Ministry of higher education and scientific research
AL-Mustaqbal University college
Department of medical physics

Analytical chemistry

Lecture 8

# Spectroscopy ,Ultraviolet-visible spectrophotometric analysis

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# Introduction to Spectroscopy

**What is spectroscopy?**

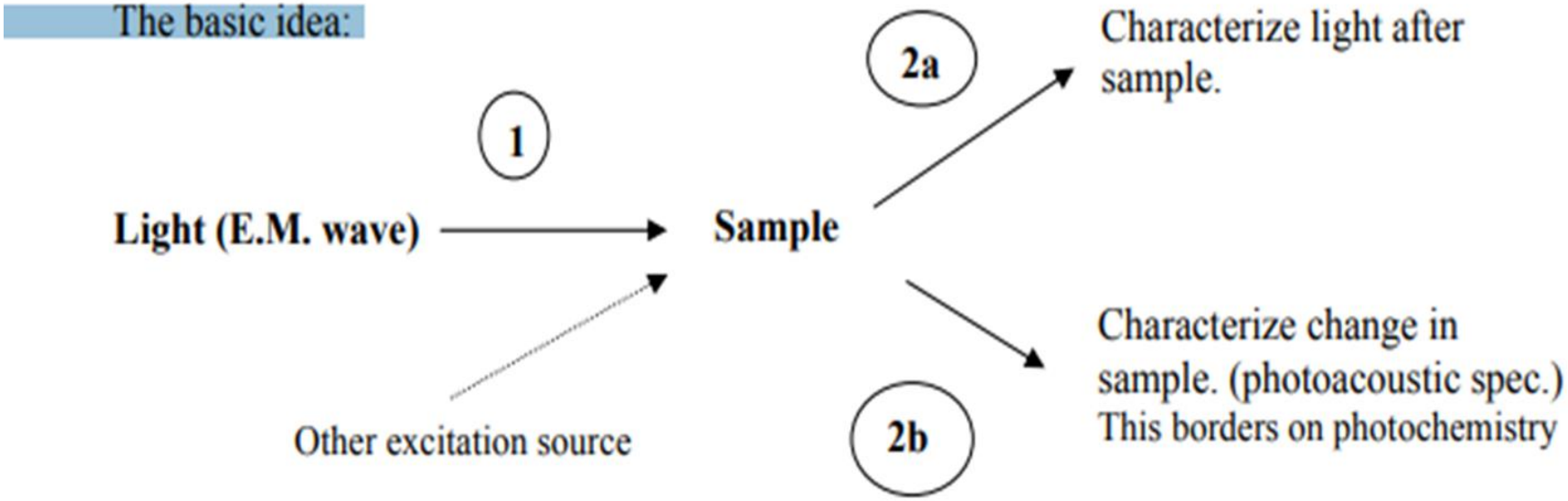
- ✓ Studying the properties of matter through its interaction with different frequency components of the electromagnetic spectrum.
- ✓ With light, you aren't looking directly at the molecule—the matter—but its "ghost." You observe the light's interaction with different degrees of freedom of the molecule.
- ✓ Each type of spectroscopy—different light frequency—gives adifferent picture → the spectrum.
- ✓ Spectroscopy is a general methodology that can be adapted in many ways to extract the information you need (energies of electronic, vibrational, rotational states, structure and symmetry of molecules, dynamic information).

# What does a spectrum measure?

Interaction of light with a sample can influence the sample and/or the light. Method involves:

(1) excitation

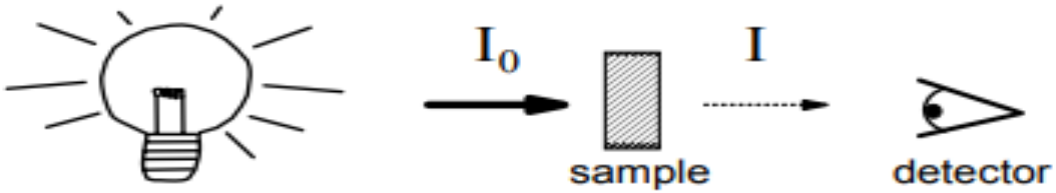
(2) detection.



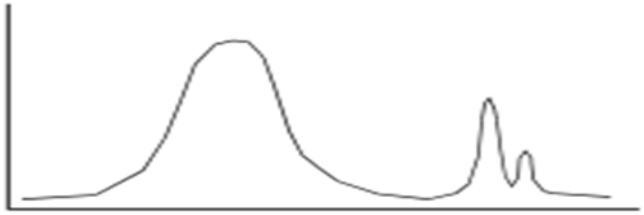
# In most spectroscopies, we characterize how a sample modifies light entering it.

