Spectroscopy and spectroscopic analysis

Ultraviolet, visible and infrared spectroscopies, the most commonly used range of wavelengths employed by chemists today. Ultraviolet radiation is commonly defined as the wavelengths from 200 to 350 nm. Around 200nm oxygen absorbs strongly, part of the process to produce ozone in the upper atmosphere, and makes measurements difficult. One solution to the problem is to evacuate the instrument, giving rise to the terminology that wavelengths from 200 to 100nm are in the ‘‘vacuum UV’’ region. The range of wavelengths of the absorption spectrum is shown in Table1.

A spectrum is a plot of some measure of the electromagnetic radiation absorbed by a sample versus the wavelength or energy of the electromagnetic radiation. For example, it is common practice to plot the absorbance versus wavelength for spectra in the ultraviolet and visible spectral regions.

Absorption Spectrophotometry

Absorption Process

When matter interacts with an energy source (heat, sound, electricity, light, etc.) some of the energy can be absorbed,

causing the particles to be elevated to different energy levels. Under certain conditions, though, the amount of energy absorbed can be controlled, and information about the chemical system can be obtained. This is the case in absorption spectroscopy.

Table 1: The range of wavelengths of the absorption spectrum.

Wavelength Region	**Wavelength Limits**	**Types of Transitions in Chemical Systems with Similar Energies**
Gamma ray	0.01 - 1 A	Nuclear proton/neutron arrangements
Ultraviolet	10 - 380 nm	Outer-shell electrons in atoms Bonding electrons in molecules
Visible	380 - 720 nm	Same as ultraviolet
Infrared	0.72 - 1000 µm	Vibrational position of atoms in molecular bonds
Microwave	0.1 - 100 cm	Rotational position in molecules Orientation of unpaired electrons in an applied magnetic field
Radiowave	1 - 1000 m	Orientation of nuclei in an applied magnetic field

Visible and Ultraviolet Spectroscopy

Visible wavelengths cover a range from approximately 400 to 800 nm. The longest visible wavelength is red and the shortest is violet. The wavelengths of what we perceive as particular colors in the visible portion of the spectrum are displayed and listed below. In horizontal diagrams, such as the one on the bottom left, wavelength will increase on moving from left to ightright, c.f. Fig. 1. If one monitors a beam of light shining through a sample containing a substance that can absorb one of the beam's wavelengths, one can obtain a plot of the amount of light absorbed, A, versus the wavelength, λ, as shown in the figure 1. This plot is known as an *absorption spectrum*, and shows which particular wavelengths of light a chemical species can absorb.

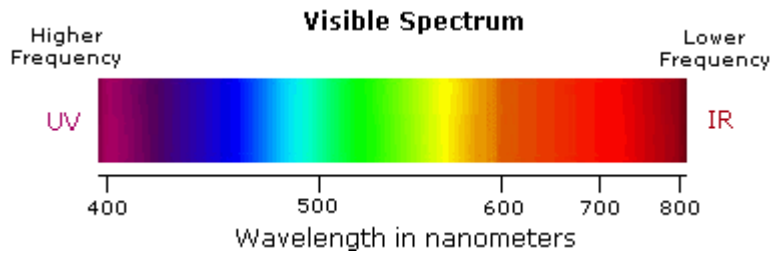


Fig. 1: The longest visible wavelength is red and the shortest is violet

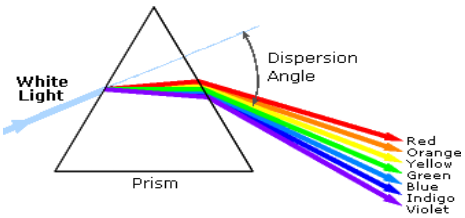


Fig.2: Dispersion of light through a prism.

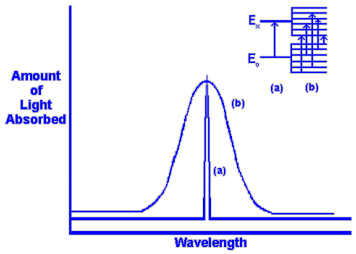


Fig. 3: Absorbed Light versus Wavelength

When one shine a light beam of a certain wavelength (λ) and initial intensity (I_0) through an absorbing sample contained in a spectrophotometer cell, the intensity of the light beam *transmitted* through the sample (I_t) is dependent on three factors. The first factor is whether the sample will absorb light at that wavelength. The second is the amount of sample which the light must pass through or, the *cell width* (b). The third factor is the *concentration of the absorbing species* in the sample solution (C).

The fraction of light transmitted, or transmittance (T), is defined as the follows.

