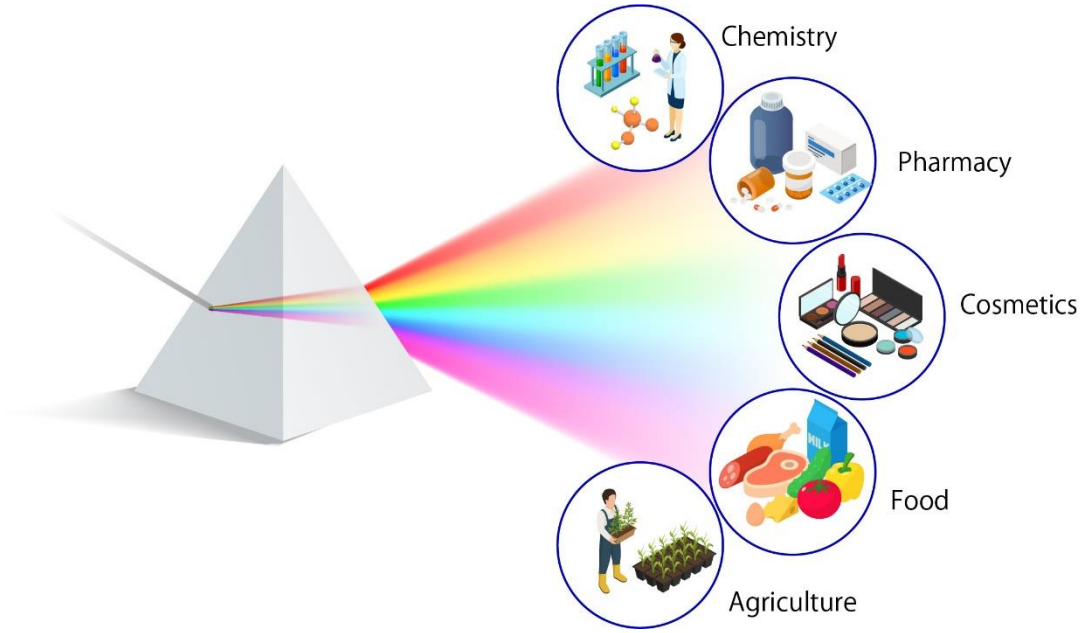
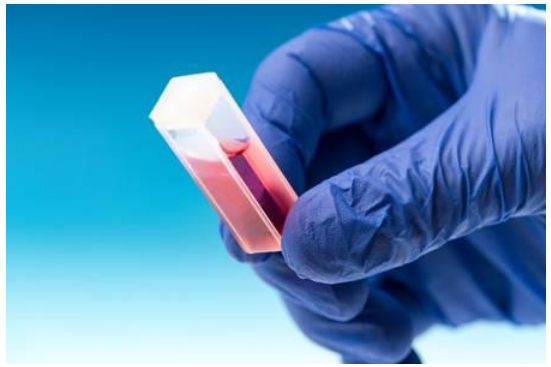




SPECTROSCOPIC METHODS

Basic Principles of Spectroscopy

Introduction to Spectroscopy

- Spectroscopy deals with the production, measurement, and interpretation of spectra arising from the interaction of electromagnetic radiation with matter.
- Spectroscopic analytical methods are based on measuring the amount of radiation produced or absorbed by molecular or atomic species of interest.
- There are many different spectroscopic methods available for solving a wide range of analytical problems.
- The methods differ with respect to the species to be analyzed (such as molecular or atomic spectroscopy), the type of radiation-matter interaction to be monitored (such as absorption, emission, or diffraction), and the region of the electromagnetic spectrum used in the analysis.

