4. Analysis of Microwave, IR and UV/vis Spectra

Applications of microwave, IR and UV/vis spectroscopy:
   analysis and identification of compounds,
   determination of molecular properties.

a) Microwave Spectroscopy
Consider a rigid diatomic molecule (rigid rotator model),

The molecule can rotate end-over-end about its centre of gravity, C.
The moment of inertia, $I$, about C is given by,

$$I = \mu \cdot r_0^2$$

where $\mu = \frac{m_1 m_2}{m_1 + m_2}$ is the reduced mass of the molecule.
For such a simple system the Schrödinger equation can be solved and the rotational energy levels (in Joules) of the molecule determined to be:

\[ E_J = \frac{\hbar^2}{8\pi^2 I} J(J + 1) \quad \text{where } J = 0,1,2,… \quad (4.2) \]

Alternatively, spectroscopists often express "energy" loosely in units of cm\(^{-1}\) and rewrite eq. 4.2 in the form:

\[ E_J = BJ(J + 1) \quad \text{cm}^{-1} \quad (4.3) \]

\[ B = \frac{h}{8\pi^2 Ic} \quad \text{cm}^{-1} \quad (4.4) \]

where \( B \) is termed the rotational constant.

The energy required for the transition from the state \( J=0 \) to \( J=1 \) can easily be calculated from eq. 4.2 to be,

\[ \Delta E_{J=0 \rightarrow J=1} = \frac{2\hbar^2}{8\pi^2 I} \quad (4.5) \]

This energy (or that of any other transition) can be measured by microwave spectroscopy. Once it is known the moment of inertia, \( I \), can be calculated and hence from eq. (4.1) the bond length of the molecule.
Example: CO, \((1/\lambda)_{0\rightarrow1} = 3.842 \text{ cm}^{-1}\)

What is the bond length of CO?

Applications of microwave spectroscopy:

- Determination of molecular structure, i.e. bond lengths, bond angles, dipole moments.
- Determination of isotopic abundances \((\Delta E\) depends on the reduced mass).

Example: \(^{12}\text{C}^{16}\text{O}\) absorbs at 3.84235 cm\(^{-1}\)
\(^{13}\text{C}^{16}\text{O}\) absorbs at 3.67337 cm\(^{-1}\)

The increased reduced mass of \(^{13}\text{C}\) increases the moment of inertia, \(I\) (eq. 4.1), and hence decreases \(\Delta E\) (eq. 4.5).
From the relative intensities of the two peaks the relative abundances of \(^{12}\text{C}\) and \(^{13}\text{C}\) can be determined.
A very large microwave spectrometer:

Parkes Radiotelescope, NSW

Used to detect interstellar molecules. Several molecules were found in space using a radiotelescope before they were discovered in the laboratory, e.g. C₂H, HCO⁺ and N₂H⁺.
b) Infrared Spectroscopy
Consider a diatomic molecule, e.g. HCl, undergoing stretching and compression. The energy involved in such an oscillation can be described classically using the theory of simple harmonic motion, e.g. for a spring, using Hooke's law:

\[ E = \frac{1}{2} k (r - r_{eq})^2 \quad (4.6) \]

\( r_{eq} \) = equilibrium bond length (m)
\( k \) = force constant (bond strength, N m\(^{-1}\))

Hooke's law describes a parabolic curve of energy. According to quantum mechanics, however, only discrete energy levels on this parabola are allowed.
Classically it can be shown that the vibration frequency, $\nu$, is related to the force constant, $k$, and the reduced mass, $\mu$, by:

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \text{ Hz} \quad (4.7)$$

Solving the Schrödinger wave equation for the simple harmonic oscillator model yields the vibrational energy levels (in Joules) of the molecule:

$$E_v = \left( v + \frac{1}{2} \right) \hbar \nu \quad \text{where } v = 0,1,2\ldots \quad (4.8)$$

Alternatively, if $E_v$ is expressed in cm$^{-1}$ and the wavenumber is used instead of frequency, the equation can be rewritten as:

$$E_v = \left( v + \frac{1}{2} \right) \cdot \frac{1}{\lambda} \text{ cm}^{-1} \quad (4.9)$$

As in the case of microwave spectroscopy, the energy required for the transition from the state $v = 0$ to $v = 1$ can easily be calculated from eq. 4.8 to be,

$$E_{v=0\rightarrow v=1} = h \nu \quad \text{Joules} \quad (4.10)$$
This energy can be measured by IR spectroscopy. Once it is known the frequency, ν, of the vibration can be calculated and hence from eq. 4.7 the force constant, k (assuming that the μ is known).

Note: Real molecules don't exactly obey the laws of simple harmonic motion. If a bond is stretched too far it will break and the atoms dissociate.

