

Spectrophotometric analytical methods

Infrared spectrophotometry

Ultraviolet spectrophotometry

F. W. Herschel (1738-1822)



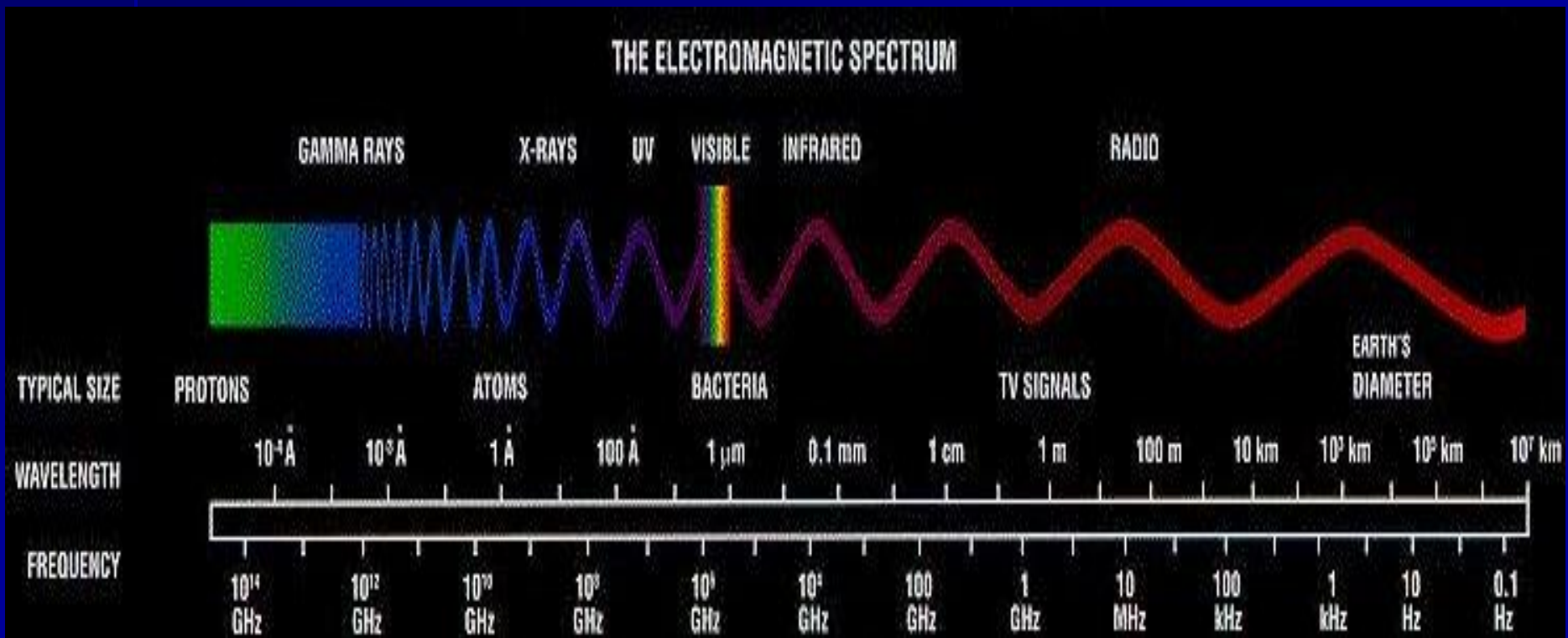
- Beam of elmag. Radiation with intensity I_0 goes through compound:
 - Absorption occurs
 - Goes without absorption – full transmittance.
 - Depends on the beam frequency and character of (non)absorbing molecule.
- Elmag. radiation is a quantum of energy – during absorption, the change of energetic state of molecule occurs : from basic state to a higher energetic level.
- If the frequency of radiation corresponds to the so called Bohr frequency condition (quantum of radiation energy corresponds to the difference between two energetic states of molecule), absorption occurs.
- Spectrum of compound = difference between energy impacting and passing through.

Types of absorption spectrometry

- Depends on the type of transition into higher energetic levels and the corresponding range of frequency of absorbed radiation.
 - Rotation state.
 - Vibration state.
 - Excitation of valence electrons.

