Chapter 2
Ultraviolet and visible spectroscopy
Molecular Spectrophotometry

- Properties of light
- Electromagnetic radiation and electromagnetic spectrum
- Absorption of light
- Beer’s law
- Limitation of Beer’s law
- Absorption of light by molecules
- Instrumentation: Spectrophotometer
- Applications: Individual species and mixtures
- Spectrophotometric titration (up to p.524 in the notes)
Spectrophotometry

• It refers to the use of light (electromagnetic radiation) to measure chemical concentrations.

• Mainly, the fundamental principles of absorption and emission of radiation by molecules or atoms and how these processes are used in quantitative analysis will be discussed.
Electromagnetic radiation

• Electromagnetic radiation or light, is a form of energy whose behavior is described by the properties of both waves and particles.

• The optical properties of electromagnetic radiation, such as diffraction and dispersion, are explained best by describing light as a wave.

• Many of the interactions between electromagnetic radiation and matter, such as absorption and emission, are better described by treating light as a particle, or photon.
• **Wave Properties of EMR** consists of oscillating electric and magnetic fields that propagate through space along a linear path and with a constant velocity.

• Oscillations in the electric and magnetic fields are perpendicular to each other, and to the direction of the wave's propagation.

Plane polarized electromagnetic radiation showing the electric field, the magnetic field and the direction of propagation.
• In a vacuum, EMR travels at the speed of light, c, which is $2.99792 \times 10^8$ m/s.

• EMR moves through a medium other than a vacuum with a velocity, v, less than that of the speed of light in a vacuum.

• The difference between v and c is small enough (< 0.1%) that the speed of light to three significant figures, $3.00 \times 10^8$ m/s, is sufficiently accurate for most purposes.
Characteristics **electromagnetic wave**

- The interaction of EMR with matter can be explained using either the electric field or the magnetic field.
- Only the electric field component will be used to discuss this matter.
- An electromagnetic wave is characterized by several fundamental properties, including its **velocity, amplitude, frequency, phase angle, polarization, and direction of propagation**.
• The interaction of EMR with matter can be explained using either the electric field or the magnetic field.

• Only the electric field component will be used to discuss this matter.

\[ A_e \] is the electric field maximum amplitude.

\[ \lambda \] is the distance between successive maxima or successive minima.
• **Frequency**, \( \nu \), is the number of oscillations in the electric field per unit time. **One oscillation/sec = one hertz (HZ)**

• **The wavelength** of an electromagnetic wave, \( \lambda \), is defined as the distance between successive maxima, or successive minima.

• For **ultraviolet and visible electromagnetic radiation** the wavelength is usually expressed in nanometers (\( \text{nm, } 10^{-9} \text{ m} \))

• The wavelength for infrared radiation is given in microns (\( \mu \text{m, } 10^{-6} \text{ m} \)).

• Wavelength depends on the electromagnetic wave's velocity, where

\[ \lambda = \frac{c}{\nu} = \frac{v}{\nu} \quad (\text{in vacuum}) \]

• **Wave number**: \( \bar{\nu} = \frac{1}{\lambda} \)
Power and Intensity of light

- Power, $P$, and Intensity, $I$, of light give the flux of energy from a source of EMR.
- $P$ is the flux of energy per unit time.
- $I$ is the flux of energy per unit time per area.
Particle Properties of Electromagnetic Radiation

• When a sample absorbs electromagnetic radiation it undergoes a change in energy.
• The interaction between the sample and the electromagnetic radiation is easiest to understand if we assume that:
  – electromagnetic radiation consists of a beam of energetic particles (packets of energy) called photons.
• When a photon is absorbed by a sample, it is "destroyed," and its energy is acquired by the sample.
Particle Properties of Electromagnetic Radiation

The energy of a photon, in joules, is related to its frequency, wavelength, or wavenumber by the following equations:

\[ E = h \nu = \frac{hc}{\lambda} = hc \nu \]

h is Planck's constant, which has a value of 6.626 \times 10^{-34} \text{ J} \cdot \text{s}. 

Electromagnetic Spectrum

- The spectrum is the written records of the EMR.

- EMR is divided into different regions based on the type of atomic or molecular transition that gives rise to the absorption or emission of photons.

- The boundaries describing the electromagnetic spectrum are not rigid, and an overlap between spectral regions is possible.
The Visible Spectrum

infrared light

700  600  500  400

Wavelength (nm)

ultraviolet light

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Wavelengths, Frequencies, and Energies of Some Regions of the Electromagnetic Spectrum

