

# **Spectro-chemical methods**

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Visible spectrometry is one of the most widely used methods of analysis. It is very widely used in clinical chemistry and environmental laboratories because many substances can be selectively converted to a colored derivative .

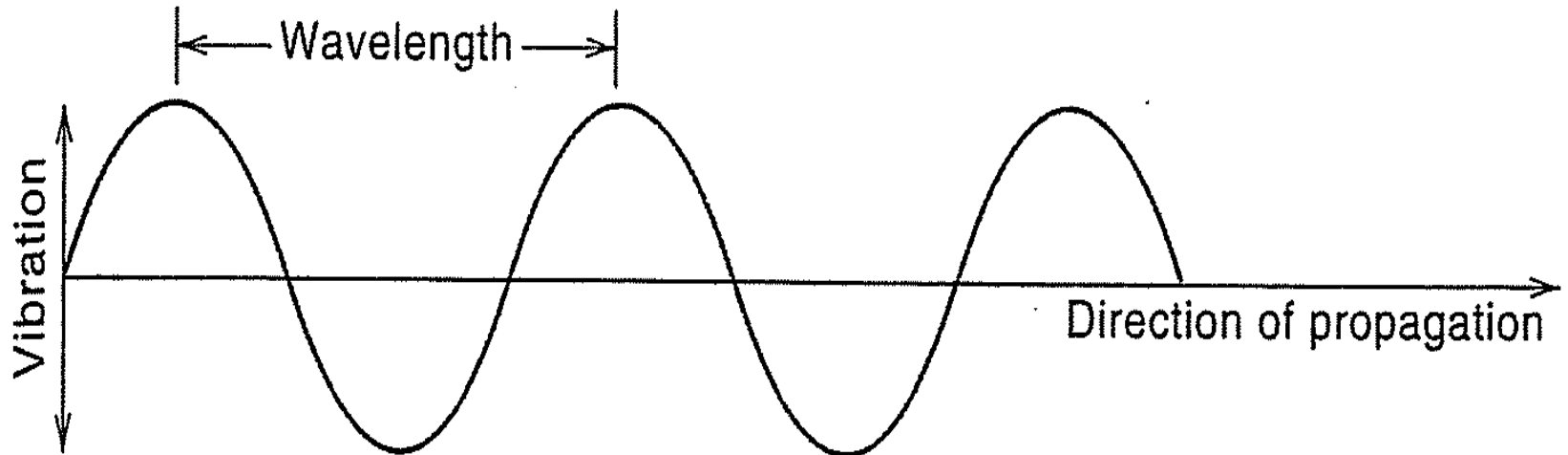
**In spectrometric methods, the sample solution absorbs electromagnetic radiation from an appropriate source, and the amount absorbed is related to the concentration of the analyte in the solution.**

A solution containing copper ions is blue because it absorbs the *complementary color* yellow from white light and transmits the remaining blue light.

# **The Electromagnetic SPECTRUM**

Wavelength, frequency, and  
wave number are interrelated

The **wavelength,  $\lambda$** , is the distance between equivalent points on the wave train,



The **frequency**,  $\nu$ , is the number of cycles of radiation passing a point in space per second. It is expressed as  $s^{-1}$ , or hertz (Hz).

The above definitions show that  
the relation between these

quantities  $\nu = c/\lambda$

Sometimes the **wavenumber**,  $\bar{\nu}$ ,

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

, is used where



## Colors of Different Wavelength Regions

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Wavelength Absorbed (nm)	Absorbed Color	Transmitted Color (Complement)
380–450	Violet	Yellow-green
450–495	Blue	Yellow
495–570	Green	Violet
570–590	Yellow	Blue
590–620	Orange	Green-blue
620–750	Red	Blue-green

The relationship between the wavelength and frequency is

$$\lambda = \frac{c}{\nu}$$

where  $\lambda$  is the wavelength in centimeters (cm),<sup>1</sup>  $\nu$  is the frequency in reciprocal seconds ( $\text{s}^{-1}$ ), or hertz (Hz), and  $c$  is the velocity of light ( $3 \times 10^{10}$  cm/s). The wavenumber is represented by  $\bar{\nu}$ , in  $\text{cm}^{-1}$ :

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

The wavelength of electromagnetic radiation varies from a few angstroms to several meters. The units used to describe the wavelength are as follows:

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

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

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

Wavelengths in the ultraviolet and visible(UV-Vis) regions are on the order of nanometers. In the infrared region(IR), they are micrometers, but the reciprocal of wavelength is often used (wave numbers, in  $\text{cm}^{-1}$  )

Electromagnetic radiation possesses a certain amount of energy. The energy of a unit of radiation, called the photon, is related to the frequency or wavelength by

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

where  $E$  is the energy of the photon in ergs and  $h$  is Planck's constant,  $6.62 \times 10^{-34}$  joule-second (J-s). It is apparent, then, that *the shorter the wavelength or the greater the frequency, the greater the energy.*

**Working ranges of the UV/Vis and IR spectra.**

UV	200–380 nm
Vis	380–780 nm
Near-IR	0.78–2.5 $\mu\text{m}$
Mid-IR	2.5–15 $\mu\text{m}$

