Vibrational Spectroscopy

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Sir Chandrasekhar Venkata Raman (1956)
## Spectroscopic Techniques

<table>
<thead>
<tr>
<th></th>
<th>Gamma</th>
<th>X-Ray</th>
<th>UV/vis</th>
<th>Infrared</th>
<th>Microwave</th>
<th>Radiowave</th>
</tr>
</thead>
<tbody>
<tr>
<td>eV</td>
<td>14000</td>
<td>8000</td>
<td>2000</td>
<td>4 - 1</td>
<td>0.1-0.01</td>
<td>10^{-4} - 10^{-5}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10^{-6} - 10^{-7}</td>
</tr>
</tbody>
</table>

- **Mössbauer**
- **XAS**
- **EXAFS**
- **ABS**
- **MCD**
- **Raman**
- **IR**
- **EPR**
- **ENDOR**
- **NMR**
1. **Introduction**

2. **Molecular Vibrations**
   - Vibrational Frequencies and Normal Coordinates
   - Physical Origin of IR Intensities
   - Physical Origin of Raman Intensities
   - Physical Origin of Resonance Raman Intensities

3. **Experimental Techniques**
   - Raman Spectroscopy
   - Resonance Raman Spectroscopy
   - Infrared Spectroscopy (FT-IR)

4. **Applications in Bioinorganic Chemistry**
   - Hemoproteins
   - Copper Proteins
   - Metal-Radical Sites
   - Mononuclear Iron-Dioxygen Interactions
Why Vibrational Spectroscopy?

- **Structural Information (IR/Raman/resonance Raman)**
  - Identification of Characteristic Vibrations
  - Isotope Shifts
  - Normal Coordinate Analysis
  - Detection of functional groups

- **Electronic Information (resonance Raman)**
  - Identification of Electronic Transitions
  - Excitation Profiles
  - Insight into Bonding

- **Mechanistic Information (IR/Raman/resonance Raman)**
  - Trapping of Short Lived Intermediates
  - Freeze Quench Techniques
  - Combination with Electrochemistry, Stopped Flow, Continuous Flow,

- **Complementary to other Techniques**
  - Not dependendent on Magnetic Properties (EPR,MCD)
  - Much higher Resolution than Absorption and CD Spectroscopy
  - Not Limited to Certain Isotopes (Mössbauer)
IR versus Raman Spectroscopy

IR Experiment

IR Light Source → Sample Cuvette → IR Detector → Reference Cuvette → Computer

\[ A = -\log(I/I_0) \propto \varepsilon dc \]

Raman Experiment

VIS Laser → Sample → VIS Detector → Computer

\[ I_{sc} = \propto \nu^4 I_0 c \]

Methyldithio acetate

![Raman Spectrum](image)
IR versus Raman Spectroscopy

- > 1000 Atoms
- Thousands of Peaks in IR+Raman Spectra
- Impossible to understand in Detail

=> Both IR and Raman are "globally sensitive" to Secondary Structure Elements (similar to CD)

- Resonance Raman Selectively Enhances Vibrational Features of Chromophoric Groups
- Resonance Raman is Orders of Magnitude More Sensitive than off-Resonance Raman
- Highly Sensitive Difference-FT-IR can Provide Information about Changes in Parts of the Protein
- In "Non-Crowded Regions" (Protein and H₂O) IR peaks may be Directly Detected (i.e., Bound CO, CN⁻, NO)
II.A. Vibrational Frequencies and Normal Modes
Potential Energy Surfaces

\[ \text{"Potential Energy"} = T_{el} + V_{el,nuc} + V_{el,el} + V_{nuc,nuc} \]  
(Solution to Time Independent, Non-relativistic Schrödinger Equation)
The Vibrations of a Diatomic Molecule

Newton's law:

\[ F = -\frac{\partial V(R)}{\partial R} = m \frac{\partial^2 R(t)}{\partial t^2} \]

Molecule:

\[ V(R) = V_0 + \left. \frac{\partial V}{\partial R} \right|_{R=R_0} (R - \bar{R}) + \frac{1}{2} \left. \frac{\partial^2 V}{\partial R^2} \right|_{R=R_0} (R - \bar{R})^2 + \ldots \]

Thus:

\[ \frac{\partial^2 R(t)}{\partial t^2} = -\frac{k}{m} (R - \bar{R}) \]

Solution:

\[ R(t) = \bar{R} + c_1 \sin \left( \frac{\sqrt{k}}{m} t + \frac{\sqrt{k}}{m} t \right) + c_2 \cos \left( \frac{\sqrt{k}}{m} t \right) \]

Characteristic Quantities:

Vibrational Frequency \( v = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \)

Reduced Mass \( m = \frac{m_A m_B}{m_A + m_B} \)

Force Constant
The Reduced Mass and Isotope Shifts

Vibrational Frequency

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \]

Reduced Mass

\[ m = \frac{m_A m_B}{m_A + m_B} \]

