

IR - Spectroscopy in Biophysics

- 1 -

- electromagnetic waves of $800 - 10^6 \mu\text{m}$ length

MIR : $2,5 - 500 \mu\text{m}$

FIR : $50 - 10^3 \mu\text{m}$

NIR : $800 \text{ nm} - 2,5 \mu\text{m}$

- selection rule: IR-active molecules display altered dipolar moment during oscillation

$$(\partial \mu_{el} / \partial r \neq 0)$$

- measurement of transmission T (not absorption)

fraction of transmitted beam: $T = \frac{I}{I_0}$
(monochromatic)

as function of $\tilde{\nu} = \frac{1}{\lambda}$

or absorbance $A = \lg \frac{I_0}{I}$ (Lambert-Beer = $\epsilon \cdot c \cdot d$)

- Approximation: bond molecules can be ^{seen as} harmonic oscillators.

1st. two-atom molecules



Hooke's Law $F_{\text{bond}} = -k \Delta r$, $\Delta r = r - r_0$
(r_0 = equilibrium distance)

classical: $F_{\text{pot}} = \frac{1}{2} k \Delta r^2$

Q.M. $E_v = h \nu_0 (v + \frac{1}{2})$ $v = 0, 1, 2, 3$

selection rule: $\Delta v = \pm 1$, $\Delta E = E_{v+1} - E_v = h\nu_0$ - 2 -

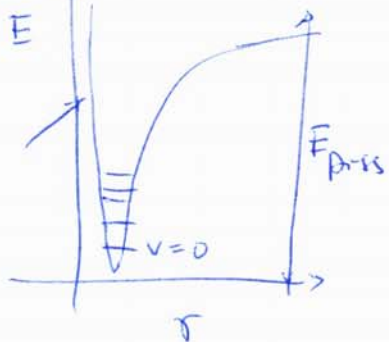
$$\nu_0 = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad \text{with } \mu = \frac{m_1 \cdot m_2}{m_1 + m_2} \quad (\text{reduced mass})$$

frequency of the oscillator \rightarrow can be taken directly from the spectrum (absorption bands) \rightarrow allows for calculation of the force constant k

- gas phase: rotational transitions induced by vibrational transitions

\Rightarrow biologically with no meaning/significance, rotational transitions not resolved

- more realistic - Morse potential



Anharmonicity,
 ΔE not equidistant

Overtones ($\Delta v > |\pm 1|$) possible

(at low probability)
 rapidly decreasing intensity)

room temperature: - only the ground level occupied
 ($E_0 = h\nu_0/2$)

- Normal modes of a polyatomic molecule:
 contributions from all atoms, in the same frequency and phase, however ~~with~~ move with different amplitudes!

→ Normal mode: no translation of the center of gravity of the molecule or rotation

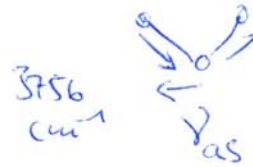
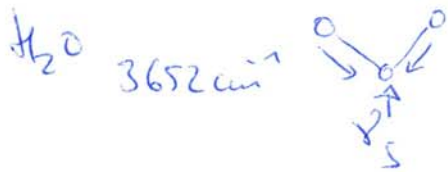
-3-

Force ~~Vibr~~ Valence / Stretching - vibrations

→ oscillation in direction of the bonds

1) symmetric

2) antisymmetric



Deformation: change of bond angle



bending
rocking
twisting
wagging

p. 349 Noll/Winter

degrees of freedom: $3N - 6$ (angular molecules)

example CO_2 : linear, symmetric ($3N - 5$)

⇒ no v_s -Band

however: deformation

- Characteristic group frequencies:
localised vibration, largely decoupled

A) two channel spectrometers:

light source Kramm-bar (oxides)
or Globar SiC

- enables correction for solvent absorption

- 1 - 10 μm thickness of sample H_2O

10 - 100 μm

D_2O

Windows: CaF_2

- detection thermocouples (T - depends
thermo potential
between metals /
semiconductors)

also $\text{Hg}_{1-x}\text{Cd}_x\text{Te}$ (MCT)

detectors, very sensitive

- dry air flow \rightarrow reduces water absorption

\rightarrow picture 351

B) Fourier transform IR spectroscopy

& - Michelson interferometer principle

\Rightarrow p. 352 picture

elements: - beam splitter

- fixed mirror

- mirror scanner (movable mirror)

-5-

⇒ Interferogram, monochromatic light

Intensity: $I(x) = 2 I_0 R T (1 + \cos(2\pi \tilde{\nu} x))$

↓
 $x =$ path difference

↑ incident light
↑ reflection / transmission of the beam splitter ($R+T=1$)

↑ wave number of the observed

polychromatic:

$$I(x) = \int 2 I_0(\tilde{\nu}) R(\tilde{\nu}) T(\tilde{\nu}) (1 + \cos(2\pi \tilde{\nu} x)) d\tilde{\nu}$$

$I \rightarrow I(\sigma)$: intensity at wavelength optical path length:

$$x \gg \lambda \Rightarrow \cos(2\pi \nu x) - \text{Terms} \approx 0$$

Interferogram - function F:

$$F(x) = I(x) - I(\sigma) = \int A(\tilde{\nu}) \cos(2\pi \tilde{\nu} x) d\tilde{\nu}$$

$$\text{with } A(\tilde{\nu}) = 2 I_0(\tilde{\nu}) R(\tilde{\nu}) T(\tilde{\nu})$$

⇒ Spectrum obtained by Fourier-Transformation.

$$A(\tilde{\nu}) = 2 \int F(x) \cos(2\pi \tilde{\nu} x) dx$$

⇒ not monochromator, but mathematical "filtering"

Measurement:

1) without sample

↳ single beam / background

2) sample spectrum

↳ T or A - spectrum calculated

advantages: - all frequencies at once

→ short scan times

- higher intensity: no apertures,

split

as dispersive instruments

better S/N - ratio

- laser or internal calibration

used → position control of the

movable mirror,

0.001 cm^{-1} precision

ATR:

attenuated total reflection

for thin films → multi-reflection

penetration depth $d_p = \frac{\lambda}{2\pi n_1 \sqrt{\sin^2 \theta - n_{21}^2}}$

$$n_{21} = n_2 / n_1$$

θ = angle of incidence

-7-) Application: phase behavior of membrane lipids

↳) Secondary structure determination of proteins
significant wave numbers: p. 365