Introduction to Spectroscopy

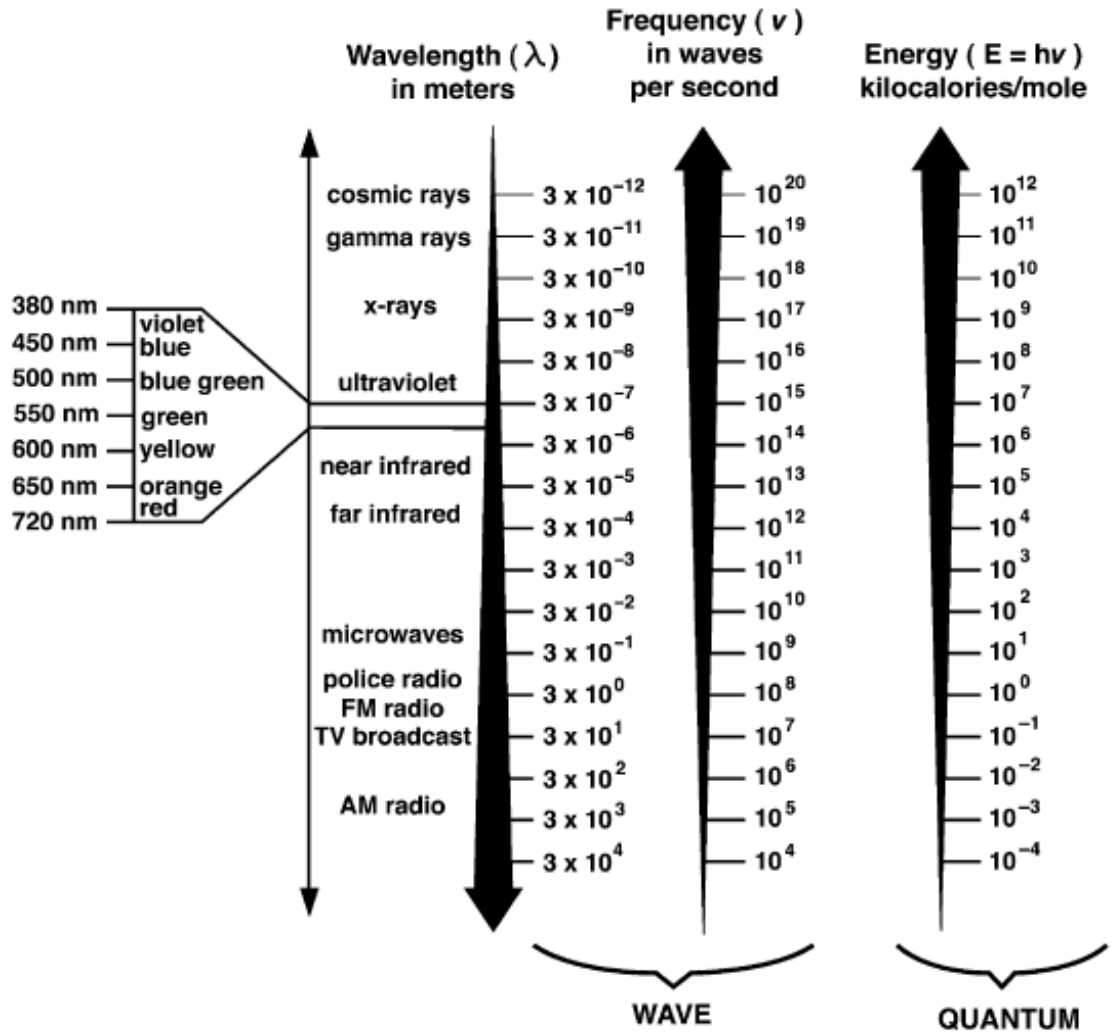
- We can classify spectroscopic methods according to the region of the electromagnetic spectrum used or produced in the measurement. The **γ-ray, X-ray, ultraviolet (UV), visible, infrared (IR), microwave, and radio-frequency (RF) regions** have been used.

- Indeed, current usage extends the meaning of spectroscopy yet further to include techniques such as **acoustic, mass, and electron spectroscopy** in which electromagnetic radiation is not a part of the measurement.

- Spectroscopic methods based on the absorption or emission of radiation in the **ultraviolet (UV), visible (Vis), infrared (IR), and radio (nuclear magnetic resonance, NMR)** frequency ranges are most commonly encountered in traditional food analysis laboratories. Each of these methods is distinct in that it monitors different types of molecular or atomic transitions.

- Spectroscopic methods are very informative and widely used for both quantitative and qualitative analyses. Spectrochemical methods have provided 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**.