■ Types of absorption spectrometry:

- Microwave spectrometry
- Infrared spectrometry
- UV/Vis spectrometry

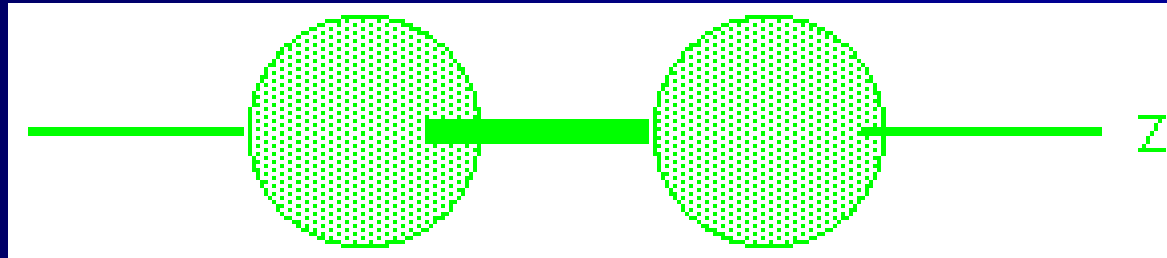


- Infrared area lies between red part of visible are and microwave area.
- IR area
 - Near (NIR).
 - Middle (fundamental).
 - Far (FIR).

Infrared spectrometry

- Molecule can move in space in different ways:
 - **In space via certain direction and velocity** – translation movement, is connected to the kinetic energy of the molecule
 - 3 translation degrees of freedom exist
 - **Rotation according to the internal axis** - rotation movement, connected to the kinetic rotation energy
 - 3 translation degrees of freedom exist

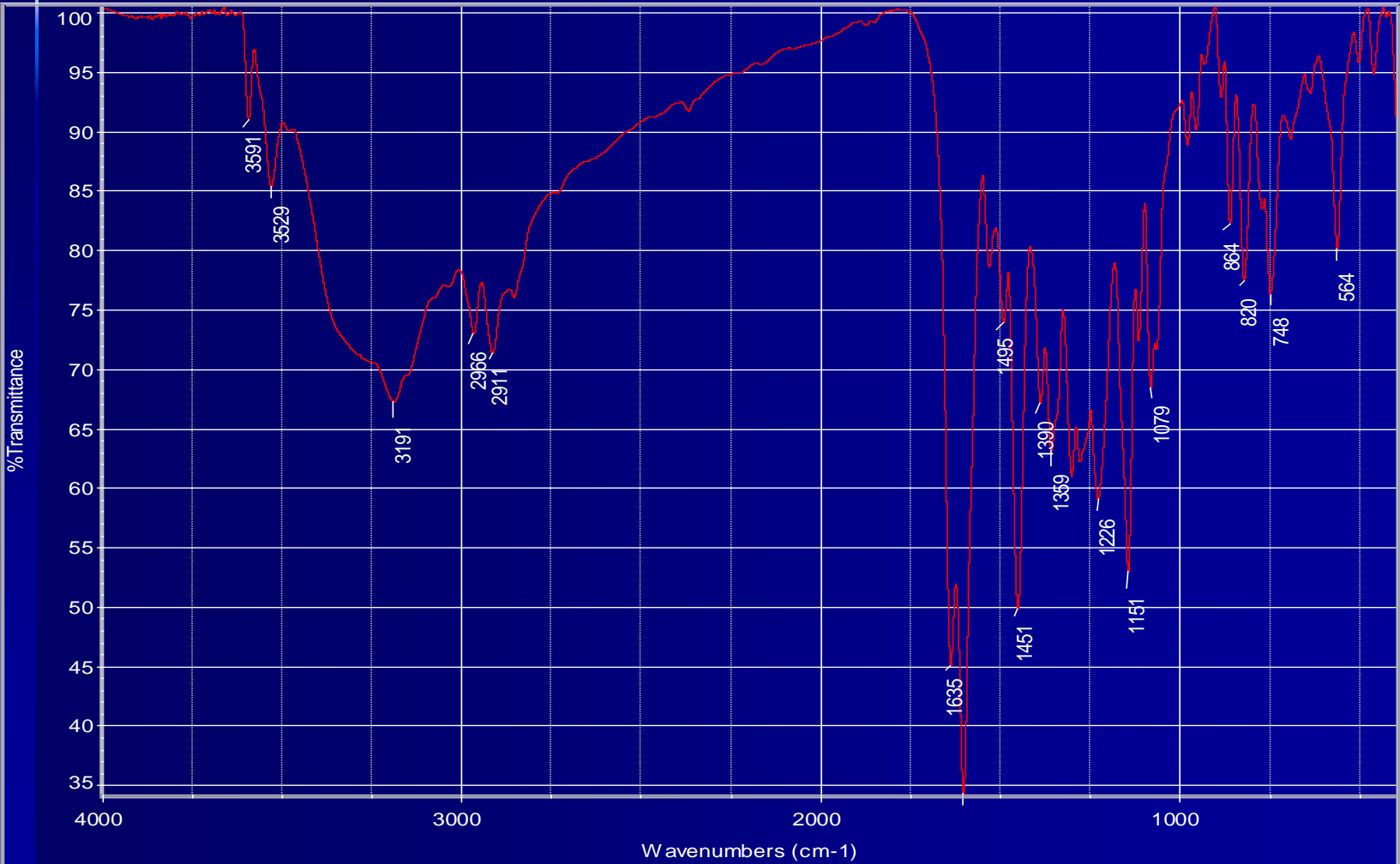
- Possibility of vibration - 1 degree of vibration degree
- Two – atomic molecule



