Chapter 4 - Experimental Techniques

Separation and purification techniques: 
Gas Chromatography (GC)

- Separate volatile components of a mixture
- Depends on the different interactions of the components in a mobile phase (carrier gas) with a stationary phase (alumina or silica)
- Characteristic retentions times
Separation and purification techniques:
Liquid Chromatography (LC)

Liquid chromatography is a separation technique in which the mobile phase is a liquid, the stationary phase is either packed inside a column or adhered to a glass plate.

- Ratio of distance travelled by the analyte to that of the solvent front is the retention factor ($R_f$)
- Equilibrium between the surface bound and solution species, preference for stationary or mobile phase is given by the equilibrium constant:
  $$ K = \frac{a_{\text{stationary}}}{a_{\text{mobile}}} $$
- Monitor separation by eye or UV absorption, under gravity flow or pressure (flash chromatography)

Separation and purification techniques:
High-Performance Liquid Chromatography (HPLC)

HPLC is type of liquid chromatography in which the mobile phase is introduced under pressure ($\approx 40$ Mpa) and the stationary phase consists of very small particles ($d = 3-10 \, \mu$m).
Recrystallization

-purification step involving the dissolution and crystallization of a solid from a solvent or solvent mixture.

Elemental Analysis: CHN analysis by combustion
Atomic absorption spectroscopy (AAS)

-quantitative determination of a metal can be carried out using atomic absorption spectroscopy (AAS) by observing the diagnostic absorption spectrum of gaseous atoms of the metal

- Specific wavelength
- Atomizer
- Standards and calibration curve
- Beer-Lambert Law
- Sensitive, limit of detection near μg/L

Thermogravimetric analysis (TGA)

The change in mass of a sample is monitored as a sample is heated at a constant rate.

Investigate: solvent of crystallization, thermal decomposition, gas uptake
Mass spectrometry

Separation of ions (atomic or molecular) according to the mass to charge ratio (m/z).

Many known techniques: electron ionization (electron impact, EI), fast atom bombardment (FAB), matrix assisted laser desorption ionization time of flight (MALDI-TOF), and electrospray ionization (ESI)

Electron ionization
- production of ions by bombarding gaseous molecules with high energy electrons
- A ‘hard’ technique that causes fragmentation of the parent molecule

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Carbon Tetrachloride

MASS SPECTRUM

NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)
**FAB mass spectrometer**

Ions are produced by bombarding the sample (neutral molecules or ionic salts) with high energy xenon or argon atoms. It is a soft technique and usually causes little fragmentation.

**MALDI-TOF mass spectrometer**

Matrix assisted laser desorption ionization time-of-flight mass spectrometry utilizes a UV laser
- A 'soft' method with little fragmentation.
Electrospray ionization (ESI) mass spectrometry
• A ‘soft’ method for neutral molecules or ionic salts.

Infrared and Raman spectroscopies

• For a vibrational mode to be IR active, it must give rise to a change in dipole moment.
• For a vibrational mode to be Raman active, it must give rise to a change in polarizability.

Molecules undergo anharmonic oscillations, but we can approximate a transition from the ground state to first vibrational state with a simple harmonic oscillator.

\[ E_v = (v+1/2)\hbar\nu \]
Reduced mass

\[ \frac{1}{\mu} = \frac{1}{m_X} + \frac{1}{m_Y} \quad \text{or} \quad \mu = \frac{m_X m_Y}{m_X + m_Y} \]

Fundamental absorption

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

where:
- \( \nu \) = fundamental vibrational frequency (Hz)
- \( k \) = force constant (N m\(^{-1}\))
- \( \mu \) = reduced mass (kg)
- c = 3.00×10\(^{10}\) cm s\(^{-1}\)

wavenumber

\[ \tilde{\nu} = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}} \]

Application of Isotopes

Infrared Spectroscopy (IR)

- **Reduced mass** and corresponding shift of absorption
- Rapid deuterium exchange reactions to investigate N-H, O-H, and C-H assignments in spectra.

