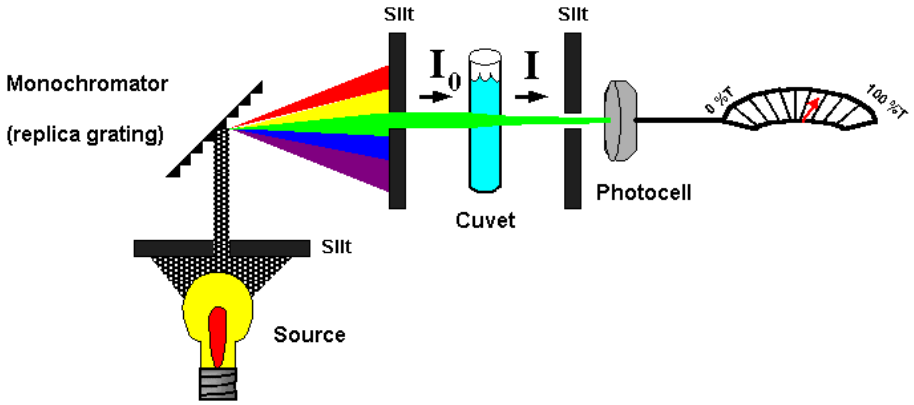
Ultra-Violet / Visible Spectroscopy- 2nd Session

Schematic Image to Understand Absorption Spectroscopy

- I_0: intensity of light incident upon sample cell
- I: intensity of light leaving sample cell



- Absorption spectroscopy measures ratio of I to I_0: $\frac{I}{I_0}$
- Transmittance = $\frac{I}{I_0}$
- Absorbance = - log Transmittance: $A = -\log T = -\log \frac{I}{I_0} = \log \frac{I_0}{I}$

Principles of Absorption Spectroscopy

- Lambert law: $I = I_0 e^{-k_1 l}$: $\frac{I}{I_0} = e^{-k_1 . l}$
- ✓ l = length of sample cell
- Beer law: $I = I_o e^{-k_2 c}$: $\frac{I}{I_0} = e^{-k_2 . c}$
- ✓ c = molar concentration of solute in sample solution
- Combination of Beer-Lambert law: $I = I_0 e^{-k_3 cl}$: $\frac{I}{I_0} = e^{-k_3 . c . l}$
- $\log_e \frac{I}{I_0} = - k_3 . c. l$
- $\log_e \frac{I_0}{I} = k_3 . c. l$
- $\log \frac{I_0}{I} = \frac{1}{2.303} k_3 . c. l$

$$A = \log(I_0/I) = \varepsilon c l$$

Beer - Lambert Law in Absorption Spectroscopy

- Combination of Beer law & Lambert law: $A = \log(I_0/I) = \varepsilon c l$

✓ ε : molar extinction coefficient = molar absorptivity: 0 to 10^6

- Assumption of Beer-Lambert law: linear correlation of Abs. to conc.

- Limitation to Beer-Lambert law:

✓ low intensity radiations

✓ scattering radiation losses: in suspension or not-transparent solutions

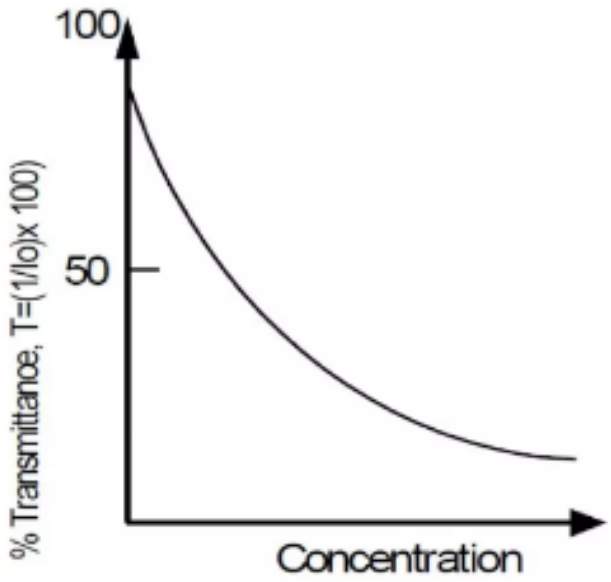
✓ reflection & interference losses: intermolecular due to conc.

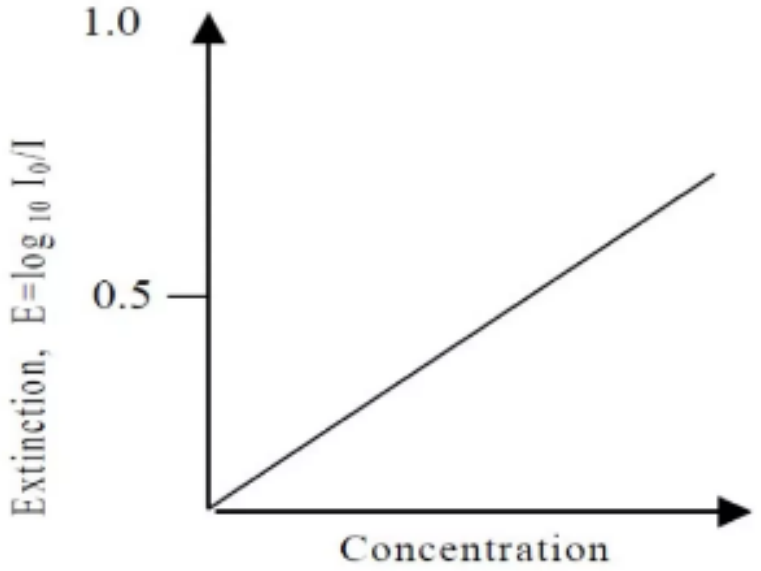
Curves of UV Transmittance & UV Absorbance to Concentration of Solute in Sample Solution