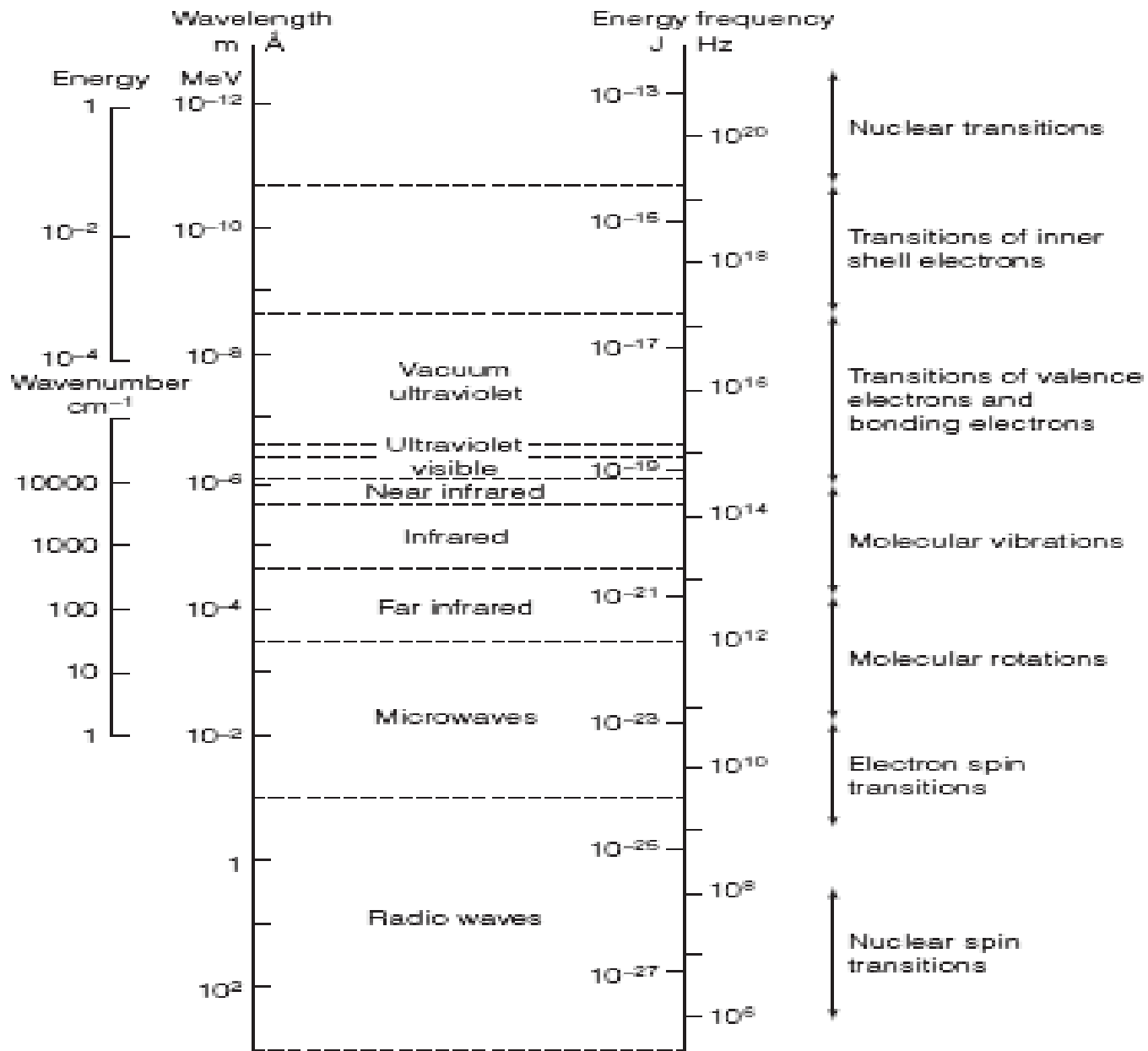


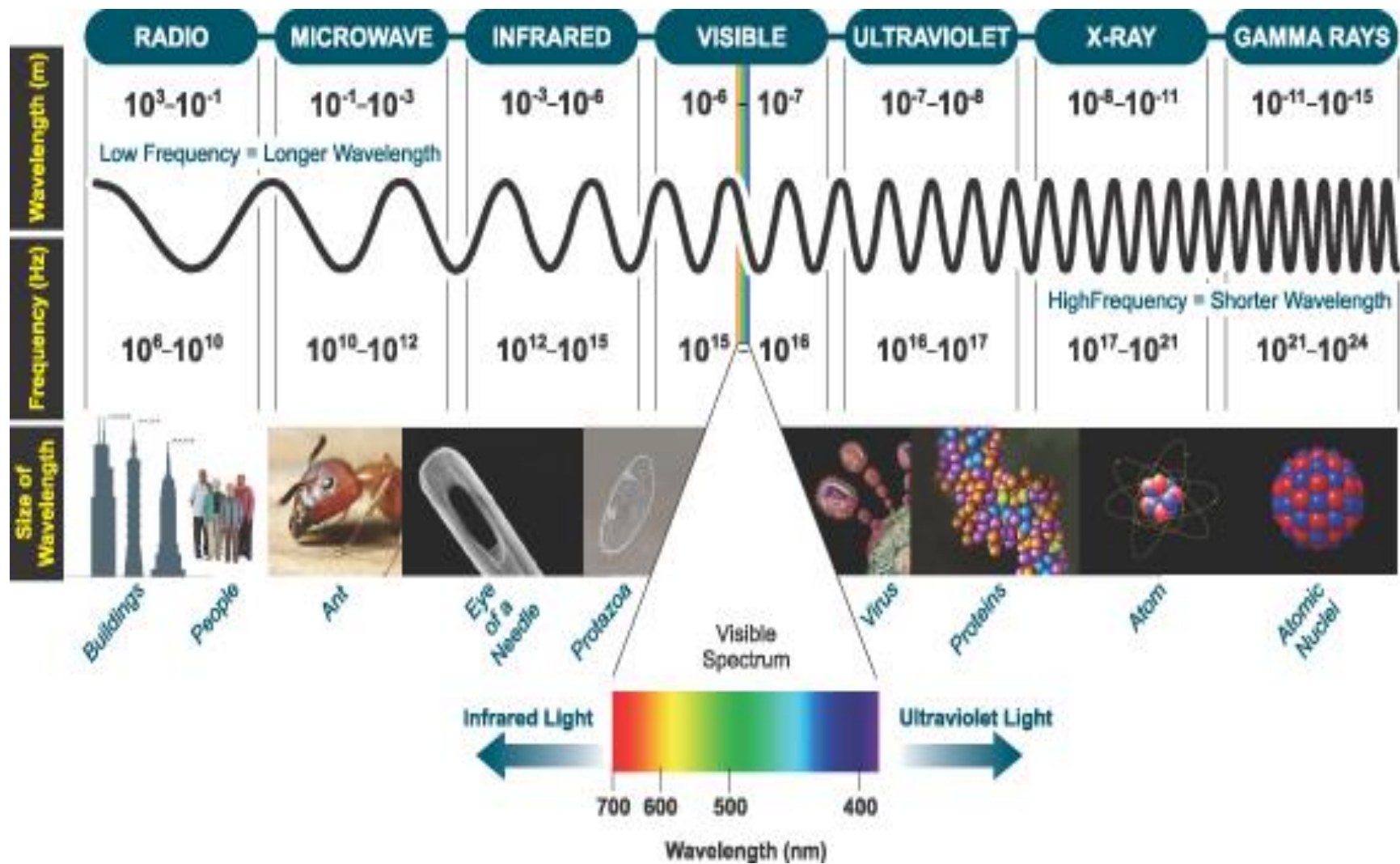
Fig. 2. The electromagnetic spectrum from  $\gamma$ -ray to radiowave.



The *ultraviolet* region extends from about 10 to 380 nm, but the most analytically useful region is from 200 to 380 nm, called the **near-ultraviolet or quartz UV region.**

Below 200 nm, the air absorbs and so the instruments are operated under a vacuum; hence, this wavelength region is called the **vacuum-ultraviolet region**.

The **visible (Vis) region** is actually a very small part of the electromagnetic spectrum, and it is the region of wavelengths that can be seen by the human eye, that is, where the light appears as a color. The visible region extends from the near-ultraviolet region (380 nm) to about 780 nm.