Visible light

<table>
<thead>
<tr>
<th>Region</th>
<th>Wavelength (m)</th>
<th>Frequency (Hz)</th>
<th>Energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-rays</td>
<td>$3 \times 10^{-16}$</td>
<td>$10^{24}$</td>
<td>$10^{13}$</td>
</tr>
<tr>
<td>X-rays</td>
<td>$3 \times 10^{-14}$</td>
<td>$10^{22}$</td>
<td>$10^{11}$</td>
</tr>
<tr>
<td>UV</td>
<td>$3 \times 10^{-12}$</td>
<td>$10^{20}$</td>
<td>$10^{9}$</td>
</tr>
<tr>
<td>Infrared</td>
<td>$3 \times 10^{-10}$</td>
<td>$10^{18}$</td>
<td>$10^{7}$</td>
</tr>
<tr>
<td>Microwaves</td>
<td>$3 \times 10^{-8}$</td>
<td>$10^{16}$</td>
<td>$10^{5}$</td>
</tr>
<tr>
<td>FM</td>
<td>$3 \times 10^{-4}$</td>
<td>$10^{14}$</td>
<td>$10^{3}$</td>
</tr>
<tr>
<td>AM</td>
<td>$3 \times 10^{-2}$</td>
<td>$10^{12}$</td>
<td>$10^{1}$</td>
</tr>
<tr>
<td>Long radio waves</td>
<td>$3 \times 10^{-1}$</td>
<td>$10^{10}$</td>
<td>$10^{-1}$</td>
</tr>
<tr>
<td>Long radio waves</td>
<td>$3 \times 10^{-3}$</td>
<td>$10^{8}$</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>Long radio waves</td>
<td>$3 \times 10^{-5}$</td>
<td>$10^{6}$</td>
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<td>Long radio waves</td>
<td>$3 \times 10^{-7}$</td>
<td>$10^{4}$</td>
<td>$10^{-7}$</td>
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<tr>
<td>Long radio waves</td>
<td>$3 \times 10^{-9}$</td>
<td>$10^{2}$</td>
<td>$10^{-9}$</td>
</tr>
<tr>
<td>Long radio waves</td>
<td>$3 \times 10^{-11}$</td>
<td>$10$</td>
<td>$10^{-11}$</td>
</tr>
</tbody>
</table>
The diagram illustrates the relationship between wavelength, frequency, type of transition, and spectral region.

- **Wavelength (m)**: The horizontal axis shows wavelength in meters, ranging from $10^{-14}$ to $10^2$.
- **Frequency (s$^{-1}$)**: The vertical axis shows frequency in cycles per second, ranging from $10^{22}$ to $10^8$.
- **Type of transition**:
  - Nuclear
  - Core-level electrons
  - Valence electrons
  - Molecular vibrations
  - Molecular rotations; electron spin
  - Nuclear spin
- **Spectral region**:
  - $\gamma$-ray
  - X-ray
  - UV
  - IR
  - Microwave
  - Radio wave

The visible spectrum is highlighted in the middle, with wavelengths ranging from 380 nm (Violet) to 780 nm (Red).
### Colors of the visible light

<table>
<thead>
<tr>
<th>$\lambda$ of maximum absorption (nm)</th>
<th>Color absorbed</th>
<th>Color observed</th>
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</thead>
<tbody>
<tr>
<td>380-420</td>
<td>Violet</td>
<td>Green-yellow</td>
</tr>
<tr>
<td>420-440</td>
<td>Violet-blue</td>
<td>Yellow</td>
</tr>
<tr>
<td>440-470</td>
<td>Blue</td>
<td>Orange</td>
</tr>
<tr>
<td>470-500</td>
<td>Blue-green</td>
<td>Red</td>
</tr>
<tr>
<td>500-520</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>520-550</td>
<td>Yellow-green</td>
<td>Violet</td>
</tr>
<tr>
<td>550-580</td>
<td>Yellow</td>
<td>Violet-blue</td>
</tr>
<tr>
<td>580-620</td>
<td>Orange</td>
<td>Blue</td>
</tr>
<tr>
<td>620-680</td>
<td>Red</td>
<td>Blue-green</td>
</tr>
<tr>
<td>680-780</td>
<td>Purple</td>
<td>Green</td>
</tr>
</tbody>
</table>
Measuring Photons as a Signal

- Spectroscopy is divided into two broad classes:
  1. Energy is transferred between a photon of electromagnetic radiation and the analyte (Absorption or Emission of radiation)
  2. Changes in electromagnetic radiation wave characteristics (changes in amplitude, phase angle, polarization, or direction of propagation).

Class 1: Absorption of radiation

- In absorption spectroscopy the energy carried by a photon is absorbed by the analyte, promoting the analyte from a lower-energy state (Ground state) to a higher-energy, (or excited) state.
- Absorbing a photon of visible light causes a valence electron in the analyte to move to a higher-energy level.
- When an analyte absorbs infrared radiation one of its chemical bonds experiences a change in vibrational energy.
• The intensity of photons passing through a sample containing the analyte is attenuated because of absorption.
• The measurement of this attenuation, which we call absorbance,
• The energy levels have well-defined values (i.e., they are quantized).
• Absorption only occurs when the photon's energy matches the difference in energy, $\Delta E$, between two energy levels.
• A plot of absorbance as a function of the photon's energy (wavelength, $\lambda$, is called an absorbance spectrum.
Ultraviolet/visible absorption spectrum for bromothymol blue

Wavelength at which absorbance is maximum

\( \lambda_{\text{max}} \)
Class 1
Emission of Radiation

- Emission of a photon occurs when an analyte in a higher-energy state returns to a lower-energy state.
- The higher-energy state can be achieved in several ways:
  - including thermal energy, radiant energy from a photon, or by a chemical reaction.
- Emission following the absorption of a photon is also called photoluminescence, and that following a chemical reaction is called chemiluminescence.
Emission (luminescence) Spectrum

(a) Sample with incident and transmitted radiation. Emission spectrum shown with peaks at $\lambda_1$ and $\lambda_2$.

(b) Energy levels $E_{21} = h\nu_{21} = hc/\lambda_{21}$, $E_2 = h\nu_2 = hc/\lambda_2$, $E_1 = h\nu_1 = hc/\lambda_1$.

