0.240	0.305	0.378

Note.

$$\frac{10^5 c}{mol\ dm^{-3}} = 0.446$$

Divide both sides by 10^5, and multiply both sides by mol dm^{-3}. This gives

$$c = \frac{c0.446\ moldm^{-3}}{10^5} = 0.446\ x\ 10^{-5} moldm^{-3} = 4.46\ x\ 10^{-6}\ moldm^{-3}$$

This is a standard way of presenting tabulated data, and it is necessary to be completely at ease in performing the above manipulation.

It is also necessary to be careful when drawing graphs of tabulated data presented in this manner, so as to get the powers of ten correct.

Using Beer's law, draw a calibration graph and determine the value of ε_λ.

The reaction of this substance with $C_2H_5O^-$ in ethanolic solution is followed spectrophotometrically. The following results are found. Plot a graph of reactant concentration against time.

$\frac{\text{time}}{\text{min}}$	0	40	80	120	140
A	0.560	0.283	0.217	0.168	0.149

Answer.

A graph of A versus c is a straight line through the origin. From the slope ε =1.79 x 10^4 dm^3 mol^{-1} cm^{-1} (Figure 10-A).

This can be used to calculate the [reactant] corresponding to each absorbance, since A= ε cd. Since d= 1.000 cm the following table can be drawn up:

$\frac{\text{time}}{\text{min}}$	0	40	80	120	140
$\frac{10^5 \times \text{conc.}}{\text{mol dm}^{-3}}$	3.13	1.58	1.21	0.939	0.832

Alternatively, values of the concentration corresponding to the measured absorbances can be read off from the calibration curve.

Figure 10-B shows the smooth curve of [reactant] versus time.

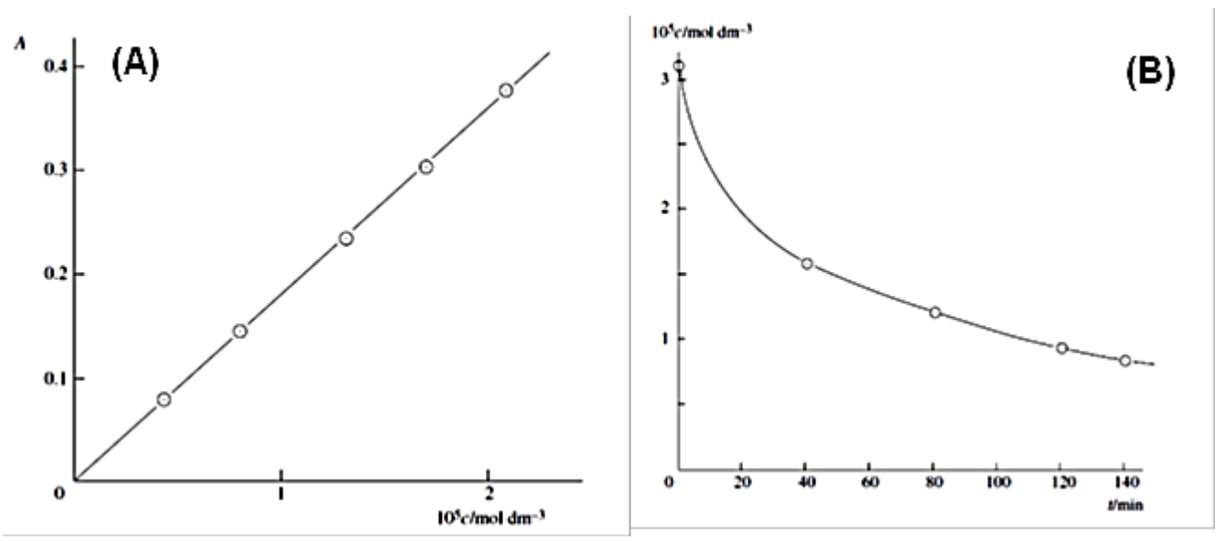


Figure 10. Beer's law plot of absorbance versus concentration (A), Graph of concentration versus time (B).