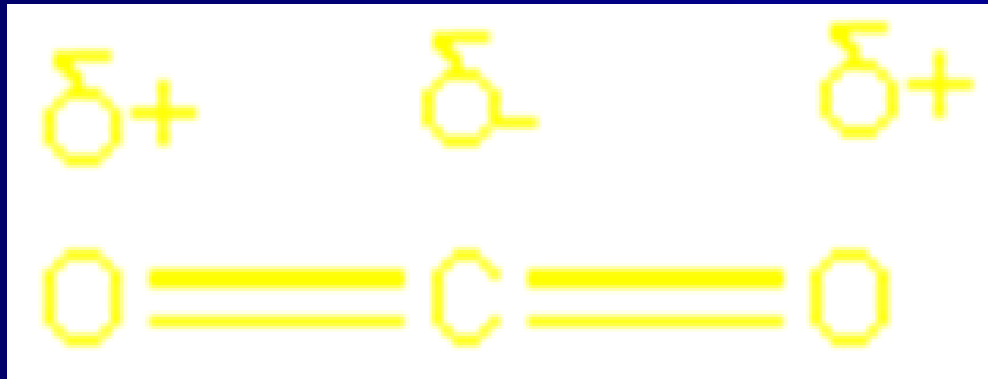
- Linear molecules: $3N-5$ degrees of freedom
- Non-linear molecules: $3N-6$ degrees of freedom

- Each of vibration movements is manifested by a certain characteristic frequency.
- Each of the vibrational movements is connected to a certain energetic state.
- Molecules can enter different (higher) energetic state by absorption of energy.
- Frequency of light beam must correspond to the frequency of vibration.
- Frequency of vibration therefore can be measured via measurement of frequency of light absorbed by the molecules of a compound.

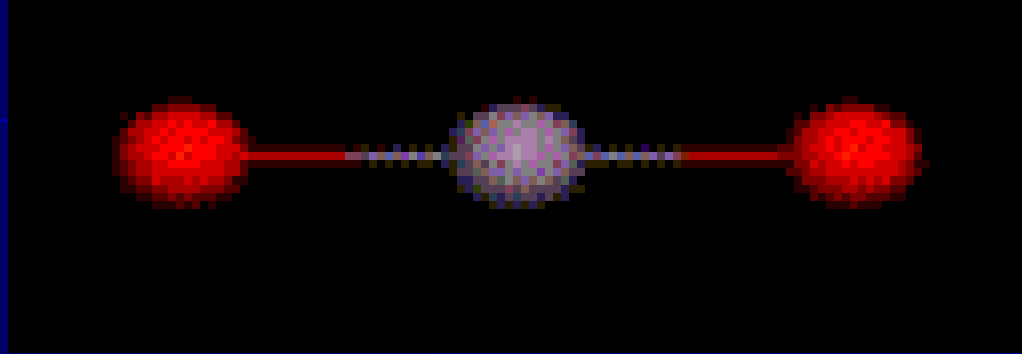
- Infrared spectrum of compound is then a graph of dependence of intensity of the IR light transmitted or absorbed by a molecule on energy of infrared light expressed in wavenumbers (cm^{-1}) or wavelength (microns). Transmittance is used in case of spectral structural analysis, absorbance for quantitative analysis of the intensity.