(c) Emission spectrum with peaks at $\gamma$.
Typical Emission Spectrum
Various spectroscopic techniques of class 1

<table>
<thead>
<tr>
<th>Type of Energy Transfer</th>
<th>Region of the Electromagnetic Spectrum</th>
<th>Spectroscopic Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>absorption</td>
<td>γ-ray</td>
<td>Mossbauer spectroscopy</td>
</tr>
<tr>
<td></td>
<td>X-ray</td>
<td>X-ray absorption spectroscopy</td>
</tr>
<tr>
<td></td>
<td>UV/Vis(^a)</td>
<td>UV/Vis spectroscopy(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>atomic absorption spectroscopy(^b)</td>
</tr>
<tr>
<td></td>
<td>infrared</td>
<td>infrared spectroscopy(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>raman spectroscopy</td>
</tr>
<tr>
<td></td>
<td>microwave</td>
<td>microwave spectroscopy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>electron spin resonance spectroscopy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>emission (thermal excitation)</td>
<td>UV/Vis(^a)</td>
<td>atomic emission spectroscopy(^b)</td>
</tr>
<tr>
<td>photoluminescence</td>
<td>X-ray</td>
<td>X-ray fluorescence</td>
</tr>
<tr>
<td></td>
<td>UV/Vis(^a)</td>
<td>fluorescence spectroscopy(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phosphorescence spectroscopy(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>atomic fluorescence spectroscopy</td>
</tr>
</tbody>
</table>

\(^a\)UV/Vis: ultraviolet and visible ranges.

\(^b\)Techniques discussed in this text.
Class 2
Changes in the EMR wave characteristics

• In this class of spectroscopy:
  – 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.
  – Several representative spectroscopic techniques are listed in the following table
Various spectroscopic techniques of class 2

<table>
<thead>
<tr>
<th>Region of the Electromagnetic Spectrum</th>
<th>Type of Interaction</th>
<th>Spectroscopic Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray</td>
<td>diffraction</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>UV/Vis(^a)</td>
<td>refraction</td>
<td>refractometry</td>
</tr>
<tr>
<td></td>
<td>scattering</td>
<td>nephelometry(^b)</td>
</tr>
<tr>
<td></td>
<td>dispersion</td>
<td>turbidimetry(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>optical rotary dispersion</td>
</tr>
</tbody>
</table>

\(^a\)UV/Vis: Ultraviolet and visible ranges.
\(^b\)Techniques covered in this text.
Sources of Energy

• All forms of spectroscopy require a source of energy.
• In absorption and scattering spectroscopy this energy is supplied by photons (EMR or light).
• Emission and luminescence spectroscopy use thermal, radiant (photon), or chemical energy to promote the analyte to a less stable, higher energy state.
Sources of Electromagnetic Radiation

• A source of electromagnetic radiation must provide an output that is both intense and stable in the desired region of the electromagnetic spectrum.

• Sources of electromagnetic radiation are classified as either continuum or line sources.

• A *continuum source* emits radiation over a wide range of wavelengths, with a relatively smooth variation in intensity as a function of wavelengths.

• *Line sources* emit radiation at a few selected, narrow wavelength ranges
# Common sources of EMR

<table>
<thead>
<tr>
<th>Source</th>
<th>Wavelength Region</th>
<th>Useful for</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂ and D₂ lamp</td>
<td>continuum source from 160–380 nm</td>
<td>UV molecular absorption</td>
</tr>
<tr>
<td>tungsten lamp</td>
<td>continuum source from 320–2400 nm</td>
<td>Vis molecular absorption</td>
</tr>
<tr>
<td>Xe arc lamp</td>
<td>continuum source from 200–1000 nm</td>
<td>molecular fluorescence</td>
</tr>
<tr>
<td>Nernst glower</td>
<td>continuum source from 0.4–20 μm</td>
<td>IR molecular absorption</td>
</tr>
<tr>
<td>globar</td>
<td>continuum source from 1–40 μm</td>
<td>IR molecular absorption</td>
</tr>
<tr>
<td>nichrome wire</td>
<td>continuum source from 0.75–20 μm</td>
<td>IR molecular absorption</td>
</tr>
<tr>
<td>hollow cathode lamp</td>
<td>line source in UV/Vis</td>
<td>atomic absorption</td>
</tr>
<tr>
<td>Hg vapor lamp</td>
<td>line source in UV/Vis</td>
<td>molecular fluorescence</td>
</tr>
<tr>
<td>laser</td>
<td>line source in UV/Vis</td>
<td>atomic and molecular absorption, fluorescence and scattering</td>
</tr>
</tbody>
</table>

*Abbreviations: UV: ultraviolet; Vis: visible; IR: infrared.*
Emission spectrum from a continuum emission source

Emission spectrum from a typical line source
Absorbance of Electromagnetic Radiation

• In absorption spectroscopy a beam of electromagnetic radiation passes through a sample.
• Much of the radiation is transmitted without a loss in intensity.
• At selected wavelengths the radiation's intensity is attenuated.
• The process of attenuation is called absorption.
• Two general requirements must be met if an analyte is to absorb electromagnetic radiation.
  – **The first requirement** is that there must be a mechanism by which the radiation's electric field or magnetic field interacts with the analyte.
  – For ultraviolet and visible radiation, this interaction involves the electronic energy of valence electrons.
  – A chemical bond's vibrational energy is altered by the absorbance of infrared radiation.
• The **second requirement** is that the energy of the electromagnetic radiation must exactly equal the difference in energy, \( AE \), between two of the analytes quantized energy states.
Molecular Orbital (MO) Theory Review

**MO Theory:** Electrons in atoms exist in atomic orbitals while electrons in molecules exist in molecular orbitals.

**Bonding MO:** A MO where electrons have a lower energy than they would in isolated atomic orbitals.

**Anitbonding MO:** A MO in which electrons have a higher energy than they would in isolated atomic orbitals.

**Ground State:** Refers to the state of lowest energy. Electrons can be promoted from a ground state to a higher excited state by input of energy.