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

**Sample attenuates light → transmission T=I/I0**

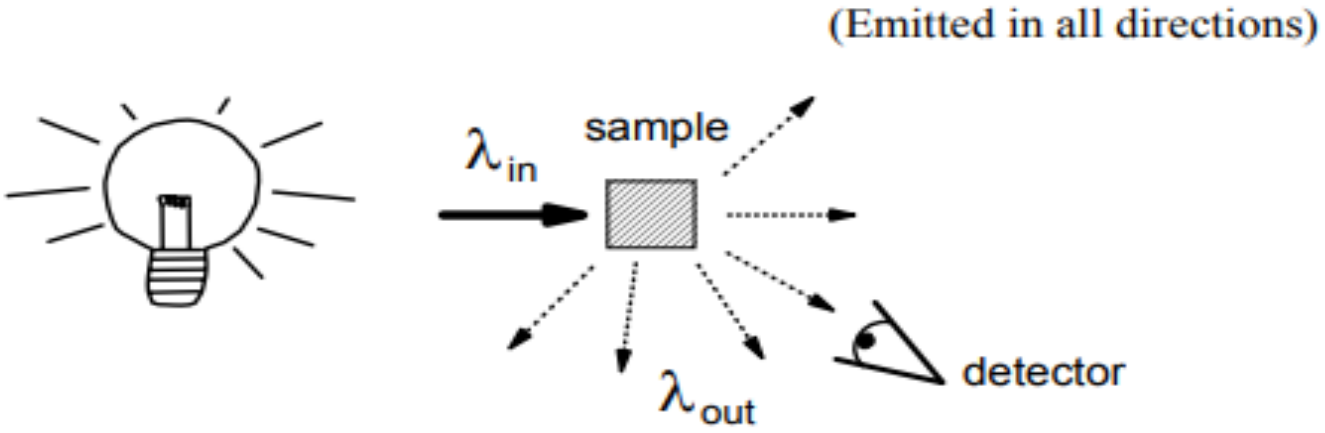



We measure the absorption of light at different frequency or wavelength.



ω/λ/ν (characteristic frequency/wavelength of light entering sample)

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

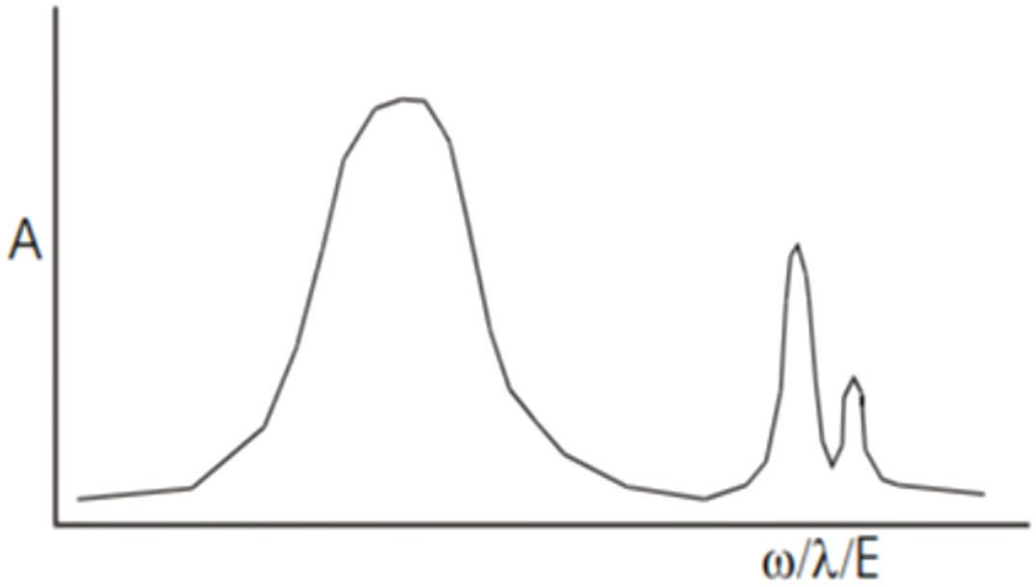



**Includes**:

I. Fluorescence (emission from excited electronic singlet states)
II. Phosphorescence (emission from excited electronic triplet states)
III. Raman Scattering (light scattering involving vibrational transition)

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

***Let's work on describing absorption.***




Let's look at a typical absorption spectrum.

# What are the axes?

1. **X-axis:** Characterizes the input light in terms of frequency-wavelength-energy.