Force Constant

Example: \( ^{16}\text{O}_2 \rightarrow ^{18}\text{O}_2 \)

\[ \nu(^{18}\text{O}_2) = 0.943 \nu(^{16}\text{O}_2) \]
### Force Constants

**Units:**

\[
k = \left. \frac{\partial^2 V}{\partial R^2} \right|_{R=R_0}, \quad [k] = \frac{\text{Energy}}{\text{Area}}
\]

**Practical Spectroscopy**

\[
[k] = \frac{10^{-18} \text{J}}{\text{Å}^2} = 10^2 \text{N m}^{-1} = \frac{\text{mdyn}}{\text{Å}}
\]

**Typical Force Constants:**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Force Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂</td>
<td>22.41</td>
</tr>
<tr>
<td>O₂</td>
<td>11.41</td>
</tr>
<tr>
<td>F₂</td>
<td>4.45</td>
</tr>
<tr>
<td>CO</td>
<td>18.55</td>
</tr>
<tr>
<td>NO</td>
<td>15.48</td>
</tr>
<tr>
<td>H₂</td>
<td>5.20</td>
</tr>
</tbody>
</table>

Force Constants Become Large if the Bonds are Strong (more correctly - if the bonds are *stiff*)

**Observed Trends:**

1. Bonds with Large Force Constants have High Dissociation Energies
2. Bonds with Large Force Constants are Short
Badgers Rule

**Observation** (Badger, R.M. (1934) *J. Chem. Phys.*, 2, 128)

Relationship between Bond Lengths and Vibrational Frequencies

\[ k \approx 1.86 \left( \bar{R} - d_{ij} \right)^3 \]

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>( d_{ij} ) (Angström)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>0.025</td>
</tr>
<tr>
<td>H</td>
<td>1st row</td>
<td>0.335</td>
</tr>
<tr>
<td>H</td>
<td>2nd row</td>
<td>0.585</td>
</tr>
<tr>
<td>H</td>
<td>3rd row</td>
<td>0.650</td>
</tr>
<tr>
<td>1st row</td>
<td>1st row</td>
<td>0.680</td>
</tr>
<tr>
<td>1st row</td>
<td>2nd row</td>
<td>0.900</td>
</tr>
<tr>
<td>1st row</td>
<td>3rd row</td>
<td></td>
</tr>
<tr>
<td>2nd row</td>
<td>2nd row</td>
<td>1.180</td>
</tr>
<tr>
<td>2nd row</td>
<td>3rd row</td>
<td></td>
</tr>
<tr>
<td>3rd row</td>
<td>3rd row</td>
<td>1.350</td>
</tr>
</tbody>
</table>
For a Morse Potential: \[ V(R) = D_e [1 - \exp(-\beta R)]^2 \]

\[ \frac{\partial^2 V(R)}{\partial R^2} = 2D_e \beta^2 \]

Qualitative Justification Of the Observed Trends
Vibrational States of a Diatomic

In the Quantum Mechanical Oscillator:
- Energy is quantized
- Can only give a probability for finding the nuclei in a certain arrangement
- There always is a zero-point energy

Key Equations:
- „Full“ wavefunction:
- „Full“ energy:
- Vibrational frequency:
- Vibrational energy:
- Vibrational wavefunction:

\[ \Psi_f = \Psi_{\text{Electronic}} \otimes \Psi_{\text{Vibrational}}^n \]

\[ E = E_{\text{Electronic}}^f + E_{\text{Vibrational}}^n \]

\[ \nu_f = \left( \frac{k}{m/4\pi^2} \right)^{1/2} \]

\[ E_{\text{Vibrational}}^n = (n + 1/2)\hbar\nu_f \]

\[ \Psi_{\text{Vibrational}}(R) = N_n \exp\left(-\frac{R^2}{4\sigma^2}\right)H_n\left(\frac{R}{\sqrt{2}\sigma}\right) \]

\[ \sigma^2 = \frac{\hbar}{4\pi m\nu} \]
Anharmonicity and ZPE Effects

1. Deviations from Equal Spacing
2. Actual Frequency is Always Lower than the Harmonic one
3. Effects are Larger the Larger the Frequency
4. Overtones become Allowed

\[ E_{vibrational}^{\ell,n} = h\nu \left( n + \frac{1}{2} \right) - x_e h\nu \left( n + \frac{1}{2} \right)^2 + \ldots \]

\[ D_0 = D_e - h\nu \]

Observable Quantity
Vibrations of Polyatomic Molecules

In a Polyatomic Molecule Many Vibrations are Possible.