The attenuation of electromagnetic radiation as it passes through a sample is described quantitatively by two separate, but related terms: transmittance and absorbance. **Transmittance** is defined as the ratio of the electromagnetic radiation's power exiting the sample, I, to that incident on the sample from the source, I_o. Multiplying the transmittance by 100 gives the percent transmittance (%*T*), which varies between 100% (no absorption) and 0% (complete absorption). All methods of detection, measure the transmittance of electromagnetic radiation. Attenuation of radiation as it passes through the sample leads to a transmittance of less than 1. Besides absorption by the analyte, several additional phenomena contribute to the net attenuation of radiation, including reflection and absorption by the sample container, absorption by components of the sample matrix other than the analyte, and the scattering of radiation. To compensate for this loss of the electromagnetic radiation's power, one use a method blank. The radiation's power exiting from the method blank is taken to be I_o. An alternative method for expressing the attenuation of electromagnetic radiation is absorbance, *A*, which is defined as Absorbance is the more common unit for expressing

the attenuation of radiation because it is a linear function of the analyte's concentration.

$$T = I/I_o$$

$$\text{Percentage of light transmitted (\% T)} = T \times 100$$

where, I_0 = initial intensity of the light beam, and I = transmitted ntensity of the light beam. The transmittance of the sample varies logarithmically with the cell width and the concentration of the absorbing species in the following way:

$$\log (1/T) = -\log T = \varepsilon, \text{ proportionally constant, } b\,C$$

The proportionality constant depends on many factors:

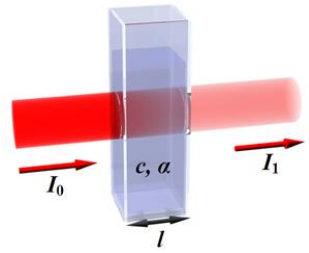
1-The chemical nature of the individual absorber,

2-The wavelength at which the measurements are being made, and

3-The units of *l* and C.

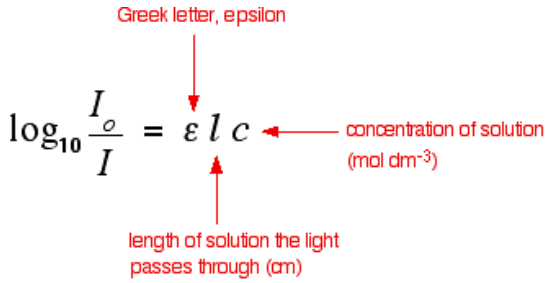
In visible absorption spectroscopy, *l* is normally measured in centimeters, cm. If C is also measured in mol/L (molar concentration, M), the proportionality constant is defined as the ***molar absorptivity*** (ε), which has units of l/M-cm. If C is measured in any other units (e.g., g/l), the constant is simply called the ***absorptivity* (a)**, whose units will depend on C. Under normal operating conditions, ε or (a) is determined

experimentally by measuring absorbance of a standard solution of known concentration.



Absorption of a monochromatic light through a cuvete of size *l*.

The relationship relates the amount of light *transmitted* through the sample to the concentration and cell width can express as follows. One frequently like to think in terms of how much light is *absorbed* by the sample, so we define a new term, ***absorbance*** (A):



Factors that determine transmitted light intensity.

$$A = \log (1/T) = -\log T$$

Thus, making all of the appropriate substitutions, one get:

$$A = \varepsilon \, l \, C \text{ (when C = mol/L or M)}$$

or,

$$A = a \, l \, C \text{ (when C = g/l or other units)}$$

This equation is known as Beer's Law, which shows the linear relationship between the absorbance and the concentration of the absorbing species.

In essence, the law states that there is a logarithmic dependence between the transmission of light through a substance and the concentration of the substance, and also between the transmission and the length of material that the light travels through. Thus if *l* and ε are known, the concentration of a substance can be deduced from the amount of light transmitted by it.

If the material is a liquid, it is usual to express the absorber concentration as a ***mole fraction*** i.e. a dimensionless fraction. The units of the absorption coefficient are thus reciprocal length (e.g. cm^{-1}). If the concentration is expressed in *moles* per unit *volume*, ε is a ***molar absorptivity*** (usually given the symbol ε) in units of $mol^{-1}\ cm^2$ or sometimes $L \cdot mol^{-1} \cdot cm^{-1}$.

In the case of a gas, the concentration may be expressed as a number density (e.g. cm^{-3}), in which case ε is an *absorption cross-section* and has units of area (e.g. cm^2).

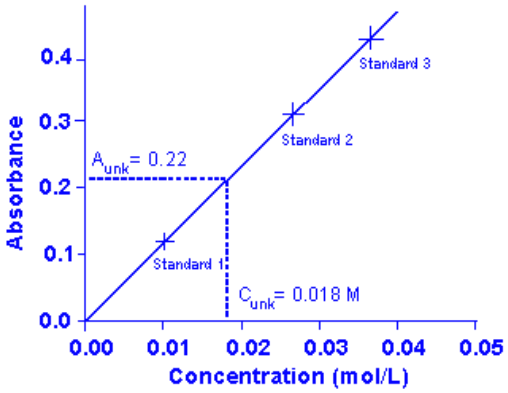
In chemical analyses, the following three methods can utilize Beer's Law:

1. *Absolute Calculations*

Absorptivity or molar absorptivity is calculated by measuring the absorbance of a standard of known concentration in a cell known cell width. This calculated value is then used to determine an unknown concentration of the same absorbing species from the absorbance measurement at that concentration.