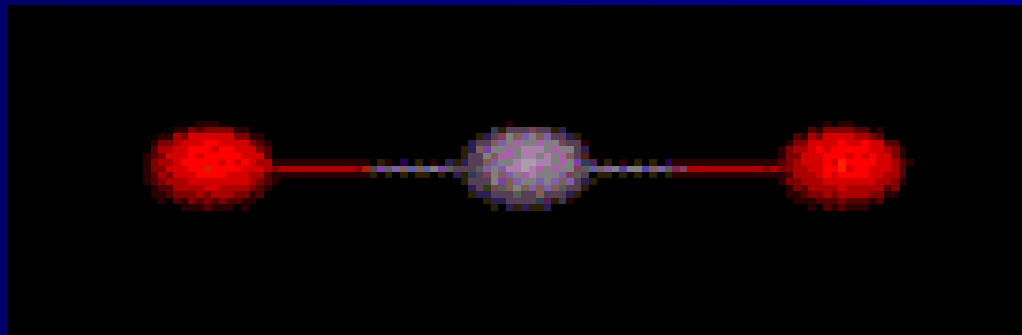
- Vibration of molecule does not directly mean absorption of infrared light.
- Molecule must possess certain dipole moment – which can be permanent (HCL) or is created by vibration of molecule (CO₂).



