Experiment 3: Fluorescence Spectroscopy
I: Introduction to steady state spectra

Prelab Lecture 30jan19
P. Callis
WHAT IS FLUORESCENCE?

ALL light comes from fluorescence, e.g., the sun, light bulbs, fire, cell phone screens, some chemical reactions, etc.

Fluorescence usually refers to a form of spontaneous emission in the Visible or UV wavelengths coming promptly from an electronically excited state (decay time ps to μs i.e., 10^{-12} to 10^{-6} s).

The most-used fluorescence probes have nanosecond decay times.

Spontaneous emission happens whenever a system is in an excited state. WHY?

ANSWER: caused by strong fluctuations in the zero-point electromagnetic field, present even in dark vacuum!
Electromagnetic radiation is emitted by **all** objects not at 0 Kelvin.

Temperature dependence is from the **Boltzmann** ratio for probability to be in an excited state:

\[
\frac{N_{\text{excited}}}{N_{\text{ground}}} = \exp\left(\frac{\Delta E}{k_B T}\right),
\]

where \( \Delta E = h\nu = \frac{hc}{\lambda} \)
Evidence: looking skyward with an IR spectromter:

IR fluorescence from the atmosphere.

Taken from the Earth’s surface looking up into a cold cloudless sky in Canada during winter. Infrared radiation emitted by the greenhouse gases is heating the Earth’s surface.

Why is CO₂ so strong compared to water??
There is much more water in air.

(Evans 2006).

(44x477)
Fluorescence of EARTH at different temperatures seen from space.

Modtran3 v1.3 upward irradiance at 20 km, U.S. Standard Atmosphere

300 ppm CO\textsubscript{2}, F\textsubscript{↑}=260.12 W m\textsuperscript{-2}

600 ppm CO\textsubscript{2}, F\textsubscript{↑}=256.72 W m\textsuperscript{-2}

\(\Delta F=3.39\) W m\textsuperscript{-2}

Average Fluorescence of EARTH

Absorbed by H\textsubscript{2}O and CO\textsubscript{2}

Absorbed by Ozone

260 K

220 K

300 K

spectral irradiance (W m\textsuperscript{-2} cm\textsuperscript{-1})

wavenumber (cm\textsuperscript{-1})
Fluorescence lifetime ~5 ns (exponential decay)

Kasha’s Rule:
Fluorescence is 99.9% from $S_1$ independent of excitation wavelength; $\sim$ mirror image of $S_1$ abs.
Beer-Lambert Law

photons + M ----> M* (electronically excited)

\[ \frac{d[\text{photon}]}{dt} = -k[M][\text{photon}] = -k[M]I \]

[photon] = light intensity = I

\[ \frac{dI}{dt} = -k[M]I \], where \( k[M] \) is a pseudo first-order rate constant for the disappearance of photons

\[ f = \frac{I}{I_0} = e^{-k[M]t} = e^{-k[M]x/c} = 10^{-\frac{-k[M]x}{2.303c}} = 10^{-\varepsilon [M]x} = 10^{-A} \]

In this context \( f = \text{fraction of photons remaining after travelling distance } x \)

i.e., \( f = \text{Transmittance} = T = 10^{-\varepsilon cx} = 10^{-A} \)

\( 10^{-A} \) is just telling you that \( A = \varepsilon cx = -\log T \)
Attenuation of the Excitation Light through Absorbance

Sample concentration & the *inner filter effect*

Guess the Absorbance

<table>
<thead>
<tr>
<th>A</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1</td>
<td>10^{-0.1}</td>
</tr>
<tr>
<td>1</td>
<td>10^{-1}</td>
</tr>
<tr>
<td>3</td>
<td>10^{-3}</td>
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<tr>
<td>10</td>
<td>10^{-10}</td>
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</tbody>
</table>

Rhodamine B

Fluorescein

= Abs. if A < 0.1
Fluorescence from single molecules under a microscope

http://www.youtube.com/watch?v=CDald68tTz0
Fig. 10.41 Energy-level diagram for infrared absorption, elastic (Rayleigh) scattering, and inelastic (Raman) scattering. Transitions between vibrational energy levels of the ground electronic state absorb infrared radiation; not all transitions are allowed. For elastic scattering the frequency of the incident light is equal to the frequency of the scattered light. For inelastic scattering the frequency of the scattered light is different from that of the incident light. The virtual state shown can have any energy; the scattering of light occurs in any region of the spectrum.