2. Working Curve Analysis

The absorbance of a series of three to five standard solutions are measured and plotted against the concentrations of these standards. This is known as a Beer's Law plot or calibration curve. The absorbance of an unknown concentration is then measured, and its concentration is determined directly from the plot. This method is more commonly used than the absolute calculation, because experimental error will average out over the number of standards.



Beer's Law Plot

3. Standard Addition Analysis

This method is used when certain matrix interferences may cause problems with the measurements. The assumption is that the interference will act equally on the analyte and on standard added to analyte. In this case, the sample is measured (A_1), and then a second portion of the sample is spiked with a standard and this is measured (A_2).

$$A_1 = abC_{unk}$$

$$A_2 = ab\left(C_{unk} + C_{spike}\right)$$

$$\frac{A_2}{A_1} = \frac{\left(C_{unk} + C_{spike}\right)}{C_{unk}}$$

$$C_{unk} = \frac{A_1 C_{unk}}{A_2 - A_1}$$

One will use the first two methods in the laboratory experiment.

Limitations of the Beer's law

According to Beer's law, a calibration curve of absorbance versus the concentration of analyte in a series of standard solutions should be a straight line with an intercept of 0 and a slope of ε*l* or a*l*. In many cases, however, calibration curves are found to be nonlinear. Deviations from linearity are divided into three categories: fundamental, chemical, and instrumental.

Fundamental Limitations to Beer's Law:

Beer's law is a limiting law that is valid only for low concentrations of analyte. There are two contributions to this fundamental limitation to Beer's law. At higher concentrations the individual particles of analyte no longer behave independently of one another. The resulting interaction between particles of analyte may change the value of ε. A second contribution is that the absorptivity, *a*, and molar absorptivity, ε, depend on the sample's refractive index. Since the refractive index varies with the analyte's concentration, the values of, a, and ε will change. For sufficiently low concentrations of analyte, the refractive

index remains essentially constant, and the calibration curve is linear.

Chemical Limitations to Beer's Law:

Chemical deviations from Beer's law can occur when the absorbing species is involved in an equilibrium reaction. Consider, as an example, an analysis for the weak acid, HA. To construct a Beer's law calibration curve, several standards containing known total concentrations of HA, C_{tot}, are prepared and the absorbance of each is measured at the same wavelength. Since HA is a weak acid, it exists in equilibrium with its conjugate weak base, A^-

$$HA + H_2O = H_3O^+ + A^- \quad (1)$$

If both HA and A^- absorb at the selected wavelength, then Beer's law is written as

$$A = \varepsilon_{HA} l C_{HA} + \varepsilon_A l C_A \qquad (2)$$

where C_{HA} and C_A are the equilibrium concentrations of HA and A^-. Since the weak acid's total concentration, C_{tot}, is

$$C_{tot} = C_{HA} + C_A \qquad (3)$$

the concentrations of HA and A^- can be written as

$$C_{HA} = \alpha_{HA} C_{tot} \qquad (4)$$

$$C_A = (1 - \alpha_{HA})\, C_{tot} \qquad (5)$$

where α_{HA} is the fraction of weak acid present as HA. Substituting equations 4 and 5 into equation 3, and rearranging, gives

$$A = (\varepsilon_{HA}\alpha_{HA} + \varepsilon_A - \varepsilon_A\alpha_{HA})lC_{tot} \qquad (6)$$

Because values of α_{HA} may depend on the concentration of HA, equation 6 may not be linear. A Beer's law calibration curve of A versus C_{tot} will be linear if one of two conditions is met. If the wavelength is chosen such that ε_{HA} and ε_A are equal, then equation 6 simplifies to

$$A = \varepsilon_A lC_{tot}$$

and a linear Beer's law calibration curve is realized. Alternatively, if α_{HA} is held constant for all standards, then equation 6 will be a straight line at all wavelengths. Because HA is a weak acid, values of α_{HA} change with pH. To maintain a constant value for α_{HA}, therefore, one need to buffer each standard solution to the same pH. Depending on the relative values of ε_{HA} and ε_A, the calibration curve will show a positive or negative deviation from Beer's law if the standards are not buffered to the same pH.

Quantitative Applications

The determination of an analyte's concentration based on its absorption of ultraviolet or visible radiation is one of the most frequently encountered quantitative analytical methods. One reason for its popularity is that many organic and inorganic compounds have strong absorption bands in the UV/vis region of the electromagnetic spectrum. In addition, analytes that do not

absorb UV/vis radiation, or that absorb such radiation only weakly, frequently can be chemically coupled to a species that does. For example, nonabsorbing solutions of Pb^{2+} can be reacted with dithizone to form the red Pb–dithizonate complex. An additional advantage to UV/vis absorption is that in most cases it is relatively easy to adjust experimental and instrumental conditions so that Beer's law is obeyed. The applications of Beer's law for the quantitative analysis of samples in environmental chemistry, clinical chemistry, industrial chemistry and forensic chemistry are numerous. Examples from each of these fields follow.