**Excited State:** Any electronic state other than the ground state.
Relative Energies of Molecular Orbitals

- Compounds containing only sigma bonds have absorptions only in the ultraviolet.
- These transitions correspond to $\sigma$-$\sigma^*$
- $n$-$\sigma^*$ transitions are common
- Compare the energy of $n$-$\sigma^*$ vs a $\sigma$-$\sigma^*$
<table>
<thead>
<tr>
<th>Transition</th>
<th>Region of Electronic Spectra</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma \rightarrow \sigma^*$</td>
<td>Vacuum ultraviolet</td>
<td>$\text{CH}_4$ at 125 nm</td>
</tr>
<tr>
<td>$n \rightarrow \sigma^*$</td>
<td>Far-ultraviolet, sometimes near-ultraviolet</td>
<td>Acetone at 190 nm; methylamine at 213 nm</td>
</tr>
<tr>
<td>$\pi \rightarrow \pi^*$</td>
<td>Ultraviolet</td>
<td>Saturated aldehydes at 180 nm</td>
</tr>
<tr>
<td>$n \rightarrow \pi^*$</td>
<td>Near-ultraviolet and visible</td>
<td>Acetone at 277 nm; nitroso-$t$-butane at 665 nm</td>
</tr>
</tbody>
</table>
Electronic transition in Formaldehyde
Example of Electronic Transitions: Absorptions

Formaldehyde

Contains both \( \pi \) and nonbonding electrons (\( n \))
Molecular Absorption

- Molecules undergo three types of quantized transitions when excited by ultraviolet, visible, and infrared radiation.

1. **Electronic transition**
   - The transition of an electron between two orbitals (the energy by the photon must be exactly the same as the energy difference between the two orbital energies) and the absorption process is called *electronic absorption*
In electronic transition, an electron from one molecular orbital moves to another orbital with an increase or decrease in the energy of the molecule.

The lowest energy electronic transition in formaldehyde involves the promotion of a non-bonding (n) electron to the anti-bonding $\pi^*$ orbital.
Singlet state and triplet state

**Singlet state**: the state in which the spins are opposed

![Singlet state diagram](image)

S\(_1\), \(\lambda\)355, UV

In general \(T_1\) is of lower energy than \(S_1\)

**Singlet state**: the state in which the spins are paired

![Singlet state diagram](image)

\(T_1\), \(\lambda\)397, visible
2. vibrational and rotational transitions

- Vibration of the atoms of the molecule with respect to one another;
- Atoms and groups of atoms within molecules can undergo various types of vibrations and each requires a discrete amount of energy to initiate or maintain.
- Also molecules can rotate around their axes a matter that requires discrete amount of energy.
Various Types of Vibrations

(a) Stretching vibrations

In-plane rocking

In-plane scissoring

(b) Bending vibrations

Out-of-plane wagging

Out-of-plane twisting
Vibrations of formaldehyde
• Thus each molecular energy state is comprised of an electronic, vibrational and rotational component such that:

\[
E_{\text{total}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}}
\]

• \(E_{\text{electronic}} > E_{\text{vibrational}} > E_{\text{rotational}}\)
Energy of a Molecule

\[ E_{\text{molecule}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}} + E_{\text{spin}} + E_{\text{translational}} \]

Our Focus

- \( E_{\text{electronic}} \) (UV/Vis)
- \( E_{\text{vibrational}} \) (IR)
Energy of a Molecule

\( E_{ \text{electronic} } \rightarrow 10^5 - 10^6 \text{ kJ/mole} \rightarrow \text{UV-Vis} \)

UV-Vis range: 200 - 700 nm

- \( E_{ \text{vibrational} } \rightarrow 10 - 40 \text{ kJ/mole} \rightarrow \text{IR} \)

Near IR: 800 - 2500 nm (5000 nm)
Mid-IR: 5000 nm - 25,000 nm (5 microns - 25 microns)

\( E_{ \text{rotational} } \rightarrow 10 \text{ kJ/mole} \rightarrow \text{microwaves} \)

\( E_{ \text{spin} } \rightarrow 10^{-3} \text{ J/mole} \rightarrow \text{Radiofrequency} \)

\( E_{ \text{translational} } \rightarrow \text{continuous} \)
Electronic transitions

(a) Molecular absorption
(b) Nonradiative relaxation
(c) Fluorescence

Resonance fluorescence

Band 1  Band 2
What happens to the absorbed energy?
• Internal Conversion (IC)
  Radiationless transition between states with same spin quantum numbers (\( S_1 \rightarrow S_0 \))

• Intersystem Crossing (ISC)
  Radiationless transition between states with different spin quantum numbers (\( S_1 \rightarrow T_1 \))

• Fluorescence
  Radiation transition between states with the same spin quantum number (\( S_1 \rightarrow S_0 \))

• Phosphorescence
  Radiation transition between states with different spin quantum number (\( T_1 \rightarrow S_0 \))
Combined electronic, vibrational, and rotational transitions

- When a molecule absorbs light having sufficient energy to cause an electronic transition, vibrational and rotational transitions—that is, changes in the vibrational and rotational states—can occur as well.