Kinetic Isotope Effect
- Probe reaction mechanisms

<table>
<thead>
<tr>
<th>Functional group</th>
<th>( \nu \text{ cm}^{-1} )</th>
<th>Functional group</th>
<th>( \nu \text{ cm}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>O–H</td>
<td>3700–3500</td>
<td>CO ligand (terminal MCO)(^1)</td>
<td>2200–1900</td>
</tr>
<tr>
<td>O–H (hydrogen bonded)</td>
<td>3500–3000</td>
<td>CO ligand (bridging M–CO)(^1)</td>
<td>1900–1700</td>
</tr>
<tr>
<td>N–H</td>
<td>3500–3200</td>
<td>NO (terminal, linear MNO)</td>
<td>1900–1650</td>
</tr>
<tr>
<td>C–H</td>
<td>3000–2850</td>
<td>NO (terminal, bent MNO)</td>
<td>1690–1525</td>
</tr>
<tr>
<td>B–H (terminal)</td>
<td>2650–2350</td>
<td>CN ligand (terminal MCN)</td>
<td>2200–2000</td>
</tr>
<tr>
<td>B–H (bridge)</td>
<td>2100–1600</td>
<td>B–Cl</td>
<td>1000–600</td>
</tr>
<tr>
<td>S–H</td>
<td>2700–2550</td>
<td>C–Cl</td>
<td>1000–600</td>
</tr>
<tr>
<td>P–H</td>
<td>2450–2273</td>
<td>Si–Cl</td>
<td>750–600</td>
</tr>
<tr>
<td>Si–H</td>
<td>2250–2100</td>
<td>N–Cl</td>
<td>800–600</td>
</tr>
<tr>
<td>Al–H</td>
<td>1800–1700</td>
<td>P–Cl</td>
<td>600–450</td>
</tr>
<tr>
<td>C=O (organic)</td>
<td>1750–1650</td>
<td>O–Cl</td>
<td>1200–700</td>
</tr>
<tr>
<td>C=N (organic)</td>
<td>1690–1630</td>
<td>S–Cl</td>
<td>750–400</td>
</tr>
</tbody>
</table>

\(^1\)See Fig. 24.2 and accompanying discussion.
IR spectra of $[\text{Co(NH}_3\text{)}_6\text{]}_2[\text{SO}_4\text{]}_3 \cdot 5\text{H}_2\text{O}$ (in red) and $[\text{Co(NH}_3\text{)}_5(\text{OSO}_3\text{})]\text{Br}$ (in green)


\[
\begin{array}{cccc}
D_m & \nu_2 (A_1^+), & \nu_2 (E), & \nu_4 (E) \\
\hline
[\text{CO}_3]^{2-} \text{ (solid CaCO}_3\text{)} & 187 & 1429\text{-}1492 \text{ broad} & 766 \\
[\text{NO}_3]^{-} \text{ (solid NaNO}_3\text{)} & 151 & 1405 & 692 \\
[\text{NO}_3]^{-} \text{ (solid KNO}_3\text{)} & 128 & 1370 & 605 \\
\hline
C_m & \nu_3 (A_1), & \nu_1 (A_1), & \nu_2 (E), & \nu_2 (E) \\
\hline
[\text{SO}_4]^{2-} \text{ } & 967 & 630 & 943 & 669 \\
[\text{CrO}_4]^{2-} \text{ } & 533 & 606 & 977 & 477 \\
\hline
D_m & \nu_2 (A_2), & \nu_2 (E), & \nu_2 (E) \\
\hline
[\text{PO}_4]^{3-} \text{ } & 150 & 321 & 161 \\
[\text{PCl}_3]^{3-} & 147 & 213 & 165 \\
\end{array}
\]

• **Rayleigh scattering**: most of light of frequency $\nu_0$ is scattered by a molecule with no change in frequency.