The Electromagnetic Spectrum

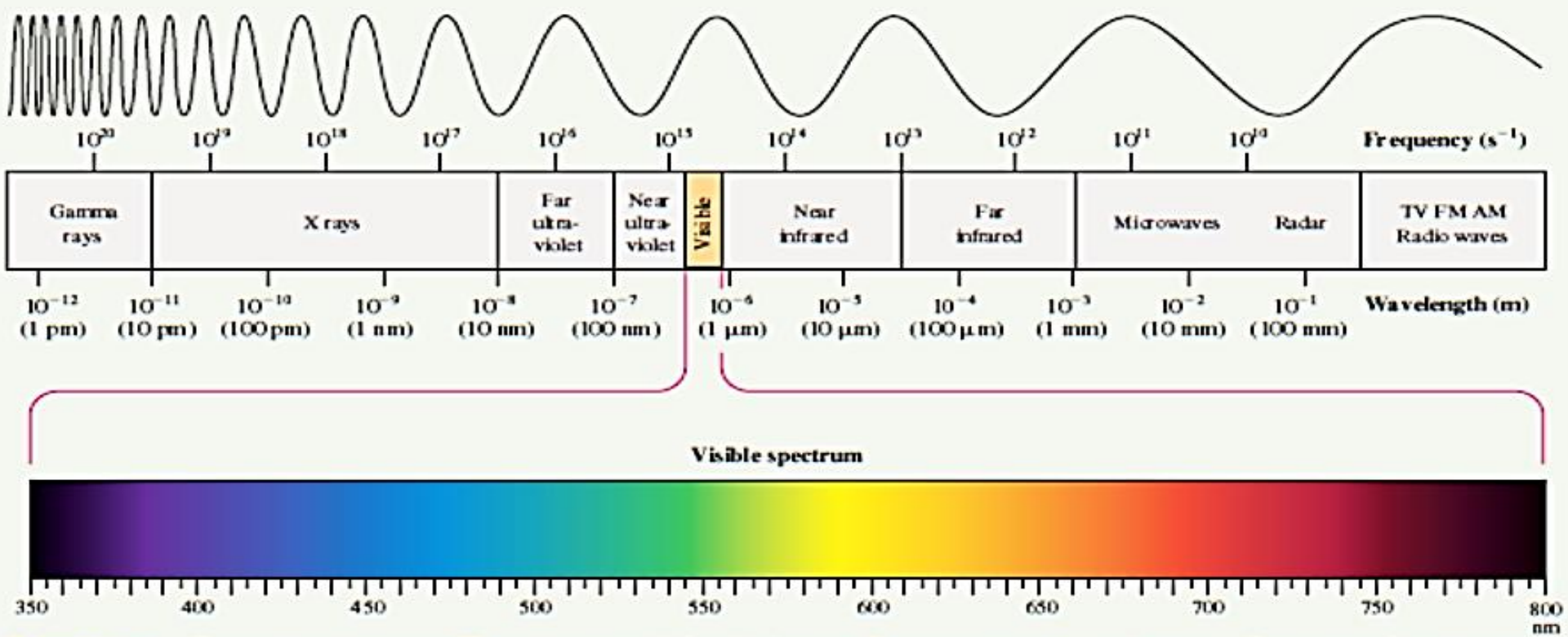


Regions of the UV, Visible, and IR Spectrum

Region	Wavelength Range
UV	180–380 nm
Visible	380–780 nm
Near-IR	0.78–2.5 μm
Mid-IR	2.5–50 μm

One easy way to recall the order of the colors in the spectrum is by the mnemonic **ROY G BIV**, which is short for **R**ed, **O**range, **Y**ellow, **G**reen, **B**lue, **I**ndigo, and **V**iolet.

The visible region of the spectrum extends from about 400 nm to almost 800 nm.



Color Plate 21 Electromagnetic spectrum. The spectrum extends from high-energy (frequency) gamma rays to low-energy (frequency) radio waves (see Section 24B-1, page 654). Note that the visible region is only a tiny fraction of the spectrum. The visible region, broken out in the lower portion, extends from the violet (~380 nm) to the red region (~800 nm). (Courtesy of Ebbing and Gammon, *General Chemistry*, 10th ed.)

The EM spectrum is generally divided into 7 regions (radio waves, microwaves, infrared (IR), visible light, ultraviolet (UV), X-rays and gamma rays), in order of decreasing wavelength and increasing energy and frequency.

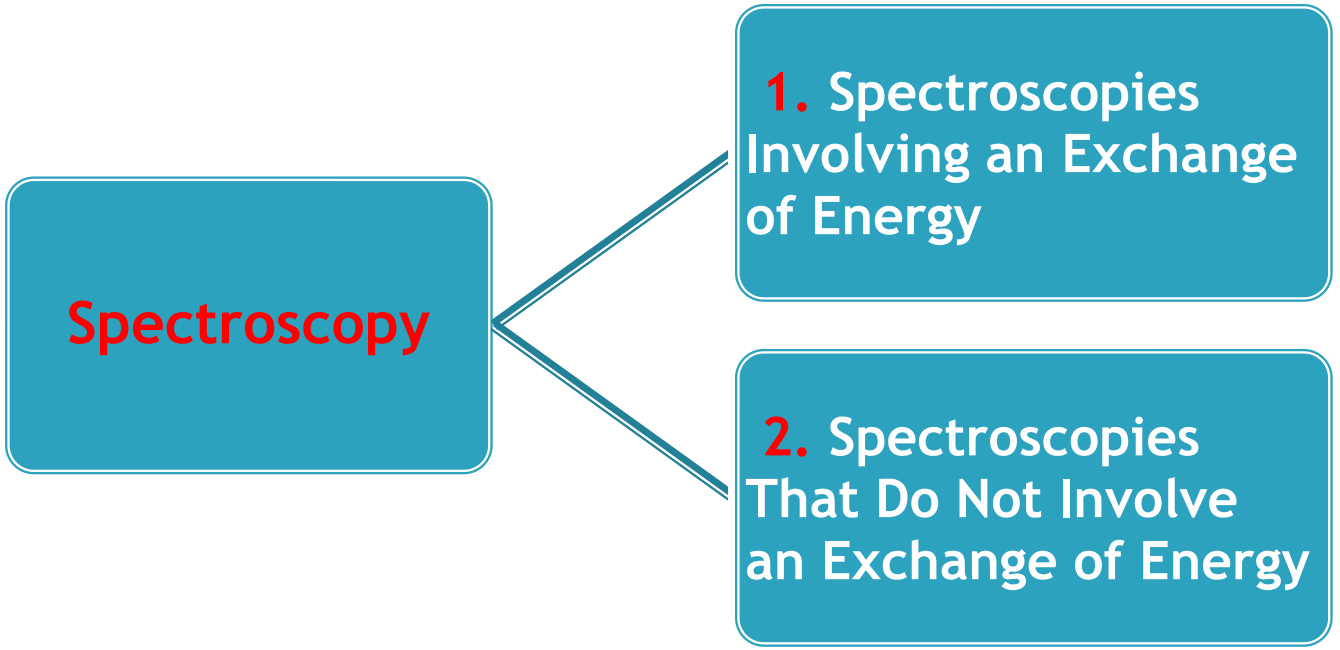
- The units commonly used for describing wavelength differ considerably in the various spectral regions.

