Analytical chemistry

Lecture №25

Introduction to Instrumental methods of analysis

Introduction:

Analytical Chemistry is the science, which deals with methods for determining the chemical composition of samples of matter (elements or compounds).

Classical methods

Involve separating the components in a sample by precipitation, extraction or distillation.

In **qualitative classical methods,** the separated components treated with reagents can yield products recognized by their colors, boiling points, melting points, solubility's in a series of solvents, odors, optical activities or their refractive index.

In **quantitative classical methods,** the amounts of components are determined by gravimetric or titration methods.

In **gravimetric analysis,** the mass of components is determined**.**

In **titrimetric analysis,** the volume or mass of a standard reagent, required to react completely with sample components is measured.

In ***classical analysis***, the signal depends on the chemical properties of the sample: a reagent reacts completely with the analyte, and the relationship between the measured signal and the analyte concentration is determined by chemical stoichioimetry. In ***instrumental analysis***, some physical property of the sample is measured, such as the electrical potential difference between two electrodes immersed in a solution of the sample, or the ability of the sample to absorb light.

Instrumental methods

In the 19th century, chemists began to exploit phenomenon other than those used for classical methods for solving analytical problems. Thus, measurement of physical properties of analysts such as-conductivity, electrode potential, light absorption or emission, mass-to-charge ratio and fluorescence, began to be used for quantitative analysis of a variety of inorganic, organic and biochemical analysis. Furthermore, highly efficient chromatographic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative and quantitative determination. These methods are called **Instrumental methods of analysis.**

Classical methods are most useful for accurate and precise measurements of analyte concentrations at the 0.1% level or higher. On the other hand, some specialized instrumental techniques are capable of detecting individual atoms or molecules in a sample! Analysis at the **ppm (µg/mL)** and even **ppb (ng/mL)** level is routine.

The advantages of instrumental methods over classical methods include:

1. The ability to perform *trace analysis.*
2. Generally, large numbers of samples may be analyzed very quickly.
3. Many instrumental methods can be automated.
4. Most instrumental methods are multi-channel techniques.
5. Less skill and training is usually required to perform instrumental analysis than classical analysis.

Because of these advantages, instrumental methods of analysis have revolutionized the field of analytical chemistry, as well as many other scientific fields. However, they have not entirely supplanted classical analytical methods, due to the fact that the latter are generally more accurate and precise, and more suitable for the analysis of the major constituents of a chemical sample. In addition, the cost of many analytical instruments can be quite high.

Instrumental analysis can be further ***classified according to the principles*** by which the measurement signal is generated.

1. ***Electrochemical methods of analysis*****,** in which the analyte participates in a redox reaction or other process. In ***potentiometric*** analysis, the analyte is part of a galvanic cell, which generates a voltage due to a drive to thermodynamic equilibrium. The magnitude of the voltage generated by the galvanic cell depends on the concentration of analyte in the sample solution. In ***voltammetric*** analysis, the analyte is part of an electrolytic cell. Current flows when voltage is applied to the cell due to the participation of the analyte in a redox reaction; the conditions of the electrolytic cell are such that the magnitude of the current is directly proportional to the concentration of analyte in the sample solution.

2. ***Spectrochemical methods of analysis*****,** in which the analyte interacts with electromagnetic radiation. Most of the methods in this category are based on the measurement of the amount of light absorbed by a sample; such ***absorption-based techniques*** *include atomic absorption,* molecular absorption, and nmr methods. The rest of the methods are generally based on the measurement of light emitted or scattered by a sample; these ***emission-based techniques*** include atomic emission, molecular fluorescence, and Raman scatter methods.

3. The technique of ***mass spectroscopy*** is a powerful method for analysis in which the analyte is ionized and subsequently detected. Although in common usage, the term “spectroscopy” is not really appropriate to describe this method, since electromagnetic radiation is not usually involved in mass spectroscopy. Perhaps the most important use of mass spectrometers in quantitative analysis is as a gas or liquid chromatographic detector. A more recent innovation is the use of an inductively coupled plasma (ICP) as an ion source for a mass spectrometer; this combination (ICP-MS) is a powerful tool for elemental analysis.

4. Although they do not actually generate a signal in and of themselves, some of the more sophisticated separation techniques are usually considered “instrumental methods.” These techniques include ***chromatography and electrophoresis.*** These techniques will separate a chemical sample into its individual components, which are then typically detected by one of the methods listed above.

5. Finally, we should note that a number of methods that are based on stoichiometry, and so must be considered “classical,” still have a significant “instrumental” aspect to their nature. In particular, the techniques of ***electrogravimetry, and potentiostatic and amperostatic coulometry*** are relatively sophisticated classical methods that have a significant instrumental component. And let us not forget that instrumental methods can be used for endpoint detection in titrimetric analysis. Even though potentiostatic titrimetry uses an instrumental method of endpoint detection, it is still considered a classical method.

Single-Channel *vs Multi-Channel Techniques*

So now we have classified analytical methods according to the method by which they generate the measurement data. Another useful distinction between analytical techniques is based on the information content of the data generated by the analysis:

• ***single-channel techniques*** will generate but a single number for each analysis of the sample.

Examples include gravimetric and potentiometric analysis. In the former, the signal is a single mass measurement (e.g., mass of the precipitate) and in the latter method the signal is a single voltage value.

• ***multi-channel techniques*** will generate a series of numbers for a single analysis. Multi-channel techniques are characterized by the ability to obtain measurements while changing some independently controllable parameter. For example, in a molecular absorption method, an absorption *spectrum may be generated, in which the absorbance of a sample is monitored as a* function of the wavelength of the light transmitted through the sample. Measurement of the sample thus produces a series of absorbance values.

Multi-channel methods have two important ***advantages*** over their single-channel counterparts:

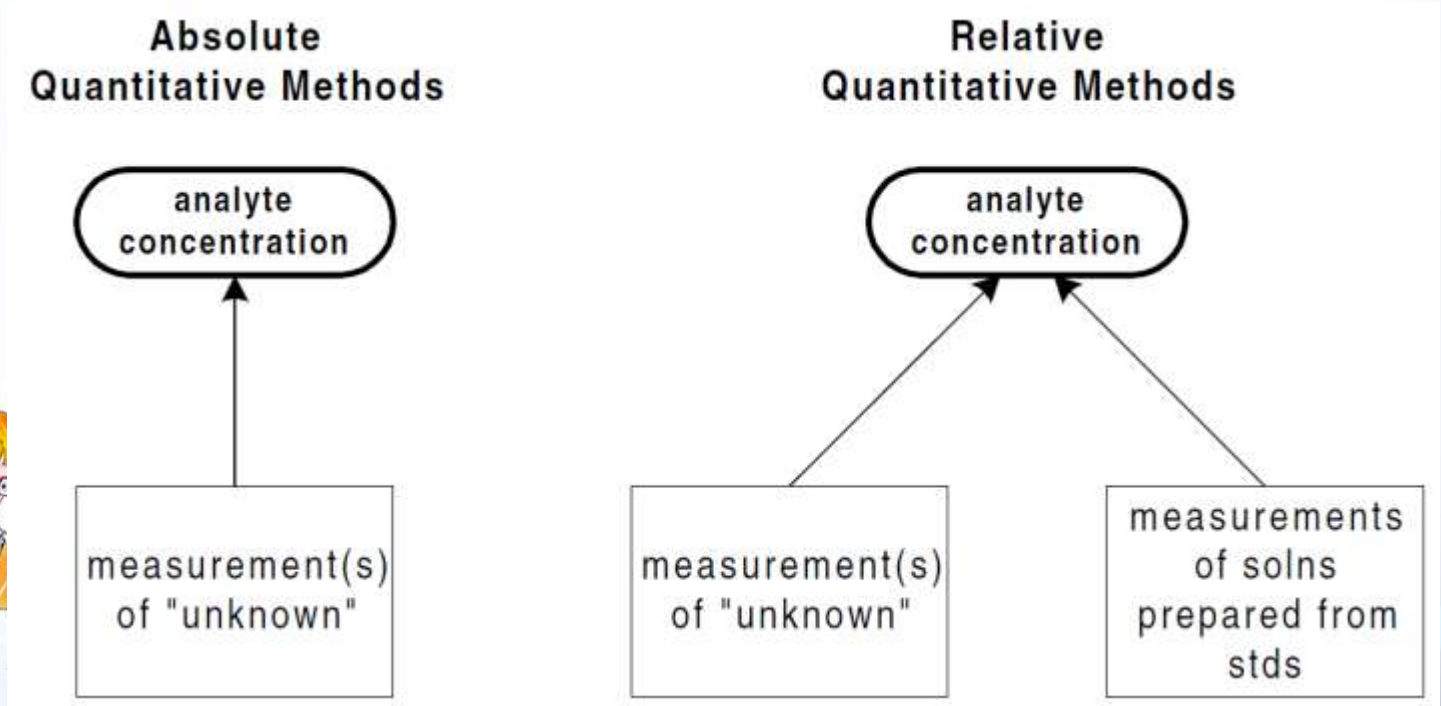
1. They provide the ability to perform *multicomponent analysis. In other words, the* concentrations of more than one analyte in a single sample may be determined.
2. Multi-channel methods can detect, and sometimes correct for, the presence of a number of types of interferences in the sample. If uncorrected, the presence of the interference will result in biased estimates of analyte concentration.

Relative vs Absolute Techniques

Another way of classifying analytical techniques is according to the method by which the analyte concentration is calculated from the data:

• in ***absolute analytical techniques,*** the analyte concentration can be calculated directly from measurement of the sample. No additional measurements are required (other than a measurement of sample mass or volume).

• in ***relative analytical techniques,*** the measurement of the sample must be compared to measurements of additional samples that are prepared with the use of analyte *standards (e.g.,* solutions of known analyte concentration).