• Some of the scattered light has frequency $\nu = \nu_0 + \nu$, where $\nu$ is the fundamental frequency of the molecule (Raman scattering).

• Raman scattering extends to lower wavenumber than routine lab IR spectroscopy, so one may observe M-L modes.

• Sensitivity may be increased by **resonance Raman spectroscopy**, where the wavelength of the laser corresponds to the wavelength of absorptions in the electronic spectrum of the compound.
**Electronic spectroscopy**

- transitions of electrons between energy levels and covers both absorption and emission spectroscopies
- HOMO-LUMO
- Broad bands are observed
- Franck-Condon approximation

### Notation for emission:
(high E level) → (low E level)
c.g. \( \pi^* \rightarrow \pi \)

### Notation for absorption:
(high E level) ← (low E level)
c.g. \( \pi^* ← \pi \)

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The UV-VIS spectrum of a CHCl₂ solution (8.8 \( \times \) 10⁻⁵ mol dm⁻³) of the copper(II) complex shown in the figure. Absorption maxima are at 252, 292, 347 (shoulder), 415 and 612 nm.

**Beer-Lambert Law**

\[ A = \varepsilon bc \]

Absorbance (A), molar extinction (\( \varepsilon \)), path length (b), concentration (c)

From a plot A vs \( \lambda \), determine: \( \lambda_{\text{max}} \) (nm), \( A_{\text{max}} \), \( \varepsilon_{\text{max}} \)

\[ A = -\log T = -\log(I/I_o) \]

\( \varepsilon_{\text{max}} \) against wavelength enables one to readily compare compounds

Compare shifts in \( \lambda_{\text{max}} \), a **bathochromic effect** (red shift, lower E) or **hypsochromic effect** (blue shift, higher E)
Sources of $^2$H and $^{13}$C

Isotopically enriched solvents needed for NMR experiments

At the anode: $2H_2O \leftrightarrow O_2 + 4H^+ + 4e^-$

At the cathode: $4H_2O + 4e^- \leftrightarrow 2H_2 + 4OH^-$

Separation Factor: $\frac{H}{D}$

K = 1.026

$^{13}CN(g) + [^{13}CN]^{-} (aq) \leftrightarrow ^{12}CN(g) + [^{12}CN]^{-} (aq)$

K = 1.012

Nuclear Spin

- A nucleus with an odd atomic number or an odd mass number has a nuclear spin.
- The spinning charged nucleus generates a magnetic field.
External Magnetic Field

When placed in an external field, spinning protons act like bar magnets.

Two Energy States

The magnetic fields of the spinning nuclei will align either *with* the external field, or *against* the field.

A photon with the right amount of energy can be absorbed and cause the spinning proton to flip.
The NMR Spectrometer
The NMR Graph

Delta Scale

chemical shift, ppm \( \delta \) = \( \frac{\text{shift downfield from TMS (in Hz)}}{\text{spectrometer frequency (in MHz)}} \)

<table>
<thead>
<tr>
<th>600 Hz</th>
<th>480 Hz</th>
<th>360 Hz</th>
<th>240 Hz</th>
<th>120 Hz</th>
<th>0 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

ppm \( \delta \)

60 MHz

<table>
<thead>
<tr>
<th>3000 Hz</th>
<th>2400 Hz</th>
<th>1800 Hz</th>
<th>1200 Hz</th>
<th>600 Hz</th>
<th>0 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

ppm \( \delta \)

300 MHz

\( \Rightarrow \)
Location of Signals

- More electronegative atoms deshield more and give larger shift values.
- Effect decreases with distance.
- Additional electronegative atoms cause increase in chemical shift.