- A Potential for a **Diatom Molecule** Leads to 1 Eigenfrequency and 1 **Vibrational Mode** (Stretching Vibration)
- In Polyatomic Molecules there are Different Forms of Movements:
  - **Stretches**
  - **Bends**:
  - **Torsions**:

- A Potential for an **N-Atomic Molecule** Leads $M=3N-6$ ($3N-5$ if the Molecule is Linear) Vibrational Frequencies ($\nu_i$) and also $M$ Vibrational Modes (=„**Normal**“ Modes, $Q_i$)
- In General **all Atoms move** in each Normal Mode which consists of Linear Combinations of „primitive“ Stretches Bends and Torsions (i.e. the Modes are **Delocalized**).
Rotations and Translations

If the Molecule Rotates or Translates as a Whole there is **NO Restoring Force** and therefore these Movements are Associated with the Vibrational Frequency Zero!

Translations

Rotations

Not a Rotation!
Normal Coordinates of Water

$\nu_1 (a_1)$  
Bending  
1595 cm$^{-1}$

$\nu_2 (a_1)$  
Symmetric Stretch  
3652 cm$^{-1}$

$\nu_3 (b_1)$  
Asymmetric Stretch  
3756 cm$^{-1}$

IR

Raman
Normal Coordinates of CO$_2$

$v_3 (\sigma_g^+)$
Antisymmetric Stretch
2349 cm$^{-1}$

$v_2 (\sigma_g^+)$
Symmetric Stretch
1337 cm$^{-1}$

$v_1 (\pi_u)$
Out of plane Bending
667 cm$^{-1}$

$v_1 (\pi_u)$
In plane Bending
667 cm$^{-1}$

„Doubly Degenerate“ Vibration
<table>
<thead>
<tr>
<th>Group</th>
<th>Compound Class</th>
<th>Frequency Range (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H</td>
<td>Alkanes</td>
<td>2965-2850</td>
</tr>
<tr>
<td></td>
<td>-CH₃</td>
<td>1450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1380</td>
</tr>
<tr>
<td></td>
<td>-CH₂</td>
<td>1465</td>
</tr>
<tr>
<td></td>
<td>Alkenes</td>
<td>3095-3010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>700-1000</td>
</tr>
<tr>
<td></td>
<td>Alkynes</td>
<td>~3300</td>
</tr>
<tr>
<td>C-C</td>
<td>Alkanes</td>
<td>700-1200</td>
</tr>
<tr>
<td>C=≡C</td>
<td>Alkenes</td>
<td>1680-1620</td>
</tr>
<tr>
<td>C=≡C</td>
<td>Alkynes</td>
<td>2260-2100</td>
</tr>
<tr>
<td>C≡O</td>
<td>Ketones</td>
<td>1725-1705</td>
</tr>
<tr>
<td></td>
<td>Aldehydes</td>
<td>1740-1720</td>
</tr>
<tr>
<td></td>
<td>Carbonic Acids</td>
<td>1725-1700</td>
</tr>
<tr>
<td></td>
<td>Esters</td>
<td>1750-1730</td>
</tr>
<tr>
<td></td>
<td>Amides</td>
<td>1700-1630</td>
</tr>
<tr>
<td></td>
<td>Anhydrides</td>
<td>1850-1800</td>
</tr>
<tr>
<td>C-O</td>
<td>Any</td>
<td>1300-1000</td>
</tr>
<tr>
<td>-O-H</td>
<td>Alcohols, isolated</td>
<td>3650-3590</td>
</tr>
<tr>
<td></td>
<td>Alcohols, H-bonded</td>
<td>3400-3200</td>
</tr>
<tr>
<td></td>
<td>Carbonic Acids</td>
<td>3300-2500</td>
</tr>
<tr>
<td>-N-H</td>
<td>Primary Amines</td>
<td>~3500</td>
</tr>
<tr>
<td></td>
<td>Secundary Amines</td>
<td>3500</td>
</tr>
<tr>
<td>C≡N</td>
<td>Nitriles</td>
<td>2260-2240</td>
</tr>
</tbody>
</table>
General Normal Coordinate Analysis

In General there will be a „Force Constant Matrix“ that is most Simply Calculated in Cartesian Displacements (e.g. with a Quantum Chemistry Program):

\[ F_{X_iY_j} = \frac{\partial^2 E}{\partial X_i \partial Y_j} \]

Masses

\[ \tilde{F}_{X_iY_j} = 15.57 \frac{F_{X_iY_j}}{\sqrt{M_A M_B}} \] (in mdyn/Å)

\[ \tilde{F}Y_I = x_I Y_I \]

Diagonalization

\[ \nu_I = 1302.78 \sqrt{x_I} \] (in cm⁻¹)

\[ Q_I = N^{-1/2} \left( M^{-1/2} Y_I \right) \]

Normalization

1. Great if you have an Accurate Force Field (Good Quantum Chemical Method/Program/Theoretician)
2. Errors Reflect the Shortcomings of the Theoretical Methodology. Hard to Fix!
Normal Coordinate Fitting

In Practice one often Wants to do an *Experimentally Motivated Analysis*. For this Reason a Suitable Fragment is Chosen.