Environmental Applications

Methods for the analysis of waters and waste waters relying on the absorption of UV/vis radiation are among some of the most frequently employed analytical methods. Many metals can be analyzed following the formation of a colored metal–ligand complex. One advantage to these spectroscopic methods is that they are easily adapted to the field analysis of samples using a filter photometer. One ligand used in the analysis of several metals is diphenylthiocarbazone, also known as dithizone. Dithizone is insoluble in water, but when a solution of dithizone in $CHCl_3$ is shaken with an aqueous solution containing an appropriate metal ion, a colored metal–dithizonate complex

forms that is soluble in $CHCl_3$. The selectivity of dithizone is controlled by adjusting the pH of the aqueous sample. For example, Cd^{2+} is extracted from solutions that are made strongly basic with NaOH, Pb^{2+} from solutions that are made basic with an ammoniacal buffer, and Hg^{2+} from solutions that are slightly acidic.

Quantitative Analysis of Mixtures

The analysis of two or more components in the same sample is straightforward if there are regions in the sample's spectrum in which each component is the only absorbing species. In this case each component can be analyzed as if it were the only species in solution. Unfortunately, UV/vis absorption bands are so broad that it frequently is impossible to find appropriate wavelengths at which each component of a mixture absorbs separately. ***Beer's law is additive***; thus, for a two-component mixture of X and Y, the mixture's absorbance, A_m, is

$$(A_m)_{\lambda 1} = (\varepsilon_X)_{\lambda 1} l C_X + (\varepsilon_Y)_{\lambda 1} l C_Y \qquad (7)$$

where λ_1 is the wavelength at which the absorbance is measured. Since equation 7 includes terms for both the concentrations of X and Y, the absorbance at one wavelength does not provide sufficient information to determine either C_X or C_Y. If one measure the absorbance at a second wavelength, λ_2,

$$(A_m)_{\lambda 2} = (\varepsilon_X)_{\lambda 2} l C_X + (\varepsilon_Y)_{\lambda 2} l C_Y \qquad (8)$$

then C_X and C_Y can be determined by solving equations 7 and 8. Of course, it is necessary to determine values for e for each component at both wavelengths. In general, for a mixture of *n* components, the absorbance must be measured at *n* different wavelengths.

Example: 1

The concentrations of Fe^{3+} and Cu^{2+} in a mixture can be determined following their reaction with hexacyanoruthenate(II), $[Ru(CN)_6]^{4-}$, which forms a purple blue complex with Fe^{3+} (λ_{max} = 550 nm), and a pale green complex with Cu^{2+} (λ_{max} = 396 nm). The molar absorptivities (M^{-1} cm^{-1}) for the metal complexes at the two wavelengths are summarized in the following table.

	ε_{550}	ε_{396}
Fe^{3+}	9970	84
Cu^{2+}	34	856

When a sample containing Fe^{3+} and Cu^{2+} is analyzed in a cell with a path length of 1.00 cm, the absorbance at 550 nm is 0.183, and the absorbance at 396 nm is 0.109. What are the molar concentrations of Fe^{3+} and Cu^{2+} in the sample?

Solution

Substituting known values into equations 7 and 8 gives

$$A_{550} = 0.183 = 9970C_{Fe} + 34C_{Cu}$$

$$A_{396} = 0.109 = 84C_{Fe} + 856C_{Cu}$$

To determine the C_{Fe} and C_{Cu} one solve the first equation for C_{Cu}

$$C_{Cu} = (0.183 - 9970C_{Fe})/34$$

and substitute the result into the second equation.

$$0.109 = 84C_{Fe} + 856((0.183 - 9970C_{Fe})/34$$

$$= 4.607 – (2.51x10^{5}) C_{Fe}$$

Solving for C_{Fe} gives the concentration of Fe^{3+} as $1.80x10^{–5}$ M. Substituting this concentration back into the equation for the mixture's absorbance at a wavelength of 396 nm gives the concentration of Cu^{2+} as $1.26x10^{–4}$ M.

Example: 2

The molar absorptivities of compounds X and Y were measured with pure samples of each. The absorptivies of X and Y are shown in the following Table

	e (M^{-1} cm^{-1})	*e* (M^{-1} cm^{-1})
λ, nm	X	Y
272	*16440*	*3870*
327	*3990*	*6420*

A mixture of compounds X and Y in a 1.000 cm cell has an absorbance of 0.957 at 272 and 0.559 at 327 nm. Find the concentrations of X and Y in the mixture.

To obtain results with good accuracy and precision the two wavelengths should be selected so that $\varepsilon_X > \varepsilon_Y$ at one wavelength

and $\varepsilon_Y < \varepsilon_X$ at the other wavelength. The optimum precision is obtained when the difference in molar absorptivities is as large as possible. One method for locating the optimum wavelengths, therefore, is to plot $\varepsilon_X/\varepsilon_Y$ as a function of wavelength and determine the wavelengths at which $\varepsilon_X/\varepsilon_Y$ reaches maximum and minimum values.

Charge-transfer spectra

Many organic and inorganic complexes exhibit charge transfer absorption (CT complex or electron-donor-acceptor) and are known as charge transfer complexes. They represent one of the most important classes of spectra for analytical chemistry since the molar absorptivities tend to be very large (ϵ_{max} > 10,000). Charge-transfer can occur in substances, usually complexes that have one moiety that can be an electron donor and another that can have a small difference in their energy levels so that the electron can be readily transferred from the donor to the acceptor orbitals and back again. When absorption occurs, an electron from a donor group is transferred to an acceptor, at the excited electronic state.