**Wavelength** $\lambda$ (nm, $\mu$m, Å),

**Frequency** $\nu$ (cycles/sec or $s^{-1}$ or Hz) $= \frac{\omega}{2\pi} = \frac{c}{\lambda}$

$\omega = 2\pi\nu$ (rad/sec) (angular frequency)

$\bar{\nu} = \omega/2\pi c = 1/\lambda$ expressed in units of $cm^{-1}$ (wavenumbers)

**Energy** $E = h\nu$ (expressed as eV or as $cm^{-1}$ using $E/hc = \nu/c$)

**Conversions**

$\bar{\nu}\ (cm^{-1}) = 10^7 / \lambda(nm)$

$\bar{\nu}\ (eV) = 1240 / \lambda(nm)$

# 2- y-axis:

## Absorption

$$A(\nu) = -\log\frac{I}{I_0} = \varepsilon(\nu)\, c\, L \qquad \text{(Beer's Law)}$$

**$I_0$ = light intensity incident on the sample**
**I = light intensity after the sample**
ε = molar decadic extinction coefficient $\left(M^{-1}cm^{-1}\right)$ – the molecular quantity
c = concentration (M)
L = sample length (cm)

This comes from assuming that the fraction of light absorbed as you propagate though the sample is proportional to the distance traversed:

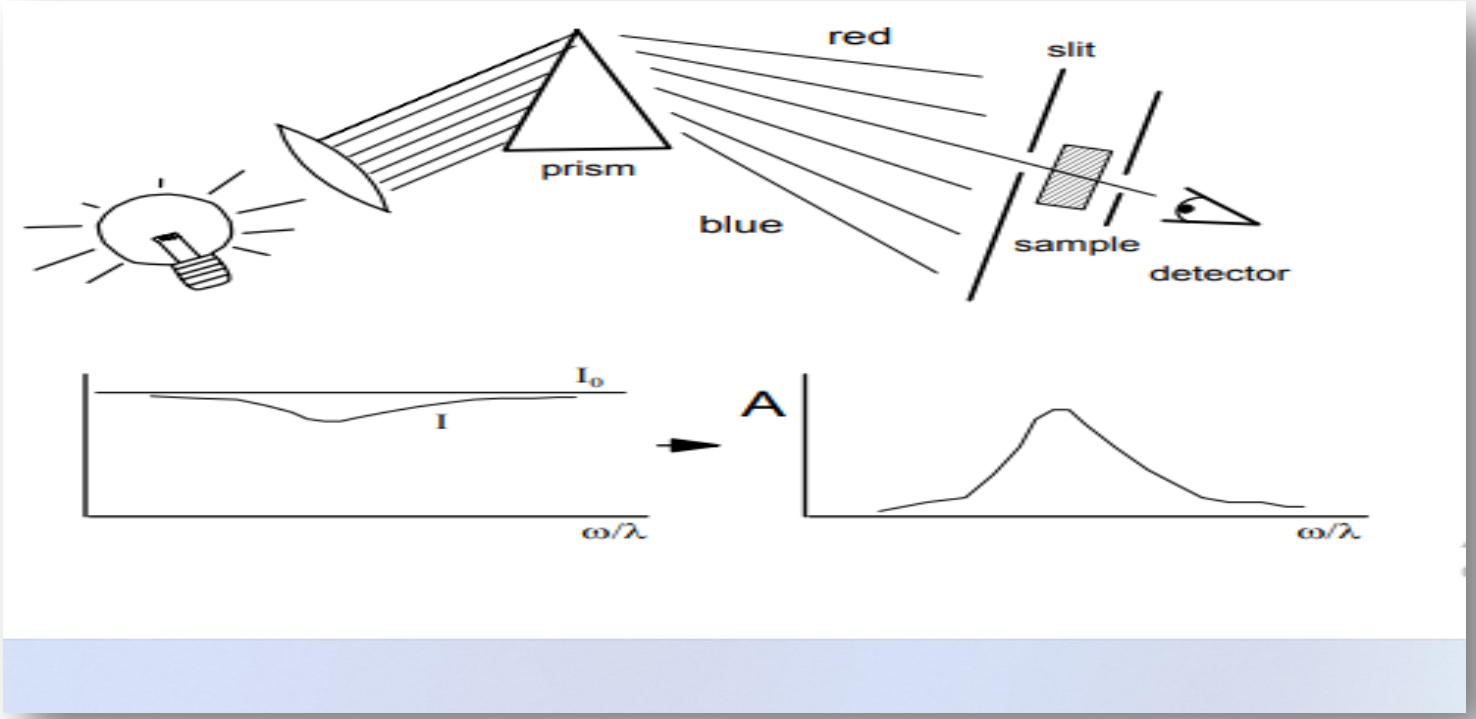
$$dI / dx = -\alpha$$

# How do your measure absorption spectra?

Measure the change of intensity of light at different frequencies as it passes through a sample.Two types of spectrometers:

1) Dispersive
2) Fourier transform

Dispersive spectrometer: Separate different frequency components

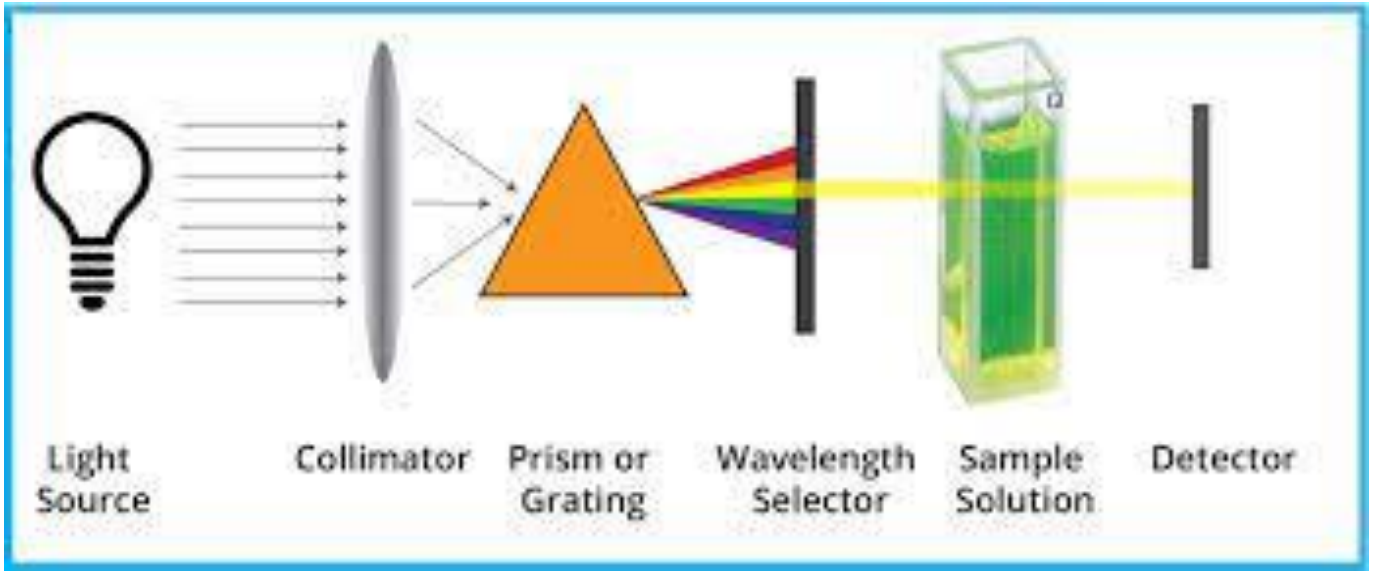


# What is a UV-Vis Spectrophotometer?

- Ultraviolet-visible (UV-Vis) spectrophotometry is a technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum.
- When incident light strikes matter it can either be absorbed, reflected, or transmitted.
- The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state.
- A UV-Vis spectrophotometer can use this principle to quantify the analytes in a sample based on their absorption characteristics.

# What is analyzed when ultraviolet-visible spectroscopy?

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample.



# How does a UV-Vis spectrophotometer work?

There are a variety of variations to the UV-Vis spectrophotometer to get more understanding of how it works we will look at the primary components, as shown below in the figure below.