## How the matter interact with light

**A qualitative picture of the absorption** of radiation can be obtained by considering the absorption of light in the visible region.

We " see " objects as colored **because they transmit or reflect** only a portion of the light in this region. When polychromatic light (white light), which contains the whole spectrum of wavelengths in the visible region, is passed through an object, the object will absorb certain of the wavelengths, leaving the unabsorbed wavelengths to be transmitted. These residual transmitted wavelengths will be seen as a color.

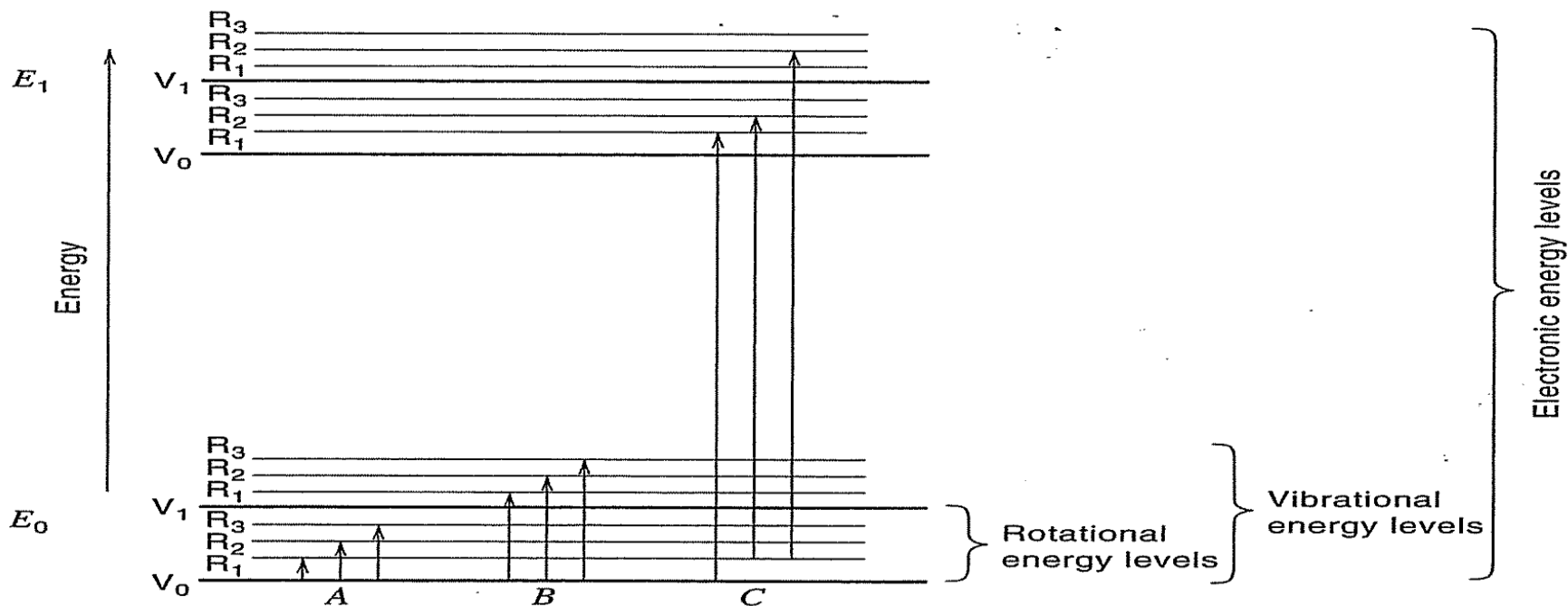
This color is **complementary** to the absorbed colors. In a similar manner, opaque objects will absorb certain wavelengths, leaving a residual color to be reflected and "seen."

As an example, a solution of potassium permanganate absorbs light in the green region of the spectrum with an absorption maximum of 525 nm, and the solution is purple.

There are three basic processes by which a molecule can absorb radiation; all involve raising the molecule to a higher internal energy level, the increase in energy being equal to the energy of the absorbed radiation ( $h\nu$ ). The three types of internal energy are **quantized**; that is, they exist at discrete levels. First, the molecule rotates about various axes, the energy of rotation being at definite energy levels, so the molecule may absorb radiation and be raised to a higher rotational energy level, in a **rotational transition**. Second, the atoms or groups of atoms within a molecule vibrate relative to each other, and the energy of this vibration occurs at definite quantized levels. The molecule may then absorb a discrete amount of energy and be raised to a higher vibrational energy level, in a **vibrational transition**. Third, the electrons of a molecule may be raised to a higher electron energy, corresponding to an **electronic transition**.



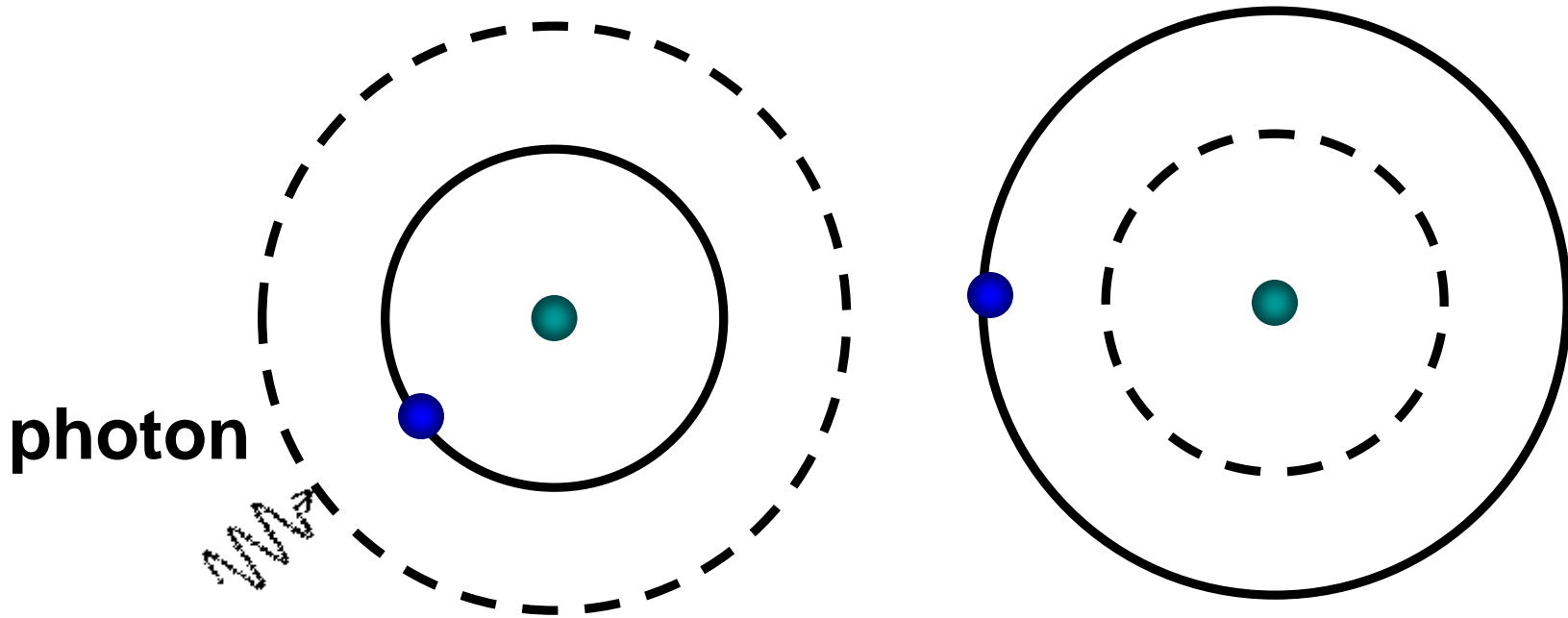
Rotational transitions occur at very long wavelengths (low energy, far infrared). Sharp line spectra are recorded.