  - The angstrom unit, A (=10^{-10} m) is convenient for X-ray.
  - The nanometer, nm (=10^{-9} m) is used with visible and ultraviolet radiation.
  - The micrometer, μm (=10^{-6} m) is used for the infrared region.

Optical Methods: Spectroscopic methods that use or produce visible, ultraviolet and infrared radiation is called optical methods.

Although the human eye is not sensitive to UV or IR radiation, this terminology arises from the common features of instruments for the three spectral regions and the similarities in the way viewing the interactions of the three types of radiation with matter.

- **Spectroscopy is possible only if the photon's interaction with the sample leads to a change in one or more of the characteristic properties of** electromagnetic radiation, including its energy, velocity, amplitude, frequency, phase angle, polarization, and direction of propagation.



- Energy is transferred between a photon of electromagnetic radiation and the analyte.

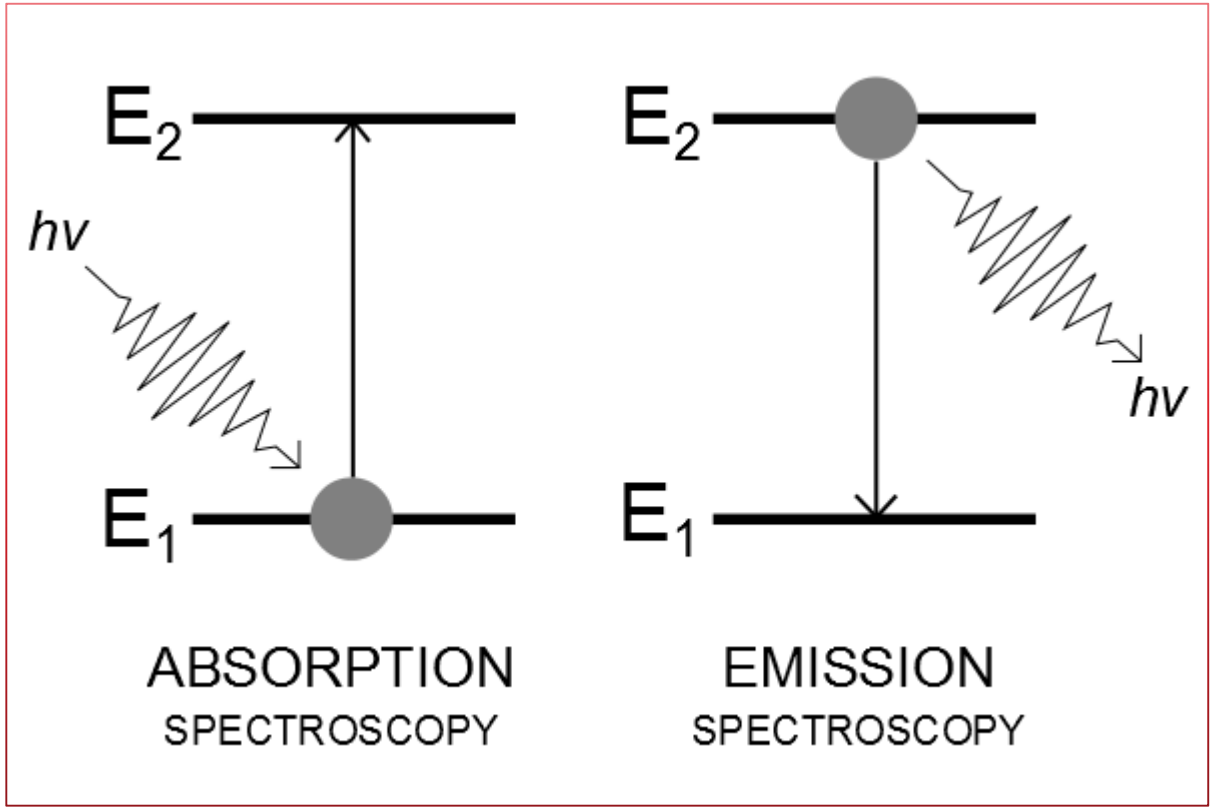
- The electromagnetic radiation undergoes a change in amplitude, phase angle, polarization, or direction of propagation as a result of its refraction, reflection, scattering, diffraction, or dispersion by the sample.