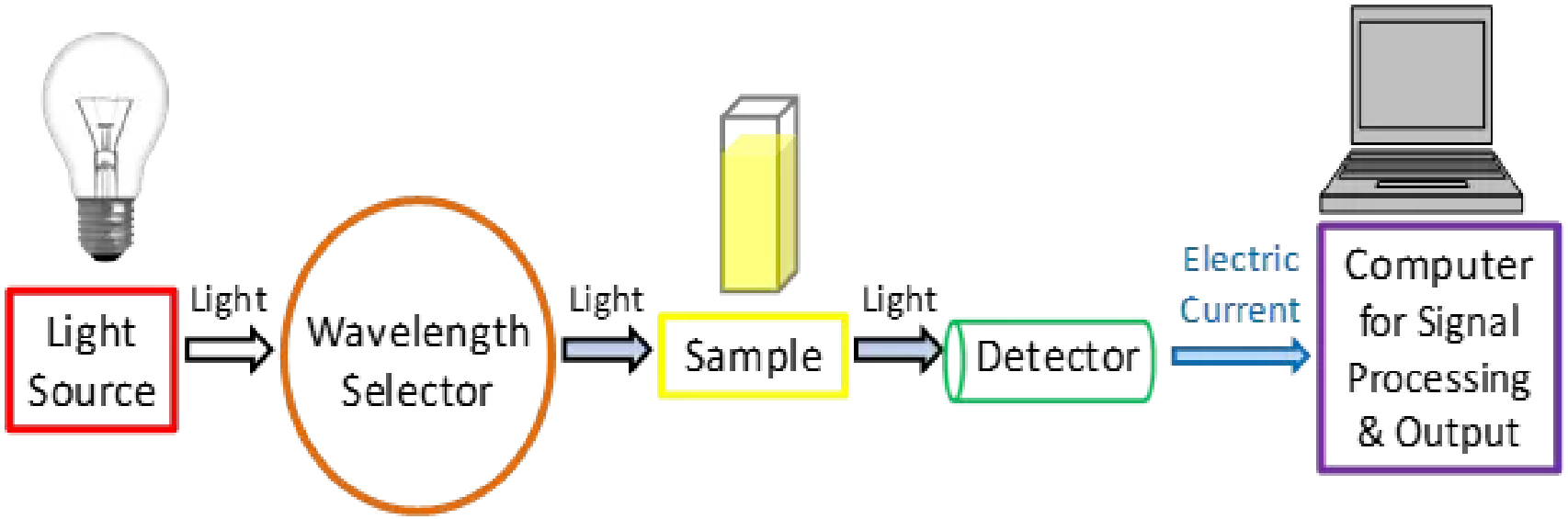



Figure: A simplified schematic of the main components in a UV-Vis spectrophotometer. Credit: Dr. Justin Tom.

# Instrumentation of UV Spectroscopy

he basic components of a spectrometer include: light source (UV and visible), monochromator (wavelength selector), sample stage, and detector.

## 1- Light source (UV and visible):

- As a light-based technique, a steady source able to emit light across a wide range of wavelengths is essential.
- A single xenon lamp is commonly used as a high intensity light source for both UV and visible ranges.
- Xenon lamps are, however, associated with higher costs and are less stable in comparison to tungsten and halogen lamps.

## 2- Monochromator (wavelength selector):

A monochromator separates light into a narrow band of wavelengths. It is most often based on diffraction gratings that can be rotated to choose incoming and reflected angles to select the desired wavelength of light.

## 3- Sample analysis :

Whichever wavelength selector is used in the spectrophotometer, the light then passes through a sample. For all analyses, measuring a reference sample, often referred to as the "blank sample", such as a cuvette filled with a similar solvent used to prepare the sample, is imperative. If an aqueous buffered solution containing the sample is used for measurements, then the aqueous buffered solution without the substance of interest is used as the reference.

**4- Detector:**

After the light has passed through the sample, a detector is used to convert the light into a readable electronic signal. Generally, detectors are based on photoelectric coatings or semiconductors.

# Advantages of UV-VIS Spectrometers

The biggest advantage for chemists and astronomers who use UV-VIS spectrometers is the accuracy of the device. Even small UV-VIS spectrometers can give extremely accurate readings, which is crucial when you are preparing chemical solutions or recording the movement of celestial bodies.

UV-VIS spectrometers are easy to use. Most UV-VIS spectrometers used in astronomy attach to telescopes. Most of the ones used in chemistry are comparable in size to electron microscopes and require the same basic skills to use. Because they are simple to operate, there is little chance of a UV-VIS spectrometer being used improperly.

# Disadvantages of UV-VIS Spectrometers

The main disadvantage of using a UV-VIS spectrometer is the time it takes to prepare to use one. With UV-VIS spectrometers, setup is key. You must clear the area of any outside light, electronic noise, or other outside contaminants that could interfere with the spectrometer's reading.

If the space has been properly prepared ahead of time, UV-VIS spectrometers are simple to use and give accurate results. However, if the space has not been properly prepared, even a small bit of outside light or vibration from a small electronic device could interfere with the results you are hoping to achieve in using a UV-VIS spectrometer.

# What are the applications of ultraviolet spectroscopy?

Ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique in many areas of science ranging from :

1. Quality control.
2. Cosmetic industry.
3. Petrochemistry.
4. Pharmaceutical research.
5. Optical components.
6. Food and agriculture.
7. Life sciences.
8. Traditional chemistry


THE KEY TO SUCCESS IS
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OBSTACLES.
مفتاح النجاح هو التركيز على
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