Classical methods of analysis are considered absolute techniques, because there is a direct and simple relationship between the signal (mass in gravimetry; endpoint volume in titrimetry) and the analyte concentration in the sample. The vast majority of instrumental methods of analysis are relative methods: the measurement of the sample solution must be compared to the measurement of one or more solutions that have been prepared using standard solutions. The most common methods of quantitation for instrumental analysis will be described shortly.

Technique	Quantity Measured	Single- or multi-channel? (*independent parameter*)	Theoretical Principle
Classical Techniques – all absolute methods[a]			
gravimetry	mass	single-channel	complete/selective rxn of analyte; composition of weighing form is known
electrogravimetry	mass	single-channel	
titrimetry (chemical indicator)	endpoint volume/mass	single-channel	complete/selective rxn of analyte; known stoichiometry of titration reaction
titrimetry (instrum endpt detection)	instrument signal	multi-channel (*volume/mass of titrant solution*)	
amperostatic coulometry	time	single-channel	complete/selective rxn of analyte; Faraday's Law, and the known stoichiometry of titration reaction
potentiostatic coulometry	current	multi-channel (*time*)	
Instrumental Techniques – all relative methods[b]			
potentiometry	potential	single-channel	thermodynamic drive to equilibrium (Nernst)
voltammetry	current	multi-channel (*working electrode potential*)	analyte diffusion controls signal (Fick's Law)
atomic absorption	attenuation of light intensity	single-channel[c]	Beer's Law
molecular absorption	attenuation of light intensity	multi-channel (*wavelength*)	
atomic emission	emitted light intensity	multi-channel (*wavelength*)	signal is proportional to excited-state concentration
molecular fluorescence	fluorescence light intensity	multi-channel[d] (*excitation wavelength* and *emission wavelength*)	

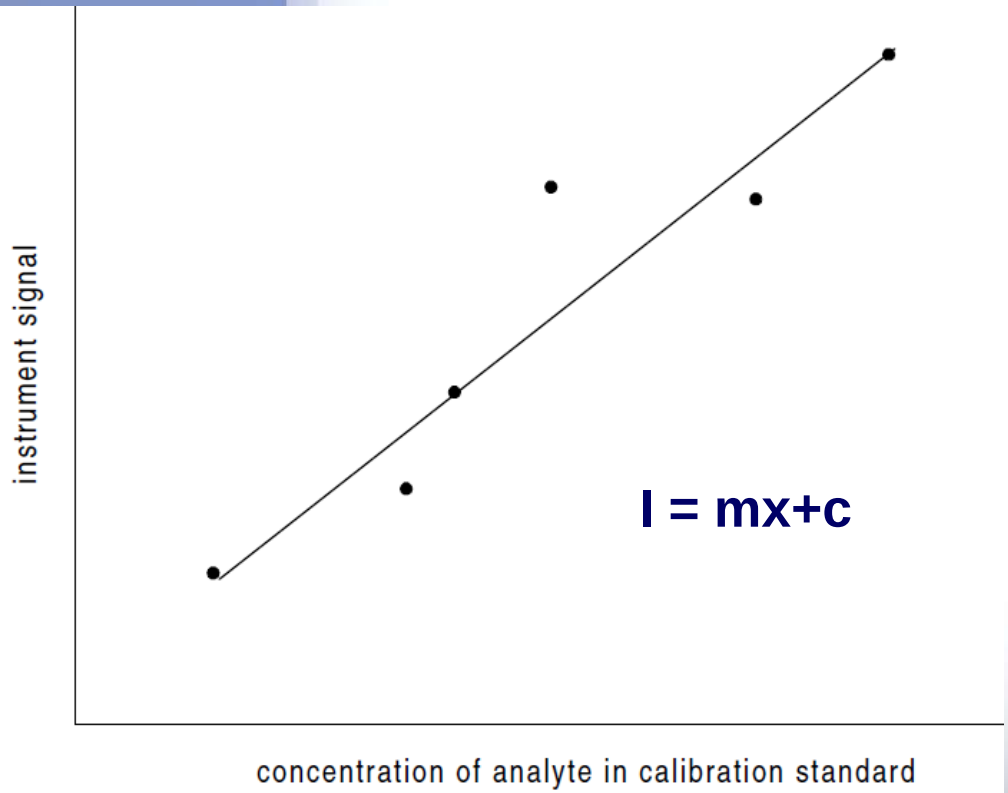
Methods of Quantitation for Instrumental Analysis

Instrumental techniques are almost all *relative in nature:* the signal obtained from the analysis of the sample must be compared to other measurements in order to determine the analyte concentration in the sample. All types of analytical methods require calibration, i.e. relating the measured analytical signal to the concentration of the analyst. To use this technique, several standards containing exactly known concentrations of the analyte are introduced into the instrument and the instrumental response is recorded.two most common methods of calibration in instrumental analysis are **(I)** the use of ***calibration curves, and (II) the method of standard additions.*** In addition, ***internal standards*** may be used in combination with either of these methods. We will now describe how these methods may be used in quantitative chemical analysis.

Calibration Curve Method

For any instrumental method used for quantitative chemical analysis, there is some functional relationship between the instrument signal, *r, and the analyte concentration, CA: r = f(CA)*

The calibration curve approach to quantitation is an attempt to estimate the nature of this functional relationship. A series of ***calibration standards are analyzed,*** and a "best-fit" line or curve is used to describe the relationship between the analyte concentration in the calibration standards and the measured signal. The following figure demonstrates the concept.



Typical calibration curve. The instrument response is measured for a series of calibration standards, which contain a known concentration of analyte. The curve is a function that describes the functional relationship between signal and concentration. linear plots are obtained. Non linear plots are due to matrix effects, interferences, instrumental drift,- - etc.

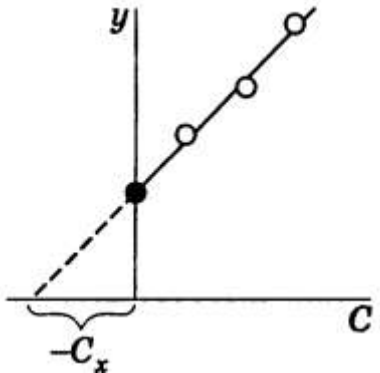
The following points should be made about this method of quantitation:

1. The central philosophy of the calibration curve method is this: *the function that describes the relationship between signal and concentration for the calibration standards also applies to any other sample that is analyzed. Any factor that changes this functional relationship will* result in a biased estimate of analyte concentration.
2. A linear relationship between signal and concentration is desirable, generally resulting in the best accuracy and precision using the fewest number of calibration standards.
3. Ideally, the analyte concentration should only be calculated by *interpolation, not by extrapolation. In other words, the analyte concentration should be within the range of* concentrations spanned by the calibration standards. If the analyte concentration in the sample is too great, then the sample may be diluted. If the analyte concentration is too small, then additional calibration standards can be prepared. For best precision, the concentration is close to the mean concentration of the calibration standards.

Standard Addition Method

Whenever there is reason to suspect that the calibration curve approach will not work due to the presence of a matrix effect, the method of standard additions may give more accurate results. In addition, to each new solution a certain volume of standard analyte solution is added (the concentration of this solution is known). The volume of added standard is kept small so that it has little effect on the matrix; presumably, the final solutions have identical sample matrices, and so *the analyte should be affected by the matrix equally in all the solutions. This is the key assumption in the standard addition method.*

In the **method of standard additions,** a known amount of a standard solution of analyte is added to one portion of the sample. The responses before and after the addition are measured and used to obtain the analyte concentration. Alternatively multiple additions are made to several portions of the sample. The standard additions method assumes a linear response. Linearity should always be confirmed, or the **multiple additions method used to** check linearity.



$$C_x = \frac{C_{доб} V_{доб} y_x}{(V_{доб} + V_x) y_{доб} - V_x y_x}.$$

$$C_x = C_{доб} \frac{y_x}{y_{доб} - y_x}.$$

Internal Standard Methods

An **internal standard** is a reference species, chemically and physically similar to the analyte. The ratio of the response of the analyte to that of the internal standard is plotted versus the concentration of analyte.

$$C_x = C_{ст} \frac{y_x}{y_{ст}}.$$

$$C_x = \frac{C_2(y_x - y_1) + C_1(y_2 - y_x)}{y_2 - y_1}$$

$$C_x = C_1 + \frac{(C_2 - C_1)(y_x - y_1)}{y_2 - y_1}.$$

Sensitivity

The sensitivity of an analytical method or instrument defined, as the ratio of the change in response (R) to the change in the quantity or concentration (C) that is measured.

$S = \Delta R / \Delta C$

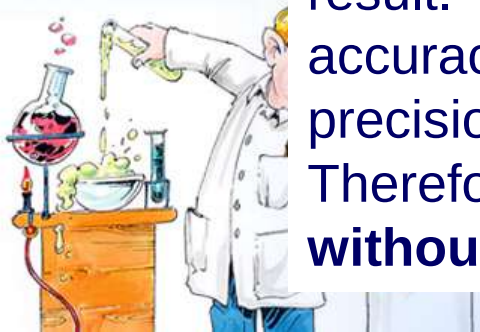
The sensitivity is dependent on type of instrument and experimental conditions.

A parameter often used to express sensitivity is the limit of detection **Detection limit**

The minimum concentration or mass of analyte that can be detected at a known confidence level. Detection limits differ widely for several analytical methods, and from one element or compound to another.

