Ministry of Higher Education
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Pharmacy department

Lecture (7)

Instrumental Methods

- instrumental methods of analysis.
- Energy states of chemical species
- Electromagnetic radiation(EM radiation or EMR).
- Beer's Law

Instrumental Methods (introduction)

- Early in the twentieth century, chemists began to exploit phenomena other than those used for classical methods (volumetric and gravimetric methods) for solving analytical problems.
- Thus, measurements of physical properties of analytes (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 analytes.
- Furthermore, highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as

The most important instrumental methods used for estimation of chemical species are: **1) Spectrochemical Methods**

2) Chromatograghic methods

✓ Spectrochemical Methods:

- **Spectrochemical methods** have provided the most widely used tools for the explanation the structure of molecular species as well as the quantitative and qualitative determination of both organic and inorganic compounds.
- **Spectroscopy** referred to a branch of science in which light (that is, visible radiation) was resolved into its components wave lengths to produce spectra.

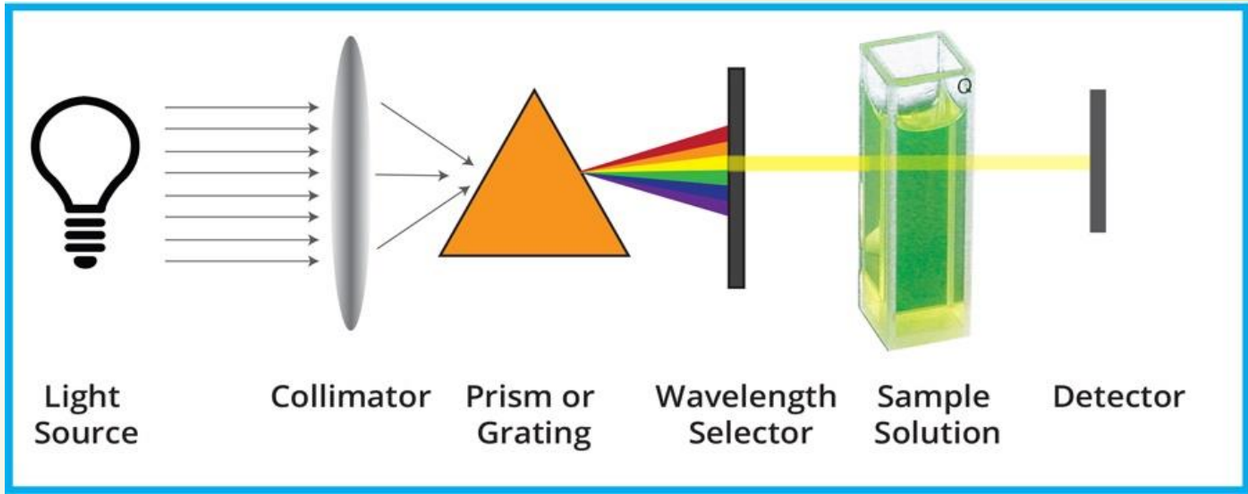
Spectroscopy use of absorption, emission or scattering of electromagnetic radiation by atoms or molecules to qualitative or quantitative study the atoms or molecules or to study physical process.

✓ There are five types of analytical spectroscopy:

- Absorbance
- Fluorescence and Phosphorescence
- Emission (atomic with flames, arcs, sparks)
- Chemiluminescence and Bioluminescence
- Scattering.

Optical instruments are spectroscopic devices that employ ultraviolet, visible and infrared radiation. Most of these instruments are made up of five components:

1. A stable source of radiant energy.
2. A wavelength selector that permits the isolation of a restricted wavelength reign.
3. One or more sample containers.
4. A radiation detector.
5. A signal processor and readout.