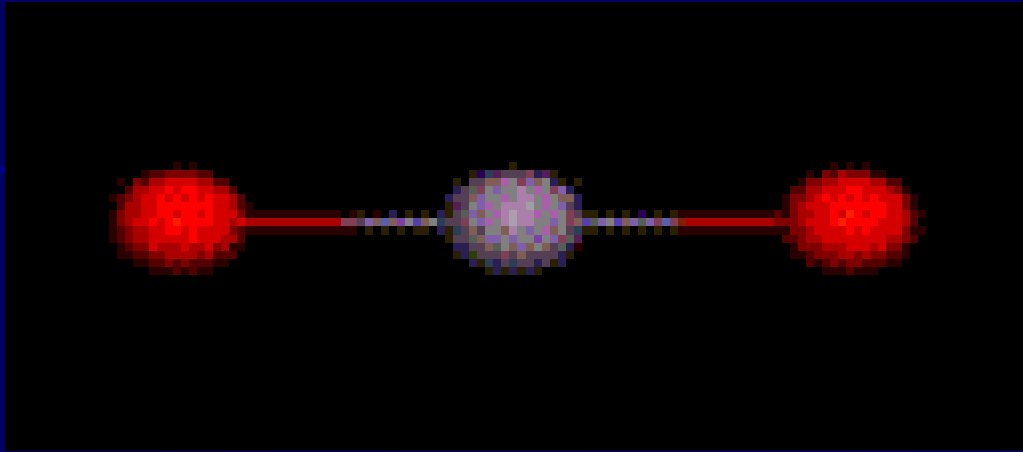
- Asymmetric stretch



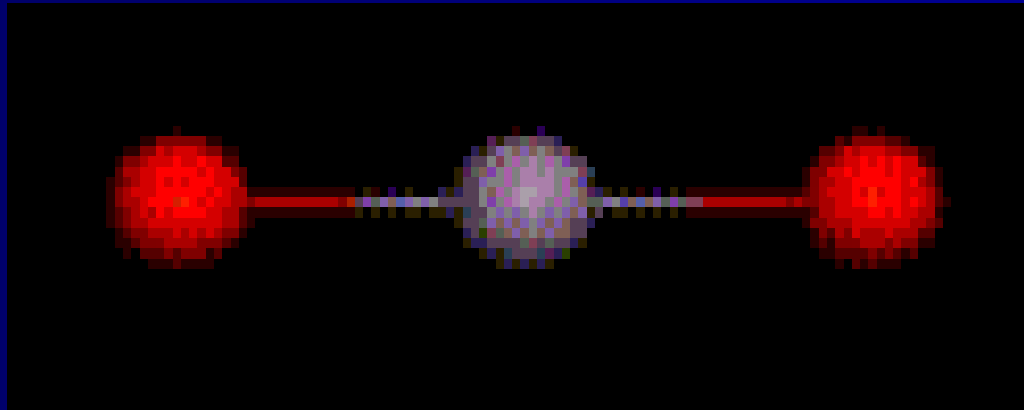
- Symmetric stretch



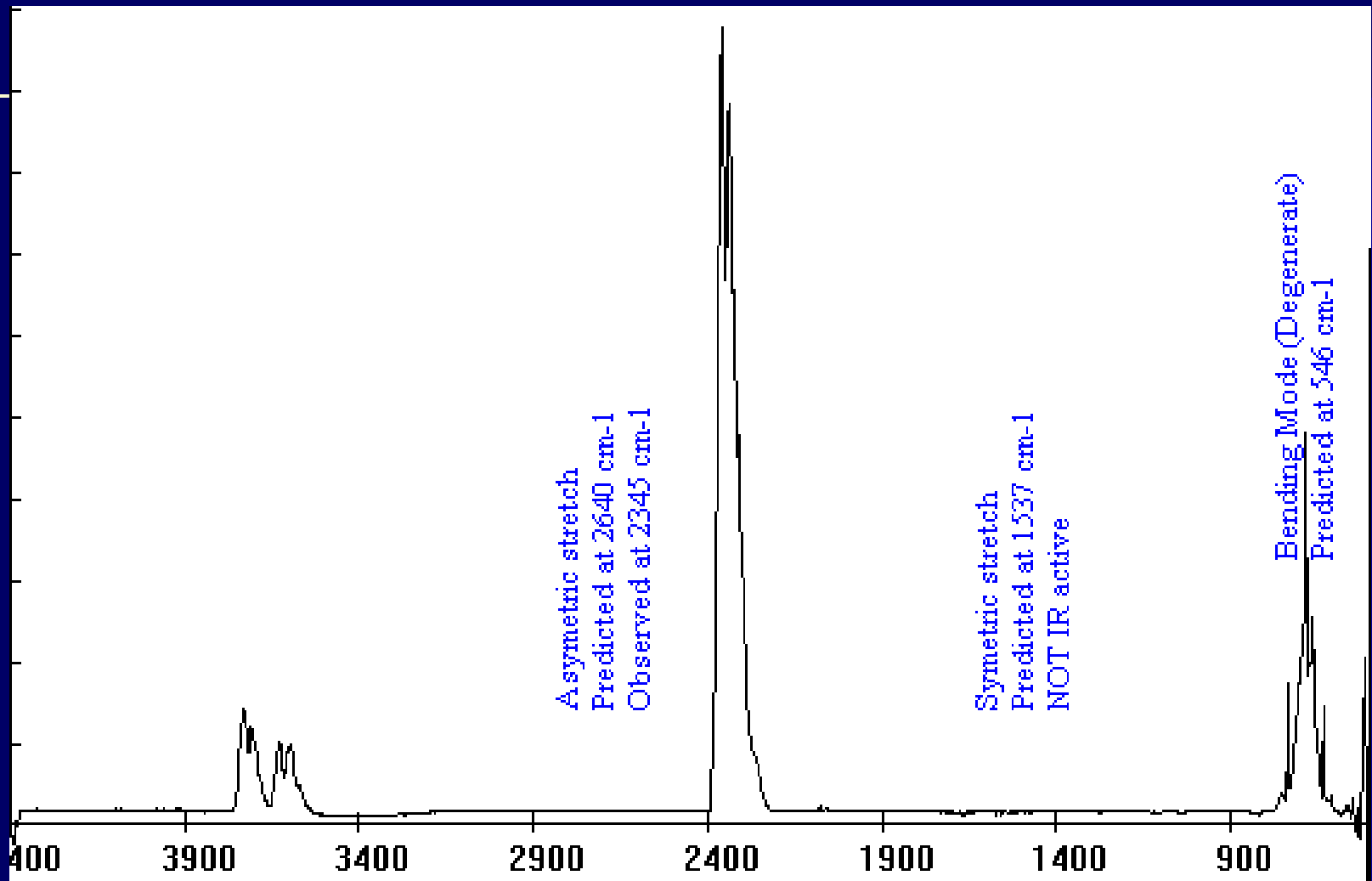
- Vertical bend



- Horizontal bend



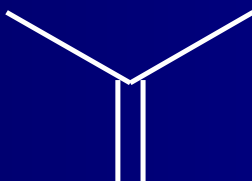
IR spectrum of carbon dioxide



Applications of infrared spectrometry in organic chemistry

- Functional groups of organic molecules contain atoms of different atomic weight connected via bonds with different strength. Different vibrational frequencies.