- The reason why electronic absorption bands are usually very broad is that many different vibrational and rotational levels are available at slightly different energies. Therefore, a molecule could absorb photons with a fairly wide range of energies and still be promoted from the ground electronic state to one particular excited electronic state.
Absorption of Light

Beer’s Law

\[ T = \frac{P}{P_0} \]
Beer’s Law

\[ P_0 = 10,000 \quad \text{and} \quad P = 5,000 \]

\[ T = \frac{P}{P_0} = \frac{5000}{10000} = 0.5 \]
Beer’s Law

\[ P_0 = 10,000 \]
\[ P = 2,500 \]

\[ T = \frac{P}{P_0} = \frac{2500}{10000} = 0.25 \]
Beer’s Law

\[ P_0 = 10,000 \]

\[ P = 1,250 \]

\[ T = \frac{P}{P_0} = \frac{1250}{10000} = 0.125 \]
Beer’s Law

\[ P_0 = 10,000 \]

\[ P = 625 \]

\[ T = \frac{P}{P_0} = \frac{625}{10000} = 0.0625 \]
Relationship between transmittance and cell thickness

<table>
<thead>
<tr>
<th>Thickness, ( b )</th>
<th>Transmittance, ( T )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
<td>1</td>
<td>0.5</td>
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<td>10</td>
<td>0.0009765625</td>
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</table>

\[
T = \frac{P}{P_0}
\]
### Relationship between absorbance and cell thickness

\[ A = -\log T = -\log \frac{P}{P_0} \]

\[ A = abc \]

<table>
<thead>
<tr>
<th>Thickness, b</th>
<th>Transmittance, T</th>
<th>( A = -\log T )</th>
</tr>
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<td>2.709</td>
</tr>
<tr>
<td>10</td>
<td>0.000976563</td>
<td>3.010</td>
</tr>
</tbody>
</table>

\( A = \text{absorbance} \)

\( a = \text{absorptivity} \)

\( b = \text{thickness} \)

\( c = \text{concentration} \)
Relation between Absorbance and Transmittance

Calculation of absorbance from transmittance

<table>
<thead>
<tr>
<th>T</th>
<th>%T</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0.000</td>
</tr>
<tr>
<td>0.9</td>
<td>90</td>
<td>0.046</td>
</tr>
<tr>
<td>0.8</td>
<td>80</td>
<td>0.097</td>
</tr>
<tr>
<td>0.7</td>
<td>70</td>
<td>0.155</td>
</tr>
<tr>
<td>0.6</td>
<td>60</td>
<td>0.222</td>
</tr>
<tr>
<td>0.5</td>
<td>50</td>
<td>0.301</td>
</tr>
<tr>
<td>0.4</td>
<td>40</td>
<td>0.398</td>
</tr>
<tr>
<td>0.3</td>
<td>30</td>
<td>0.523</td>
</tr>
<tr>
<td>0.2</td>
<td>20</td>
<td>0.699</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>1.000</td>
</tr>
<tr>
<td>0.075</td>
<td>7.5</td>
<td>1.125</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>1.301</td>
</tr>
<tr>
<td>0.01</td>
<td>1</td>
<td>2.000</td>
</tr>
<tr>
<td>0.001</td>
<td>0.1</td>
<td>3.000</td>
</tr>
</tbody>
</table>

Cell B3=100*A3
Cell C3=-log(A3)

\[ A = - \log T = abc \]
## Table 7.2. Spectrophotometry Nomenclature

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Definition</th>
<th>Name Not Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>$A$</td>
<td>$-\log T$</td>
<td>Optical density (O.D.), extinction, absorbancy</td>
</tr>
<tr>
<td>Absorptivity</td>
<td>$a$</td>
<td>$= A/bc^a$</td>
<td>Absorbancy index, absorbing index, extinction coefficient</td>
</tr>
<tr>
<td>Path length</td>
<td>$b$</td>
<td>Internal cell or sample length, in cm</td>
<td>$l$ or $d$</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>$\epsilon$</td>
<td>$= A/bc^b$</td>
<td>Molar absorbancy index, molar extinction coefficient, molar absorption coefficient</td>
</tr>
<tr>
<td>Transmittance</td>
<td>$T$</td>
<td>$I/I_0^c$</td>
<td>Transmittancy, transmission $m\mu$ (millimicron), $\mu$ (micron)</td>
</tr>
<tr>
<td>Wavelength unit</td>
<td>nm</td>
<td>$10^{-9}$ m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\mu$m</td>
<td>$10^{-6}$ m</td>
<td></td>
</tr>
<tr>
<td>Absorption maximum</td>
<td>$\lambda_{max}$</td>
<td>Wavelength at which a maximum absorption occurs</td>
<td></td>
</tr>
</tbody>
</table>

a. The concentration is in grams per liter.
b. The concentration is in moles per liter.
c. The ratio of radiant power transmitted to radiant power incident.
<table>
<thead>
<tr>
<th>Term and Symbol*</th>
<th>Definition</th>
<th>Alternative Name and Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiant power $P, P_0$</td>
<td>Energy of radiation (in ergs) impinging on a 1-cm$^2$ area of a detector per second</td>
<td>Radiation intensity $I, I_0$</td>
</tr>
<tr>
<td>Absorbance $A$</td>
<td>$\log \frac{P_0}{P}$</td>
<td>Optical density $D$; extinction $E$</td>
</tr>
<tr>
<td>Transmittance $T$</td>
<td>$\frac{P}{P_0}$</td>
<td>Transmission $T$</td>
</tr>
<tr>
<td>Path length of radiation† $b$</td>
<td>—</td>
<td>$l, d$</td>
</tr>
<tr>
<td>Absorptivity† $a$</td>
<td>$\frac{A}{bc}$</td>
<td>Extinction coefficient $k$</td>
</tr>
<tr>
<td>Molar absorptivity‡ $\epsilon$</td>
<td>$\frac{A}{bc}$</td>
<td>Molar extinction coefficient</td>
</tr>
</tbody>
</table>