1. Normal Coordinate Fitting is *Fairly Involved* (→Specialists 😊)
   - Many Parameters → Underdetermined Equations!
   - Depends on Subjective Choice of Model System
2. Often *Strong Simplications* are Necessary.
3. Results may Apply to larger *Classes of Compounds*

\[ \text{O=Mn-O-H} \]

Choose „Relevant“ Internal Coordinates \( S_I \)

„Guess“ Force Constant Matrix

Include Masses and Geometrical Parameters („FG“ Matrix)

Get Frequencies + Normal Modes

Fit to Experimentally Obs. Freq. (+Int.)
II.B. Vibrational Intensities
IR and Raman Transitions lead from one Vibrational State on a given Electronic Potential Energy Surface to Another Vibrational States:

- **Fundamentals**: \( n=0 \rightarrow n'=1 \), \( \Delta E=\hbar \nu \)
- **„Hot“ Bands**: \( n=1 \rightarrow n'=n\pm1 \), \( \Delta E=\hbar \nu \)
- **Overtones**: \( n=0 \rightarrow n'=2,3,... \), \( \Delta E=2\hbar \nu, 3\hbar \nu,... \)
- **Combination Bands**: \( n_1=0, n_2=0 \rightarrow n_1'=1, n_2'=1 \), \( \Delta E=\hbar \nu_1+\hbar \nu_2 \)
Physical Principles of IR and Raman Spectroscopy
Overtone and Combination Bands

![Graph showing Raman shift against frequency for various chemical bonds with a molecular structure of a complex compound depicted. The graph indicates the relationship between overtone and combination bands in vibrational spectroscopy.](image-url)
Physical Principle of IR Spectroscopy

Molecular Origin of Infrared Spectra

- Transfer of Infrared Energy to Vibrating Dipole

Vibrating heteronuclear diatomic molecule $\rightarrow$ Vibrating dipole

Oscillating IR electric field

$\nu_{\text{vibration}} = \nu_{\text{dipole}} = \nu_{\text{IR field}}$

(a)

(b)

- Infrared Absorption Intensity

$I_{IR}^{1/2} \propto |\mu|_{v',v} \equiv \langle v' | \mu | v \rangle \propto (\partial \mu / \partial Q)_0$

Dipole transition moment

Change of dipole moment due to normal vibration, $Q$
Physical Principle of Raman Spectroscopy

Polarizability:

\[ \alpha_{pq} = \sum_{n=0}^{\infty} \frac{\langle \Psi_0 | \hat{\mu}_p | \Psi_n \rangle \langle \Psi_n | \hat{\mu}_q | \Psi_0 \rangle}{E_n - E_0} \]

\[ h\nu_1 \quad h\nu_2 = h\nu_1 \pm \Delta\nu \]
Raman vs. Resonance Raman Spectroscopy

If the Incident Light is Close to an **Electronic Transition Energy** one needs the Frequency Dependent Polarizability (FDP):

\[
\alpha_{pq}(\nu) = \sum_{n=0}^{\infty} \frac{\langle \Psi_0|\hat{\mu}_p|\Psi_n\rangle \langle \Psi_n|\hat{\mu}_q|\Psi_0\rangle}{E_n - E_0 - \hbar\nu + i\Gamma} + \frac{\langle \Psi_0|\hat{\mu}_p|\Psi_n\rangle \langle \Psi_n|\hat{\mu}_q|\Psi_0\rangle}{E_n - E_0 + \hbar\nu + i\Gamma}
\]

Thus, if: \( E_I - E_0 \approx \hbar\nu \) The \( I^{th} \) Excited State Dominates the FDP and:

\[
I_{Raman}^{1/2}(Q_n) \propto \sum_{p,q} \frac{\partial \alpha_{pq}(\nu)}{\partial Q_n} = \frac{1}{i\Gamma} \sum_{p,q} \frac{\partial}{\partial Q_n} \langle \Psi_0|\hat{\mu}_p|\Psi_I\rangle \langle \Psi_I|\hat{\mu}_q|\Psi_0\rangle - \alpha_{pq}(\nu) \left( \frac{\partial (E_I - E_0)}{\partial Q_n} \right)_{Q=0} \frac{1}{i\Gamma}
\]

**Transition Dipole Moment** \( (I_{ABS} \approx M_{0n}^2) \)

**Damping Factor** (Linewidth)

**Transition Energy**

**Non-Resonant Energy**

**Resonant Energy**

**Smaller Term** (Non Totally Symmetric Modes)

**Larger Term** (Totally Symmetric Modes)

Most Resonance Raman Spectra are Dominated by Totally Symmetric Vibrations (Those that do not Break the Symmetry of the Molecule)

Very good to Observe Stretching Vibrations
Summary of Theoretical Aspects

1. **Force Constants** Measure the Stiffness of Internal Motions (*Stretches, Bends, Torsions*).