Systematic error

The difference between the true result and the experimental result. Calibration with blank samples can correct for it The accuracy of a result is therefore dependent both upon the precision of the measurement and on the systematic error. Therefore, **a method or measurement may be precise without being accurate.**



Future of Modern Instrumentation

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

1. Improved Performance. As is traditional in analytical chemistry, a better understanding of the science of measurements leads to improvements in the sensitivity, selectivity, application of instrumentation to complex sample matrices, and innovative configurations of instruments. Many of these developments are driven by the needs of unique problems, such as the study the dynamics of chemical reactions or the mapping of small inhomogeneities in the surface composition of a polymer. Most certainly, probe microscopy instruments that image three-dimensional micro- and nano-scale surfaces and subsurfaces will continue to evolve.
2. Miniaturized Instruments. One of the major trends in modern instrumental development is to create smaller, robust and low cost instruments. Developments in light sources (diode lasers), polymeric materials, optics, microelectrical mechanical systems (MEMS), electronics, and microfabrication technologies have impacted instrument design. Laboratory instruments occupy a much smaller footprint than their predecessors. Small, portable instruments and sensors with few or no moving parts allow the instrument to come to the sample in locations such as deep, ocean environments, rather than bringing the sample to the lab for analysis.
3. Remote and Process Analysis. Monitoring chemical processes in harsh environments, at high temperatures, and for different manufacturing processes is an expanding area in instrumental analysis. For example, the pharmaceutical industry uses instruments that can monitor mixing, drying, and tableting processes in real time, allowing for better quality control. Continual development of more powerful methods for these applications can be expected.
4. Computers. Computers are now integral components of almost every instrument, where they control measurement parameters as well as the collection, processing, storage and display of data. Parallel and multiplexing data allows simultaneous, real-time collection of information on multiple analytes. Systems which integrate the preparation and sample introduction with measurements are now common and will become more intelligent in the future, increasing productivity and reducing operator intervention. Expert systems used for data interpretation and advances in wireless communication will also impact future instrumental methods.
5. Bioanalytical Methods.

Introduction to Spectrochemical Methods

In a situation whereby the sample is made to interact with a wide spectrum of wavelengths in a given zone of electromagnetic radiation, consequently giving rise to a collection of measurement signals as a function of wavelength is termed as a **spectrum,** ultimately putting forward the most common terminology **spectrochemical analysis or spectroscopy.**

Spectroscopy: is the study of the interaction between matter and radiant energy.

Spectroscopic analytical methods are based on measuring the amount of radiation **absorbed (absorption spectroscopy) or produced (emission spectroscopy) by molecular (molecular) or atomic (atomic)** species of interest.

Spectrochemical methods provide 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.

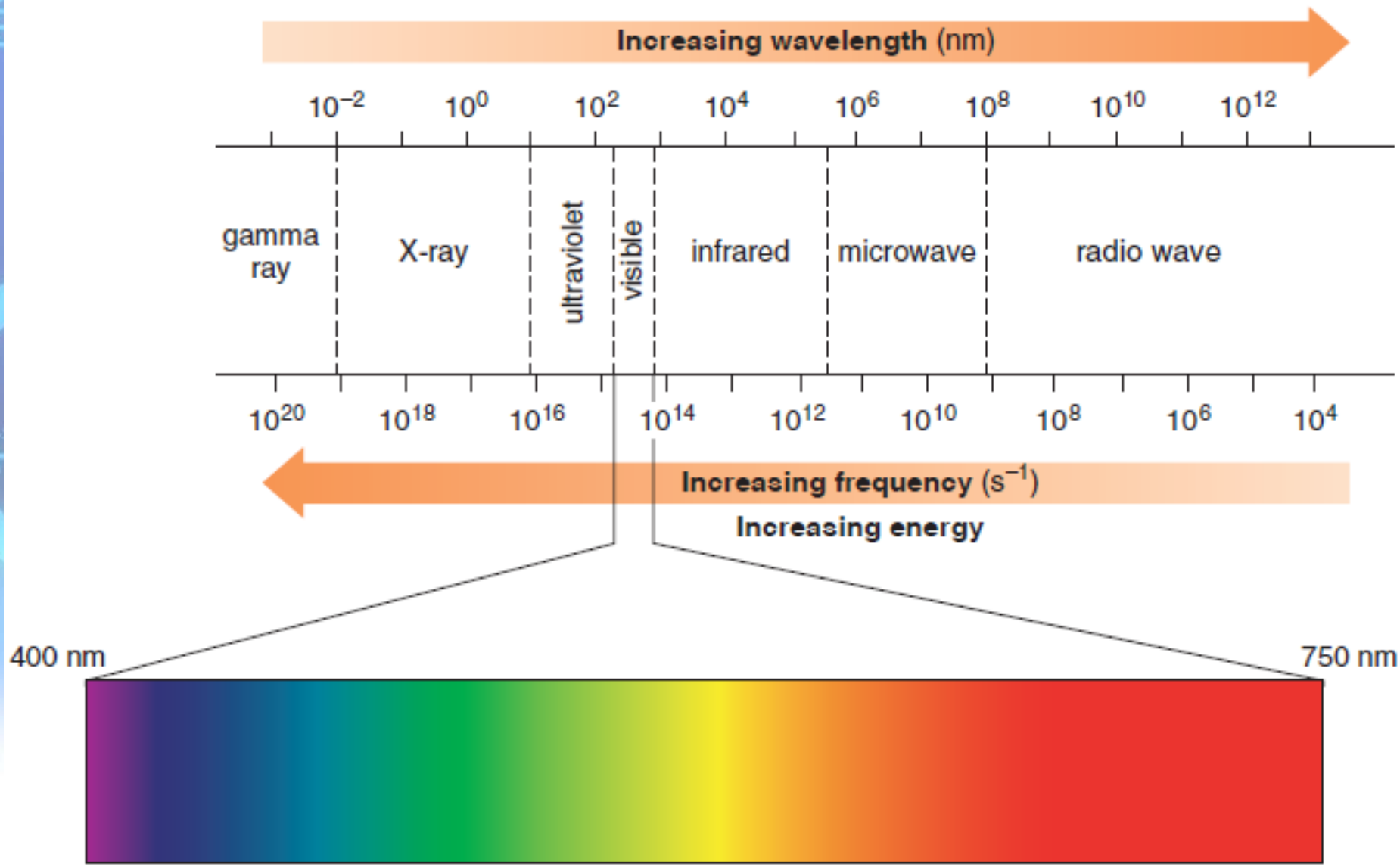
Who Was the First Spectroscopist?

Johannes Marcus Marci of Kronland (1595–1667) in Eastern Bohemia is likely the first spectroscopic scientist. He was interested in the phenomenon of the rainbow and performed experiments to explain it. He published a book in ca. 1648 whose title, roughly translated, is *The Book of Thaumas, about the Heavenly Rainbow and the Nature of the Colors* That Appear and Also about Its Origin. He described the conditions responsible for the production of rainbows and wrote about the production of a spectrum by passing a beam of light through a prism. The phenomenon (and the rainbow) was correctly explained as being due to the diffraction of light. Newton, over 20 years later, performed experiments similar to Marci's and provided a more rigorous explanation of the colors of the rainbow. Although he is credited more often, Marci was the first!

Isaac Newton showed that the white light from the sun could be dispersed into a continuous series of colors. He coined the word "spectrum."

The electromagnetic spectrum is composed of a large range of wavelengths and frequencies (energies). It varies from the highly energetic gamma rays to the very low energy radio-waves. The entire range of radiation is commonly referred to as the ***electromagnetic spectrum*****.**

Electromagnetic Spectrum



Spectrochemical methods that use not only visible but also ultraviolet and infrared radiation are often called **optical methods** in spite of the fact that the human eye is not sensitive to UV or IR radiation.

Classify spectroscopic methods according to region of the electromagnetic spectrum involved

Type spectroscopy	Type of transitions	Wavelength range
Gamma rays	Nuclear	$(10^{-10}$ -$10^{-14})$ m
X-rays	Inner K-and L-shell electrons	(10^{-9}) –(6×10^{-12}) m
Ultraviolet rays	Valence and middle-shell electrons	(3.8×10^{-7})-(6×10^{-10}) m
Visible	Valence electrons	$(7.8– 3.8)\times10^{-7}$
Infrared	Molecular vibrations and rotations	(10^{-3}) –(7.8×10^{-7})m
Microwave	Molecular rotations	0.3m-1mm
Radio waves		Few km-0.3 m

Properties of Electromagnetic Radiation

- Electromagnetic radiation is energy
- Electromagnetic radiation can be described as a wave (wavelength, frequency, velocity, and amplitude)
- Electromagnetic radiation can be treated as discrete packets of energy or particles called ***photons or quanta.***
- These dual views of radiation as particles and waves are not mutually exclusive but complementary.