Representative Spectroscopic Techniques Involving an Exchange of Energy

Type of Energy Transfer	Region of the Electromagnetic Spectrum	Spectroscopic Technique
Absorption	γ-ray	Mossbauer spectroscopy
	X-ray	X-ray absorption spectroscopy
	UV/Vis	**UV/Vis spectroscopy**
		Atomic absorption spectroscopy
	Infrared	**Infrared spectroscopy**
		Raman spectroscopy
	Microwave	Microwave spectroscopy
		Electron spin resonance spectroscopy
	Radio waves	**Nuclear magnetic resonance spectroscopy**
Emission (thermal excitation)	UV/Vis	Atomic emission spectroscopy
Photoluminescence	X-ray	X-ray fluorescence
	UV/Vis	**Fluorescence spectroscopy**
		Phosphofluorescence spectroscopy
		Atomic fluorescence spectroscopy

Representative Spectroscopic Techniques That Do Not Involve an Exchange of Energy

Region of the Electromagnetic Spectrum	Type of Interaction	Spectroscopic Technique
X-ray	diffraction	X-ray diffraction
UV/Vis	refraction	refractometry
	scattering	nephelometry
		turbidimetry
	dispersion	optical rotary dispersion



Absorption spectroscopy: a photon is **absorbed** ("lost") as the molecule is raised to a higher energy level.

Emission spectroscopy: a photon is **emitted** ("created") as the molecule falls back to a lower energy level.

Ultraviolet, Visible and Fluorescence Spectroscopy

Ultraviolet, Visible and Fluorescence Spectroscopy

- Spectroscopy in the ultraviolet-visible (UV-Vis) range is one of the most commonly encountered laboratory methods in food analysis.
  - Quantification of macrocomponents (total carbohydrates by the phenol-sulfuric method)
  - Quantification of microcomponents (thiamin by the thiochrome fluorometric procedure)
  - Rancidity test (lipid oxidation status by the thiobarituric acid test)
  - Surveillance test (enzyme-linked immunoassays)
- In each of these cases, the analytical signal for which the assay is based is either the emission or absorption of radiation in the UV-Vis range.
- The analytical signal;
  - inherent in the analyte (i.e. the absoption of radiation in the visible range by pigments)
  - a result of a chemical reaction involving the analyte (i.e. the colorimetric copper-based Lowry method for the analysis of soluble protein)

- Electromagnetic radiation in the UV-Vis portion of the spectrum (wavelength): ~200-700 nm

  UV range: 200-350 nm

  Vis range: 350-700 nm

Spectrum of Visible Radiation

Wavelength (nm)	*Color*	*Complementary hue**
<380	Ultraviolet	
380-420	Violet	Yellow-green
420-440	Violet-blue	Yellow
440-470	Blue	Orange
470-500	Blue-green	Red
500-520	Green	Purple
520-550	Yelow-green	Violet
550-580	Yellow	Violet-blue
580-620	Orange	Blue
620-680	Red	Blue-green
680-780	Purple	Green
>780	Near-Infrared	

UV range: Colorless to the human eye

Different wavelengths in the visible range: each have a characteristic color, ranging from violet at the short wavelength end of the spectrum to red at the long wavelength end of the spectrum.

*Complementary hue refers to the color observed for a solution that shows maximum absorbance at the designated wavelength assuming a continuous spectrum "white" light source.

Spectroscopy in the UV-Vis range

- Absorbance Spectroscopy
- Fluorescence Spectroscopy

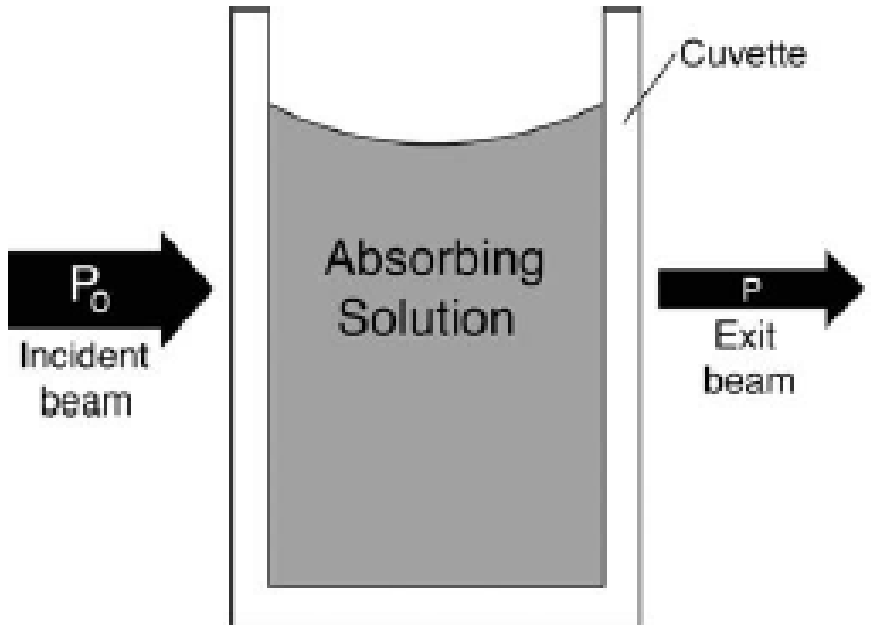
Ultraviolet and Visible Absorption Spectroscopy

Ultraviolet and Visible Absorption Spectroscopy

Basis of Quantitative Absorption Spectroscopy

- Objective of quantitative absorption spectroscopy: determination of the concentration of analyte in a given sample solution.
- The determination is based on the measurement of the amount of light absorbed from a reference beam as it passes through the sample solution.
- In some cases, the analyte may naturally absorb radiation in the UV–Vis range, such that the chemical nature of the analyte is not modified during the analysis.
- In other cases, analytes that do not absorb radiation in the UV–Vis range are chemically modified during the analysis, converting them to a species that absorbs radiation of the appropriate wavelength.
- In either case, the presence of analyte in the solution will affect the amount of radiation transmitted through the solution and, hence, the **relative transmittance** or **absorbance** of the solution may be used as an index of **analyte concentration**.