- Transmittance = $\frac{I}{I_0}$
- Absorbance = - log Transmittance:

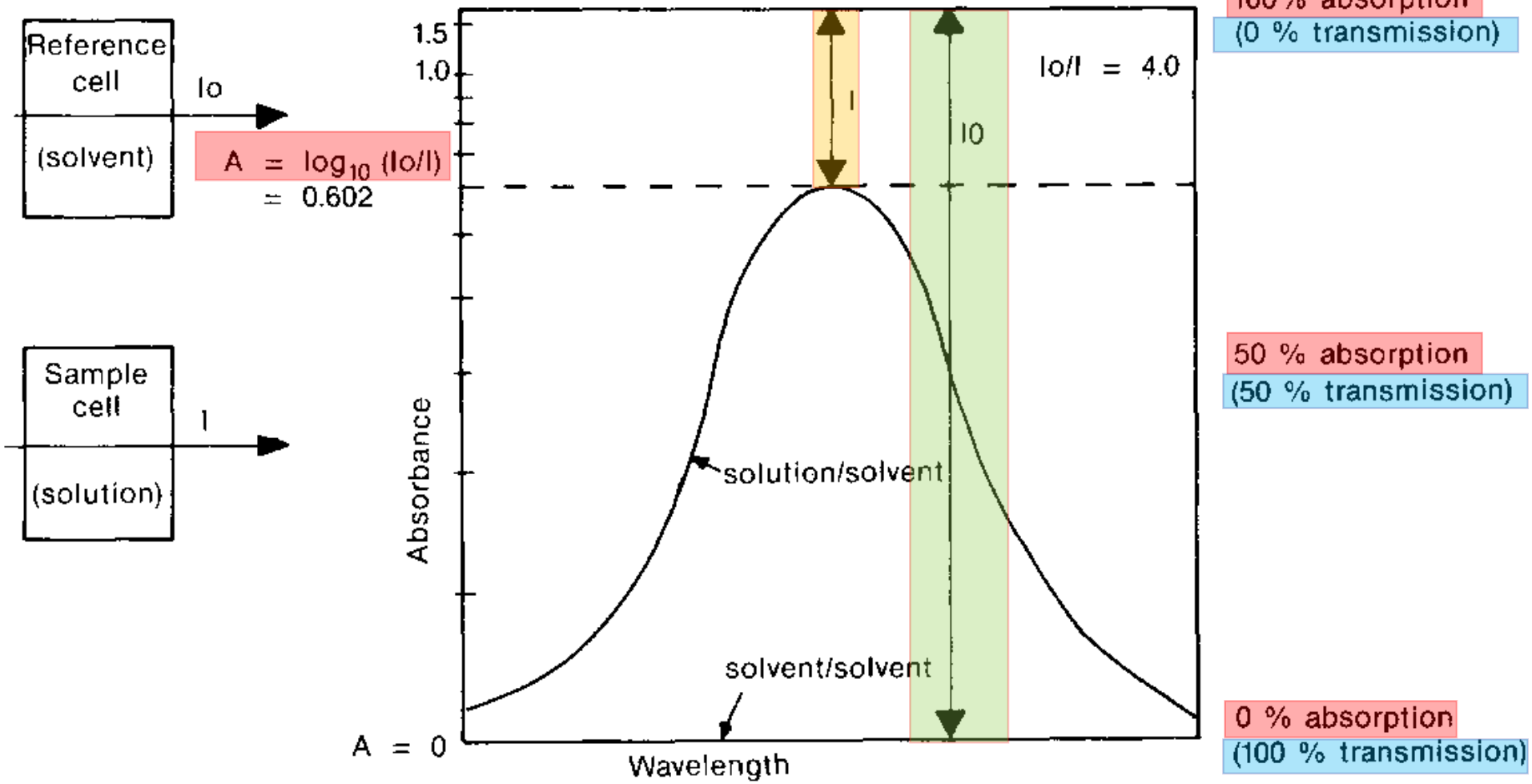
✓ $A = -\log T = -\log \frac{I}{I_0} = \log \frac{I_0}{I}$

- What is logic range for A?





Interpretation of UV Absorption Spectrum



UV Spectrophotometer: Output Data

- UV Absorption Spectrum: UV plot or UV curve:

✓ absorbance is plotted to λ:

- Reporting items:

✓ A or logA by AUC

✓ ε or log ε

✓ λ_{max}

$\lambda_{max} = 230$ nm	$\log \varepsilon = 4.2$
272	3.1
282	2.9

Figure 7.4 is the actual spectrum that corresponds to these data.