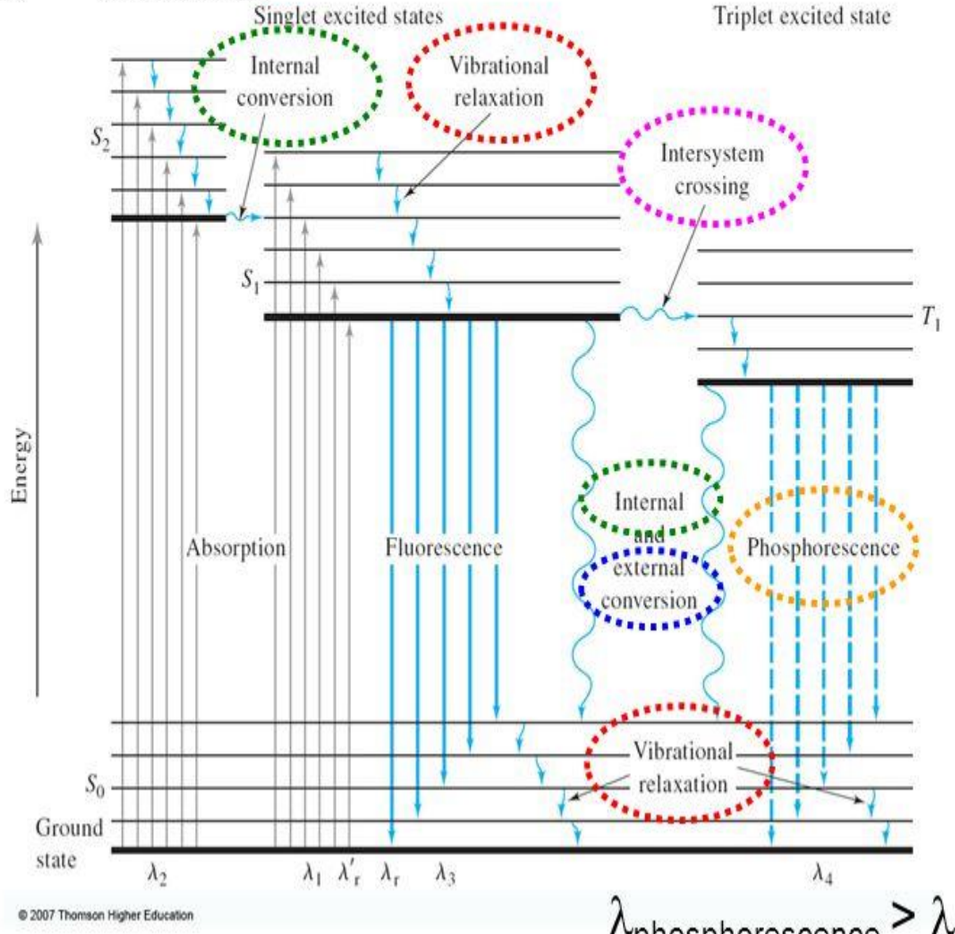


❖ Energy states of chemical species

- The interaction of radiation with matter can cause redirection of the radiation and/or transitions between the energy levels of the atoms or molecules.
- A transition from a lower energy level to a higher energy level with transfer of energy from the radiation field to the atom or molecule is called **absorption**.
- A transition from a higher energy level to a lower energy level is called **emission** if energy is transferred to the radiation field or nonradiative decay if no radiation is emitted. Redirection of light due to its interaction with matter is called **scattering**, and may not occur with transfer of energy, i.e., the scattered radiation has a slightly different or the same wavelength.

