
Instrument:
light source: common energy range of 1200 cm\(^{-1}\) (near IR) and 200 cm\(^{-1}\) (far UV)
sample space: room and LN2 temperature for EAS and LHe for MCD
magnet: 1T for Cu(II), at least 7 T for Fe(II)/Fe(III) - top of the line 11.5T
(Vendors: Cary for UV/VIS, Jasco for CD, Oxford Instruments for superconductormagnets)

Reminder:
- depending on the nature of the excited valence electrons
  a) d→d ligand field transition (electron hole(s) in the d-manifold)
  b) L→d ligand-to-metal charge transfer (donation)
  c) d→L metal-to-ligand charge transfer (backdonation)
  d) L→L ligand-to-ligand charge transfer (ligands with \(\pi\)-electrons)
- UV/VIS - color change indicates changes in the energy levels of the orbitals ⇒ change in electronic structure ⇒ change in geometric structure ⇒ change in chemical function

1. Electronic absorption spectroscopy (EAS):
- intensities: parity/Laporté forbidden (vibronic allowed); spin forbidden (spin orbit allowed)
- oscillator strength
  \[ f_{\text{exp}} = \text{const} \int \epsilon(\nu) d\nu \]  
  (area under the absorption band)
  \[ f_{\text{calc}} = \text{const} \nu^2 \left[ \int \Psi_g \hat{M} \Psi_e^* d\tau \right]^2 \]  
  (transition dipole moment integral)
- for light (\(\lambda >> \) radius of electron on orbit)
  \[ \hat{M}_{\text{total}} = \hat{M}(\text{electric dipole}) + \hat{M}(\text{magnetic dipole}) + \hat{M}(\text{electric quadrupole}) + ... \]
- information content:
  a) band intensity gives the nature of the transition
  b) Tanabe-Sugano diagram/matrix for ligand field transitions (probing the site geometry)
  c) Molecular orbital for charge transfer transitions (probing the metal-ligand bonding)

• problems: overlapping features, intensities for LF transitions are weak
2. Circular dichroism (CD) spectroscopy:
- measure the difference ($\Delta \varepsilon$ as a function of $\nu$) between the LCP and RCP light absorption ⇒ sign and intensity information (can help resolution of overlapping bands in EAS)
- optically active molecules (with C$_n$ or D$_n$ symmetry): EAS looks the same for enantiomers, while CD gives mirror images for LCP and RCP
- protein structure: gives different absorption bands for $\alpha$-helix and $\beta$-sheets ⇒ can be quantitative ($\%\alpha, \%\beta$)
- rotational strength:
  \[
  f_{\text{exp}} = \text{const} \int \frac{\Delta \varepsilon(\nu)}{\nu} \, d\nu \\
  f_{\text{calc}} = \text{const} \int \Psi_g^* \hat{M}_{\text{elec.dipole}}(x, y, z) \Psi_e \, d\tau \cdot \int \Psi_g^* \hat{M}_{\text{mag.dipole}}(R_x, R_y, R_z) \Psi_e \, d\tau
  \]
  allowed transition must be both electric and magnetic dipole allowed
- Kuhn anisotropy factor: $\Delta \varepsilon / \varepsilon = \text{CD/EAS} = f/R > 0.01$ for magnetic allowed transition

3. Magnetic circular dichroism (MCD) spectroscopy:
- application of a static, longitudinal magnetic field (|| to the propagation of the light)
- baseline is the CD spectrum (0T MCD)
- magnetic field induces Zeeman splitting ($g\beta H M_J$) for ground and excited states:
- equal population of both ground state sublevels ($kT \sim g\beta H$) ⇒ form EAS ground vs. excited state is $\sim 10000$ cm$^{-1}$
- form EPR splitting is $\sim 10$ cm$^{-1}$
  A term - weak signal
  - $M_J = +1/2$ less populated at LN2 or even less at LHe
  - also vibronic coupling is reduced
  ⇒ C term - strong signal
  - non-degenerate ground (zero field splitting) or excited states (small distortion of E or T orbitals) ($E_K - E_e = 0$ back to A or C-terms) ⇒
  B term strong signal w/ or w/o temperature dependence
• Information content (most intense is the C-term for paramagnetic complexes):
  a) generally (C\textsubscript{1} symmetry) ground states are orbitally non-degenerate, but degenerate respect to spin, therefore \(\Delta M_J = \pm 1\) requires change in the orbital angular momentum (\(\Delta M_L = \pm 1\) and \(\Delta M_S = 0\))
  b) the electric dipole allowed transition moments is along \(x, y,\) or \(z\) (1 dimensional), while the MCD C-term \(\sim g_M M_y + g_y M_x M_z + g_x M_y M_z\) requires two perpendicular moments
  c) in order to observe an allowed transition need to mix ground and excited states via spin-orbit coupling (similar to EPR deviation of g-values from ligand field-only, free electron value)
  d) spin-orbit coupling can mix two excited states with transition moments in different directions
  e) magnitude of spin-orbit coupling for 1st row TM atoms is \(\sim 500-1000\) cm\(^{-1}\), while for O,N is about \(\sim 100\) cm\(^{-1}\), and for P,S,Cl \(\sim 200-400\) cm\(^{-1}\) \(\Rightarrow\) intense for LF transitions
  f) \(C_0/D_0 = \frac{\text{MCD}}{\text{EAS}} = \frac{\Delta \varepsilon}{\varepsilon} \sim 0.1\) for LF and \(\sim 0.01\) for CT transitions

4. Case Study: Perturbation by the axial ligand determines the cupredoxin active site (refs. on class website)
   Two protein solutions: blue and green - different optical spectra

   ![blue Cu](image)
   ![green Cu](image)

   **abs. max 600 nm**
   **abs. max 460 nm**

   from EPR we know
   \[ g_{\text{blue},||} = 2.226, \quad g_{\text{blue},\perp} = 2.050, \quad \text{and} \quad A_{\text{blue},||}^\text{Cu} = 63 \times 10^{-4} \text{ cm}^{-1} \rightarrow \text{Cu } 3d_{x^2-y^2} \text{ SOMO} \]
   \[ g_{\text{green},||} = 2.195, \quad g_{\text{green},\perp} = 2.040, \quad \text{and} \quad A_{\text{blue},||}^\text{Cu} = 73 \times 10^{-4} \text{ cm}^{-1} \]
   \(^{33}\)S and \(^{14}\)N labelled EPR and ENDOR shows superhyperfine interactions for both samples
bands 5-7 are LF using $C_0/D_0$ ratios;
- from the sign of the MCD features - the orbitals can be assigned as $z^2$, $xy$, $xz+yz$, $xz-yz$
- from comparison to models and other spectroscopy: site geometry is trigonal pyramidal for blue and
tetragonal for green (larger spread of the LF transitions), the intense bands are Cu-S(Cys) $\pi$ and $\sigma$ CT for
blue and green, respectively,