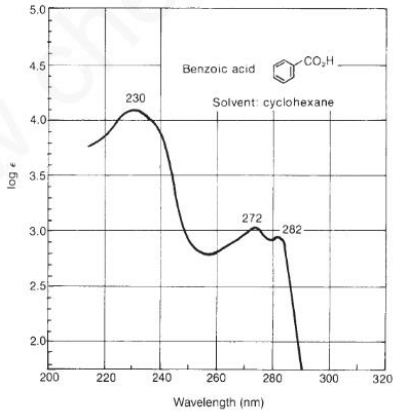
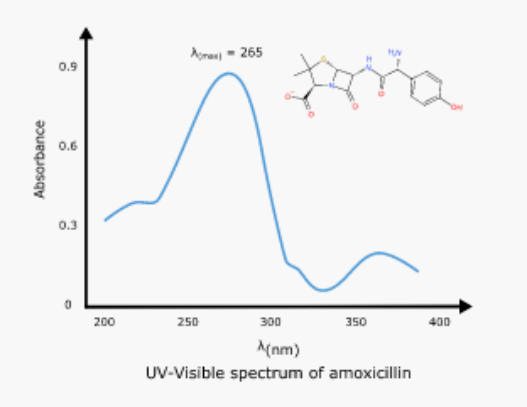


FIGURE 7.4 Ultraviolet spectrum of benzoic acid in cyclohexane. (From Friedel, R. A., and M. Orchin, *Ultraviolet Spectra of Aromatic Compounds*, John Wiley and Sons, New York, 1951. Reprinted by permission.)

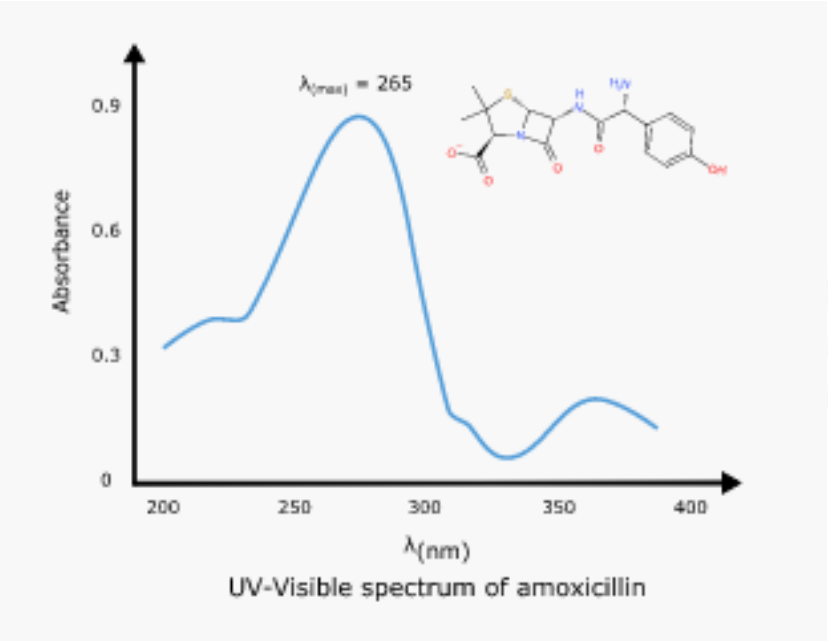
- To do qualitative & quantitative analysis



UV-Visible spectrum of amoxicillin

Two Examples for UV Absorption Curves

- Benzoic acid: λ_{max}: ?
- Amoxicillin: λ_{max}: ?



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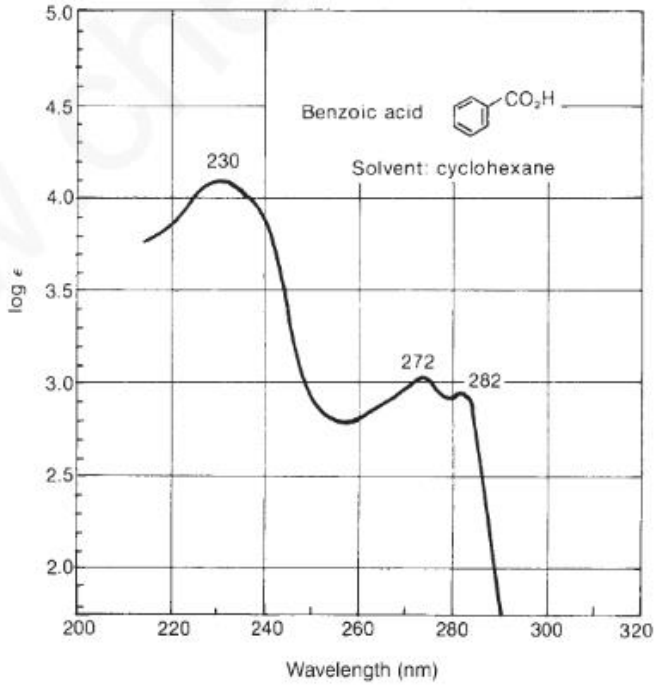

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Calibration Curve or Standard Curve in UV Spectroscopy

- Find λ_{max} for the target known compound:

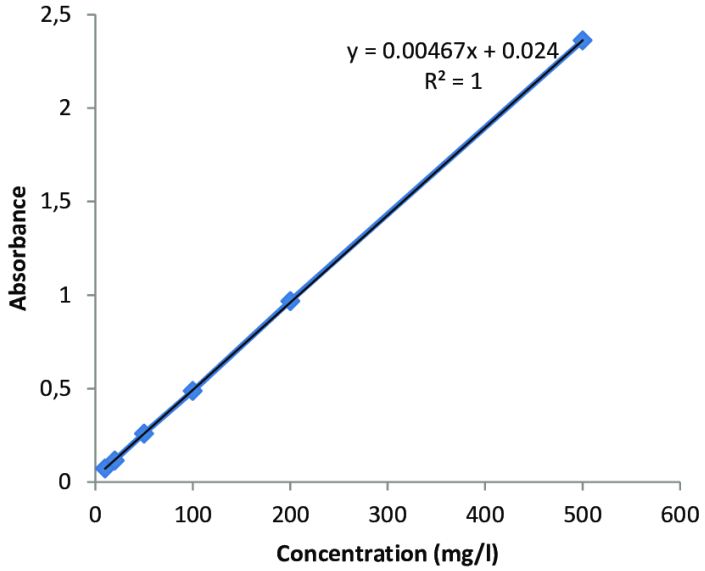
✓ from reference text or by running UV primary scan.

- Prepare serial concentrations of the target compound in a suitable solvent.
- Run UV for each of concentrations: consider reliability & reproducibility:

✓ for at least three times individually: obtain absorption data

- Calculate mean of readout absorbances for each concentration.
- Plot mean of absorbances to the correlated concentration:

✓ calibration curve or standard curve

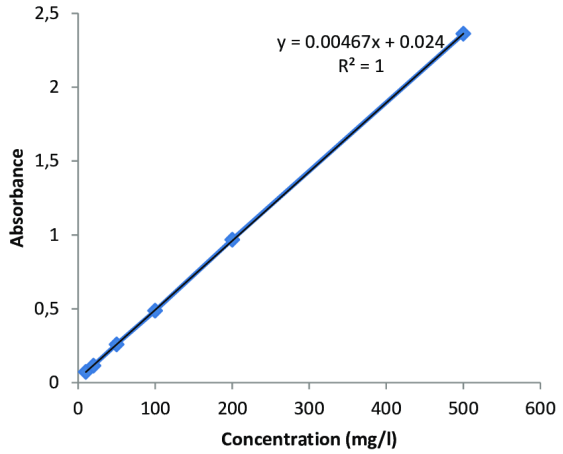


Calibration Curve or Standard Curve in UV Spectroscopy- Contd.

- Calculate the equation for the curve:

✓ Y= aX + b Abs.= a. Conc. + b

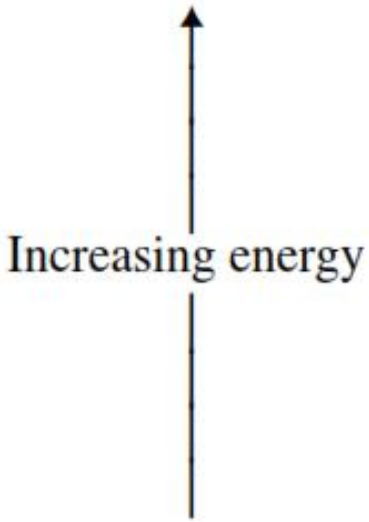
- Calculate regression constant (R or R^2) for the obtained curve.



- Run UV scan for unknown concentration of the same compound in a simple solution or mixture solution.
- using the equation of standard curve, find the concentration for the sample with unknown concentration of the compound.

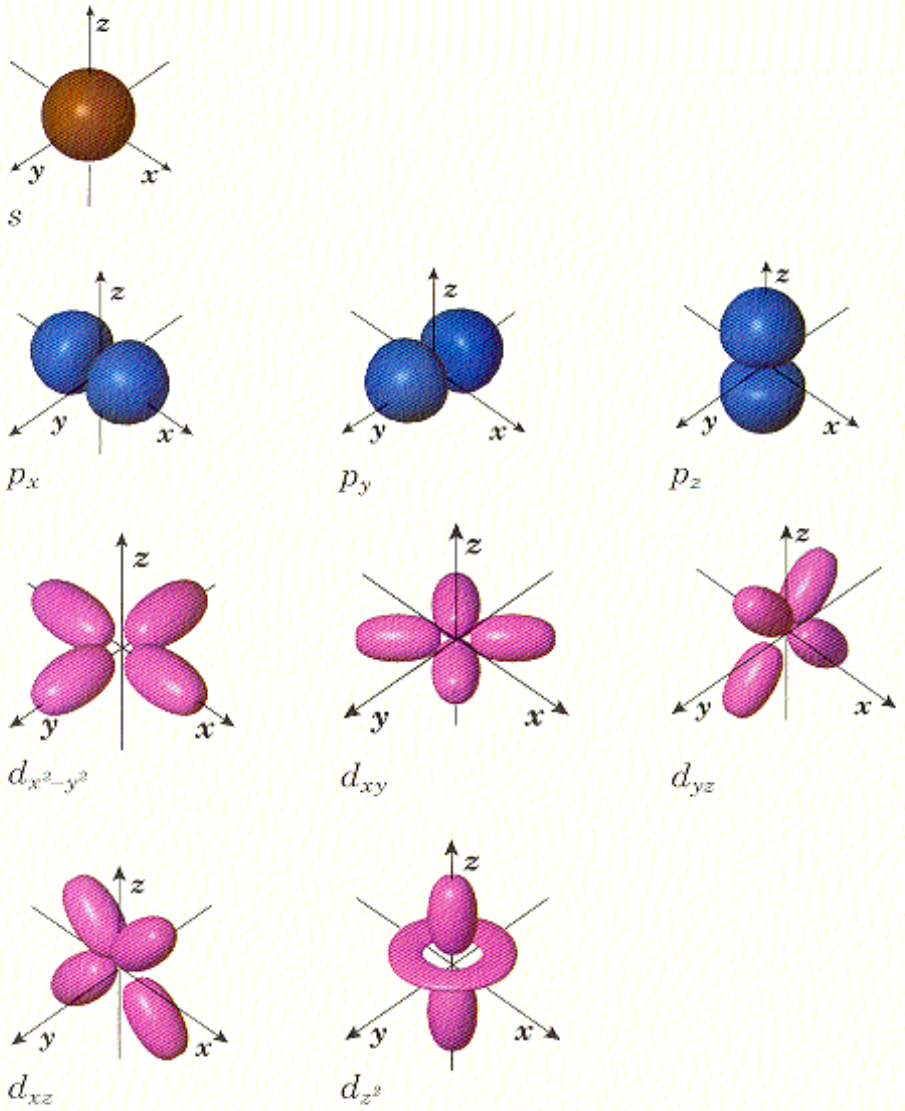
Possible Electron Transitions for Some of Functional Groups

