Spectroscopy

Quantization of energy levels

<table>
<thead>
<tr>
<th>Gamma rays</th>
<th>X-rays</th>
<th>UV</th>
<th>IR</th>
<th>Microwaves</th>
<th>Radiowaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>High energy</td>
<td>X-rays</td>
<td>UV</td>
<td>IR</td>
<td>Microwaves</td>
<td>Radiowaves</td>
</tr>
<tr>
<td>Low λ</td>
<td>UV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High ν</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X-Ray

- high energy
- electronic energy levels of inner-shell electrons
- Elemental analysis – used to determine the elemental composition of a sample (usually a solid)
- Transition energies are slightly dependent on oxidation state of the Element
- Need expensive, specialized instrumentation
- X-ray crystallography – atomic distances from diffraction patterns

UV-visible

- probes outer electronic energy levels
- Simple instrument, fairly inexpensive
- “White light” is defracted off a monochrometer and light of a very narrow range of wavelengths is directed through a slit and subsequently through the liquid sample. The transmittance of the light is measured with a photomultplier tube (Fig 21-12)
- Used as a universal detector (for example: on the end of an HPLC)
- Spectra are influenced by solvent and are usually broad
- Non-specific; it is difficult to confidently identify a compound by its UV spectrum

Infrared

- probes vibrational and rotational energy levels
- Detectors very sensitive to bench-top environment (detects heat!) and therefore, are very noisy. Instead of using a monochrometer,
light of all wavelengths within an IR/microwave range are passed through the sample at once. The beam is modulated using an interferometer, and the intensity of light is measured as a function of time. A mathematical manipulation called a Fourier transform is performed on the data to create a plot of intensity vs frequency of light (or a normal spectrum). We will learn the details of how the interferometer functions in lecture. The advantages of this method is that one spectrum is detected in a couple of seconds, and many spectra can be averaged together to increase the S/N (signal-to-noise ratio).

Spectral peaks are narrower and more defined than in UV absorption spectra. The spectra can be used to indicate the presence of certain functional groups, such as hydroxyls, amines, esters, acids, amides, etc. Fourier transform does not provide the same benefits in the UV that are obtained in the IR (this is due mostly to the noisy IR detectors).

**Microwave**

Used to heat samples (and food). Water molecules are excited to higher rotational energy levels. When the excited water molecules relax back to the ground rotational state, energy is released as heat.

**Radio waves**

NMR - radio waves are used to probe nuclear spin states of certain Nuclei. Nuclei that have a spin quantum number of $\frac{1}{2}$ ($^1$H, $^{13}$C, $^{19}$F, $^{31}$P) split into two nearly equally populated spin states in the presence of a magnetic field. The energy difference between the two states in the radio frequency range, and the absolute magnitude of the energy difference is directly proportional to the magnetic field strength. With a 4.69 T magnet (a common instrument), the absorption frequency for a proton is $\approx 200$ MHz. The actual frequency at which a given proton absorbs depends upon its local electronic environment (this is referred to as a shielding effect). We usually represent these differences as a “chemical shift”, which represents the shielding effect as the difference (in terms of ppm) between the frequency of the nuclei being investigated and standard nuclei, such as the protons in TMS.

Resonance frequency of a particular proton = $v_0 = (\gamma/2\pi) B_0(1-\sigma)$

$\gamma =$ magnetogyric ratio (different for different elements, $^1$H, $^{13}$C, etc.)

$B_0 =$ applied magnetic field (in the z-axis)

$\sigma =$ screening constant (depends on local electronic environment)

$\delta =$ chemical shift = $(\sigma_{\text{ref}} - \sigma_{\text{sam}}) \times 10^6$ ppm
NMR is an important tool for determining chemical structure, however, it suffers from low sensitivity (you generally need at least a µmol of material). The low sensitivity is due to two factors. The first is the fact that both the ground state and excited state are populated to almost the same extent (the ground state just a little more so), and absorption can only occur until the populations are exactly equal. Secondly, most of the other isotopes with spin quantum numbers of ½ besides ¹H are of low natural abundances.