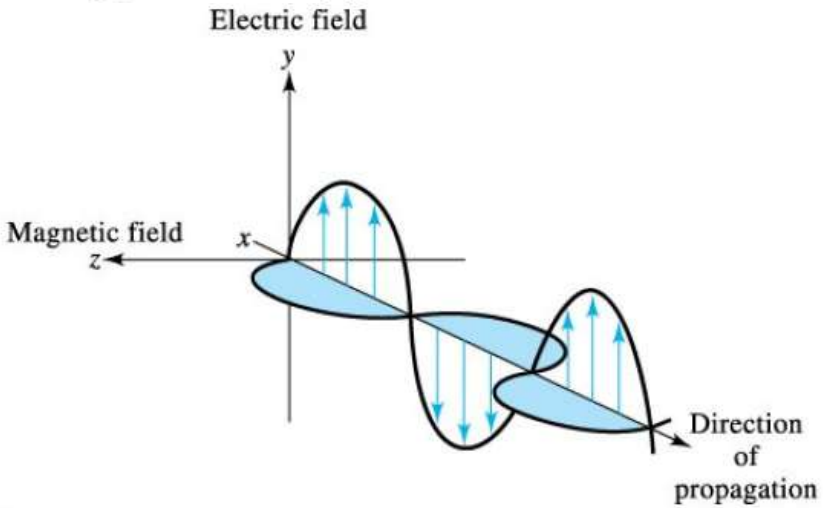
wavenumber

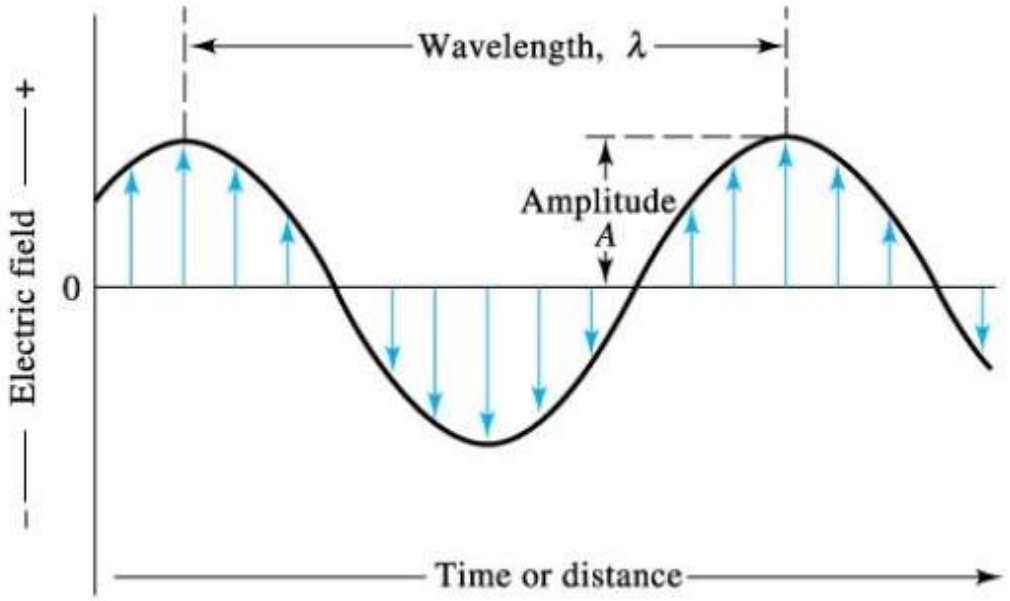
$$\bar{\nu} = \frac{1}{\lambda}$$

Wavelength (Greek lambda; λ): the distance from one wave maximum to the next, it is generally measured in micrometers (μm; 10^{-6} m) or nanometers (nm; 10^{-9} m).

Frequency (Greek nu; ν): the number of waves that pass by a fixed point per unit time, usually given in reciprocal seconds (s^{-1}), or hertz (Hz), 1 Hz = 1 s^{-1}

$$\nu = \frac{C}{\lambda} = \bar{\nu}\, C$$





Wavelengths in the ultraviolet and visible regions are on the order of nanometers. In the infrared region, they are micrometers, but the reciprocal of wavelength is often used (wavenumbers, in cm^{-1}).

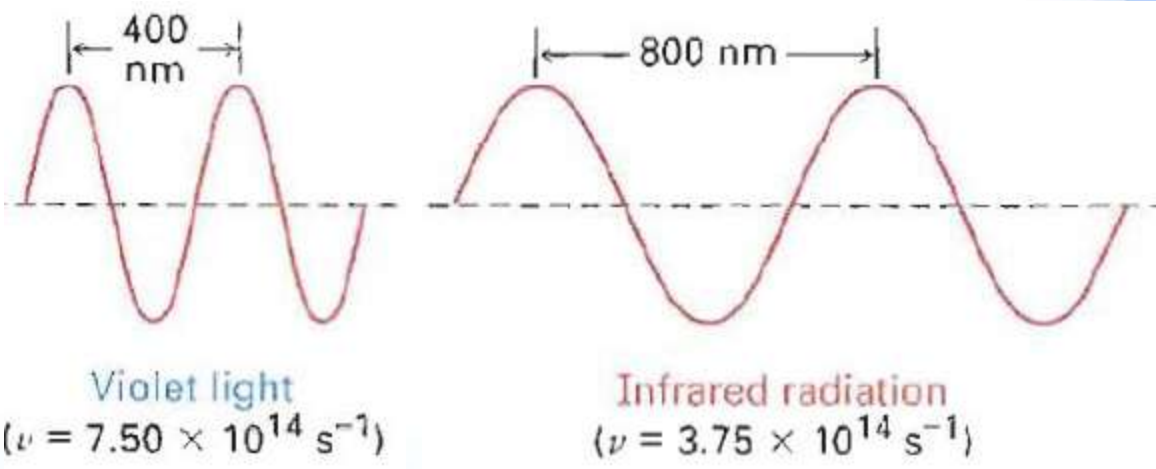
The **amplitude** of an electromagnetic wave is a vector quantity that provides a measure of the electric or magnetic field strength at a maximum in the wave.

The **period** of an electromagnetic wave is the time in seconds for successive maxima or minima to pass a point in space.

$$\text{Å} = \text{angstrom} = 10^{-10}\ \text{meter} = 10^{-8}\ \text{centimeter} = 10^{-4}\ \text{micrometer}$$

$$\text{nm} = \text{nanometer} = 10^{-9}\ \text{meter} = 10\ \text{angstroms} = 10^{-3}\ \text{micrometer}$$

$$\mu\text{m} = \text{micrometer} = 10^{-6}\ \text{meter} = 10^{4}\ \text{angstroms}$$



These relationship means that if the wavelength is longer, the frequency is lower.

The particle Nature of Light: Photons

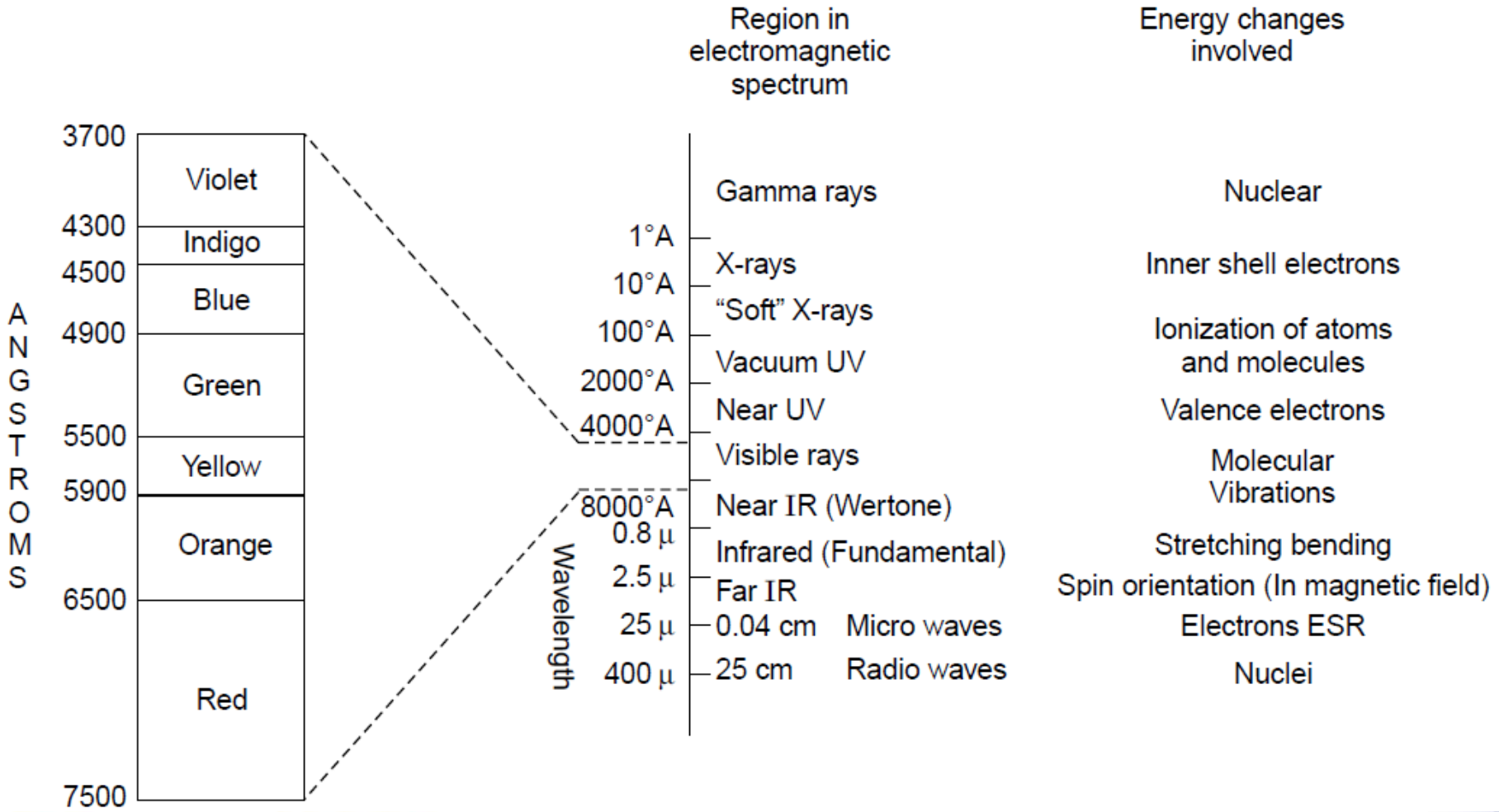
Energy of photon is related to its wavelength, or frequency, or wavenumber by

$$E = h\nu = \frac{hc}{\lambda}$$

Where, E = energy of the photon (ergs)
v = frequency of electromagnetic radiation (Hz)
h = plank's constant = 6.624×10^{-27}
The higher the frequency, the higher the energy of radiation (i.e.) a photon of high frequency (short wavelength) has higher energy content than one of lower frequency (longer wavelength).

The intensity of a beam of radiation is proportional to the number of photons and is independent of the energy of each photon. Since energy per unit time is power, Intensity is often referred as the radiant power emitted by the source.

A **photon** is a particle of electromagnetic radiation having zero mass and an energy of h_{υ}.



Working ranges of the UV/Vis and IR spectra.

UV	190–380 nm
Vis	380–780 nm
Near-IR	0.78–2.5 μm
Mid-IR	2.5–15 μm

- The electromagnetic spectrum covers a very wide range of energies (frequencies) and wavelengths.
- From 1019 Hz (g-rays) to 103 Hz (radio waves).
- Different quantum changes (energy level changes) in atoms/molecules are caused by radiation with different frequencies.