Molecular energy level diagrams aka Jablonski diagrams

$\lambda_{fluorescence} > \lambda_{absorbance}$



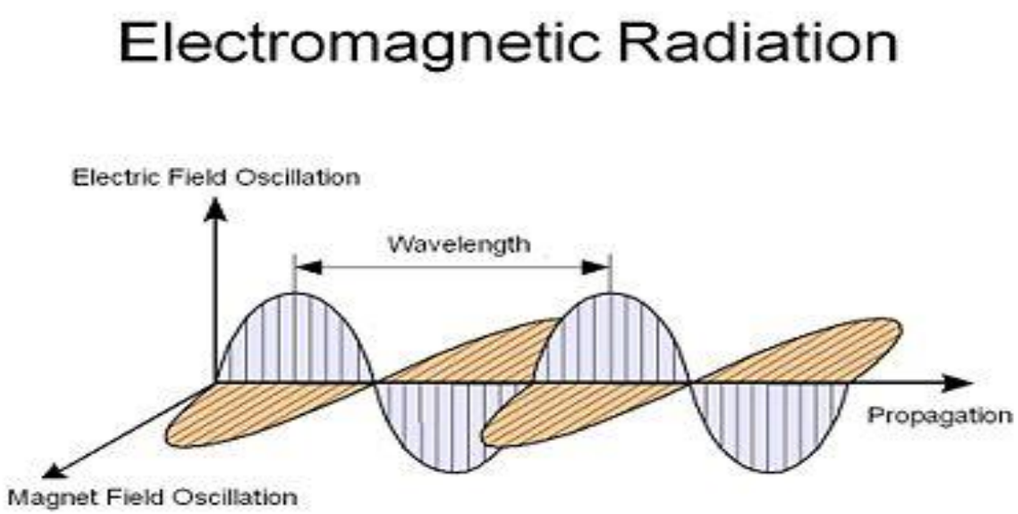
$\lambda_{phosphorescence} > \lambda_{fluorescence}$

✓ Electromagnetic radiation(EM radiation or EMR)

- EMR is a form of energy emitted and absorbed by charged particles which exhibits wave-like behavior as it travels through space. EMR has both electric and magnetic field components, which oscillate in phase perpendicular to each other and perpendicular to the direction of energy and wave propagation.
- EMR is made up of packets of energy called photons (or quanta).The energy of a photon depends upon the frequency of the radiation and is given by:

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

E = energy of the photon (ergs)
h =Planck's constant =6.626×10^{-34} J s
ν = frequency (s^{-1} or Hz)
c=velocity of light (c = 3×10^{10} cm s^{-1})
λ = wavelength(cm).



❖ UV-Vis spectra

Working ranges of the UV-Vis spectra, including: (**UV, 200-380 nm** and **Vis, 380-780 nm**)

Light is a form of electromagnetic radiation. When it falls on a substance, three things can happen:

- The light can be reflected by the substance
- It can be absorbed by the substance
- Certain wavelengths can be absorbed and the remainder transmitted or reflected.
- Absorption spectrometry is based on the absorption of photons by the analyte. (the analyte is the molecule being studied), This absorption reduces the number of photons in the beam of light, thereby reducing the intensity of the light beam.

A **spectrophotometer** is employed to measure the amount of light that a sample absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector.

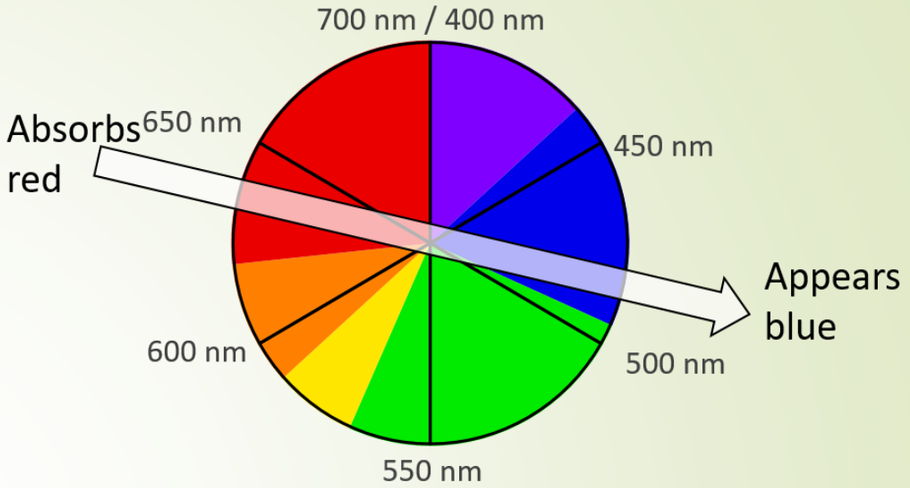
Absorption spectrometry, including:

- ✓ Molecular absorption spectrometry.
- ✓ Atomic absorption spectrometry.