One example is the well-known, deep-red color of the iron (III) thiocyanate ion. The process appears to be:

$$(Fe^{3+}SCN^{-})^{2+} + h\nu \rightarrow (Fe^{2+}SCN)^{2+}$$

an electron from the thiocyanate is excited to an orbital of iron, effectively reducing it to iron (II) and the thiocyanate radical. The electron rapidly returns to the thiocyanate to repeat the process.

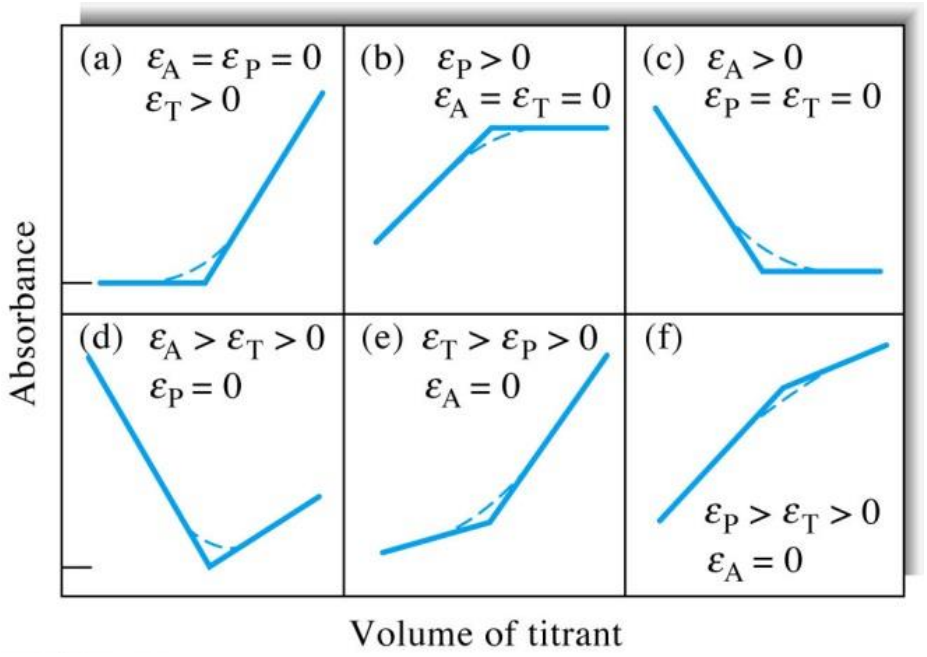
Photometric Titrations

The change in absorbance during a titration in which one of the reactants or products absorbs in the visible or UV region can be used to locate the end point. Plots of absorbance as a function of volume of titrant added give two straight line portions of differing slope intersecting at the end-point. Curvature near the end point is caused by incomplete reaction, whilst curvature elsewhere is caused by apparent deviations from the Beer's law or by dilution errors. Corrections for changes in volume will eliminate the last problem, but using a concentrated titrant is simpler. Photometric titrations have advantages to other titrations or to direct measurements:

1-Data near the end-point are unimportant provided straight-line portions can be established and extrapolated. Very dilute solutions can be titrated.

2-Precision and accuracy are better than for a direct absorbance measurement as the titration curve averages the data; relative precision is around 0.5%.

The method is particularly suited to complexometric titrations of mixtures or where there is no suitable visual indicator.



Possible shapes of photometric titration curves.

Complexation Reactions and Titrations

Complex-formation reactions are widely used in analytical chemistry. One of the first uses of these reagents was for titrating cations. In addition, many complexes are coloured or absorb ultraviolet radiation; the formation of these complexes is often the basis for spectrophotometric determinations. The most useful complex forming reagents are organic compounds that contain several electron donor groups that form multiple covalent bonds with metal ions. The selectivity of a ligand for one metal ion over another relates to the stability of the complexes formed. The higher the formation constant of a metal-ligand complex, the

better the selectivity of the ligand for the metal relative to similar complexes formed with other metals.

Titration with Complexing Agents

Complexation reactions have many uses in analytical chemistry; their application is in complexometric titrations. Here, a metal ion reacts with a suitable ligand to form a complex, and the equivalence point is determined by a suitable instrumental method. The formation of soluble inorganic complexes is not widely used for titration but the formation of precipitates is the basis for many important determinations.

The progress of a complexometric titration is generally illustrated by a titration curve, which is usually a plot of pM = -log[M] as a function of the volume of titrant added. Most often in complexometric titrations the ligand is the titrant and the metal ion the analyte, although occasionally the reverse is true. Many precipitation titrations use the metal ion as the titrant. Most simple inorganic ligands are unidentate, which can lead to low complex stability and indistinct titration end points.

As titrants, multidentate ligands, particularly those having four or six donor groups, have two advantages over their unidentate counterparts. ***First***, they generally react more completely with cations and thus provide sharper end points. ***Second***, they ordinarily react with metal ions in a single-step

process, whereas complex formation with unidentate ligands usually involves two or more intermediate species.

