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

Prelab Lecture 2feb17
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WHAT IS FLUORESCENCE?

ALL light comes from fluorescence, e.g., the sun, light bulbs, fire, cell phone screens, 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 (not in true ground state). WHY?

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

Fig. 18.2. Spectral intensity distribution of Planck’s black-body radiation as a function of wavelength for different temperatures. The maximum of the intensity shifts to shorter wavelengths as the black-body temperature increases.

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 \( \Delta E = \frac{hc}{\lambda} \)

Sun (looks white)

200 nm Where N2 and O2 start to absorb

400 nm

500 nm

700 nm
Evidence: looking skyward with an IR spectrometer:

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 CO$_2$ so strong compared to water??

There is much more water in air.

(Evans 2006).
CO₂

Fluorescence of EARTH

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

300 ppm CO₂, F↑=260.12 W m⁻²
600 ppm CO₂, F↑=256.72 W m⁻²
ΔF=3.39 W m⁻²
Fluorescence lifetime \( \sim 5 \) ns (exponential decay)

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

\(\text{photon} + M \rightarrow M^* \) (electronically excited)

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

\([\text{photon}] = \text{light intensity} = I\)

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

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

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*

**Rhodamine B**
Fluorescein

Absorbance

= Abs. if $A < 0.1$

https://en.wikipedia.org/wiki/Fluorescein
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.
How do we measure fluorescence and scattering?
Horiba Fluorimeter

Optical layout

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.
Excitation Monochromator

150 W Xenon Arc

Parabolic mirror focuses light on slit

Reference Detector

Signal Detector

Emission Monochromator

Beam Splitter

Cell

Slit

Fluorescence
Absorbance

= 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

Lowest triplet state (unpaired spins)

Fluorescence: \(~ 5 \text{ ns}~\)

Phosphorescence

Slow, forbidden \((10^{-6} \text{ to } 10 \text{ seconds})\)

Requires rigid, oxygen free environment

\[ S_1 \text{ abs} \]

\[ S_1 \text{ fluor} \]

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 = \(1/(\text{sum of rate constants})\)

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

\[
d[\text{excited molecules}]/dt = -k \ [\text{excited molecules}] \\
\frac{d(\text{Intensity})}{dt} = -k \ (\text{Intensity})
\]

1st order reaction

Solution to this differential equation?

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

or

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

\( \tau = \text{“lifetime”} = 1/k \)

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

Highest Occupied Molecular Orbital

LUMO
Lowest Unoccupied Molecular Orbital (electron excited)

Excited State (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)
Quenching by iodide ion

Iodide ion collides; (has higher HOMO) will quench fluorescence
Electron transfer from I\textsuperscript{-} to indole makes a radical pair that cannot fluoresce. (would violate Pauli exclusion)

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