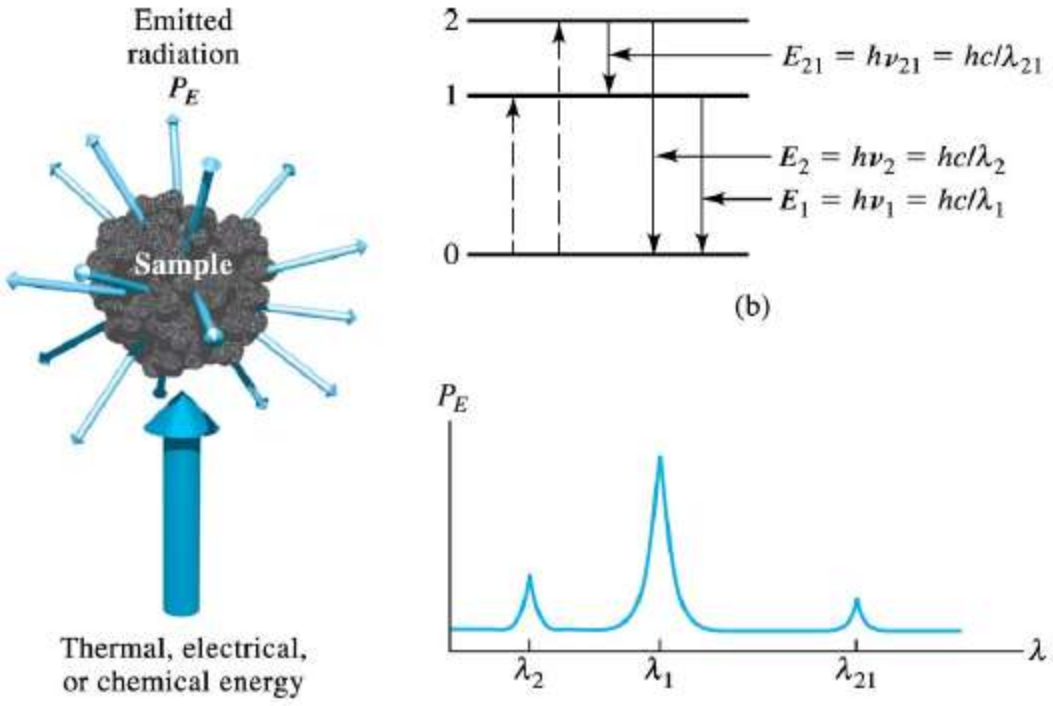
Spectroscopic Measurements

• Interactions of radiation with mater is used to get information about a sample.

• Sample is stimulated by applying energy (heat, electrical energy, light, particles, or a chemical reaction.)

• The analyte in the sample is predominately in its lowest energy or *ground state.* The stimulus causes some analyte species to undergo a transition to a higher-energy or excited state**.**

• We obtain information about the analyte either by measuring the electromagnetic radiation emitted as it returns to the ground state or by measuring the amount of electromagnetic radiation absorbed during excitation.



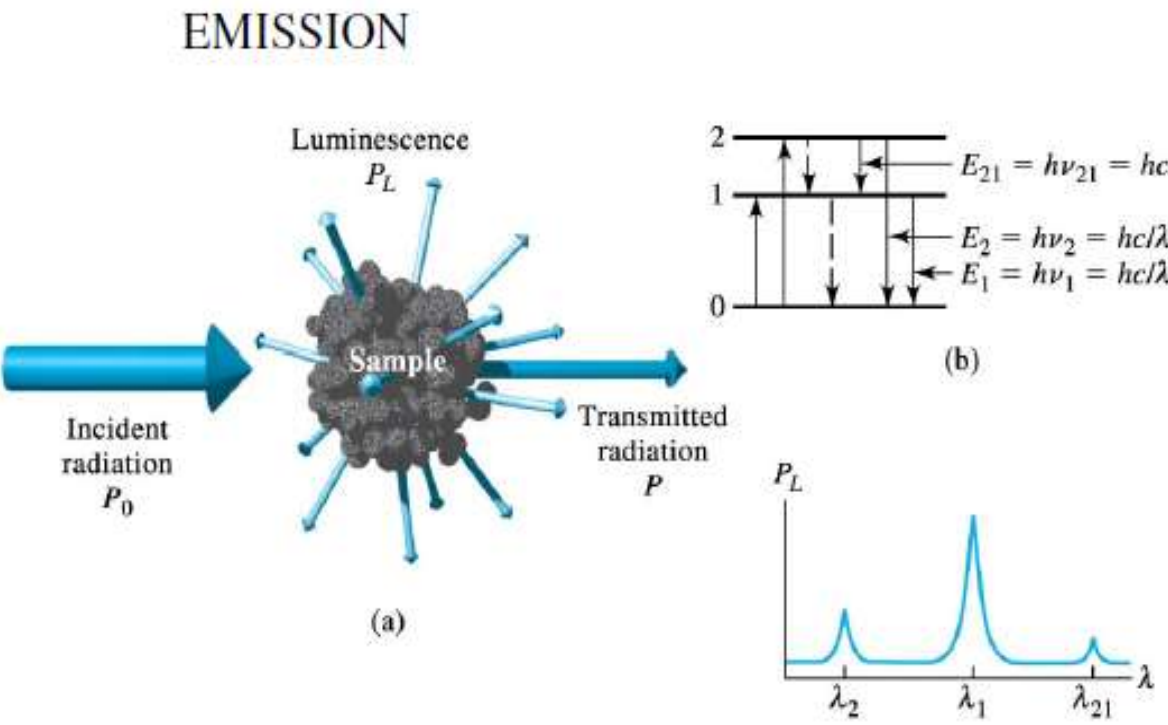
• ***Emission spectroscopy*** usually refers to methods in which the stimulus is heat or electrical energy.

• ***chemiluminescence spectroscopy*** refers to excitation of the analyte by a chemical reaction.

• Measurement of the radiant power emitted as the analyte returns to the ground state can give information about its identity and concentration.

• The results of such a measurement are often expressed graphically by a spectrum, a plot of the emitted radiation as a function of frequency or wavelength.

• When sample is stimulated by irradiation some of the incident radiation is absorbed and promotes some analyte species to an excited state.

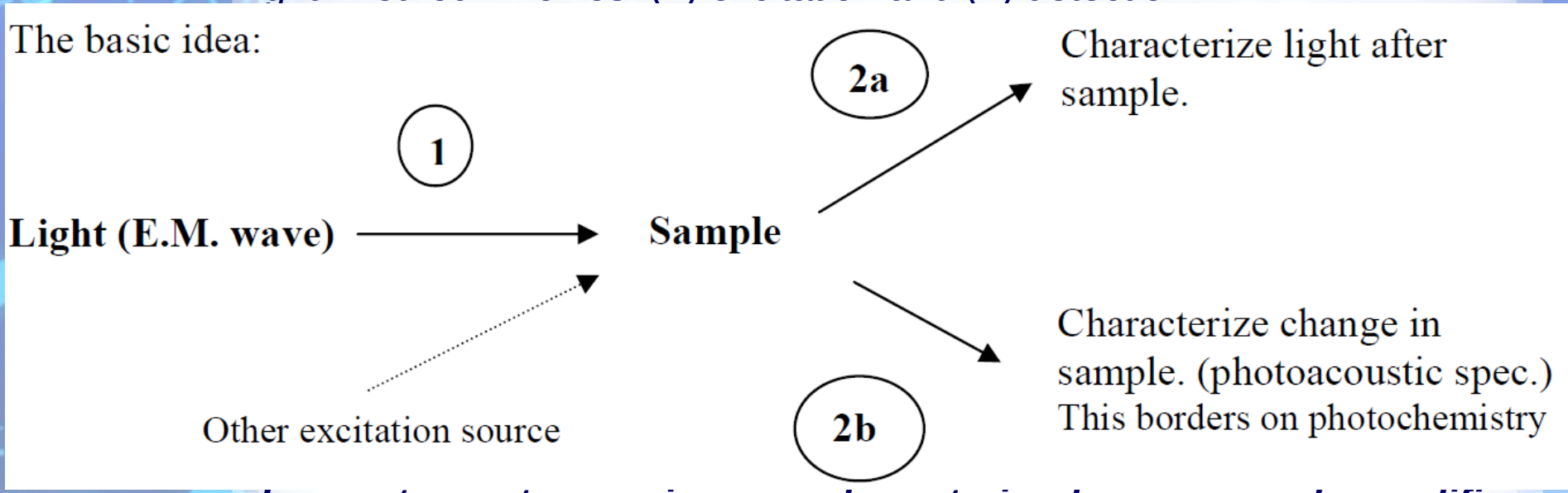


In ***absorption spectroscopy,*** the amount of light absorbed as a function of wavelength is measured, which can give qualitative and quantitative information about the sample.

In ***photoluminescence spectroscopy the emission*** of photons at different wavelength is after absorption. These are called ***fluorescence and phosphorescence spectroscopy.***

What does a spectrum measure?

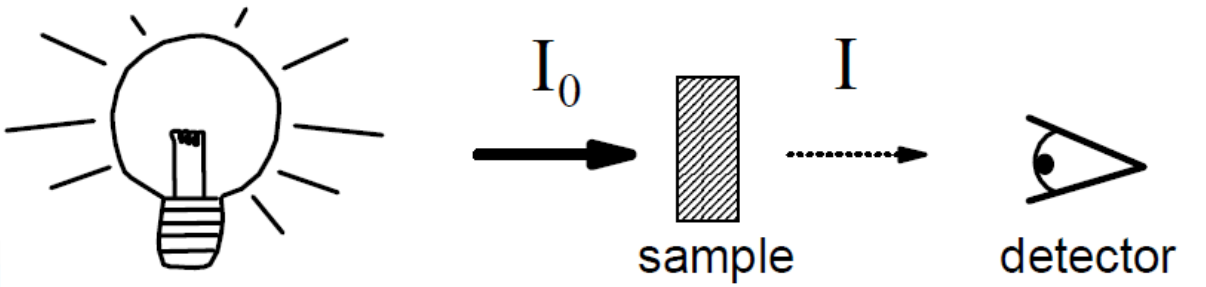
Interaction of light with a sample can influence the sample and/or the light. Method involves: (1) excitation and (2) detection.