Atomic spectroscopy

Atomic spectroscopy includes all analytical techniques that employ the emission and/or absorption of electromagnetic radiation by individual atoms. There are three kinds of emission spectra:

1. Continuous spectra, which are emitted by incandescent solids

2. Line spectra, which are characteristic of atoms that have been excited and are emitting their excess energy

3. Band spectra, which are emitted by excited molecules

The specific wavelength of the radiation (emitted or absorbed) identifies the element. The intensity of emitted (or absorbed) radiation at the specific wavelength is proportional to the amount of the element present. Three techniques can be used to observe the atomic vapor that is produced when a sample solution is nebulized and passed into a flame. These are atomic-absorption spectrometry (AAS), flame emission spectrometry (FES), and atomic fluorescence spectrometry (AFS). Of these, AAS and FES are the most widely used.

Atomic-emission spectroscopy and spectrometry use either electrical discharges or plasmas for excitation of emission spectra. Flame spectrometric methods, qualitative and quantitative, can be applied to clinical materials (serum, plasma,

and biological fluids), soils, plant materials, plant nutrients, and samples of inorganic and organic substances.

Instrumentation for Flame Spectrometric methods

The basic instrumentation for flame atomic-emission spectrometric methods requires these items:

1. An atom source, a flame
2. A monochromator to isolate the specific wavelength of light to be used
3. A detector to measure the light transmitted
4. Electronics to treat the signal and a data logging device to display the results.

For work in atomic absorption spectrometry, a primary light source must be added, either a hollow-cathode lamp or an electrodeless discharge lamp.

Atom Sources

The atom source must produce free atoms of analytical material from the sample. A high-energy acetylene–nitrous oxide flame (2800°C) is usually employed; its function is to rapidly desolvate and to efficiently dissociate the analyte in the sample in order to minimize interferences. This is particularly true if an oxide of the analyte has high dissociation energy. A lower-temperature acetylene–air flame (2400°C) is sometimes desired

when an element is too easily ionized in a hotter flame. The sample is introduced as an aerosol into the flame. The flame burner head is aligned so that it intersects the light path of the spectrophotometer.

Nebulization

In both FES and AAS, the sample solution is introduced as an aerosol (fine mist) into the flame. Pneumatic nebulization is the technique used in most flame spectrometric determinations. A cloud of droplets of varying diameters is produced within an expansion chamber that precedes the burner. The larger droplets are removed from the sample stream upon collision with multivaned flow spoilers or the wall surfaces. Some nebulizer units break up the original droplets into smaller droplets. The final aerosol, now a fine mist, is combined with the oxidizer–fuel mixture and carried into the burner. Only a small percentage (usually 2% to 3%) of the nebulized analyte solution reaches the flame.

Atomization

The flame atomization processes are complex. Everything leading to the production of free atoms must take place in the few milliseconds that correspond to the upward movement of the molecules and atoms through the flame gases. The atomization

step must convert the analyte within the aerosol into free analyte atoms for AAS and flame atomic-emission spectrometry.

For AAS the following sequence of events occurs in rapid succession.

1. ***Desolvation of the aerosol:*** The water, or other solvent, is vaporized, leaving minute particles of dry salt.
2. ***Vaporization of the resulting particles*:** At the high temperature of the flame, the dry salt is vaporized (converted to gaseous molecules).
3. ***Dissociation of gaseous molecules***: Part or all of the gaseous molecules are dissociated to give neutral atoms.

For flame atomic emission, two more events must occur.

1. ***Excitation of atoms* (*and molecules*):** The neutral metal atoms are excited (and sometimes ionized) by the thermal energy of the flame. (Excitation occurs in AAS by thermal collisions as well as by absorption of radiation from the light source.)
2. ***Emission*:** From the excited electronic level(s) of the atom, a reversion takes place to the ground electronic state with the emission of light whose wavelength is characteristic of the element and whose intensity is proportional to the amount of analyte element present.

Spectrophotometers. The spectrophotometers are similar to those used in visible-ultraviolet instruments.

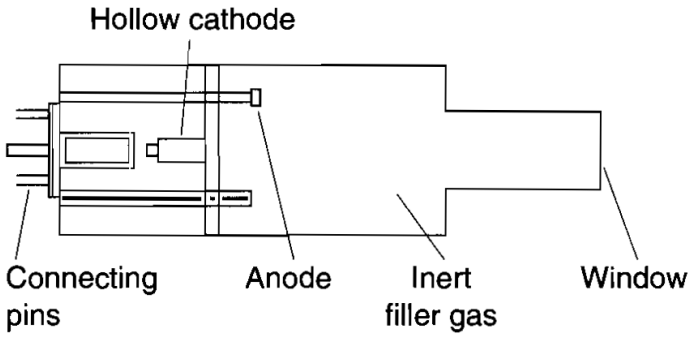
Atomic-Absorption Spectrometry

Elemental analysis at the trace or ultratrace level can be performed by a number of analytical techniques; however, atomic spectroscopy remains the most popular approach. AAS measures the concentrations of ground-state atoms by quantifying the absorption of spectral radiation that corresponds to allowed transitions from the ground to excited states.