**Fig. 16.3.** Energy level diagram illustrating energy changes associated with absorption of electromagnetic radiation: *A*, pure rotational changes (far infrared); *B*, rotational–vibrational changes (near infrared); *C*, rotational–vibrational–electronic transitions (visible and ultraviolet).  $E_0$  is electronic ground state and  $E_1$  is first electronic excited state.

# Absorption/Excitation

A photon is absorbed, the electron goes to an excited state.

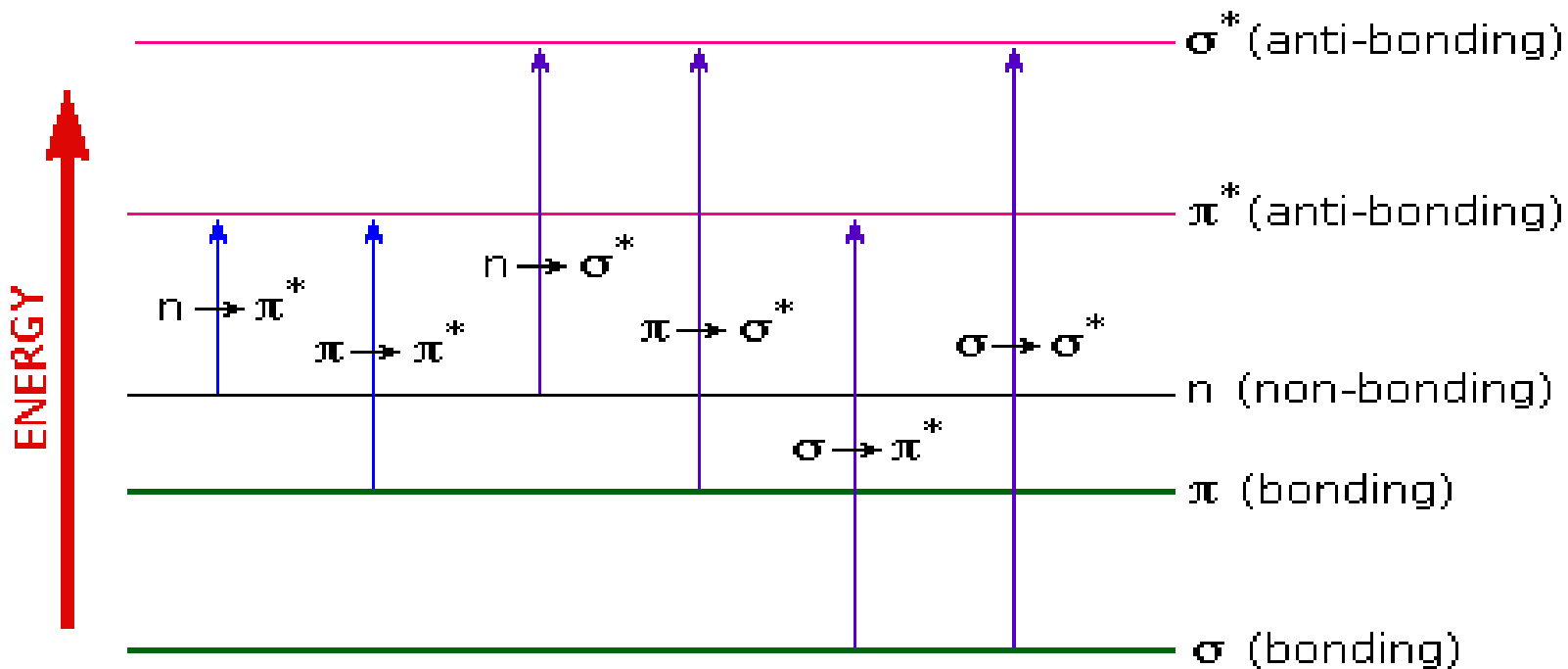


Before

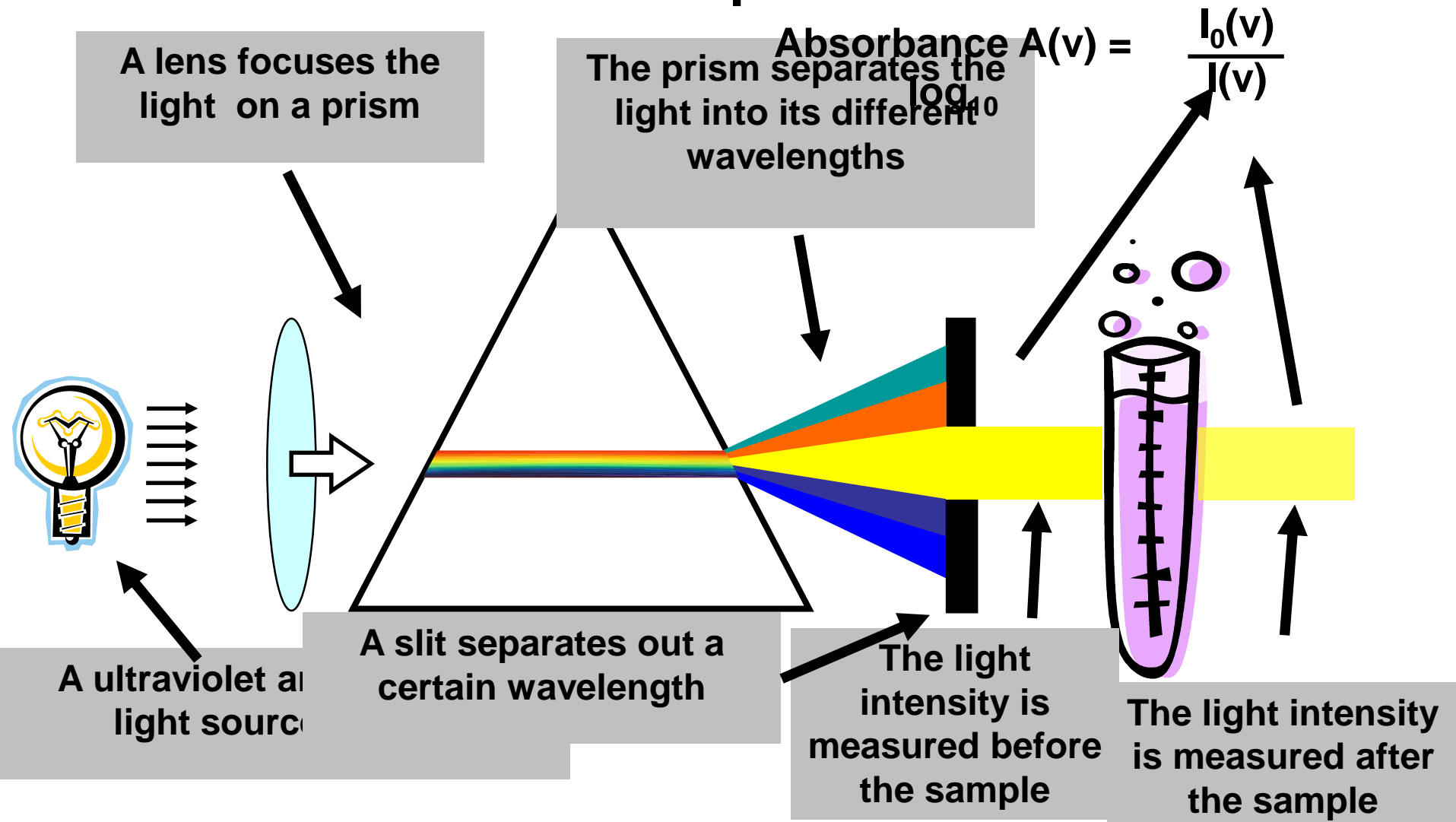
After



Fig. 4. Schematic of molecular orbitals or energy levels.

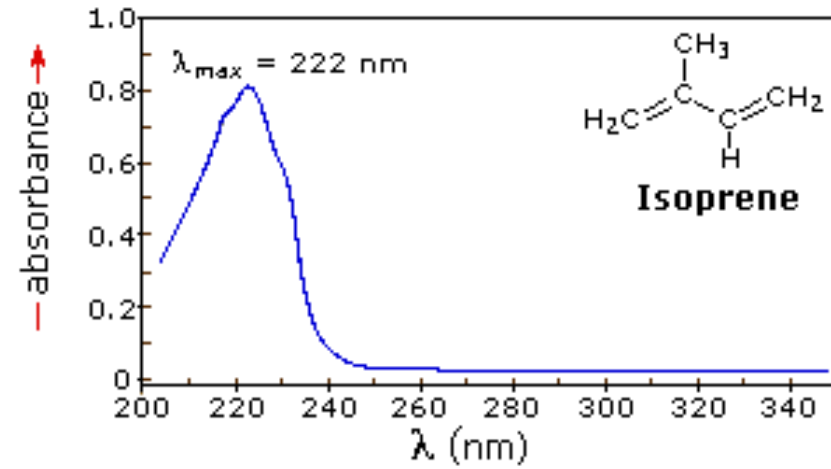
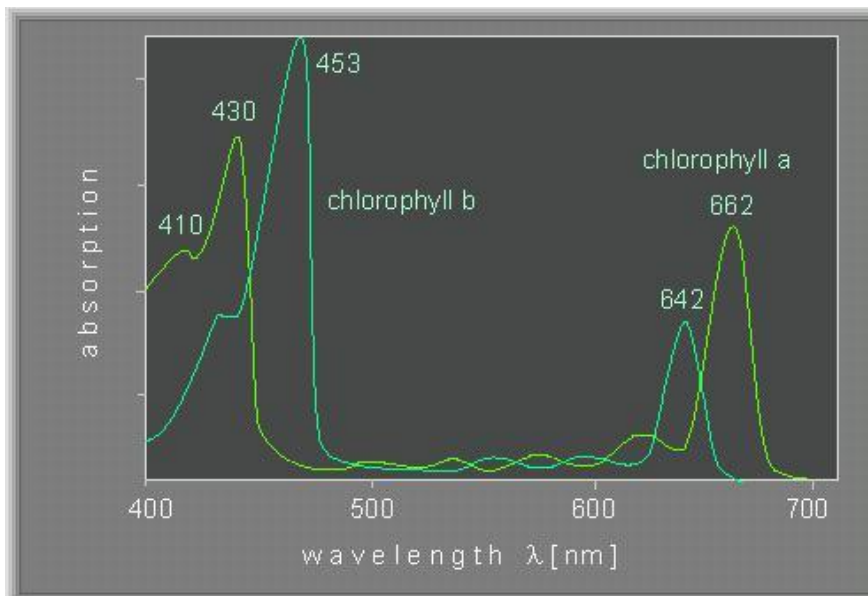


# UV-visible spectrometer



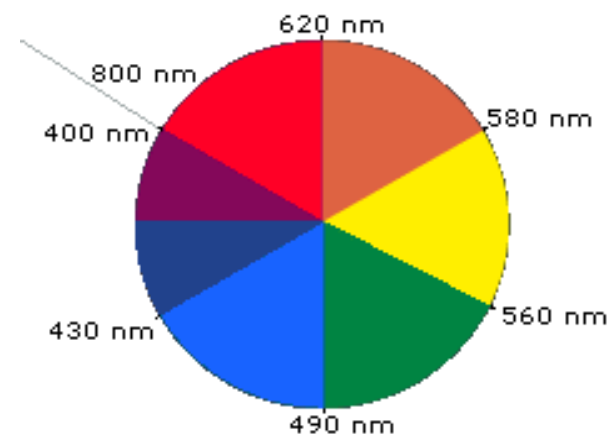
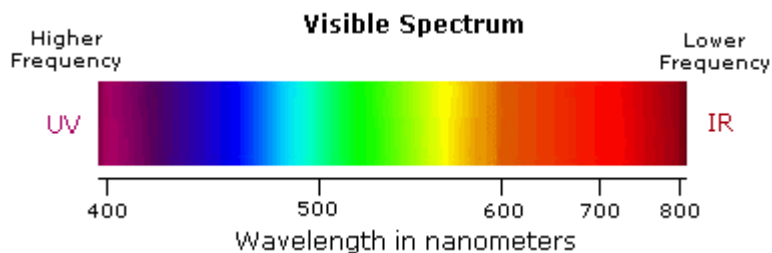
# Absorption Spectrum