In most spectroscopies, we characterize how a sample modifies light entering it.

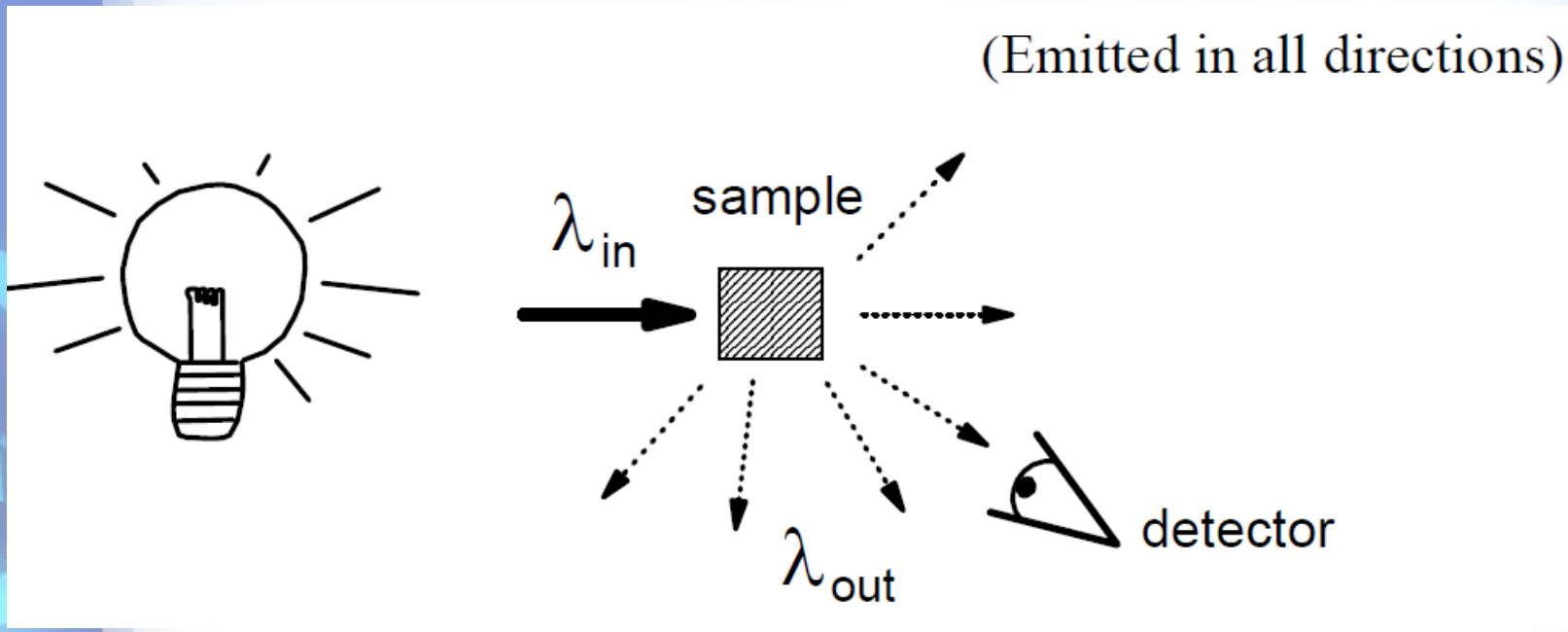
1) Absorption: Change in intensity **I** of incident light

Sample attenuates light → transmission $\mathbf{T=I/I_0}$

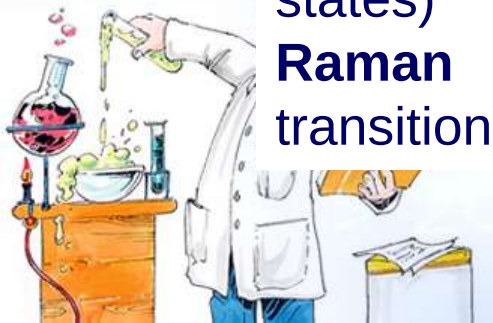


We measure the absorption of light at different frequency or wavelength.

2) Emission: Excitation induces emission of light from the sample (usually of different frequency).



Includes: **Fluorescence** (emission from excited electronic singlet states)
Phosphorescence (emission from excited electronic triplet states)
Raman Scattering (light scattering involving vibrational transition)

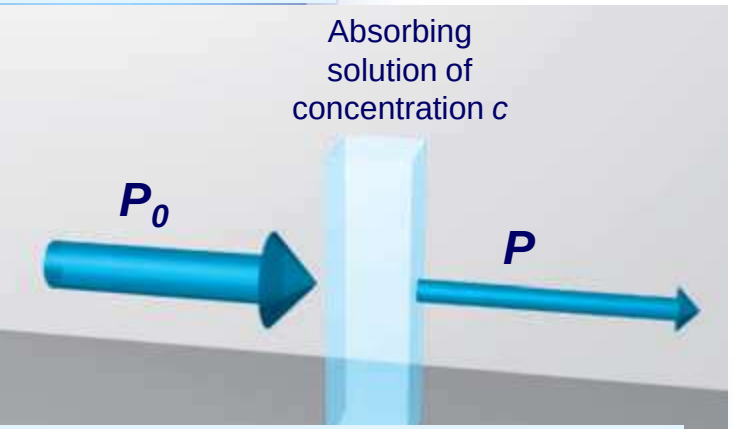


3) Optical Rotation: Change of phase of light incident on sample (rotation of polarization)

Absorption of Radiation

Every molecular species is capable of absorbing its own characteristic frequencies of electromagnetic radiation. This process transfers energy to the molecule and results in a decrease in the intensity of the incident electromagnetic radiation. Absorption of the radiation thus attenuates the beam in accordance with the absorption law. The absorption law, also known as the **Beer-Lambert law or just Beer's law,** tells us quantitatively how the amount of attenuation depends on the concentration of the absorbing molecules and the path length over which absorption occurs. As light traverses a medium containing an absorbing analyte, the intensity decreases as the analyte becomes excited.

$$T = P/P_0$$



The attenuation (decrease in intensity) of a parallel beam of monochromatic radiation as it passes through an absorbing solution of thickness b cm and concentration c moles per liter. Because of interactions between the photons and absorbing particles, the radiant power of the beam decreases from P_0 to P. The ***transmittance T*** of the solution is the fraction of incident radiation transmitted by the solution. Transmittance is often expressed as a percentage and called the ***percent transmittance.***

The **absorbance** A of a solution is related to the transmittance in a logarithmic manner.

$$A = -\log T = -\log \frac{P}{P_0} = \log \frac{P_0}{P}$$

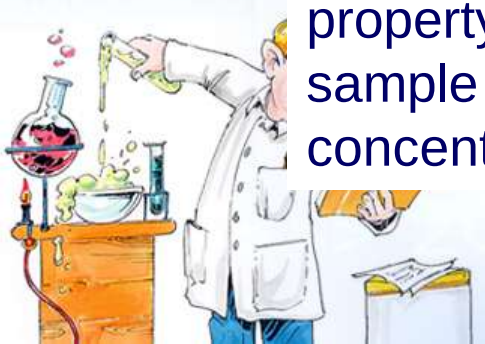
Beer's Law

According to Beer's law, absorbance A is directly proportional to the concentration of the absorbing species c and the pathlength b of the absorbing medium

$$A = \varepsilon b c$$

Here, a is a proportionality constant called the **absorptivity.** Because absorbance is a unitless quantity, the absorptivity must have units that
cancel the units of b and c. If, for example, c has the units of grams per liter (g L^{-1}) and b has the units of centimeters (cm), **molar absorptivity** has the units of liters per gram centimeter (L g^{-1} cm^{-1}).

Note:- Absorptivity (ϵ) is a property of a substance (intensive property), whereas absorbance (A) is a property of a particular sample (extensive property) and will therefore vary with the concentration and length of light path through the container.

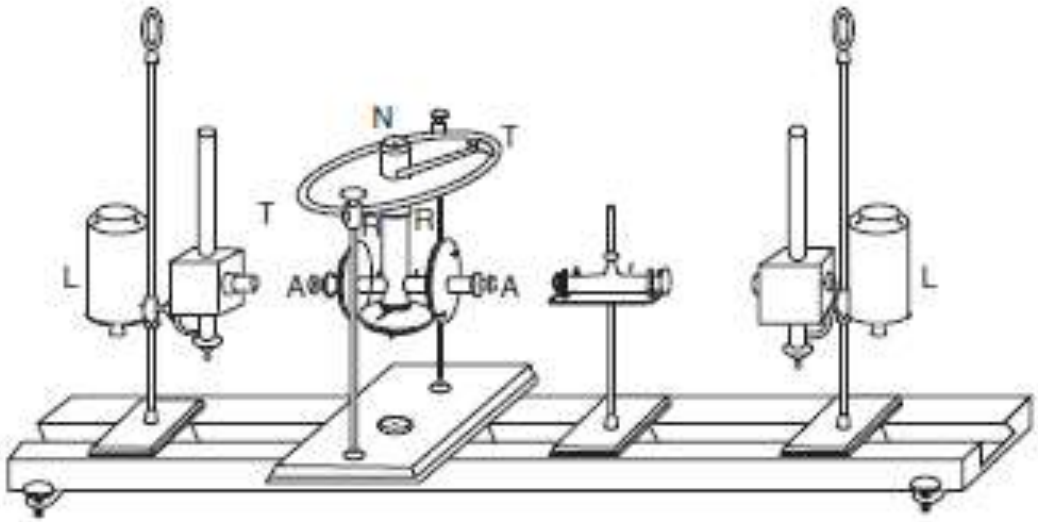


Pierre Bouguer (1698–1758) was a French Mathematician, geophysicist, geodesist and astronomer. He is widely credited as the “Father of Naval Architecture”. Son of Jean Bouguer, Regius Professor of hydrography, Brittany, Pierre succeeded him in 1713. In 1727 he defeated Leonhard Euler (one of the most eminent 18th century mathematicians) to win the French Academy of Science prize for his paper “On the Masting of Ships”. In his 1729 treatise on light lost by passage through the atmosphere, Bouguer clearly described the exponential loss of light with distance, although it will remain for Lambert to put that explicitly as an equation. Bouguer was one of the first to attempt quantitative photometry: he was the first to quantitatively compare light intensity from the sun and the full moon. Bouguer was one of the few Frenchmen of his time honored as a Fellow of the Royal Society (1750).