Light Sources

Hollow-Cathode Lamps.

The basic components of a shielded-type hollow-cathode lamp are an anode, a cathode, and either a glass or quartz exit window, all sealed in a Pyrex cylinder filled with an inert gas (argon or neon) at low pressure. Neon gas provides a greater intensity of emitted element lines; argon is used only when a neon emission line lies in close proximity to a resonance line of the cathode element. An anode wire is positioned alongside the cylindrical cathode. The cathode is a hollow cylinder of the element whose spectrum is to be produced, or in some cases, an alloy or carefully selected mixture of metals that does not spectrally interfere.



Hollow-cathode lamps emit light by the following process. The fill gas is ionized when an electrical potential is applied between the anode and the cathode. The positively charged ions collide with the negatively charged cathode and dislodge individual metal atoms in a process known as sputtering. These gaseous metal atoms are excited through impact with fill-gas ions, and light of the specific wavelengths for that element is emitted when the atom decays from the excited atomic state back to a less excited state or the ground electronic state.

Questions:

1-Sketch a figure for the amperometric titration of lead

2- Sketch a figure for the colouremetric titration of ferrous ions

3- Sketch a figure for the colouremetric titration of cynide ions

4-Sketch a figure for the colouremetric titration of hydrochloric acid

5- Sketch a figure for the colouremetric titration of permenganate ions

Chromatography

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture.

Chromatography terms

Analyte is the substance to be separated during chromatography.

Analytical chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a sample.

Stationary phase is a phase that is covalently bonded to the support particles or to the inside wall of the column tubing.

Chromatogram is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.

Chromatograph is equipment that enables a sophisticated separation, e.g. gas chromatographic or liquid chromatographic separation.

Chromatography is a physical method of separation that distributes components to separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction.

Eluate is the mobile phase leaving the column. Eluent is the solvent that carries the analyte.

Eluotropic series is a list of solvents ranked according to their eluting power.

Immobilized phase is a stationary phase that is immobilized on the support particles, or on the inner wall of the column tubing.

Mobile phase is the phase that moves in a definite direction. It may be a liquid (LC) or a gas (GC). The mobile phase consists of the sample being separated / analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromotagraphy and the sample being separated. The mobile phase moves through the chromatographic column (the stationary phase) where the sample interacts with the stationary phase and is separated.

Preparative chromatography is used to purify sufficient quantities of a substance for further use, rather than analysis.

Retention time is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.

Sample is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.

Solute refers to the sample components in partition chromatography.

Solvent refers to any substance capable of solubilizing another substance, and especially the liquid mobile phase in liquid chromatography.

Stationary phase is the substance fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography

Detector refers to the instrument used for qualitative and quantitative detection of analytes after separation.

Chromatography is based on the concept of partition coefficient. Any solute partitions between two immiscible solvents. When one makes a solvent immobile (by adsorption on a solid support matrix) and another mobile it results in most common applications of chromatography.

Chromatographic Techniques

Column chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary

phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

Planar chromatography

Planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (**paper chromatography**) or a layer of solid particles spread on a support such as a glass plate (**thin layer chromatography**). Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase. The specific **Retention factor** (R_f) of each chemical can be used to aid in the identification of an unknown substance.

Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of ***chromatography paper*.** The paper is placed in a jar containing a

shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

Thin layer chromatography

Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of **adsorbent** like silica gel, alumina, or cellulose on a flat, inert **substrate.** Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification, high-performance TLC can be used.

Displacement chromatography

The basic principle of displacement chromatography is: A molecule with a high affinity for the chromatography matrix (the displacer) competes effectively for binding sites, and thus displace all molecules with lesser affinities. There are distinct

differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired for maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than “peaks”. Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

Techniques by physical state of mobile phase

Gas chromatography

Gas chromatography (GC), also sometimes known as gas-liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatographic separation is always carried out in a column, which is typically "packed" or "capillary". Packed columns are the routine work horses of gas chromatography, being cheaper and easier to use and often giving adequate performance. Capillary columns generally give far superior resolution and although more expensive are becoming widely used, especially for complex mixtures. Both types of column are made from non-adsorbent and chemically inert materials. Stainless steel and glass are the usual materials for packed columns and quartz or fused silica for capillary columns.

Gas chromatography is based on a **partition equilibrium** of analyte between a solid or viscous liquid stationary phase (often a liquid silicone-based material) and a mobile gas (most often helium). The stationary phase is adhered to the inside of a small-diameter (commonly 0.53 - 0.18 mm inside diameter) glass or fused-silica tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC

make it unsuitable for high molecular weight biopolymers or proteins (heat denatures them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring and remediation, and industrial chemical fields. It is also used extensively in chemistry research.

Liquid chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as **high performance liquid chromatography** (HPLC).

In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Methods in which the stationary phase is more polar than the mobile phase (e.g., toluene as the mobile phase, silica as the stationary phase) are termed normal phase liquid chromatography (NPLC) and the opposite (e.g., water-methanol mixture as the mobile phase and

C18 = octadecylsilyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC).