<table>
<thead>
<tr>
<th>TABLE 13.3 Chemical Shifts of the Chloromethanes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reassembled</strong></td>
</tr>
<tr>
<td>H—Cl</td>
</tr>
<tr>
<td>H-CH—C—Cl</td>
</tr>
<tr>
<td>H-CH—C—Cl</td>
</tr>
<tr>
<td>H-CH—C—Cl</td>
</tr>
</tbody>
</table>

*Note: Each chlorine atom added changes the chemical shift of the remaining methyl proton by about 2 to 3 ppm. These changes are mainly additive.*

Typical Chemical Shifts, $^1H$

<table>
<thead>
<tr>
<th>Type of Proton</th>
<th>Approximate $\delta$</th>
<th>Type of Proton</th>
<th>Approximate $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkane (—CH$_3$)</td>
<td>0.9</td>
<td>C=C—CH$_3$</td>
<td>1.7</td>
</tr>
<tr>
<td>alkane (—CH$_2$—)</td>
<td>1.3</td>
<td>Ph—H</td>
<td>7.2</td>
</tr>
<tr>
<td>alkane (—CH—)</td>
<td>1.4</td>
<td>Ph—CH$_3$</td>
<td>2.3</td>
</tr>
<tr>
<td>O—CH$_3$</td>
<td>2.1</td>
<td>R—CHO</td>
<td>9–10</td>
</tr>
<tr>
<td>C=O</td>
<td>2.5</td>
<td>R—COOH</td>
<td>10–12</td>
</tr>
<tr>
<td>R—CH$_3$—X</td>
<td>3–4</td>
<td>R—OH</td>
<td>variable, about 2–5</td>
</tr>
<tr>
<td>(X = halogen, O)</td>
<td></td>
<td>Ar—OH</td>
<td>variable, about 4–7</td>
</tr>
<tr>
<td>&gt;C=C&lt;</td>
<td>5–6</td>
<td>R—NH$_2$</td>
<td>variable, about 1.5–4</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Natural abundance (%)</td>
<td>δ</td>
<td>Frequency of observation / MHz (referred to $^1$H at 100 MHz)</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------</td>
<td>---</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>$^1$H</td>
<td>&gt;99.9</td>
<td>100</td>
<td>15.35</td>
</tr>
<tr>
<td>$^3$H</td>
<td>0.041</td>
<td>15.35</td>
<td></td>
</tr>
<tr>
<td>$^7$Li</td>
<td>7.6</td>
<td>38.9</td>
<td>LiCl (1 H in H$_2$O)</td>
</tr>
<tr>
<td>$^{13}$B</td>
<td>80.1</td>
<td>32.1</td>
<td>Fe$_2$(B-OR)$_3$</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>1.1</td>
<td>25.1</td>
<td>SiMe4</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>0.04</td>
<td>15.5</td>
<td>H$_2$O</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>100</td>
<td>94.0</td>
<td>CCl$_4$</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>100</td>
<td>26.48</td>
<td>NaCl (1 H in H$_2$O)</td>
</tr>
<tr>
<td>$^{27}$Al</td>
<td>100</td>
<td>26.1</td>
<td>[Al(OH)$_3$]$^{3-}$</td>
</tr>
<tr>
<td>$^{28}$Si</td>
<td>4.67</td>
<td>19.9</td>
<td>SiMe4</td>
</tr>
</tbody>
</table>

1. The operating frequency of an instrument is defined by the field of the magnet and is designated by the frequency at which the $^1$H nuclei of SiMe$_4$ resonate.
2. It is important to quote the reference when reporting NMR spectra since alternative references may be used.
Chemical Shift ($\delta$)

Intensity

$\text{CH}_3\text{-CH}_2\text{-I}$

Coupling to the first $^1H$
$(2 \times \frac{1}{2} + 1 = 2)$

Coupling to the second $^1H$

Coupling to the third $^1H$
Doublet: 1 Adjacent Proton
NMR spectroscopy: a factual résumé.