Absorption Process

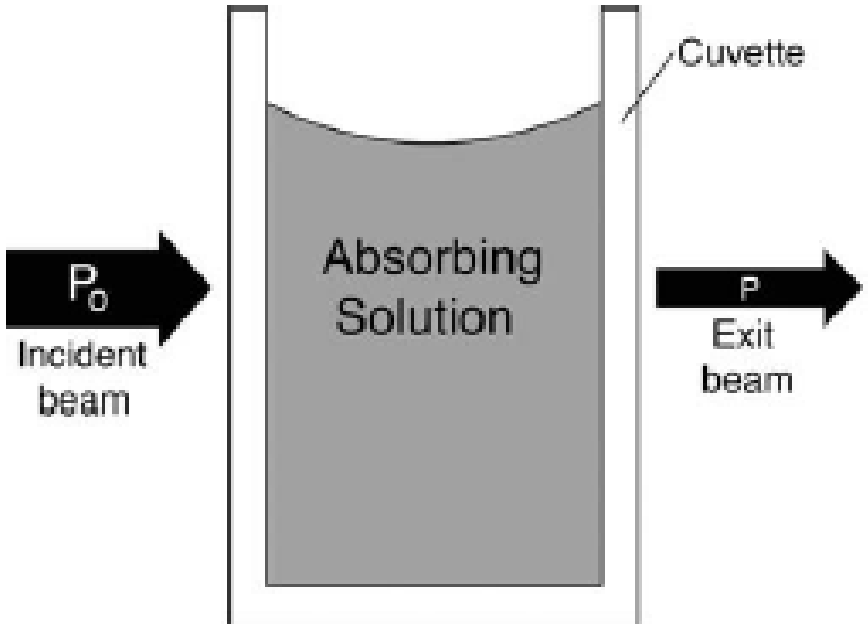


Attenuation of a beam of radiation
as it passes through a cuvette containing an absorbing solution

1. The solution to be analyzed is contained in an absorption cell and placed in the path of radiation of a selected wavelength(s).
2. The amount of radiation passing through the sample is then measured relative to a reference sample.
3. The relative amount of light passing through the sample is then used to estimate the analyte concentration.

- **When light passes through a sample, some of the light may be absorbed and the remainder transmitted through the sample.**

Absorption Process



$$P_0 > P$$

- **The decrease in radiant power** as the beam passes through the solution is due to **the capture (absorption)** of photons by the absorbing species.
- The relationship between the power of the incident and exiting beams typically is expressed in terms of either the **transmittance** or the **absorbance** of the solution.

$$T = P/P_0$$

$$\%T = (P/P_0) \times 100$$

T = transmittance
P_0 = radiant power of beam incident on absorption cell
P = radiant power of beam exiting the absorption cell
%T = percent transmittance

- The terms *T* and *%T* express the fraction of the incident light absorbed by the solution.
- Nonlinear relationship between transmittance and concentration

  [*T*, *%T*] α [1/(concentration of the the absorbing analyte in the sample solution)]

- A second term used to describe the relationship between *P* and *P*0 is **absorbance** (*A*).

$$A = \log(P_0/P) = -\log T = 2 - \log \%T$$

A = absorbance
T = transmittance
%T = percent transmittance

- Linear relationship between absorbance and concentration

  [*A*] α [concentration of the the absorbing species in the sample solution]

Beer's Law

The relationship between the absorbance of a solution and the concentration of the absorbing species is known as **Beer's law**.

$A = abc$

A = absorbance, (no units)

c = concentration of absorbing species, (M, mM, mg/ml, %)

b = path length through solution, (cm)

a = absorptivity, [(cm)−1 (concentration)−1]

- The **absorptivity**, ***a***, of a given species is a **proportionality constant** dependent on the molecular properties of the species.
- The absorptivity is wavelength dependent and may vary depending on the chemical environment (pH, ionic strength, solvent, etc.) the absorbing species is experiencing.