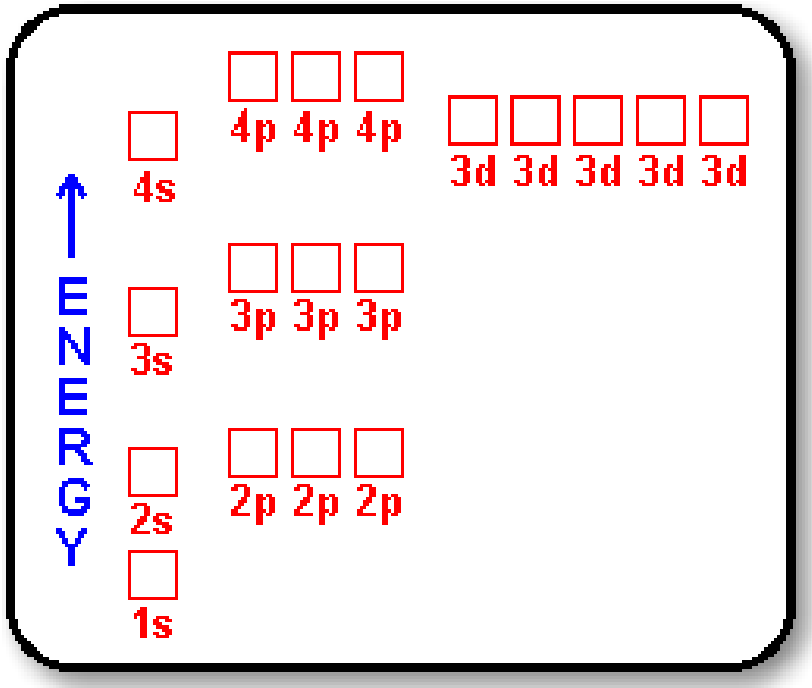
- $E = h \times \upsilon = h \times \frac{1}{\lambda}$



$\sigma \longrightarrow \sigma^*$	In alkanes
$\sigma \longrightarrow \pi^*$	In carbonyl compounds
$\pi \longrightarrow \pi^*$	In alkenes, carbonyl compounds, alkynes, azo compounds, and so on
$n \longrightarrow \sigma^*$	In oxygen, nitrogen, sulfur, and halogen compounds
$n \longrightarrow \pi^*$	In carbonyl compounds

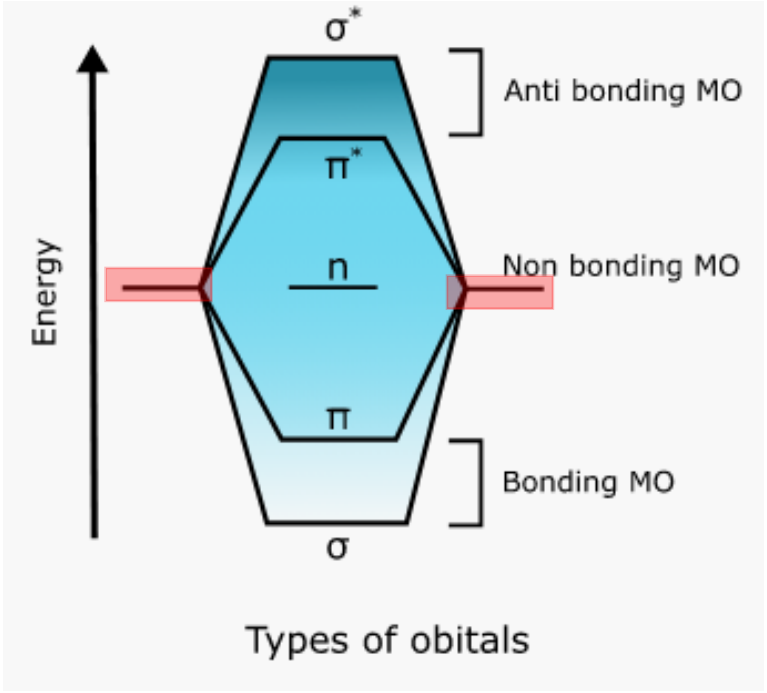
Types of Atomic Orbitals: S, P & d





Atomic orbitals & Molecular Orbitals (MOs)