- Example:



- Carbonyl: 1700 cm^{-1}
- Hydroxyl: $3200\text{-}3400\text{ cm}^{-1}$

- In organic chemical analysis IR spectrum is divided into three areas:

1. 4000-1300 cm^{-1}

2. 1300-900 cm^{-1}

3. 900-600 cm^{-1}

1. Region of functional groups and bond vibrations.

2. Fingerprint region.

3. Region of absorptions of benzene nucleus.

■ Most important chosen frequencies:

Type of bond	Type of compound	Range of frequencies
C-H	Alkanes	2960-2850(s) stretch 1470-1350(v) scissoring and bending
	Alkenes	3080-3020(m) stretch 1000-675(s) bend
C-O	Alcohols, ethers, carboxylic acids, esters	1260-1000(s) stretch
C=O	Aldehydes, ketones, carboxylic acids, esters	1760-1670(s) stretch
O-H	Primary alcohols	3640-3160(s,br) stretch
	Alcohols and phenols with hydrogen bridge	3600-3200(b) stretch
	Carboxylic acids	3000-2500(b) stretch

Usage:

1. Structural analysis.
2. Identification of unknown compounds in combination with further methods.
3. Identification of unknown compounds by comparison with standard or spectral library.
4. Purity.
5. Quantitative analysis.
6. Control of chemical reaction processes.

Preparation of sample for IR spectrometry

- Liquid sample:
 - Liquid compound or dissolved and diluted sample
 - Better not to dilute – interaction of solvent and compound.
 - Very short optical pathway (0,1 mm)-cuvette or two desks.
 - Dilution into $\sim 0,2$ M solution (CCl_4 , CS_2 , CHCl_3).
 - Cuvette or film.

■ Solid samples:

1. Method of so called Mull (nujol method)

- Sample after grinding is mixed with a small amount of a non-volatile solvent – forms a paste, which is applied between two disks.
- Comfortable, faster, no extreme conditions.

2. Method of KBr tablet (pastill method)

- KBr does not absorb in IR over 250 cm^{-1} .
- Necessary absence of humidity.
- Better in case of thermostable compounds, better for homogenizable material, better spectra.

Ultraviolet spectrophotometry

- Definition of terms:

λ – wavelength

ν – wavenumber

ν – frequency

c – velocity of light in vacuum

h – Planck constant $6,61 \times 10^{-34}$ J/s

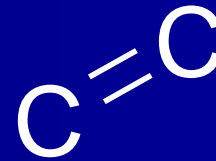
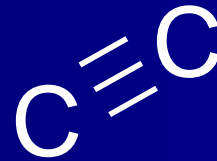
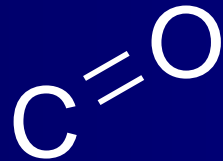
- Energy absorbed by molecule is quanted and must fulfill Bohr resonance condition.

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

$$E = h.\nu$$

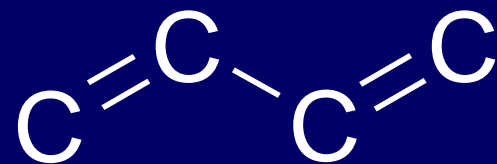
General principles of absorption of elmag. radiation in area 200 - 900 nm

- For absorption are responsible some structural elements called chromophores.
- Chromophores – isolated short bonds



- Prevalently $\pi - \pi^*$ transitions
- Further absorbing groups - auxochromes: causing
 - Bathochromic shift (red)
 - Hypsochromic shift (blue)
 - Hyperchromic shift
 - Hypochromic shift

- Conjugated systems



- Effect of environment and solvent

- Change of position and/or intensity of absorption band

- Chemical influence

- Formation of complexes

- Formation of tautomer

- Polarity of solvent and compound alone

- Dissociation

- Energetic levels:
 - During absorption of elmag. radiation electrons come from one energetic state into second, molecule comes from basic energetic state into excited.
 - Interpretation of electronic transitions with help of molecular orbitals
 - Electrons are classified according to the energy:
 - $\sigma < \pi < n$
 - σ - bond electron
 - π - π electron of double and triple bonds
 - n - electron of free electron pair
 - Most common transitions:
 - $n - \pi^*$
 - $\pi - \pi^*$
 - $\sigma - \pi^*$

■ Selection rules:

- Drive the change or stability of transition.
 1. During absorption of quantum of energy only one electron can come into excited state.
 2. Spin quantum number cannot be changed during absorption of energy.

■ Lambert-Beer law

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