Proton-Coupled $^{13}$C Spectra

<table>
<thead>
<tr>
<th>3 protons</th>
<th>2 protons</th>
<th>1 proton</th>
<th>0 protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_3\text{C}-\text{H}$</td>
<td>$\text{H}_2\text{C}-\text{H}$</td>
<td>$\text{H}_1\text{C}-\text{H}$</td>
<td>$\text{H}_0\text{C}-\text{H}$</td>
</tr>
<tr>
<td>$n + 1 = 4$</td>
<td>$n + 1 = 3$</td>
<td>$n + 1 = 2$</td>
<td>$n + 1 = 1$</td>
</tr>
</tbody>
</table>

Catherine E. Housecroft and Alan G. Sharpe: *Inorganic Chemistry* 2e © Pearson Education 2005
Proton-coupled $^{13}$C spectra for ethyl phenylacetate.

Proton-decoupled $^{13}$C NMR spectrum for ethyl phenylacetate.

The 162 MHz $^{31}$P NMR spectra of PPh$_3$ and O=PPh$_3$. 
The 100 MHz NMR spectrum of butanone.

Paramagnetically shifted $^1$H NMR spectra.
The 162 MHz $^{31}$P NMR spectrum of a salt of [PF$_6$]$^-$ consists of a binomial septet. The value of $J_{PF}$ can be measured between any pair of adjacent lines in the signal.

The 128 MHz $^{11}$B NMR spectrum of a solution of NaBH$_4$ in CD$_3$C(O)CD$_2$. The value of $J_{BH}$ can be measured between any pair of adjacent lines in the signal.
The 162 MHz $^{31}\text{P}{}^1\text{H}$ NMR spectrum of the adduct PhMe$_2$P:BH$_3$. The four-line pattern is not a binomial quartet but an approximate 1:1:1:1 multiplet.

The 376 MHz $^{19}\text{F}$ NMR spectrum of $[\text{XeF}_5]^-$
Berry pseudo-rotation interconverts one trigonal bipyramidal structure into another via a square-based pyramidal transition state. The numbering scheme illustrates that axial and equatorial sites in the trigonal bipyramid are interchanged.
Under an applied magnetic field, $B_0$, the interaction between an unpaired electron and the magnetic field results in a splitting of the energy levels (the **Zeeman electronic effect**).

\[ \Delta E = g \mu_B B_0 \]

where $g =$ Lande g-factor (g-value), $B_0 =$ applied magnetic field (in Tesla, T), $\mu_B =$ Bohr magneton ($1 \mu_B = \frac{eh}{4\pi m_e} = 9.2740 \times 10^{-24} \text{ J T}^{-1}$)

The g-value is given by ratio $2\mu_e/\mu_0$ where $\mu_e$ is the electron magnetic moment ($9.2848 \times 10^{-24} \text{ J T}^{-1}$). $g = 2.0023$ for a free electron.

The typical appearance of a simple EPR spectrum is shown by the blue line. This is the first derivative of the absorption peak (shown in red).
Typical line shapes for EPR spectra of an isotropic system (blue line), an anisotropic system with axial symmetry (red line), and an anisotropic system (green line).

(a) Coupling between an unpaired electron on a Co$^{2+}$ ion with the $^{59}$Co nucleus (100%, $I = 7/2$) gives rise to an 8-line hyperfine splitting pattern for the EPR signal (microwave frequency = 9.785 GHz). (b) Coupling between an unpaired electron on a Cu$^{2+}$ ion with the $^{63}$Cu and $^{65}$Cu nuclei (69.2% and 30.8%, respectively, both $I = 3/2$) produces two superimposed 4-line splitting patterns. $A$ and $A^*$ are the hyperfine coupling constants.
Mössbauer Spectroscopy

<table>
<thead>
<tr>
<th>Nucleus observed</th>
<th>Natural abundance /%</th>
<th>Ground spin state</th>
<th>Excited spin state</th>
<th>Radioisotope source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{57}\text{Fe}$</td>
<td>2.2</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$^{57}\text{Co}$</td>
</tr>
<tr>
<td>$^{115}\text{Sn}$</td>
<td>8.6</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$^{119}\text{Sn}$</td>
</tr>
<tr>
<td>$^{99}\text{Ru}$</td>
<td>12.7</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$^{100}\text{Rh}$</td>
</tr>
<tr>
<td>$^{117}\text{Ag}$</td>
<td>100</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
<td>$^{119}\text{Sn}$</td>
</tr>
</tbody>
</table>

$^a$ $m_i$ = metastable.