- Can be a plot of transmittance or Absorbance versus wavelength
- Transmittance graph shows a dip at the absorption frequency
- Absorbance graph shows a peak at the absorption frequency



# Chromophore

- Functional group of atoms that absorb electromagnetic radiation usually in the UV or visible region
- Responsible for the color of a molecule
- The molecules color is the complementary of whatever wavelength the chromophore absorbs
- Example: absorption of 420-430 nm light makes the molecule yellow



# Quantitative Calculations

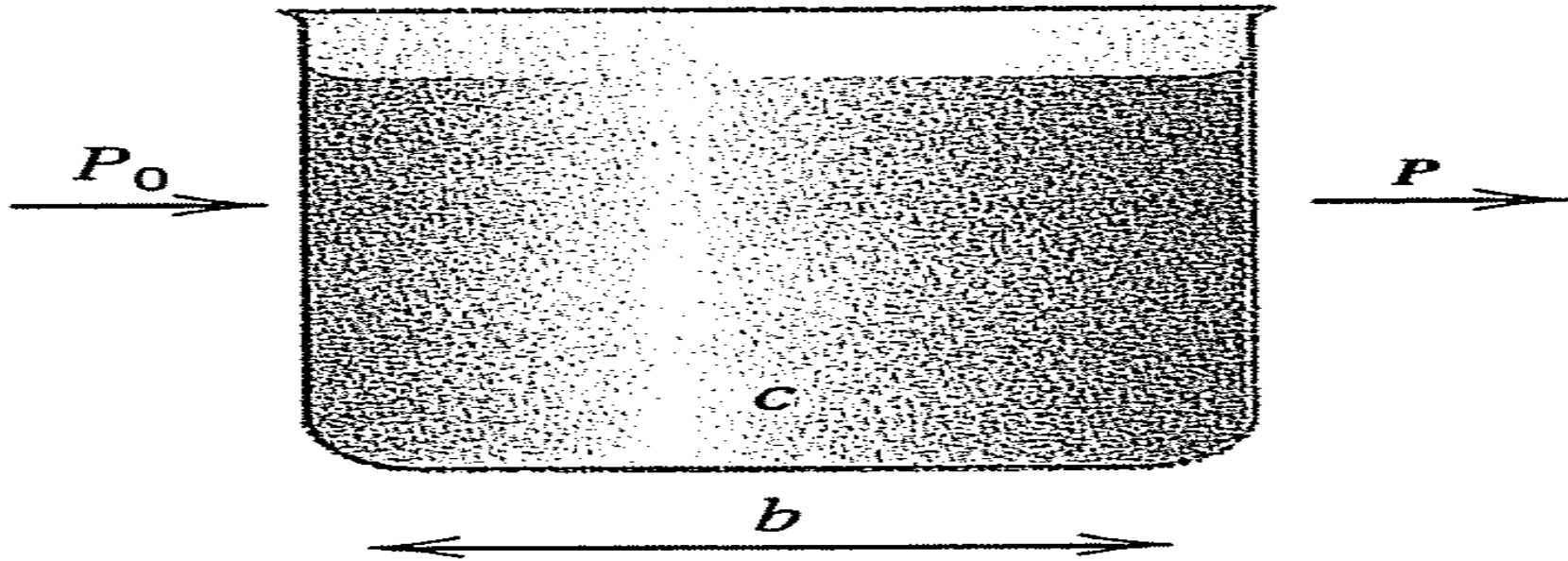
The fraction of radiation absorbed by a solution of an absorbing analyte can be quantitatively related to its concentration.

**Beers law — relating the amount of radiation absorbed to concentration**

The amount of monochromatic radiation absorbed by a sample is described by the'

Beer - Bouguer – Lambert law, commonly called Beer ' s law.





**Fig. 16.9.** Absorption of radiation.  
 $P_0$  = power of incident radiation,  
 $P$  = power of transmitted radiation,  
 $c$  = concentration,  $b$  = pathlength.

$$T = \frac{P}{P_0} = 10^{-kb} \quad (16.4)$$

where  $k$  is a constant and  $T$  is called the **transmittance**, the fraction of radiant energy transmitted. Putting this in logarithmic form,

$$\log T = \log \frac{P}{P_0} = -kb \quad (16.5)$$

In 1852, Beer [A. Beer, *Ann. Physik Chem.*, **86** (1852) 78] and Bernard [F. Bernard, *Ann. Chim. et Phys.*, **35** (1853) 385] each stated that a similar law holds for the dependence of  $T$  on the concentration,  $c$ :

$$T = \frac{P}{P_0} = 10^{-k'c} \quad (16.6)$$

where  $k'$  is a new constant, or

$$\log T = \log \frac{P}{P_0} = -k'c \quad (16.7)$$

Combining these two laws, we have Beer's law, which describes the dependence of  $T$  on both the pathlength and the concentration:

$$\boxed{T = \frac{P}{P_0} = 10^{-abc}} \quad (16.8)$$

where  $a$  is a combined constant of  $k$  and  $k'$ , and

$$\log T = \log \frac{P}{P_0} = -abc \quad (16.9)$$

It is more convenient to omit the negative sign on the right-hand side of the equation and to define a new term, **absorbance**:

$$A = -\log T = \log \frac{1}{T} = \log \frac{P_0}{P} = abc \quad (16.10)$$

where  $A$  is the absorbance. This is the common form of Beer's law. Note that it is the *absorbance* that is directly proportional to the concentration.

The percent transmittance is given by

$$\% T = \frac{P}{P_0} \times 100 \quad (16.11)$$

Equation 16.10 can be rearranged. Since  $T = \% T/100$ ,

$$A = \log \frac{100}{\% T} = \log 100 - \log \% T$$

Or

$$A = 2.00 - \log \% T$$

and

$$\% T = \text{antilog}(2.00 - A)$$

$$A = \epsilon bc \quad (16.13)$$

where  $c$  is now in *moles per liter*. The cell pathlength in ultraviolet and visible spectrophotometry is often 1 cm;  $\epsilon$  has the units  $\text{cm}^{-1} \text{mol}^{-1} \text{L}$ , while  $a$  has the units  $\text{cm}^{-1} \text{g}^{-1} \text{L}$ . The absorptivity  $a$  may be used with units other than g/L and, for example, concentrations may be expressed in ppm. But the recommended units for publication are as just described. Beer's law holds strictly for monochromatic radiation since the absorptivity varies with wavelength.

## Example 16.1

A sample in a 1.0-cm cell is determined with a spectrometer to transmit 80% light at a certain wavelength. If the absorptivity of this substance at this wavelength is 2.0, what is the concentration of the substance?