2. **Normal Modes and Vibrational Frequencies** Describe the „Eigenvibrations“ (Distortions) of the Molecule. They Depend on the **Force Constants** (different for each Electronic State!), the **Masses** of the Atoms and the **Geometry**.

3. **Isotope Shifts** of Vibrational Frequencies Occur if the **Masses of Atoms** are Changed.

4. **Normal Coordinate Analysis** is the Combined Theoretical and Experimental Determination of the Force Field.

5. **IR Intensities** Depend on the **Change of Dipole Moment** During the Normal Vibrations.

6. **Raman Intensities** Depend on the **Change of Polarizability** During the Normal Vibrations (Infinite Summation over Excited States).

7. **Resonance Raman Intensities** Depend on the **Distortion of the Molecule** in the Electronically Excited State Reached by Laser Photon (Transition Energy Gradients) and the **Transition Dipole Moments** (like Absorption Spectra).
## Summary of IR versus Raman

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infrared Spectroscopy</th>
<th>Raman Spectroscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectroscopic phenomenon</td>
<td>Absorption of light: $h\nu_{IR} = \Delta E_{vibr}$</td>
<td>Inelastic scattering of light: $h\nu_0 - h\nu_{sc} = \Delta E_{vibr}$</td>
</tr>
<tr>
<td>Allowed transition</td>
<td>$\Delta \nu = +1, +2, +3, \ldots$</td>
<td>$\Delta \nu = \pm 1, \pm 2, \pm 3, \ldots$</td>
</tr>
<tr>
<td></td>
<td>(transitions for $\Delta \nu = +2, +3, \ldots$</td>
<td>(transitions for $\Delta \nu = +2, +3, \ldots$</td>
</tr>
<tr>
<td></td>
<td>i.e., overtones are considerably less</td>
<td>i.e., overtones are considerably less</td>
</tr>
<tr>
<td></td>
<td>conspicuous than in IR)</td>
<td>conspicuous than in IR)</td>
</tr>
<tr>
<td>Excitation</td>
<td>Polychromatic IR radiation</td>
<td>Monochromatic radiation ($\nu_0$) in the UV, visible,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or near IR</td>
</tr>
<tr>
<td>Molecular origin</td>
<td>Dipole moment: $\mu = qr$</td>
<td>Induced dipole moment: $P = \alpha E$</td>
</tr>
<tr>
<td>Requirement for vibrational activity</td>
<td>Change in dipole moment during vibration: $(\partial \mu / \partial Q_k)_0 \neq 0$</td>
<td>Change in polarizability during vibration: $(\partial \alpha / \partial Q_k)_0 \neq 0$</td>
</tr>
<tr>
<td>Band intensity</td>
<td>$I_{IR}^{1/2} \propto (\partial \mu / \partial Q_k)_0$</td>
<td>$I_{R}^{1/2} \propto (\partial \alpha / \partial Q_k)_0$</td>
</tr>
<tr>
<td>Frequency measurement</td>
<td>Absolute: $\nu_{vibr} = \nu_{IR}$</td>
<td>Relative to the excitation frequency: $\nu_{vibr} = \nu_0 - \nu_{sc}$</td>
</tr>
<tr>
<td>Readout signal</td>
<td>Comparative: transmittance ($T = \Phi_s / \Phi_0$)</td>
<td>Absolute: radiant power or intensity of scattered</td>
</tr>
<tr>
<td></td>
<td>or absorbance ($A = -\log T$)</td>
<td>radiation</td>
</tr>
<tr>
<td>Spectral plot</td>
<td>Linear in %T or logarithmic in $A$ vs. wavenumber (cm$^{-1}$)</td>
<td>Linear: Raman intensity vs. wavenumber shift (cm$^{-1}$)</td>
</tr>
<tr>
<td>Dominant spectral feature</td>
<td>Vibrations destroying molecular symmetry: antisymmetric</td>
<td>Vibrations preserving molecular symmetry: symmetric</td>
</tr>
<tr>
<td></td>
<td>stretching and deformation modes</td>
<td>stretching modes</td>
</tr>
<tr>
<td>Inactive molecule</td>
<td>Homonuclear diatomics</td>
<td>None</td>
</tr>
<tr>
<td>Centrosymmetric molecule</td>
<td>Only &quot;$u$&quot;-symmetry modes active</td>
<td>Only &quot;$g$&quot;-symmetry modes active</td>
</tr>
<tr>
<td>Medium</td>
<td>Water is a strong absorber and is a poor solvent for IR</td>
<td>Water is a weak scatterer and is a good solvent for</td>
</tr>
<tr>
<td></td>
<td>studies</td>
<td>Raman studies</td>
</tr>
</tbody>
</table>