Johann Heinrich Lambert (1728–1777) was a Swiss mathematician. Son of a tailor, he was invited at the age of 36 by Euler for a professorship at the Prussian Academy of Sciences in Berlin, where he flourished. Lambert was among the most versatile scientists of his time, contributing to mathematics, map projection, physics, philosophy, and astronomy, making a multitude of theoretical and practical contributions in his short 49 years. He invented the first practical hygrometer. In 1760, in his treatise *Photometria, Lambert* quantitatively formulated the laws of light absorption, while acknowledging the prior work of Bouguer.

Bouguer in 1729 (P. Bouguer, *Essai d’otique sur la gradation de la lumier, Paris, 1729)* and Lambert in 1760 (J. H. Lambert, *Photometria, Ausburg, 1760) recognized that* when EMR is absorbed, the power of the transmitted radiation decreases exponentially.

The photometer of August Beer (1825–1863) described nearly a century after Lambert. *Annalen der Physik und Chemie* ***86 (1852) 78.*** Beer clearly showed experimentally that the attenuation of light by passage through a solution containing an absorber is related both to the length of the solution through which light passes and on the absorber concentration. Arguably, of the three men whose names are associated with the law of light absorption, Beer's contribution is the least. Yet we call this Beer's law! For a more detailed discussion, see Fred H. Perrin, "Whose Absorption Law?" *Journal of the Optical Society of America,* ***38*** (1948) 72. Beer was born in Trier, Germany. He studied mathematics and natural sciences and received his doctoral degree under the tutelage of Julius Plűcker in Bonn in 1848. Beer became a lecturer in 1850. In 1854, he published his book "Einleitung in die hȍhere optik" (Introduction to the Higher Optics). Beer became Professor of mathematics at Bonn in 1855. He died in Bonn in 1863.



Important Terms and Symbols Employed in Absorption Measurements

Term and Symbol*	Definition	Alternative Name and Symbol
Incident radiant power, P_0	Radiant power in watts incident on sample	Incident intensity, I_0
Transmitted radiant power, P	Radiant power transmitted by sample	Transmitted intensity, I
Absorbance, A	$\log(P_0/P)$	Optical density, D; extinction, E
Transmittance, T	P/P_0	Transmission, T
Path length of sample, b	Length over which attenuation occurs	l, d
Absorptivity†, a	$A/(bc)$	α, k
Molar absorptivity‡, ε	$A/(bc)$	Molar absorption coefficient

Applying Beer's Law to Mixture

Beer's law also applies to solutions containing more than one kind of absorbing substance. Provided that there are no interactions among the various species, the total absorbance for a multicomponent system is the sum of the individual absorbances. In other words,

$A_{total} = A_1 + A_2 + \ldots A_n = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \ldots + \varepsilon_n bc_n$

where the subscripts refer to absorbing componets 1, 2, …, n.

Limits to Beer's Law

There are few exception to the linear relationship between absorbance and pathlength at a fixed concentration. We frequently observe deviations from the direct proportionality between absorbance and concentration where b is a constant. Some of these deviations, called ***real deviations,*** are fundamental and represent real limitations to the law. Others occur as a consequence of the manner in which the absorbance measurements are made or as a result of chemical changes associated with concentration changes. These deviations are called ***instrumental deviations and chemical deviation*** respectively.

Real Limitations to Beer's Law

Beer's law describes the absorption behavior of dilute solutions only and is a limiting law. At concentrations exceeding about 0.01 M, the average distances between ions or molecules are diminished to the point where each particle affects the charge distribution, and thus the extent of absorption of its neighbors. The occurrence of this phenomenon causes deviations from the linear relationship between absorbance and concentration. When ions are in close proximity, the molar absorptivity of the analyte can be altered because of electrostatic interactions, which can lead to departures from Beer's law.

Chemical Deviations

Deviations from Beer's law appear when the absorbing species undergoes association, dissociation, or reaction with the solvent to give products that absorb differently from the analyte. The extent of such departures can be predicted from the molar absorptivities of the absorbing species and the equilibrium constants for the equilibria involved. Unfortunately, we are usually unaware that such processes are affecting the analyte, so compensation is often impossible. Typical equilibria that give rise to this effect include monomer dimer equilibria, metal complexation equilibria where more than one complex is present, acid/base equilibria, and solvent-analyte association equilibria.

Instrumental Deviations

The need for monochromatic radiation and the absence of stray radiation are practical factors that limit the applicability of Beer's law. Beer's law strictly applies only when measurements are made with monochromatic source radiation. If the band selected corresponds to a region in which the absorptivity of the analyte is essentially constant, departures from Beer's law will be minimal. Many molecular bands in the UV/visible region fit this description. To avoid deviation, it is advisable to select a wavelength band near the wavelength of maximum absorption where the analyte absorptivity changes little with wavelength. Stray radiation, commonly called ***stray light,*** is defined as radiation from the instrument that is outside the nominal wavelength band chosen for the determination. This stray radiation is often the result of scattering and reflection off the surfaces of gratings, lenses or mirrors, filters, and windows. When measurements are made in the presence of stray light, the observed absorbance is given by

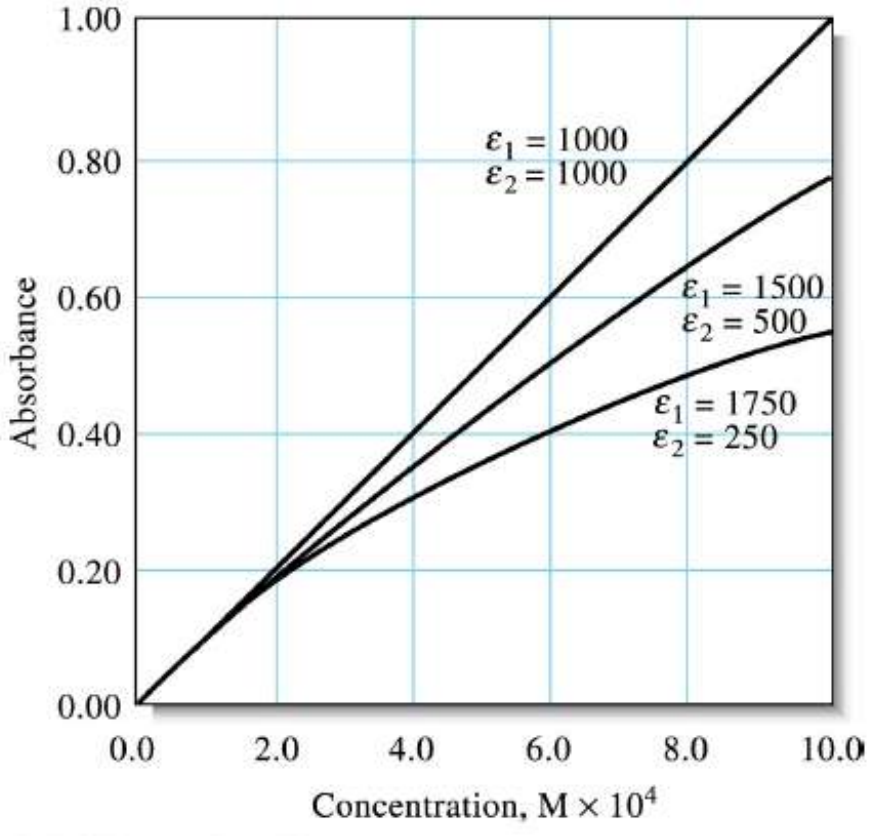
$$A' = \log\left(\frac{P_0 + P_s}{P + P_s}\right)$$

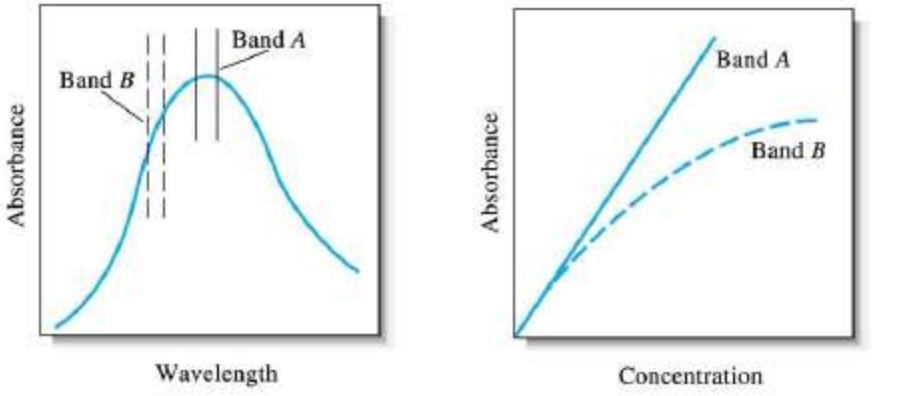
where P_s is the radiant power of the stray light.