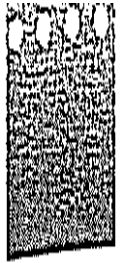
## Solution

The percent transmittance is 80%, and so  $T = 0.80$ :

$$\log \frac{1}{0.80} = 2.0 \text{ cm}^{-1} \text{ g}^{-1} \text{ L} \times 1.0 \text{ cm} \times c$$

$$\log 1.25 = 2.0 \text{ g}^{-1} \text{ L} \times c$$

$$c = \frac{0.10}{2.0} = 0.050 \text{ g/L}$$



## Example 16.2

A solution containing 1.00 mg ion (as the thiocyanate complex) in 100 mL was observed to transmit 70.0% of the incident light compared to an appropriate blank.

(a) What is the absorbance of the solution at this wavelength? (b) What fraction of light would be transmitted by a solution of iron four times as concentrated?

## Solution

(a)  $T = 0.700$

$$A = \log \frac{1}{0.700} = \log 1.43 = 0.155$$

(b)  $0.155 = ab(0.0100 \text{ g/L})$

$$ab = 15.5 \text{ L/g}$$

Therefore,  $A = 15.5 \text{ L/g} (4 \times 0.0100 \text{ g/L}) = 0.620$

$$\log \frac{1}{T} = 0.620$$

$$T = 0.240$$

The absorbance of the new solution could have been calculated more directly:

$$\frac{A_1}{A_2} = \frac{abc_1}{abc_2} = \frac{c_1}{c_2}$$

$$A_2 = A_1 \times \frac{c_2}{c_1} = 0.155 \times \frac{4}{1} = 0.620$$

## Example 16.3

Amines,  $\text{RNH}_2$ , react with picric acid to form amine picrates, which absorb strongly at 359 nm ( $\epsilon = 1.25 \times 10^4$ ). An unknown amine (0.1155 g) is dissolved in water and diluted to 100 mL. A 1-mL aliquot of this is diluted to 250 mL for measurement. If this final solution exhibits an absorbance of 0.454 at 359 nm using a 1.00-cm cell, what is the formula weight of the amine? What is a probable formula?

## Solution

$$A = \epsilon bc$$

$$0.454 = 1.25 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L} \times 1.00 \text{ cm} \times c$$

$$c = 3.63 \times 10^{-5} \text{ mol/L}$$

$$\frac{(3.63 \times 10^{-5} \text{ mol/L})(0.250 \text{ L})}{1.00 \text{ mL}} \times 100 \text{ mL} = 9.08 \times 10^{-4} \text{ mol in original flask}$$

$$\frac{0.1155 \text{ g}}{9.08 \times 10^{-4} \text{ mol}} = 127.2 \text{ g/mol}$$

## Example 16.4

Chloroaniline in a sample is determined as the amine picrate as described in Example 16.3. A 0.0265-g sample is reacted with picric acid and diluted to 1 L. The solution exhibits an absorbance of 0.368 in a 1-cm cell. What is the percentage chloroaniline in the sample?

### Solution

$$A = \epsilon bc$$

$$0.368 = 1.25 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L} \times 1.00 \text{ cm} \times c$$

$$c = 2.94 \times 10^{-5} \text{ mol/L}$$

$$(2.94 \times 10^{-5} \text{ mol/L})(127.6 \text{ g/mol}) = 3.75 \times 10^{-3} \text{ g chloroaniline}$$

$$\frac{3.75 \times 10^{-3} \text{ g chloroaniline}}{2.65 \times 10^{-2} \text{ g sample}} \times 100\% = 14.2\%$$

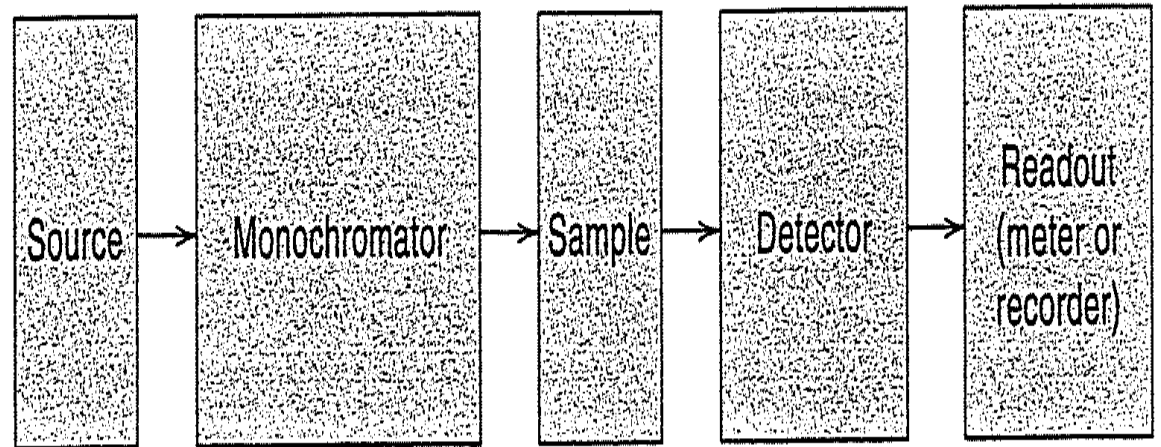
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## 16.8 Spectrometric Instrumentation

A **spectrometer** or **spectrophotometer** is an instrument that will resolve polychromatic radiation into different wavelengths. A block diagram of a spectrometer is shown in Figure 16.12. All spectrometers require (1) a **source** of continuous radiation over the wavelengths of interest, (2) a **monochromator** for selecting a



Fig. 16.12. Block diagram of spectrometer.