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$h$, Planck's constant; $\Delta E_{vibr}$, energy difference of vibrational levels; $\nu$, photon frequency; $\Delta \nu$, change in vibrational quantum number; $q$, charge; $r$, charge spacing; $\alpha$, molecular polarizability; $E$, electric field; $\Phi_s$ and $\Phi_0$, radiant powers transmitted by the sample and reference cells, respectively; $Q_k$, vibrational normal coordinate $(k \leq 3N - 6)$; "$g$" and "$u$", normal modes of vibration symmetric (gerade) and antisymmetric (ungerade) with respect to the molecular center of inversion.
III. Experimental Aspects
Experimental Raman Setup

- Laser
- Sample
- Monochromator
- CCD
- Photon Counting
- Detection Systems

Laser Light

Variable Frequency Laser Light
Lasers (Light Amplification by Stimulated Emission)

1. **Initial Photons** are created by Spontaneous Emission due to Heating or Electrical Discharge.
2. The emitted Photons **Stimulate** other Atoms (Molecules, Ions) to **Emitt** Photons of the Same Frequency *in Phase* \( (A^*+hv \rightarrow A+2hv) \)
3. As these Photons are absorbed by neighbouring Atoms Population **Inversion** is achieved.
4. By putting the Atoms (Molecules, Ions) between two Mirrors a Coherent **Photon Avalanche** is Created.
5. A Small Percentage of Photons is „Liberated“ through the **Output Coupler**.
Sample Compartments

(a) Capillary
Sealed or Flow

(b) Cylinder
Spin

(c) NMR Tube
Spin or Stir

(d) Pellet
Spin

(e) Low Temperature
Small sample of e.g., frozen solution Matrix

(f) Electrochemical
Electrode or Bulk

(g) Microscope
Small area, e.g., single crystal
Sample Compartments

Rotating Cell
- Reflection Shield
- Motor
- Position Adjustment
- From Laser
- Liquid Sample In Spinning Cell
- Beam Stopper
- Prism
- To Detector

Liquid N₂ Dewar
- Solid or Frozen Sample
- Collection And Focussing Optics
- To Detector
- From Laser
Collecting Optics

Depolarizer

Focussing Lens

Collecting Lens

Entrance Slit

Sample
Diffraction Grating is a Dispersive Element used to separate light into single wavelength contributions. Interference of incoming and outgoing light waves produces an interference pattern with maxima if:

$$d \sin(\Theta) = m\lambda$$

**Resolving Power of a Grating**

$$\frac{\lambda}{|\Delta\lambda|} = mN$$

- More Grooves = More Resolution
- Longer Wavelength = Higher Resolution

→ Use Smaller Grating at Longer Wavelengths
Detection Systems (CCD, PMT)

Each Pixel

- Metal
- Insulator
- Silicon Crystal

Pixel-Array, i.e. 1340 x 400

Pixel Position $\propto$ Frequency

Dispersed light

High Conversion Efficiency (40-60%)
Low Noise Levels
Simultaneous Detection of Large Parts of the Spectrum!

Cathode (e.g. GaAs)

High Voltage

Incoming Light

Photo-Electron

Secondary Electrons

Dynodes of Progressively Positive Potential

Current

Anode

• High Conversion Efficiency (40-60%)
• Low Noise Levels
• Simultaneous Detection of Large Parts of the Spectrum!
Possible Experimental Complications („Dirty Laundry Slide“)

1. Decomposition due to Overheating
2. Photodissociation of Bonds
3. Fluorescence Background
4. Exceedingly Weak Signals
5. Features Obscured by Solvent Peaks
6. External Artifacts (Cosmic Rays, Other Light Sources)
7. Plasma Lines from the Laser
Frequency Calibration

**CH₃CN, 298K**

- 375
- 911
- 1366
- 2240

**Na₂SO₄ Solid, 298K**

- 444
- 625
- 639
- 1092
- 1121
- 1141
- 1205
- 1226
- 1266
- 1312/1333
- 1361
- 1394
- 1456
- 1553
- 1589
- 1610
Intensity Calibration

Peak of Internal Standard:
- Frequency
- Intensity

Can Construct an „Excitation Profile“ i.e. the Intrinsic RR Intensity $P(\nu)$ as a Function of Excitation Wavelength

\[
\begin{align*}
\frac{I_{\text{sample}}}{I_{\text{Standard}}} & \propto \frac{c_{\text{sample}}\nu^4 I_0(\nu)}{c_{\text{standard}}\nu^4 I_0(\nu)} \frac{P_{\text{sample}}^{\text{RR}}(\nu)}{P_{\text{standard}}^{\text{RR}}} \\

P_{\text{sample}}^{\text{RR}}(\lambda) &= \text{const} \times \frac{I_{\text{sample}}}{I_{\text{Standard}}}
\end{align*}
\]
Experimental Setup for IR Spectroscopy

- The Whole Spectrum is Measured at the Same Time
- Very Good Signal/Noise due to High Light Throughput
- High Precision of Frequencies if Calibrated with a Laser (~0.1-0.01 cm⁻¹)