\( n \times 1000 \text{ times enhanced} \)
How do we measure fluorescence and scattering?
1 Xenon arc-lamp and lamp housing
1a Xenon-lamp power supply
1b Xenon flash lamp (FluoroMax®-4P only)
2 Excitation monochromator
2a & 2b Slits
3 Sample compartment
4 Emission monochromator
4a & 4b Slits
5 Signal detector (photomultiplier tube and housing)
6 Reference detector (photodiode and current-acquisition module)
Host computer (not on diagram)

**Illuminator (xenon arc-lamp, 1)**

The continuous light source is a 150-W ozone-free xenon arc-lamp. Light from the lamp is collected by a diamond-turned elliptical mirror, and then focused on the entrance slit of the excitation monochromator. The lamp housing is separated from the excitation monochromator by a quartz window. This vents heat out of the instrument, and protects against the unlikely occurrence of lamp failure.
150 W Xenon Arc

Parabolic mirror focuses light on slit

1a

Excitation Monochromator

1b

2a

2b

2

Beam Splitter

Cell

Slit

Grating

4a

4b

Signal Detector

Reference Detector

Fluorescence

Emission Monochromator
Absorbance

Emission

Excitation

= Abs. if A < 0.1

https://en.wikipedia.org/wiki/Fluorescein
Fluorescence lifetime ~5 ns (exponential decay)

Kasha’s Rule:
Fluorescence is 99.9% from $S_1$ independent of excitation wavelength; ~mirror image of $S_1$ abs.
Phosphorescence

$S_1$
(1st excited state)

$S_0$
(ground state)

Intersystem crossing, ns

Fluorescence:
$\sim 5$ ns

Lowest triplet state
(unpaired spins)

Phosphorescence
Slow, forbidden ($10^{-6}$
to 10 seconds)

Requires rigid,
oxygen free
environment

$S_1$ abs $\rightarrow$ $S_1$ fluor $\rightarrow$ Phos

Wavelength
Fluorescence Quantum Yield

4.2.3 Fluorescence Quenching by Iodide

1. Calculate the quantum yield for each concentration of the iodide, \([Q]\), using numbers from the Theory document and:

\[
\Phi_f = \frac{k_{rad}}{k_{rad} + k_{ic} + k_{isc} + k_q[Q]}
\]

Fluorescence Lifetime = 1/(sum of rate constants)

\[
= \frac{1}{(k_{rad} + k_{ic} + k_{isc} + k_q[Q])} = \tau_f
\]
What is fluorescence lifetime?

\[ \frac{d[\text{excited molecules}]}{dt} = -k \ [\text{excited molecules}] \]
\[ \frac{d(\text{Intensity})}{dt} = -k \ (\text{Intensity}) \quad \text{1st order reaction} \]

Solution to this differential equation?

Fluor. intensity at time \( t \) = (Fluor. Intensity at time 0) x \( e^{-kt} \)

or \( \tau \) = (Fluor. Intensity at time 0 x) \( e^{-t/\tau} \)

\( \tau = \text{“lifetime”} = 1/k \)

\( \tau = \text{inverse of 1st order rate constant} \)
“Quenching”
Ground State

Highest Occupied Molecular Orbital
Lowest Unoccupied Molecular Orbital (electron excited)

Excited State (fluorescing state)

Excited State (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)
Iodide ion collides; (has higher HOMO) will quench fluorescence
Electron transferred from I⁻ to indole makes a radical pair that cannot fluoresce.
(would violate Pauli exclusion)

Radical Pair (can’t fluoresce)

Electron transferred from iodide to vacancy in HOMO of ring
i.e., QUENCHING