Mössbauer effect (ME)
(recoilless gamma-ray emission and absorption)

Mössbauer Spectroscopy: $\text{Fe}_3(\text{CO})_{12}$

- First prepared 1907, but the structure eluded chemists for almost 50 years
- Molecular formula established 1930, three $\text{Fe(CO)}_4$ units per molecule
- Over the next 30 years a variety of structures were proposed, an example being a high-symmetry structure (incorrect)

- Early X-ray diffraction studies were unsuccessful because of disorder in the molecular structure
- IR spectroscopic studies indicated two types of carbonyl ligands
  - Terminal
  - Bridging
The solid state structure of Fe$_3$(CO)$_{12}$ as determined by X-ray diffraction methods. The molecule contains two Fe environments by virtue of the arrangement of the CO groups.

**Diffraction Methods**

Schematic representation of the interaction of X-rays with layers of atoms in a crystal. This leads to the derivation of Bragg’s equation:

\[ 2d \sin \theta = n \lambda \]
Single crystal X-ray diffraction

Single crystal growth - relies on lowering the solubility of the compound in solution (via evaporation, cooling, diffusing a solvent in which compound is insoluble into a solution of the compound in a second solvent, diffuse a vapor of volatile solvent into a solution of the compound in a second solvent).


Crystal structure of organic ligand (b) 123 K and (c) 293 K with ellipsoids plotted at a 50% probability level.
Polycrystalline Powder

Lattice points at corners of unit cell
Motif inside unit cell
Neither falls on a lattice point
Atoms from neighboring unit cells can bond
The interconnected hexagonal rings become apparent when we connect atoms from neighboring unit cells.

Begin with a single hexagonal unit cell containing a motif of two carbon atoms.

Tile unit cells together to create the crystal structure.

Powder X-ray diffraction

A powder may be composed of many small and finely ground crystals, known as crystallites.

Identifying bulk sample, screening, structure refinement, structure solution
- Crystal structure, coordination, bond distances, bond angles

Calculated powder pattern for 4'-azido-2,2':6',2''-terpyridine
**Electron diffraction**

**Gas phase electron diffraction**: electrons scattered by electric fields of atomic nuclei in gas phase molecules

- Electrons at 50 kV ($\lambda = 5.5$ pm) and obtain a diffraction pattern
- Obtain intramolecular bond parameters

![Experimental (dots) and calculated (solid line) radial distributions for SiMe₄.](image)

Solids may also be examined using electron diffraction. Low-energy electron diffraction (LEED) is useful for the study of surfaces on solids.

**Computational Methods**

**Hartree-Fock Theory** – Approximate solutions to Schrodinger equation, converge to self-consistency. Considering only valence electrons, a number of semi-empirical methods CNDO (complete neglect of differential overlap, INDO (intermediate neglect of differential overlap), MNDO (modified neglect of diatomic overlap), AM1 (Austin model 1), and PM3 (parametric method 3).

**Density Functional Theory (DFT)** – Focus on the electron density distribution in the system instead of many electron wavefunctions. Several levels BLYP, B3LYP.

**Huckel MO Theory** – Works well for $\pi$-systems of unsaturated organic molecules. Extended Huckel theory for most hydrocarbons.

**Molecular mechanics (MM)** – Uses strain energy (no quantum mechanical basis). Conformation of a molecule changes until the strain energy is minimized. AMBER and CHARMM methods. Generally applied to small (discrete molecules) and large (nucleic acids and proteins).