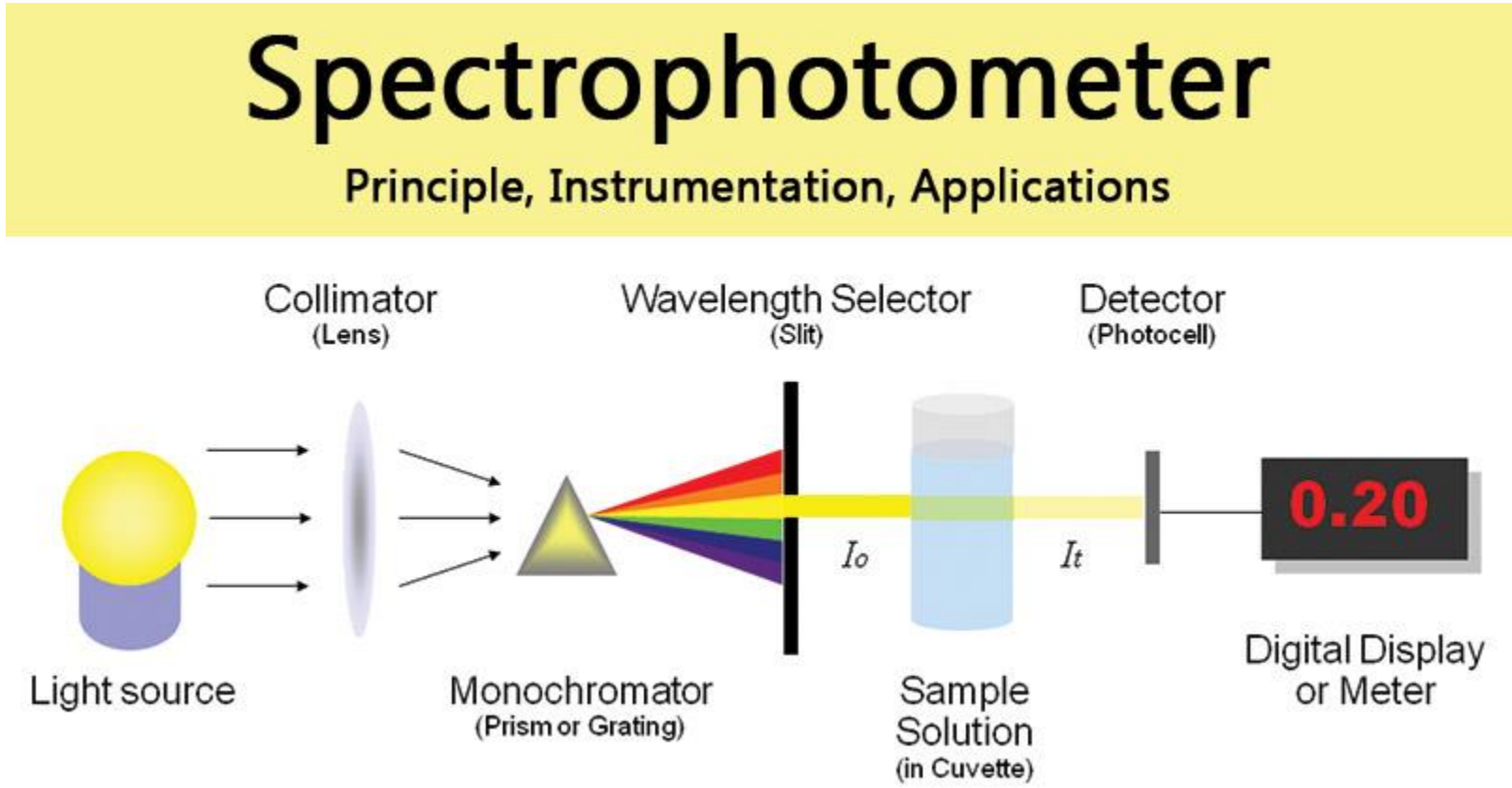
Beer's Law expressed in terms of the molar absorptivity

$A = \varepsilon bc$

c = concentration in **units of molarity**

ε = molar absorptivity, [(cm)−1 (M)−1]

Instrumentation-Spectrophotometer



Five essential components of a basic spectrophotometer:

1. The light source
2. The monochromator
3. The sample/reference holder
4. The radiation detector
5. The readout device

Instrument Design

- There are two major classes of devices: single beam and double beam.
- A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample.
- A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted.