- An important **principle** of spectrophotometry is that "***every substance absorbs or transmits certain wavelengths of radiant energy but not other wavelengths***".
- The absorption or transmission of specific wavelengths is characteristic for a substance, and a spectral analysis serves as a "***fingerprint***" of the compound.

Table 1: Colors of different wavelength regions

Wavelength absorbance (nm)	Color absorbed	Color observed (Complement)
380-435	Violet	Yellow-green
435-480	Blue	Yellow
480-490	Blue-green	Orange
490-500	Green-blue	Red
500-560	Green	Purple
560-580	Yellow-green	Violet
580-595	Yellow	Blue
595-650	Orange	Blue-green
650-750	Red	Green-blue



- For example, chlorophyll always absorbs red and violet light, while it transmits yellow, green, and blue wavelengths. The transmitted and reflected wavelengths appear green-the color your eye “sees.”
- A solution contains copper ions is blue because it absorbs the complementary color yellow from white light and transmits the remaining blue light (Table 1).

Transmittance, Absorbance and The Beer-Lambert law

We define **transmittance (T)** as the ratio of the amount of light transmitted to the amount of light that initially fell on the surface.

P◦ is the radiant power from the source and**, P** is the radiant power transmitted by the sample.

$$T = \frac{P}{P_\circ} = \frac{intensity\ of\ transmitted\ light}{intensity\ of\ incidant\ light}$$

$$\%T = \frac{P}{P_\circ} \times 100$$

Absorbance (A) is defined as the negative logarithm of the transmittance, and you will note that absorbance and transmittance bear an inverse relationship.

$$\text{Absorbance} = -\log T = -\log \frac{P}{P_\circ}$$

$$A = 2 - \log \%T$$

Beer-Lambert law, "for monochromatic radiation, absorbance is directly proportional to the path length b through the medium and the concentration c of the absorbing species". These relationships are given by

Where

$$A = a\ b\ c$$

- **A** is a dimensionless number.
- **a** the proportionality constant, is called the **absorptivity**. It is a constant for a given substance, provided the temperature and wavelength are constant. It has units of L/g • cm.
- **b** and **c** have the usual units of length (cm) and concentration (g/L).

Absorptivity depending on:

1-Nature of substance. 2-Wavelength. 3- Path length of radiation in solution. 4-Type of solvent.

Note:

$$\mathbf{A = \varepsilon\ b\ c}$$

If **b** and **c** have the units of length (cm) and concentration (mol/L), the absorptivity (a) is called the **molar absorptivity** and is given the special symbol **ε**. Thus, where ε has the unit L mol^{-1} cm^{-1}.

Example:
A solution containing 4.48 ppm $KMnO_4$ (M.wt =158 g/mol) has transmittance of 0.309 in a 1 cm cell at 520nm .Calculate the molar absorptivity.

Solution:

Absorbance = - log T = - log 0.309=0.510

$$ppm = M \times M.wt \times 1000 \Longrightarrow M = \frac{ppm}{M.wt \times 1000} \Longrightarrow M = \frac{4.48}{158 \times 1000} = 2.83 \times 10^{-5} \ mol/L$$

$$\varepsilon = \frac{A}{bc} = \frac{0.510}{1 \times 2.83 \times 10^{-5}} = 1.8 \times 10^{4} \ L\ mol^{-1}\ cm^{-1}$$

Limitations to Beer's Law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- ✓ Deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity.
- ✓ Scattering of light due to particulates in the sample.
- ✓ Changes in refractive index at high analyte concentration.
- ✓ Shifts in chemical equilibria as a function of concentration.
- ✓ Non-monochromatic radiation.
- ✓ stray light.

H.W.:

1- Define: spectrometry, EMR, Transmittance, Beer’s law, spectrophotometer.

2- What is the mechanism of Absorption Spectrometry?

3- Derive the following law: $A = 2 - \log \%T$

4- What is the Beer’s Law?

5- What is the difference between absorption and emission of EMR?

6- Why a solution contains copper ions is appeared blue?


Thank You!