- Number of Mos in molecule
- Bonding MOs: σ; π
- Non-bonding MOs: n
- Anti-bonding MOs: σ*; π*

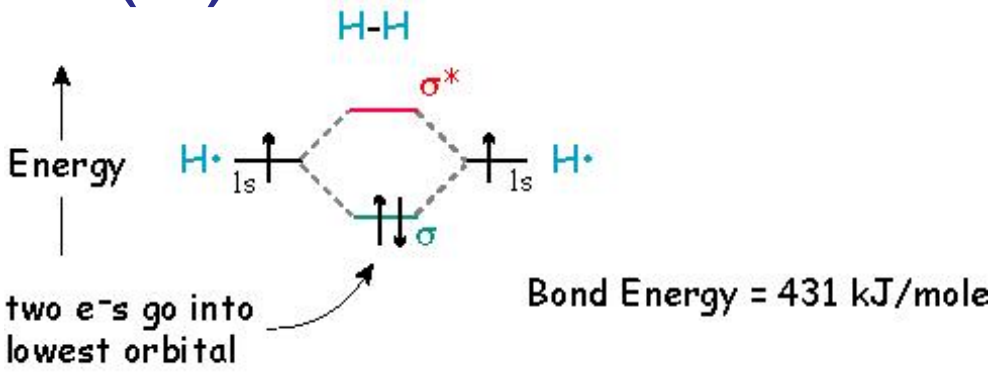


- HOMO & LUMO:
- HOMO: High Occupied Molecular Orbital
- LUMO: Lowest Unoccupied Molecular Orbital

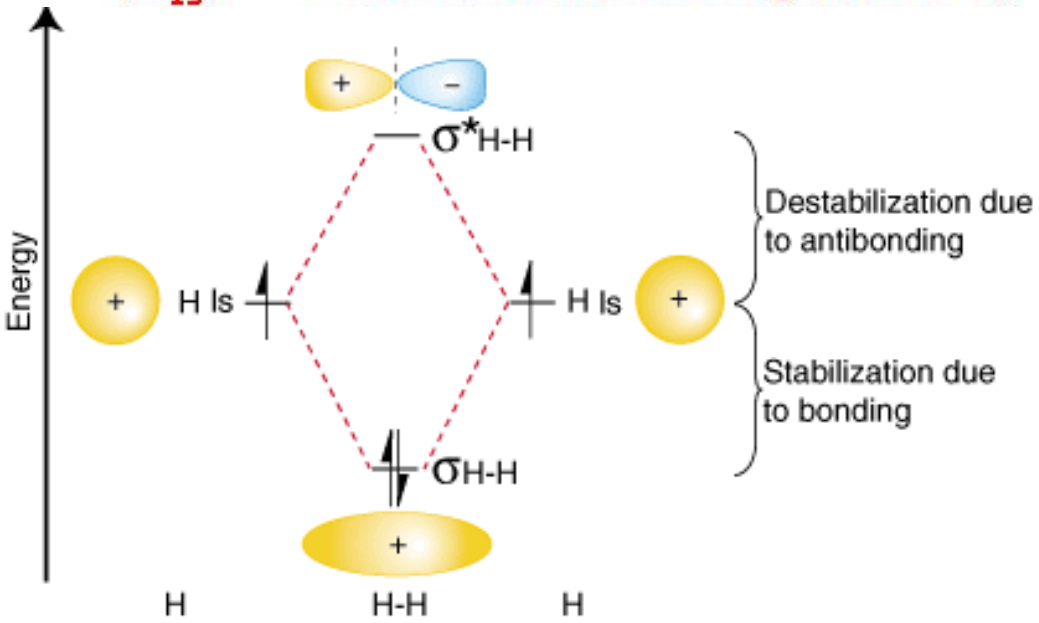
✓ electron transition is preferred from HOMO to LUMO