Characteristics of UV-Vis Absorbing Species

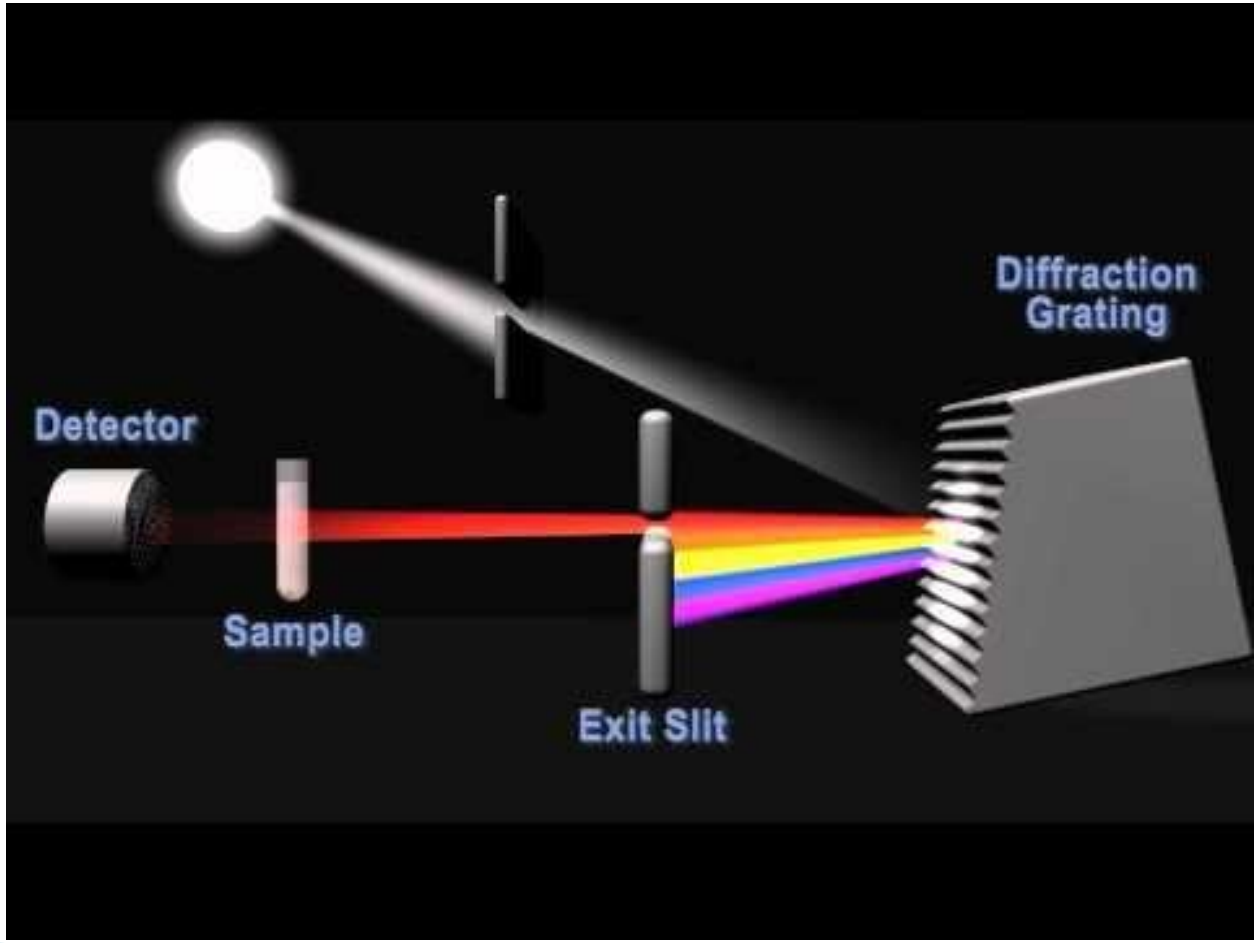
Representative Absorption Maxima Above 200nm for Select Functional Groups

Chromophore	*Example*	λ_{max}[a]	ε_{max}[b]
Nonconjugated systems			
R–CHO	Acetaldehyde	290	17
R_2–CO	Acetone	279	15
R–COOH	Acetic acid	208	32
R–$CONH_2$	Acetamide	220	63
R–SH	Mercaptoethane	210	1200
Conjugated systems			
$R_2C{=}CR_2$	Ethylene	<200	–
R–CH=CH–CH=CH–R	1,3 Butadiene	217	21,000
R–CH=CH–CH=CH–CH=CH–R	1,3,5 Hexatriene	258	35,000
–11 conjugated double bonds–	β-Carotene	465	125,000
$R_2C{=}CH{-}CH{=}O$	Acrolein (2-propenal)	210	11,500
		315	14
HOOC–COOH	Oxalic acid	250	63
Aromatic compounds[c]			
C_6H_6	Benzene	256	200
C_6H_5OH	Phenol	270	1450
C_8H_7N	Indole	278	2500

How does a spectrophometer work?

1. A lamp provides the source of light.
2. The beam of light strikes the diffraction grating, which works like a prism and separates the light into its component wavelengths.
3. The grating is rotated so that only a specific wavelength of light reaches the exit slit.
4. Then the light interacts with the sample.
5. From this point, the detector measures the transmittance and absorbance of the sample.
6. Transmittance refers to the amount of light that passes completely through the sample and strikes the detector.
7. Absorbance is a measurement of light that is absorbed by the sample.
8. The detector senses the light being transmitted through the sample and converts this information into a digital display.

Access link: https://www.youtube.com/watch?v=pxC6F7bK8CU



Fluorescence Spectroscopy

Fluorescence Spectroscopy

- Molecular fluorescence methods are based on the measurement of radiation emitted from excited analyte molecules as they relax to lower energy levels.
- The analytes are raised to the excited state as a result of photon absorption.
- The processes of photon absorption and fluorescence emission occur simultaneously during the assay.
- Quantitative fluorescence assays are generally 1–3 orders of magnitude more sensitive than corresponding absorption assays.
- Like absorption assays, under optimal conditions there will be a direct linear relationship between the fluorescence intensity and the concentration of the analyte in the unknown solution.
- Most molecules do not fluoresce and, hence, cannot be assayed by fluorescence methods.

Fluorescence Spectroscopy

- The instrumentation used in fluorescence spectroscopy is composed of essentially the same components as the corresponding instrumentation used in UV–Vis absorption spectroscopy. However, there are definite differences in the arrangement of the optical systems used for the two types of spectroscopy.

- In fluorometers and spectrofluorometers, there is a need for two wavelength selectors, one for the **excitation beam** and one for the **emission beam**.