For small concentrations of analyte, *P_s is significantly smaller than P_0 and P_T, and* the absorbance is unaffected by the stray radiation. At higher concentrations of analyte, however, *P_s is no longer significantly smaller than P_T and the absorbance is* smaller than expected. The result is a negative deviation from Beer's law.

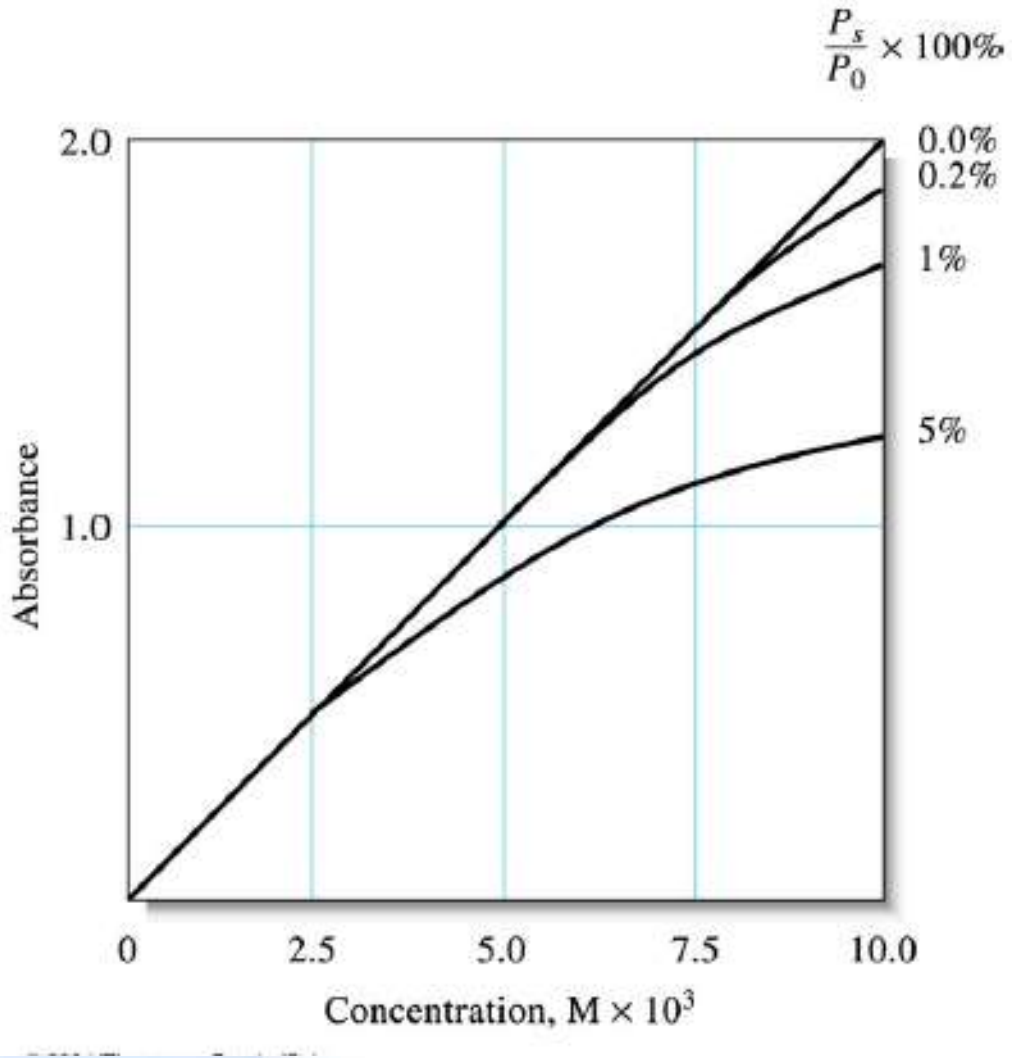
Deviations from Beer's law with polychromatic radiation.

Stray light always causes the apparent absorbance to be lower than the true absorbance. The deviations due to stray light are most significant at high absorbance values. Because stray radiation levels can be as high as 0.5% in modern instruments, absorbance levels above 2.0 are rarely measured unless special precautions are taken or special instruments with extremely low stray light levels are used. Another deviation is caused by mismatched cells. If the cells holding the analyte and blank solutions are not of equal pathlength and equivalent in optical characteristics, and intercept will occur in the calibration curve. This error can be avoided either by using matched cells or by using a linear regression procedure to calculate both the slope and intercept of the calibration curve.



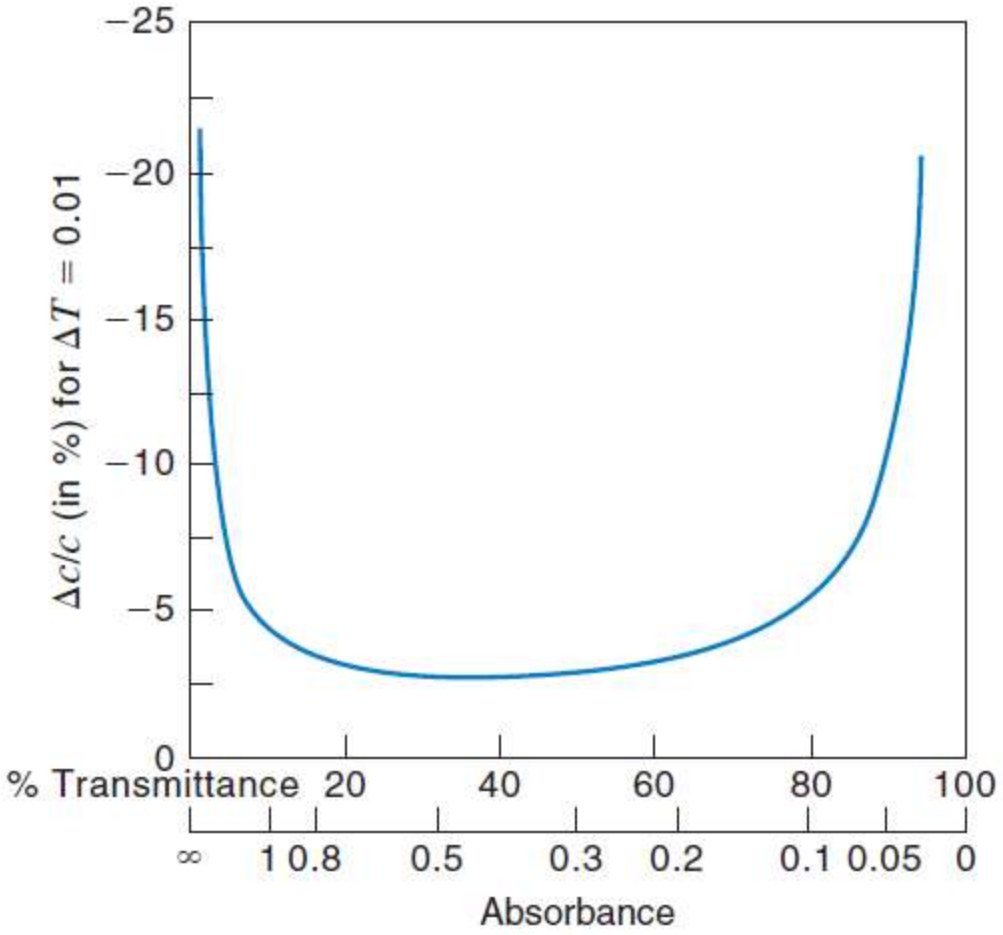


The effect of polychromatic radiation on Beer's law. In the absorption spectrum at the top, the absorptivity of the analyte is seen to be nearly constant over Band *A from* the source. Note in the Beer's law plot at the bottom that using Band *A gives* a linear relationship. In the spectrum, band *B coincides with a region of the* spectrum over which the absorptivity of the analyte changes. Note the dramatic deviation from Beer's law that results in the lower plot.



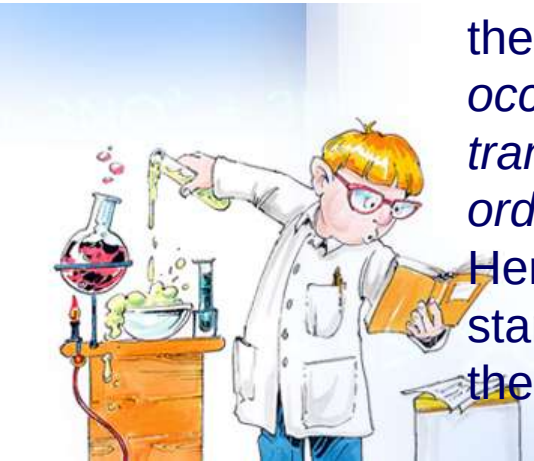
Deviation from Beer's law caused by various levels of stray light. Note that absorbance begins to level off with concentration at high stray light levels. Stray light always limits the maximum absorbance that can be obtained because, when the absorbance is high, the radiant power transmitted through the sample can become comparable to or lower than the stray light level.

This error can be avoided either by using carefully matched cells or by using a linear regression procedure to calculate both the slope and intercept of the calibration curve. In most cases, linear regression is the best strategy because an intercept can also occur if the blank solution does not totally compensate for interferences. Another way to avoid the mismatched-cell problem with single beam instruments is to use only one cell and keep it in the same position for both blank and analyte measurements. After obtaining the blank reading, the cell is emptied by aspiration, washed, and filled with analyte solution.



Relative concentration error as function of transmittance for 1% uncertainty in % *T.*

For minimum error, the sabsorbance should fall in the 0.1 to 1 range.



the minimum occurs at 36.8% *T, a nearly constant minimum error occurs* over the range of 20 to 65% *T (0.7 to 0.2 A). The percent transmittance should fall* within 10 to 80% *T (A = 1 to 0.1) in order to prevent large errors in spectrophotometric* readings. Hence, samples should be diluted (or concentrated), and standard solutions prepared, so that the absorbance falls within the optimal range.