**light interacts with matter**

processes
- reflection
- transmittance
- absorption
- refraction
- scattering

Absorption of light

\[ E = \frac{hc}{\lambda} = h\nu \]

Energy
- Plank’s constant
- Speed of light
- Wavelength
- Frequency

Wave propagation (sin waves)

Wavelength \( \lambda \)
- Frequency \( \nu \)
- In phase / out of phase

Beer’s Law

\[ A = ebc \]

\( e \) = molar absorptivity
- \( b \) = path length
- \( c \) = concentration

Limitations (fails at high concentrations)

Your unknown should always fall in range of your unknowns
UV-vis spectroscopy

Organics

Energy diagram, sigma, pi, non-bonding

n → σ* (150-200 nm) low to intermediate molar absorptivities
n → π* (200-300 nm) low to intermediate molar absorptivities (blue shift)
π → π* (200-700 nm) intermediate to high molar absorptivities (red shift)

Blue shift – shift to shorter wavelengths with increasingly polar solvents lowers n
Red shift - shift to longer wavelengths with increasingly polar solvents lowers π*

Spectral characteristics

Broad spectra
  Overlapping vibrational energy levels
  Solvent effects

As a result UV spectroscopy is good for quantitation but not qualitative analysis (can not identify compound based on UV spec.)

Analysis of mixtures
  2 component
  Multi-component (hook up with an HPLC)

Soluble metal complexes (d-orbital electrons absorb in visible)

Octahedral / square planar geometry

UV-vis instrumentation

Source
Slit
Monochrometer - diffraction
Sample holder
Detector – photomultiplier tube
Atomic spectroscopy in UV-Vis region

AA
AE (multi-component)
Very narrow lines
Hollow cathode lamp
ICP source

Matrix effects and the use of Std. Addition

Fluorescence spectroscopy
mostly in UV and X ray experiments
can be either atomic or molecular

Upon adsorption of light by a molecule or atom, the molecule or atom promotes an electron to a higher electronic energy state and eventually relax back to its initial state. To relax back to the ground electronic state, the exited molecule (or atom) must lose energy. It may do this through non-radiative or radiative processes. In non-radiative processes the absorbed energy is lost as heat (IR radiation). In radiative processes, relaxation takes place through the emission of energetic photons. The dominant process depends upon the relative rates of the two processes.

Molecular fluorescence in the UV
The process of emitting a photon upon relaxation from an excited singlet electronic state back to the ground electronic state is called fluorescence. Most molecules do not fluoresce, because the rate of non-radiative decay is much faster than the rate of fluorescence. However molecules that have conjugated double bond systems tend
to have slow non-radiative decay rates and therefore fluorescene becomes the dominant relaxation process. The extent to which a molecule fluoresces is represented in terms of its quantum yield, $\phi$.

$$
\phi = \frac{k_f}{k_f + k_{NR}},
$$
where $k_f$ is the fluorescence rate constant and $k_{NR}$ is the non-radiative rate constant.

Sensitivity of fluorescence

A fluorometer measures the emission at a $90^\circ$ angle to the incoming excitation. As a result, for a blank the number of photons striking the detector is essentially zero. Therefore, the signal for your analyte sample is measured against a zero background. Contrast this experiment to the one that takes place in a typical UV absorption experiment (such as a Beer’s law quantitative analysis). In an absorption experiment, when the blank is analyzed, essentially all of the light is transmitted and the maximum amount photons are bombarding the detector. For dilute analyte, you are measuring the ratio of photons detector for the blank and the photon detected for the sample. There ratio is very close to 1, and the error in the concentration as determined from Beer’s Law is very large. Essentially, the background noise of the absorption experiment is much greater. As a consequence, fluorescence techniques are generally 2-6 orders of magnitude greater than absorption techniques.

Fluorescent tagging

For analytes that do not fluoresce, you can sometimes covalently attach a fluorescent tag. By doing so, you can use a fluorimeter to detect your analyte with much greater sensitivity.

<table>
<thead>
<tr>
<th>absorption</th>
<th>fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_0 = 1.00 \pm 0.01$</td>
<td>$P_0 = 1.00 \pm 0.01$</td>
</tr>
<tr>
<td>$P = 0.98 \pm 0.01$</td>
<td>$F = 0.0200 \pm 0.0001$</td>
</tr>
</tbody>
</table>

Error using logs – for $y = \log x$, $s_y = (1/\ln(10)) * (s_x/x)$

- Log $P_0/P = 0.0088 \pm 0.0064$
- $s_y = (1/\ln(10)) * (0.015/1.02) = 0.0064$
- Relative error in concentration is 74 %
\[ F = K'(P_0 - P) = 2.3 \; K' \; ebcP_0 \]

\[ c = F/(2.3 \; K' \; ebP_0) \]

relative error in concentration is 1.1%

**IR and NMR spectroscopy**

IR- probes vibrational and rotational levels with IR
NMR- proves nuclear spins with radio waves