† May be expressed in g/L, or in other specified concentration units; $b$ may be expressed in cm or in other units of length.
‡ $a$ is expressed in mol/L; $b$ is expressed in cm.
Absorbance and Transmittance Spectra

% Transmission Spectrum
Absorbance Spectrum

\[ A = -\log T = abc \]
Absorbance Spectra and Concentration

Absorbance Spectra

\[ A = -\log T = abc \]
Absorbance and Concentration: Beer's Law

- When monochromatic EMR passes through an infinitesimally thin layer of sample, of thickness $dx$, it experiences a decrease in power of $dP$.
- The fractional decrease in power is proportional to the sample's thickness and the analyte's concentration, $C$. 

![Diagram showing the decrease in power of EMR through a sample]
Thus,

\[
\frac{-dP}{P} = \alpha C \, dx
\]

- where \( P \) is the power incident on the thin layer of sample,
  and \( \alpha \) is a proportionality constant.
- Integrating the left side of equation from \( P = P_o \) to \( P = P_T \),
  and the right side from \( x = 0 \) to \( x = b \), where \( b \) is the sample's overall thickness,

\[
- \int_{P=P_0}^{P=P_T} \frac{dP}{P} = \alpha C \int_{x=0}^{x=b} dx
\]

gives

\[
\ln \left( \frac{P_0}{P_T} \right) = \alpha b C
\]
• Converting from ln to log and substituting \( \log \frac{p_o}{p_T} \) by \( A \) (absorbance) gives

\[
A = abC
\]

Where \( a \) is the analyte absorptivity with units of \( \text{cm}^{-1}\text{conc}^{-1} \).

• When concentration is expressed using molarity the absorptivity is replaced by molar absorptivity.

• The absorptivity and molar absorptivity give, in effect, the probability that the analyte will absorb a photon of given energy.

• As a result, values for both \( a \) and \( \varepsilon \) depend on the wavelength of electromagnetic radiation.
### Predicting Concentrations from Absorbance Spectra

<table>
<thead>
<tr>
<th>Conc, Micro-M</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.00</td>
<td>0.162</td>
</tr>
<tr>
<td>60.00</td>
<td>0.330</td>
</tr>
<tr>
<td>90.00</td>
<td>0.499</td>
</tr>
<tr>
<td>120.00</td>
<td>0.660</td>
</tr>
<tr>
<td>150.00</td>
<td>0.840</td>
</tr>
<tr>
<td>unknown</td>
<td>0.539</td>
</tr>
</tbody>
</table>

**Regression equation**

- **slope**: 0.00562
- **Intercept**: -0.0076
- **Conc of unknown**: 97.25978648
Absorption Spectra of Mixtures Containing $n$ components

\[
A_{\lambda_1} = a_{\lambda_1} bc_1 + a_{\lambda_1} bc_2 + a_{\lambda_1} bc_3 + \cdots + a_{\lambda_1} bc_n
\]

\[
A_{\lambda_2} = a_{\lambda_2} bc_1 + a_{\lambda_2} bc_2 + a_{\lambda_2} bc_3 + \cdots + a_{\lambda_2} bc_n
\]

\[
\cdots \cdots
\]

\[
A_{\lambda_n} = a_{\lambda_n} bc_1 + a_{\lambda_n} bc_2 + a_{\lambda_n} bc_3 + \cdots + a_{\lambda_n} bc_n
\]
Absorption Spectra of Mixtures
Containing n components
Constant pathlength

\[ A_{\lambda_1} = k_{\lambda_1} c_1 + k_{\lambda_1} c_2 + k_{\lambda_1} c_3 + \cdots + k_{\lambda_1} c_n \]

\[ A_{\lambda_2} = k_{\lambda_2} c_1 + k_{\lambda_2} c_2 + k_{\lambda_2} c_3 + \cdots + k_{\lambda_2} c_n \]

\[ \vdots \]

\[ A_{\lambda_n} = k_{\lambda_n} c_1 + k_{\lambda_n} c_2 + k_{\lambda_n} c_3 + \cdots + k_{\lambda_n} c_n \]
Limitations to Beer’s Law

• Ideally, according to Beer's law, a calibration curve of absorbance versus the concentration of analyte in a series of standard solutions should be a straight line with an intercept of 0 and a slope of $ab$ or $\varepsilon b$.

• In many cases, calibration curves are found to be nonlinear.

• Deviations from linearity are divided into three categories: fundamental, chemical, and instrumental.
Fundamental Limitations to Beers Law Beer's law

- Beer’s law is a limiting law that is valid only for low concentrations of analyte.

  1. At higher concentrations the individual particles of analyte no longer behave independently of one another.
     - The resulting interaction between particles of analyte may change the value of \( a \) or \( \varepsilon \).