A real molecule is better described as an anharmonic oscillator. The energy curve is better is described by an asymmetric Morse curve:

\[
D_{eq} = \text{spectroscopic dissociation energy}
\]

\[
D_o = \text{chemical dissociation energy}
\]
For real molecules, therefore, a correction must be made for anharmonicity to derive accurate $k$ values,

<table>
<thead>
<tr>
<th></th>
<th>Force constant, $k$ (N m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>966</td>
</tr>
<tr>
<td>HCl</td>
<td>516</td>
</tr>
<tr>
<td>HBr</td>
<td>412</td>
</tr>
<tr>
<td>CO</td>
<td>1902</td>
</tr>
</tbody>
</table>

What is the reason for the different $k$ values?

So far we have only discussed diatomic molecules, which only undergo bond stretching. However, IR radiation can also excite bond bending, e.g. H$_2$O
Rather than the determination of molecular parameters, however, the main use of IR spectroscopy is in chemical analysis, i.e. the identification of functional groups and compounds.

### Characteristic Group Frequencies

<table>
<thead>
<tr>
<th>Group</th>
<th>$1/\lambda \ (\text{cm}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-O-H stretch</td>
<td>3600</td>
</tr>
<tr>
<td>N-H stretch</td>
<td>3400</td>
</tr>
<tr>
<td>C-H stretch</td>
<td>2800-3000</td>
</tr>
<tr>
<td>S-H stretch</td>
<td>2580</td>
</tr>
<tr>
<td>C=O stretch</td>
<td>1750-1600</td>
</tr>
<tr>
<td>C≡C stretch</td>
<td>1650</td>
</tr>
<tr>
<td>C≡C stretch</td>
<td>2220</td>
</tr>
<tr>
<td>-CH$_3$ umbrella bend</td>
<td>1375</td>
</tr>
<tr>
<td>-CH$_3$ asymmetric bend</td>
<td>1470</td>
</tr>
<tr>
<td>C=S</td>
<td>1100</td>
</tr>
</tbody>
</table>

Fingerprint Region : 1400-700 cm$^{-1}$

Within the fingerprint region the IR absorbance is not determined solely by characteristic groups but also by skeletal vibrations, e.g. whether a molecule has linear chains, branched chains or ring structures. This leads to a very complex spectrum, which is characteristic for each molecule and can be used as a "fingerprint" for identification.
Example:

Thioacetic acid is formed by replacing one of the oxygen atoms of acetic acid CH$_3$COOH with a sulphur atom.

Here is the spectrum. Which O has been replaced: the carbonyl O or the hydroxyl O?

Can you identify any other groups?
Another important application of IR spectroscopy is the identification of hydrogen bonding.

Hydrogen bonding significantly shifts vibration frequencies, e.g. the OH stretch of H₂O

<table>
<thead>
<tr>
<th>vibration</th>
<th>gas phase</th>
<th>liquid water</th>
</tr>
</thead>
<tbody>
<tr>
<td>symmetric</td>
<td>3567 cm⁻¹</td>
<td>3000-3500 cm⁻¹</td>
</tr>
<tr>
<td>antisymmetric</td>
<td>3756 cm⁻¹</td>
<td>3400-3700 cm⁻¹</td>
</tr>
</tbody>
</table>

The OH bond length increases in liquid water from 0.957 Å (gas phase) to 0.97 Å, i.e. the OH bond is weakened by hydrogen bonding and less energy is needed to excite it.

IR transmission spectrum of isopropyl alcohol
c) UV/visible Spectroscopy

Since all organic compounds exhibit UV/visible absorption, UV/visible spectroscopy is rarely used as a means of identification of compounds, i.e. apart from identifying whether or not π-bonding is present (\(\pi \rightarrow \pi^*\), \(n \rightarrow \pi^*\) transitions).

The major use of UV/visible spectroscopy in biological science is to monitor the absorbance of intrinsic or extrinsic probes of biological processes. Few examples:

- Assays of enzyme-substrate or hormone-receptor binding,
- Kinetics of enzyme reactions,
- DNA or protein folding and unfolding.

Many biological molecules contain UV/visible absorbing chromophores, whose absorbance wavelength and intensity are sensitive to their environment,

- e.g. tryptophan, phenylalanine, tyrosine (proteins)
- guanine, cytosine, thymine, adenine (DNA)
- porphyrin (haemoglobin, chlorophyll)
- retinal (rhodopsin)
Example: $\pi \rightarrow \pi^*$ transition of the amide linkage of poly-L-lysine

Random coil (———), pH 6.0, 25°C
β-Sheet (———), pH 10.8, 52°C
α-Helix ( - - - - ), pH 10.8, 25°C

Why are electronic transitions so sensitive to their environment?