- Quantic energy distance between electron layers

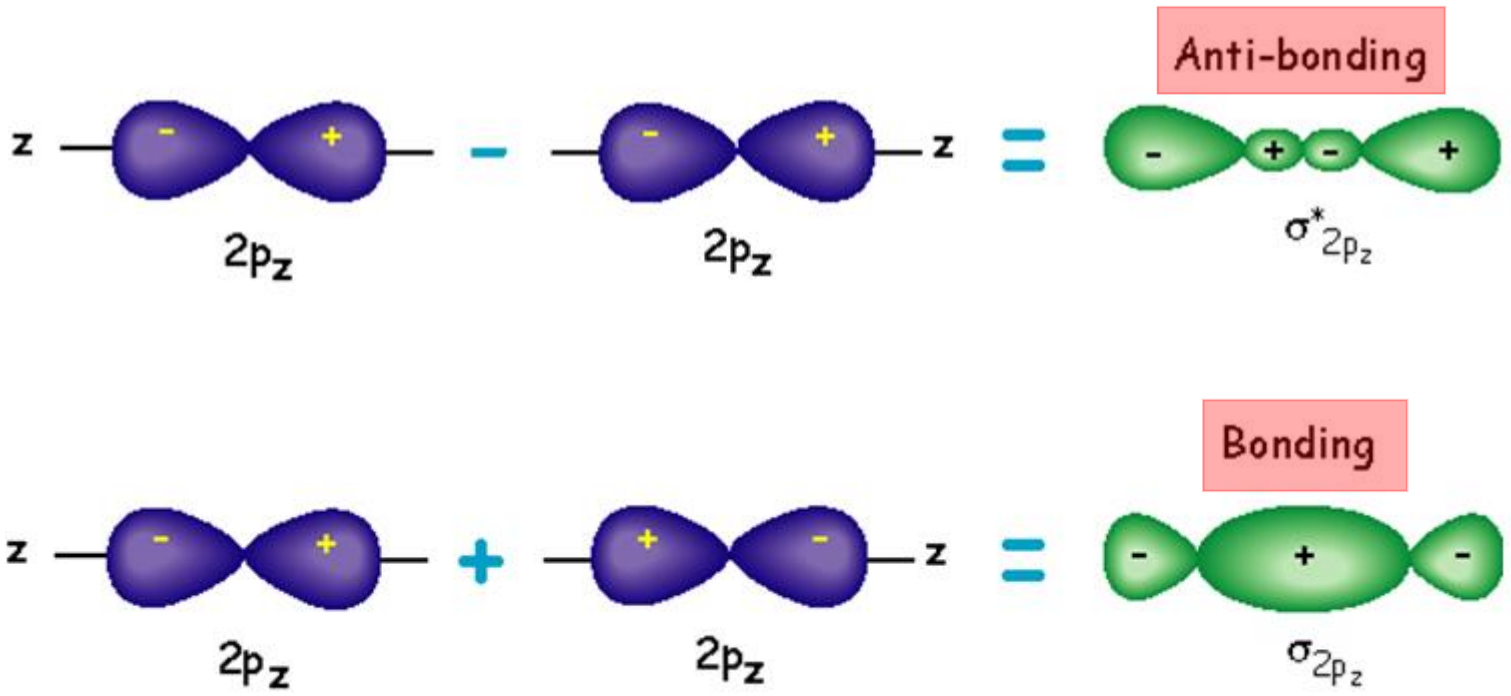
Atomic Orbitals to Provide σ(S) & σ*(S) Molecular Orbitals for H_2



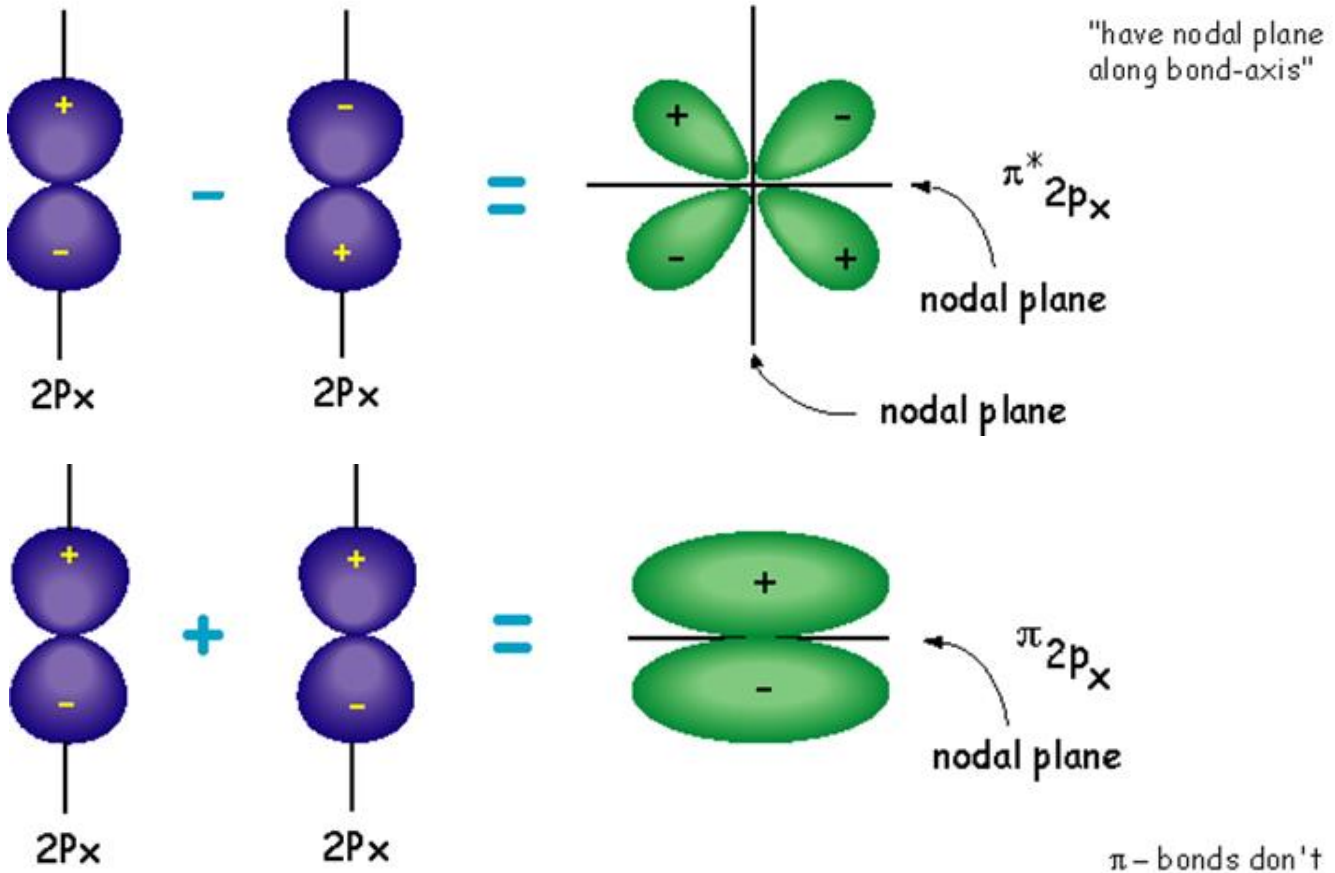
$(\sigma_{1s})^2$ Molecular electronic configuration of H_2