  2. The absorptivity, \( a \), and molar absorptivity, \( \varepsilon \), depend on the sample's refractive index.
     - Since the refractive index varies with the analyte's concentration, the values of \( a \) and \( \varepsilon \) will change.
     - For sufficiently low concentrations of analyte, the refractive index remains essentially constant, and the calibration curve is linear.
Chemical Limitations to Beer's Law

- Chemical deviations from Beer's law can occur when the absorbing species is involved in an equilibrium reaction.
- Consider, as an example, the weak acid, $\text{HA}$.
- To construct a Beer's law calibration curve, several standards containing known total concentrations of $\text{HA}$, $C_{\text{tot}}$, are prepared and the absorbance of each is measured at the same wavelength.
- Since $\text{HA}$ is a weak acid, it exists in equilibrium with its conjugate weak base, $\text{A}^-$.

\[
\text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{A}^-
\]
• If both HA and A\(^{-}\) absorb at the selected wavelength, then Beer’s law is written as

\[
A = \varepsilon_{HA} b C_{HA} + \varepsilon_{A} b C_{A}
\]

where \(C_{HA}\) and \(C_{A}\) are the equilibrium concentrations of HA and A\(^{-}\). Since the weak acid's total concentration, \(C_{\text{tot}}\), is

\[
C_{\text{tot}} = C_{HA} + C_{A}
\]

The concentration of HA and A\(^{-}\) can be written as

\[
C_{HA} = \alpha_{HA} C_{\text{tot}}
\]

\[
C_{A} = (1 - \alpha_{HA}) C_{\text{tot}}
\]

Where \(\alpha_{HA}\) is the fraction of weak acid present as HA
Because values of $\alpha_{HA}$ may depend on the concentration of HA, equation may not be linear. A Beer's law calibration curve of $A$ versus $C_{tot}$ will be linear if one of two conditions is met.

1. If the wavelength is chosen such that $\varepsilon_{HA}$ and $\varepsilon_A$ are equal, then equation simplifies to

$$A = \varepsilon \ b \ C_{tot}$$

and a linear curve is realized.
2. Alternatively, if $\alpha_{HA}$ is held constant for all standards, then equation will be a straight line at all wavelengths.

- Because HA is a weak acid, values of $\alpha_{HA}$ change with pH.
- To maintain a constant value for $\alpha_{HA}$, therefore, we need to buffer each standard solution to the same pH.
- Depending on the relative values of $\varepsilon_{HA}$ and $\varepsilon_A$, the calibration curve will show a positive or negative deviation from Beer’s law if the standards are not buffered to the same pH.
Instrumental Limitations to Beer's Law

• There are two principal instrumental limitations to Beer's law.

1. Beer’s law is strictly valid for purely monochromatic radiation; that is, for radiation consisting of only one wavelength.
   – even the best wavelength selector passes radiation with a small, but finite effective bandwidth.
   – Using polychromatic radiation always gives a negative deviation from Beer's law, but is minimized if the value of $\varepsilon$ is essentially constant over the wavelength range passed by the wavelength selector.
   – For this reason, it is preferable to make absorbance measurements at a broad absorption peak.
Effect of wavelength on the linearity of a Beer’s law calibration curve
2. Stray Radiation

- Stray radiation arises from imperfections within the wavelength selector that allows extraneous light to "leak" into the instrument.
- Stray radiation adds an additional contribution, $P_{\text{stray}}$, to the radiant power reaching the detector; thus

$$A = \log \frac{P_0 + P_{\text{stray}}}{P_T + P_{\text{stray}}}$$

- For small concentrations of analyte, $P_{\text{stray}}$ is significantly smaller than $P_0$ and $P_T$, and the absorbance is unaffected by the stray radiation.
- At higher concentrations of analyte, $P_{\text{stray}}$ 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.
Instrument Designs for Molecular UV/Vis Absorption

Filter Photometer

- Molecular UV/Vis absorption is measured using an absorption or interference filter to isolate a band of radiation.
- The filter is placed between the source and sample to prevent the sample from decomposing when exposed to high-energy radiation.
- A filter photometer has a single optical path between the source and detector and is called a single-beam instrument.
- The instrument is calibrated to 0% T while using a shutter to block the source radiation from the detector.
- After removing the shutter, the instrument is calibrated to 100% T using an appropriate blank.
• In comparison with other spectroscopic instruments, photometers have the advantage of being relatively inexpensive, rugged, and easy to maintain. Another advantage of a photometer is its portability, making it a useful instrument for conducting spectroscopic analyses in the field.

• A disadvantage of a photometer is that it cannot be used to obtain an absorption spectrum.
Spectrometer/spectrophotometer

- The simplest spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator.
- Single-beam spectrophotometers are calibrated and used in the same manner as a photometer.
- One common example of a single-beam spectrophotometer is the Spectronic-20.
- It has a fixed effective bandwidth of 20 nm.
- Because its effective bandwidth is fairly large, this instrument is more appropriate for a quantitative analysis than for a qualitative analysis.
- Other single-beam spectrophotometers are available with effective band-widths of 2-3 nm.
- Fixed-wavelength single-beam spectrophotometers are not practical for recording spectra since manually adjusting the wavelength and re-calibrating the spectrophotometer is time-consuming.
Block diagram for a single beam fixed wavelength spectrophotometer
Figure 13-7  (a) The Spectronic 20 spectrophotometer and (b) its optical diagram.  (Courtesy of Spectronic Instruments, Inc., Rochester, NY.)
Double-beam spectrophotometer
Double-beam spectrophotometer