**Fourier Transform**

In absorption spectroscopy (whether it is with UV, IR, radio waves (NMR), or X-Rays) the goal of the experiment is to measure the absorption of light (photons) as a function of wavelength (frequency). So, the plot that we seek is a plot of absorption vs. frequency (data in the frequency domain). In typical UV experiments, a range of UV/Vis light is dispersed using a monochrometer, so that the absorption can be directly measured as a function of wavelength.

However, in the IR and NMR range it is advantageous in terms of analysis time, spectral resolution, and signal-to-noise ratio (S/N) to acquire analytical signal as a function of time and then perform a mathematical function called a Fourier transform to convert to the time domain data to the frequency domain data that we seek. In this type of experiment the incoming light is not dispersed with a grating. Instead photons of a whole range of energies are allowed to interact with the sample, simultaneously, and the signal (light striking the detector) is measured as a function of time. The signal vs. time data is called an interferogram. A Fourier transform is a mathematical function that converts from the time domain to the frequency domain. A key component to this type of experiment is at time = 0s all of the light is in phase. Gradually, because the frequencies of the light waves vary across a continuum, the light waves begin to constructively interfere, producing an interferogram.

**FT-IR**

To measure a time domain spectra, it is necessary to measure the signal at time intervals that will enable one to characterize the frequencies of the light being investigated. Remember light behaves as a sine wave and to characterize the frequency of a sine wave you need to measure at least 2-4 points per cycle. In IR spectroscopy we are interested in investigating photons with a range of the frequencies from \(1.5 \times 10^{13} \) – \(9 \times 10^{13} \) s\(^{-1}\). That means detector would have to
measure at least a sampling rate of \(2\times(9\times10^{13}) \approx 2\times10^{14}\) Hz. Electronically, this is very demanding and is just not possible. To solve this problem we employ a Michelson Interferometer. This is the device that contains a fixed and moving mirror that I had discussed in class. It provides a replica of the signal vs. time data on a time scale that is spread over a much longer time period.

\[
f = \frac{(2\times v_m)}{c} \nu = 10^{-10} \nu = 20,000\text{ Hz}
\]

where \(f\) is the replicated frequency at the “new, longer” time scale, \(v_m\) is the velocity of the moving mirror, \(c\) is the speed of light, and \(\nu\) is the frequency of the original light wave. Plugging in the required sampling rate of \(\approx 2\times10^{14}\) Hz calculated above gives a new sampling rate of 20000 Hz, which is electronically manageable. Thus, using a Michelson interferometer, the sampling rate of 20000 Hz at the detector can satisfy the requirement necessary to provide a characteristic interferogram.

**FT-NMR**

As stated above, nuclei with spin states of ½ split into two spin states under the influence of a magnetic field. The classical way of understanding what is going on with the experiment is to envision the nuclei precessing around the axis (z axis) parallel to the applied magnetic field. This means they are rotating around this axis much like the wobbling of a spinning top (envision the center of the nuclei as the point of origin for the spinning top). This motion causes the magnetic moment of the nuclei to either be aligned with or against the applied magnetic field. Application of a RF (radio frequency pulse) perpendicular to the applied magnetic
field (along the x axis) causes some of the nuclei that are aligned with the magnetic field to begin to flip, such that their magnetic moments would be aligned against the applied field. The length of the pulse determines how far they proceed in this flipping process. Using a 90° pulse, this transition occurs to the half-way point. At this point the magnetic moments of the excited nuclei are bunched up and are aligned with the axis (x axis) perpendicular to the applied field (parallel to the RF pulse). They begin to precessing (still about the z axis, but) in the x-y plane. The RF coil that produced the pulse is used as the detector. As the precessing nuclei approach the receiver coil a current is induced in the coil that is measured by the detector. The induced current is measured as a function of time. At first the current is high because the precessing nuclei are bunched up (in phase). As they begin to precess at slightly different frequencies (according to their chemical shifts), they become out of phase, resulting in an interferogram. Eventually the excited nuclei relax back to their initial positions of precessing about the z-axis. The Fourier transform of the interferogram produces an NMR spectrum that contains the information of which radio frequencies were absorbed by our sample.