Techniques by separation mechanism

Ion exchange chromatography

Ion exchange chromatography (usually referred to as ion chromatography) uses an ion exchange mechanism to separate analytes based on their respective charges. It is usually performed in columns but can also be useful in planar mode. Ion exchange chromatography uses a charged stationary phase to separate charged compounds including anions, cations, amino acids, peptides, and proteins. In conventional methods the stationary phase is an ion exchange resin that carries charged functional groups that interact with oppositely charged groups of the compound to retain. Ion exchange chromatography is commonly used to purify proteins using FPLC.

Size-exclusion chromatography

Size-exclusion chromatography (SEC) is also known as **gel permeation chromatography** (GPC) or **gel filtration chromatography** and separates molecules according to their size (or more accurately according to their hydrodynamic diameter or hydrodynamic volume). Smaller molecules are able to enter the

pores of the media and, therefore, molecules are trapped and removed from the flow of the mobile phase. The average residence time in the pores depends upon the effective size of the analyte molecules. However, molecules that are larger than the average pore size of the packing are excluded and thus suffer essentially no retention; such species are the first to be eluted. It is generally a low-resolution chromatography technique and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins, especially since it can be carried out under native solution conditions.

Special techniques

Reversed-phase chromatography

Reversed-phase chromatography (RPC) is any liquid chromatography procedure in which the mobile phase is significantly more polar than the stationary phase. It is so named because in normal-phase liquid chromatography, the mobile phase is significantly less polar than the stationary phase. Hydrophobic molecules in the mobile phase tend to adsorb to the relatively hydrophobic stationary phase. Hydrophilic molecules in the mobile phase will tend to elute first. Separating columns

typically comprise a C8 or C18 carbon-chain bonded to a silica particle substrate.

Two-dimensional chromatography

In some cases, the chemistry within a given column can be insufficient to separate some analytes. It is possible to direct a series of unresolved peaks onto a second column with different physico-chemical (Chemical classification) properties. Since the mechanism of retention on this new solid support is different from the first dimensional separation, it can be possible to separate compounds that are indistinguishable by one-dimensional chromatography. The sample is spotted at one corner of a square plate, developed, air-dried, then rotated by 90° and usually redeveloped in a second solvent system.

Pyrolysis gas chromatography

Pyrolysis gas chromatography mass spectrometry is a method of chemical analysis in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry.

Pyrolysis is the thermal decomposition of materials in an inert atmosphere or a vacuum. The sample is put into direct contact with a platinum wire, or placed in a quartz sample tube,

and rapidly heated to 600–1000 °C. Depending on the application even higher temperatures are used. Three different heating techniques are used in actual pyrolyzers: Isothermal furnace, inductive heating (Curie Point filament), and resistive heating using platinum filaments. Large molecules cleave at their weakest points and produce smaller, more volatile fragments. These fragments can be separated by gas chromatography. Pyrolysis GC chromatograms are typically complex because a wide range of different decomposition products is formed. The data can either be used as fingerprint to prove material identity or the GC/MS data is used to identify individual fragments to obtain structural information. To increase the volatility of polar fragments, various methylating reagents can be added to a sample before pyrolysis.

Countercurrent chromatography

Countercurrent chromatography (CCC) is a type of liquid-liquid chromatography, where both the stationary and mobile phases are liquids. The operating principle of CCC equipment requires a column consisting of an open tube coiled around a bobbin. The bobbin is rotated in a double-axis gyratory motion (a cardioid), which causes a variable gravity (G) field to act on the column during each rotation. This motion causes the column to see one partitioning step per revolution and components of the sample separate in the column due to their partitioning coefficient

between the two immiscible liquid phases used. There are many types of CCC available today. These include HSCCC (High Speed CCC) and HPCCC (High Performance CCC). HPCCC is the latest and best performing version of the instrumentation available currently.

References

Christian, G. D.; Callis, J. B. eds. *Trace Analysis and Spectroscopic Methods for Molecules.* Wiley-Interscience: New York, 1986.

Robert, B D, Braun, Introduction to instrumental analysis McGraw-Hill (New York) 1987

Ingle, J. D.; Crouch, S. R. *Spectrochemical Analysis.* Prentice-Hall: Englewood Cliffs, N.J., 1988

Robinson, J.W. ; Frame, E.M.S. ; Frame, G.M. Undergraduate instrumental analysis, 6th ed., New York : Dekker 2005

Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis.* Saunders College Publishing: Philadelphia, 1998.

Welz, B. *Atomic Absorption Spectroscopy.* VCH: Deerfield Beach, FL, 1985.

Willard, H.H.; Merritt, L.L. Jr.; Dean, J.A.; Settle, F.A. Jr., Instrumental methods of analysis, 7th edition, USA 1988

Vandecasteele, C.; Block, C. B. *Modern Methods for Trace Element Determination.* Wiley: Chichester, England, 1994.

Van Loon, J. C. *Analytical Atomic Absorption Spectroscopy.* Academic Press: New York, 1980.