Atomic Orbitals to Provide σ(P) & σ(P)* as Molecular Orbitals



Atomic Orbitals to Provide π(P) & π*(P) as Molecular Orbitals



Types of Energy Levels for Electrons in Molecules

- Electronic energy
- Vibrational energy
- Rotational energy

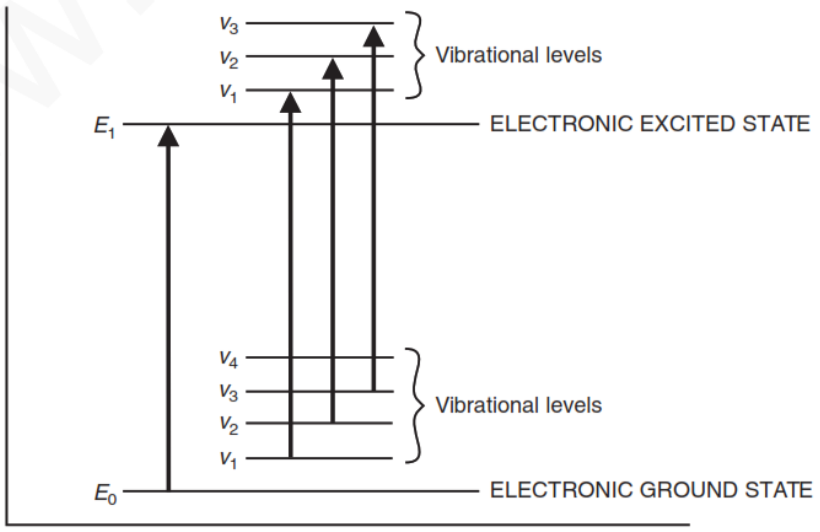


FIGURE 7.3 Electronic transitions with vibrational transitions superimposed. (Rotational levels, which are very closely spaced within the vibrational levels, are omitted for clarity.)

- $\Delta E_{total} = \Delta E_{electronic} + \Delta E_{vibrational} + \Delta E_{rotational}$

Electron Levels & Electron Transitions for Molecular Orbitals

- Types of electron transitions:

✓ σ ⟶ σ*

✓ π ⟶ π*

✓ σ ⟶ π*

✓ n ⟶ π*

✓ n ⟶ σ*

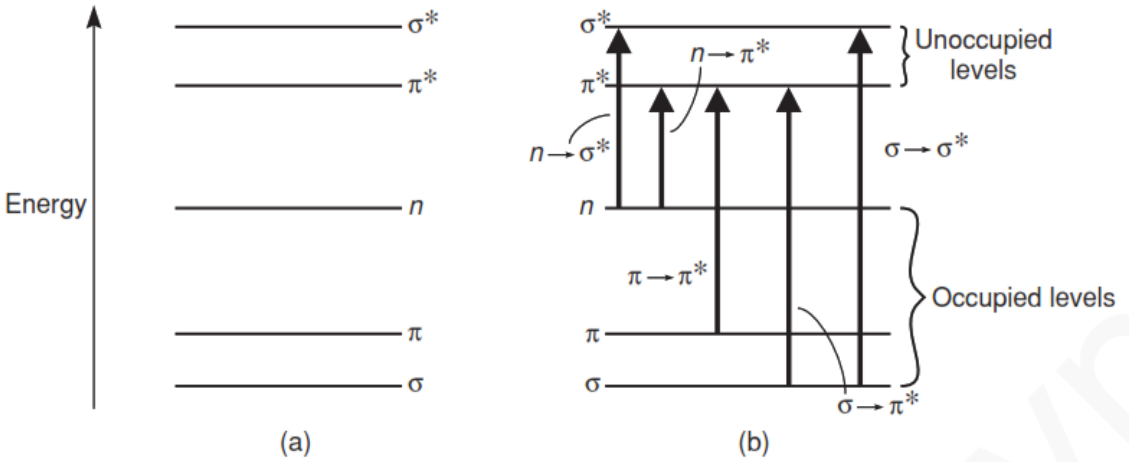


FIGURE 7.2 Electronic energy levels and transitions.

- According to the required energy:

$$\sigma - \sigma^* > n - \sigma^* > \pi - \pi^* > n - \pi^*$$

- Selection rules
- Allowed transitions
- Forbidden transitions