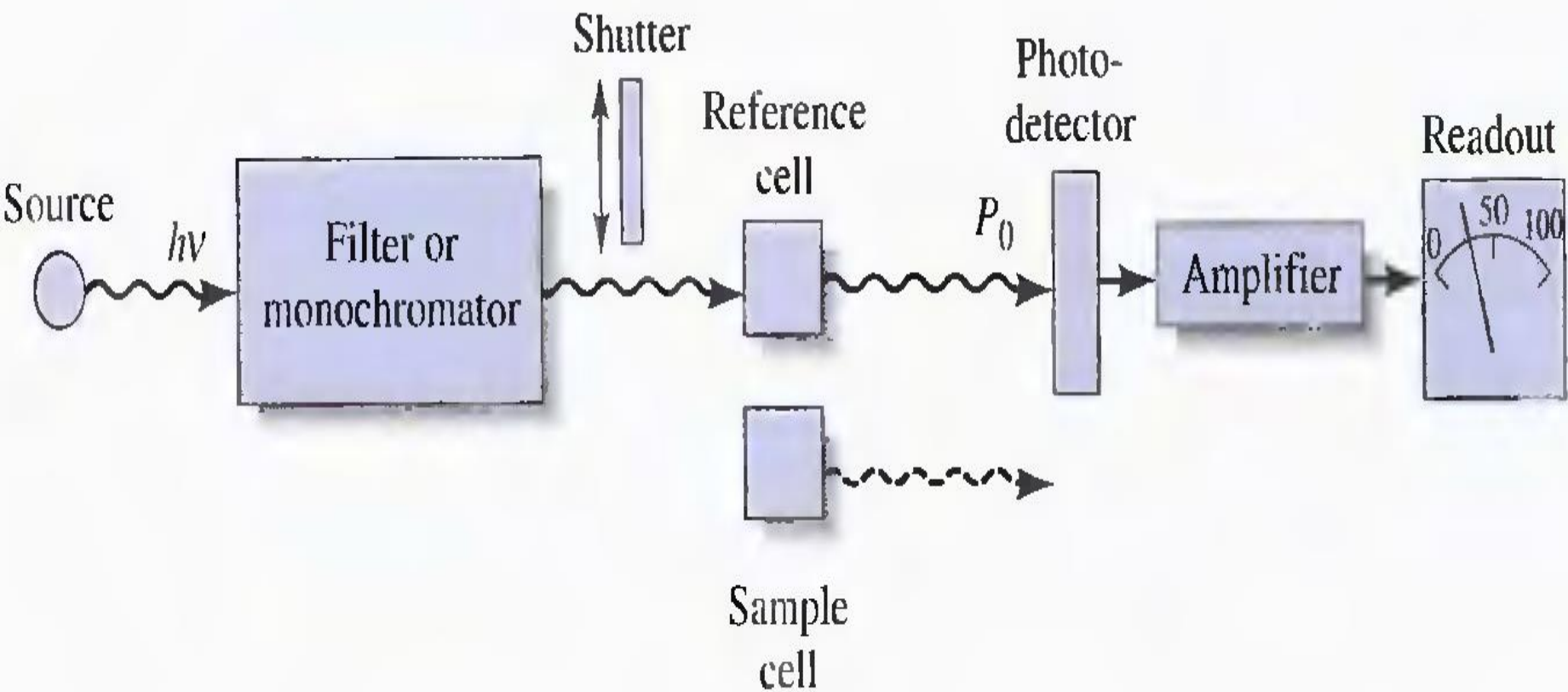
narrow band of wavelengths from the source spectrum, (3) a sample cell, (4) a **detector**, or transducer, for converting radiant energy into electrical energy, and (5) a device to read out the response of the detector. The sample may precede or follow the monochromator. Each of these, except the readout device, will vary depending on the wavelength region.

Sources for:

Vis—incandescent lamp

UV— $H_2$  or  $D_2$  discharge tube

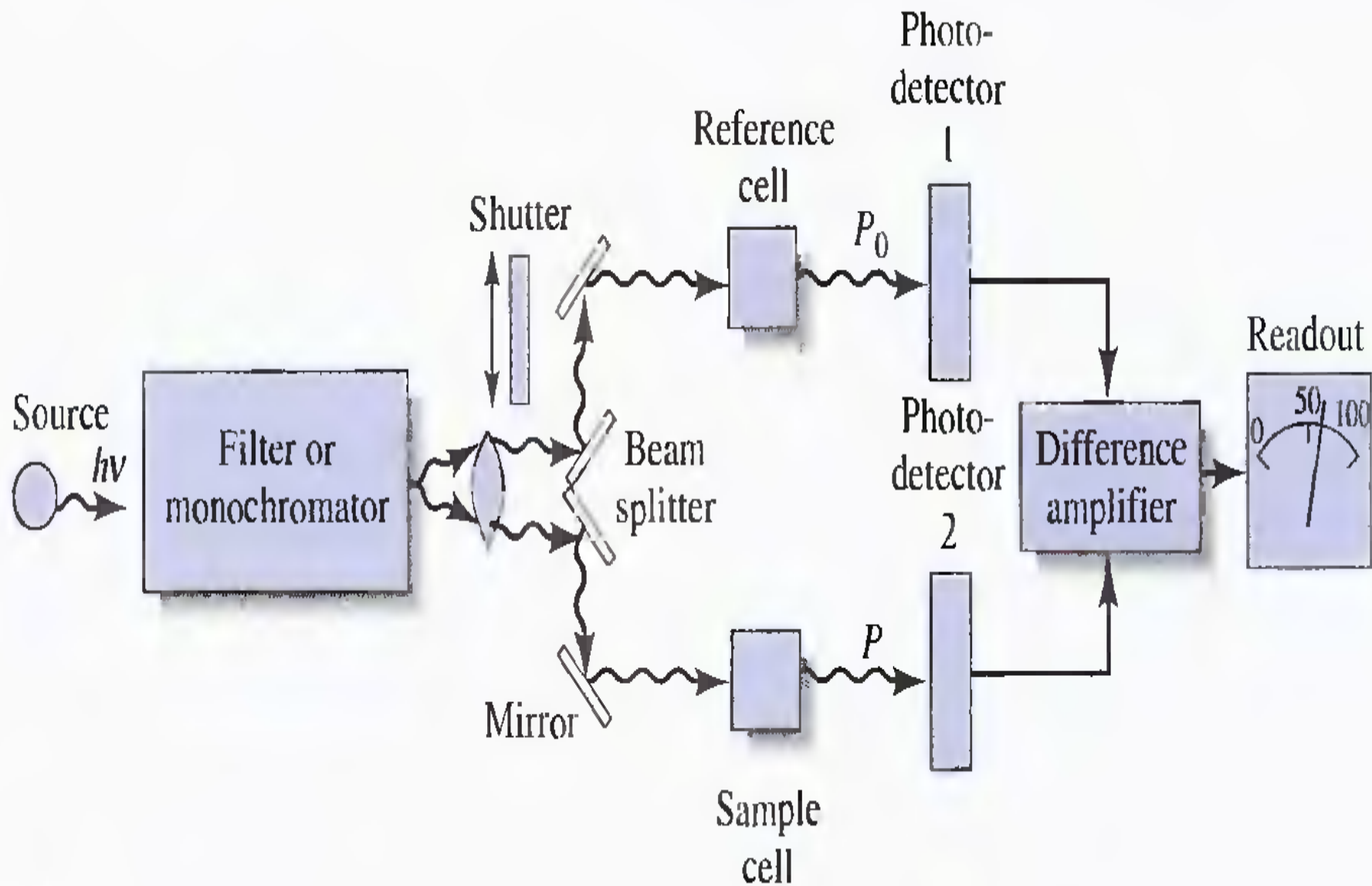
IR—rare earth oxide or silicon  
carbide glowers



(a)

**CD-ROM Exercise:**

Exploration of How Single and Double Beam Absorption Spectrophotometers Work.



Thank for your attention