• A chopper is used to control the radiation's path, alternating it between the sample, the blank, and a shutter.
• The signal processor uses the chopper's known speed of rotation to resolve the signal reaching the detector into that due to the transmission of the blank ($P_o$) and the sample ($P_T$).
• By including an opaque surface as a shutter it is possible to continuously adjust the 0% T response of the detector.
• The effective bandwidth of a double-beam spectrophotometer is controlled by means of adjustable slits at the entrance and exit of the monochromator.
• Effective band-widths of between 0.2 nm and 3.0 nm are common.
• A scanning monochromator allows for the automated recording of spectra.
• Double-beam instruments are useful for both quantitative and qualitative analyses.
• Previous designs use only one detector and can monitor a single wavelength at a time.
• A linear photodiode array consists of multiple detectors, or channels, allowing an entire spectrum to be recorded in as little as 0.1 s.
• The Source radiation passing through the sample is dispersed by a grating.
• The linear photodiode array is situated at the grating’s focal plane, with each diode recording the radiant power over a narrow range of wavelengths.
Sample Compartment (Cell)

- The sample compartment for the instruments provides a light-tight environment that prevents the loss of radiation, as well as the addition of stray radiation.
- Samples are normally in the liquid or solution state and are placed in cells constructed with UV/Vis-transparent materials, such as quartz, glass, and plastic.
- Quartz or fused-silica cells are required when working at wavelengths of less than 300 nm where other materials show a significant absorption.
- The most common cell has a pathlength of 1 cm, although cells with shorter (>1 mm) and longer pathlengths (<10 cm) are available.
- Cells with a longer pathlength are useful for the analysis of very dilute solutions or for gaseous samples.
Typical Uv/Vis Cells

- The highest quality cells are constructed in a rectangular shape, allowing the radiation to strike the cell at a 90° angle, where losses to reflection are minimal.
- These cells, which are usually available in matched pairs having identical optical properties, are the cells of choice for double-beam instruments.
Fiber optic probes

• In some circumstances it is desirable to monitor a system without physically removing a sample for analysis. This is often the case, for example, with the on-line monitoring of industrial production lines or waste lines,

• With the use of a fiber-optic probe it is possible to analyze samples in situ.

• A simple example of a remote-sensing, fiber-optic probe is shown in the Figure and consists of two bundles of fiber-optic cable.

• One bundle transmits radiation from the source to the sample cell, which is designed to allow for the easy flow of sample through it.

• Radiation from the source passes through the solution, where it is reflected back by a mirror.

• The second bundle of fiber-optic cable transmits the nonabsorbed radiation to the wavelength selector.
Fiber optic probes
UV and Visible Detectors

- UV and Visible Detectors work on the basis of the **photoelectric effect**: light ejects an electron from a metal surface.
- **A vacuum phototube** converts a light flux into an electrical current, and is useful for detecting high levels of light.
- **A photomultiplier** converts a single photon into a current pulse, and is useful for detecting low levels of light.
- **Photodiodes** are based on the promotion of electrons from the valence band to the conduction band of semiconductors, and are useful for detecting both high and low levels of light.
Experimental setup to show the photoelectric effect

Photo electric effect

When light shines on a metal surface, the surface emits electrons. For example, you can start a current in a circuit just by shining a light on a metal plate. Why do you think this happens?
The answer:
It is known that light is made up of electromagnetic waves, and that the waves carry energy. So if a wave of light hit an electron in one of the atoms in the metal, it might transfer enough energy to knock the electron out of its atom.

- Number of photoelectrons ejected is proportional to light intensity.
- Each metal has a different threshold frequency below which no photoelectrons are produced.
- A range of energies are produced but the maximum value depends on colour (frequency).
Photoelectric Effect

• Because metals contain free electrons they can absorb UV and visible radiation
• If the energy of the absorbed photon is greater than the work function of the metal, an electron is ejected into the vacuum
• Alkali metals are commonly used in detectors
• Mixtures of alkali metals can give $\lambda_0$ as high as 750 nm

<table>
<thead>
<tr>
<th>metal</th>
<th>Li</th>
<th>Na</th>
<th>K</th>
<th>Rb</th>
<th>Cs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\omega$</td>
<td>2.9 eV</td>
<td>2.75</td>
<td>2.3</td>
<td>2.16</td>
<td>2.14</td>
</tr>
<tr>
<td>$\lambda_0$</td>
<td>428 nm</td>
<td>451</td>
<td>539</td>
<td>574</td>
<td>579</td>
</tr>
</tbody>
</table>
Vacuum Phototube

- A metallic surface with a low work function is placed inside an evacuated tube.
- When light interacts with the metal, electrons are photo-ejected.
- By placing a 90 V electric potential between the photocathode and anode, the electrons are drawn to the anode. The resultant current is measured by a micro-ammeter.
Photomultipliers

Several electrons for each incident electron
Numerous electrons for each photon
Quartz envelope
Grill
Radiation, $h\nu$
Photoemissive cathode
Anode, $\sim 10^7$ electrons for each photon

(a)

900 V dc

900 V dc

Quartz envelope
Anode

Numbered dynodes shown above
To readout
Amplifier

Cathode
A photomultiplier is nothing more than a vacuum phototube followed by an electron multiplier.

The secondary electron emitters are called dynodes and are made from a beryllium alloy. The number of secondary electrons varies from 3 to 5. For an average of 4, the gain of the multiplier shown above is $4^{10} = 10^6$. This is a current of $1.6 \times 10^{-13}$ A per photon.