IR-Spectrum = Measurement with and without the Sample
IV. Bioinorganic Examples
Protein Secondary Structure

Protein FT-IR Spectra

- Amide I (C=O stretch)
- Amide II (C-N-H bend)

Wavenumber

α-Helix vs β-Sheet

1650-1660
1620-1640

Follow Protein Folding

unfolded

Folded (β-sheet)

(poly-lysine)
IR Kinetics - Bacteriorhodopsin

Cytoplasm

Extracellular

H^+

H^+

M→N
N→BR
N→O

Lys216–C^+\text{N}\text{H}

hv

L→M

AH^+

13-cis retinal

\tilde{\nu} = 1186 \text{ cm}^{-1}

Asp-85

\tilde{\nu} = 1762 \text{ cm}^{-1}

Time Resolved FT-IR
Hydrogenase

\[ \text{H}_2 \rightarrow 2\text{H}^+ + 2\text{e}^- \]
Hemoproteins – Spin and Oxidation State

Oxidation State Marker
- Fe(III) : $\nu_4 \approx 1375$ cm$^{-1}$
- Fe(II) : $\nu_4 \approx 1360$ cm$^{-1}$
- Fe(IV) : $\nu_4 \approx 1380$ cm$^{-1}$

But note (Fe(II)NO,CO $\nu_4 \approx 1375$ cm$^{-1}$)

Spin State and Core-Size Marker
- Low-Spin: $\nu_2 \approx 1580-1590$ cm$^{-1}$
Freeze Quenching
- $N_3^-$ Binding to Myoglobin -
Hemoproteins – Axial Ligands

Exogenous Ligands

- NO Binding to Ferric Hemoproteins Studied by UV-RR (244 nm)
- Met-Hb
- Met-Hb-$^{14}\text{N}^{16}\text{O}$
- Met-Hb-$^{15}\text{N}^{16}\text{O}$
- Difference Spectrum

Endogenous Ligands

- Detection of $\nu$(Fe-His) in Myoglobin
- DeoxyMb ($^{56}\text{Fe}$)
- DeoxyMb ($^{54}\text{Fe}$)
Hemoproteins – Reaction Intermediates

Cytochrome c Oxidase

Expected Intermediates

O$_2$  H$_2$O

Experimental Setup

Expected Results

ν(Fe=O)  ν(Fe-O$_2$)
Phenolates and Phenoxy Radical

Free Ligand Radical

$\nu_{7a}$ $\nu(C_1-O)$ $\nu_{8a}$ $\nu(C_5-C_3)$

Absorption

Resonance Raman

Fe(III) $\rightarrow$ $[\text{Fe(L)}]^{0,1+,2+,3+}$

-3H$^+$

571 nm 418 nm 514 nm
Galactose Oxidase – Detecting Radicals

![Diagram of Galactose Oxidase](image)

Chemical structures:
- $R-H_2C-OH$
- $R-HC=O$

Phenolates and Phenoxyls

Graph showing absorption spectra with peaks at different wavelengths.

Active Oxidase:
- Peaks at 1170, 1249, 1375, 1416, 1439, 1487, 1500, 1595 cm$^{-1}$.
Copper Proteins - The Cu\textsubscript{Z} Site

\[ \text{Cu}_A + \text{Cu}_Z \]

Proves Cu-S Involvement in Cu\textsubscript{Z}
(note that in this case this had been proven before by MCD and Biochemistry in Norwich)
Oxygen Activation in Mononuclear Iron Proteins

Oxygen Activation Pathway:

\[ \text{[Fe}^{\text{III}}\text{O}_2^-] \quad \text{[Fe}^{\text{II}}\text{O}_2] \]

\[ \text{Fe} \quad \text{O}_2 \]

\[ \text{Fe}^{\text{III}}\text{O}_2^- \]

\[ \text{Fe}^{\text{III}}\text{O}_2 \]

\[ \text{Fe}^{\text{III}}\text{OH} \]

\[ \text{Fe}^{\text{III}}\text{-OOH} \]

(S=1/2 or S=5/2)

products

Substrate Activation Pathway:

\[ \text{[Fe}^{\text{III}}\text{S}] \]

\[ \text{O}_2 \]

\[ \text{Fe} \quad \text{S} \quad \text{O}_2 \]

\[ \text{Fe}^{\text{III}}\text{SO}_2 \]

products
Electronic Structure of the Side-On Fe(III) Peroxo Bond

\[ \text{H}_2\text{O}_2 \rightarrow \cdot \text{OOH} + \text{H}^+ \]

\[ \text{[Fe(EDTA)(OH)]}^{2-} \quad \text{[Fe(EDTA)(O_2)]}^{3-} \quad \text{[Fe(EDTA)]}^- \]

Absorption+MCD

Raman

Orbitals

\[ \pi^* \quad \sigma \quad \pi^* \]

\[ ^{18}\text{O}^{16}\text{O} \quad ^{18}\text{O}^{18}\text{O} \]
Resonance Raman Spectra of $[\text{Fe(EDTA)(O}_2\text{)]}^{3-}$
Excitation Profile Analysis for \([\text{Fe(EDTA)}(\text{O}_2)]^{3-}\)
MCD Spectra of $[\text{Fe(EDTA)}(\text{O}_2)]^{3-}$
Assignment of Absorption Bands of [Fe(EDTA)(O$_2$)]$^{3-}$
Electronic Structure Insights: $[\text{Fe(EDTA)(O}_2\text{)}]^{3-}$
Fe(III) Side On Peroxo versus End-On Hydroperoxo

\[
\begin{align*}
\text{Fe} & - \text{O} \quad \text{O} \\
\text{S} &= 5/2
\end{align*}
\]

\[
\begin{align*}
\nu(\text{Fe-O}) &\quad 459 \\
\nu(\text{O-O}) &\quad 816
\end{align*}
\]

\[
\begin{align*}
\text{Fe} &- \text{O} - \text{OR} \\
\text{S} &= 5/2
\end{align*}
\]

\[
\begin{align*}
\nu(\text{Fe-O}) &\quad 469 \\
\nu(\text{O-O}) &\quad 842/876
\end{align*}
\]

\[
\begin{align*}
\text{Fe} &- \text{O} \quad \text{OH} \\
\text{S} &= 1/2
\end{align*}
\]

\[
\begin{align*}
\nu(\text{Fe-O}) &\quad 626 \\
\nu(\text{O-O}) &\quad 789
\end{align*}
\]

<table>
<thead>
<tr>
<th>Bond Type</th>
<th>Reactivity</th>
<th>Fe(II) +</th>
<th>[FeO]^{2+} +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-O_{\pi}</td>
<td>weak</td>
<td>HOO•</td>
<td>HO•</td>
</tr>
<tr>
<td>Fe-O_{\sigma}</td>
<td>strong</td>
<td></td>
<td>moderate</td>
</tr>
<tr>
<td>O-O</td>
<td>strong</td>
<td></td>
<td>weak</td>
</tr>
<tr>
<td>Reactivity</td>
<td>unreactive</td>
<td>Fe(II) +</td>
<td>[FeO]^{2+} +</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{Raman Shift (cm}^{-1}\text{)} \\
459 &\quad 816 \\
469 &\quad 842/876 \\
626 &\quad 789
\end{align*}
\]
Summary and Conclusions

1. FT-IR and Raman are Useful for Studying Protein Secondary Structure
2. FT-IR is Highly Suitable for Time Resolved Measurements
3. FT-IR is sensitive for Difference Spectroscopy and Fingerprinting in regions where the protein does not Strongly Absorb
4. RR is extremely Sensitive and is highly Specific in Enhancing only Vibrations Coupled to the Chromophore
5. RR Provides very Powerful Fingerprints
6. RR yields Detailed Electronic and Structural Information
7. RR can be Combined with Freeze-Quench or Flash-Flow Techniques to Study Kinetics
8. RR can be Combined with Electrochemistry (i.e. Surface Enhanced Resonance Raman Spectroscopy, SERR)
These are three very good and highly pedagogical reviews written by leading experts in the field:

  The classic text on vibrational spectroscopy at an introductory level. Describes Normal coordinate analysis in detail.

  This is one of the many good introductory texts in vibrational spectroscopy and group theory.

- **Spiro, T.G. (Ed.)** Biological Applications of Raman Spectroscopy. Wiley Interscience, Volumes 1-3, **1988**
  This series of books is highly recommended and gives many very detailed reviews that describe the application of Raman Spectroscopy in biochemistry.


  These two references describe the application of the so-called „time-dependent“ theory of resonance Raman spectroscopy which is very useful for the analysis of excitation profiles.
Time Dependent Theory of RR Spectroscopy

Resonance Raman Intensity (E. Heller):

\[ I_{kR}^{RR}(E_L) \propto E_L E_S^3 \left| \sum J M_0^{2J} \int_0^\infty e^{i t \left( E_L - E_j^{(0)} \right)} \left[ e^{i \omega t} - \frac{1}{\Delta_k} \right] \right| \prod_p e^{-\frac{1}{2} \left( \Delta_p' \right)^2 \left\{ -\exp(-i \omega_p t) \right\}} \right|_t dt \]

Absorption Intensity

Excited State Distortion

Absorption Intensity:

\[ I_{ABS}^{ABS}(E_L) \propto E_L \sum J M_0^{2J} \int_{-\infty}^\infty e^{i t \left( E_L - E_j^{(0)} \right)} \left[ e^{i \omega t} - \frac{1}{\Delta_p} \right] \prod_p e^{-\frac{1}{2} \left( \Delta_p' \right)^2 \left\{ -\exp(-i \omega_p t) \right\}} dt \]