Chapter 15 Molecular Luminescence Spectrometry

Problems: 1, 2, 3, 4, 5, 6, 8, 11
Observe light that is emitted as molecule returns to ground state

*Photoluminescence* - excited state obtained by molecule absorbing a photon of light
differentiate between 2 luminescent mechanisms
  - Electron spin does not change
    - Fluorescence
      - very short lived state
      - emission occurs $<10^{-6}$ sec after absorption
  - Electron does change spin state in transition
    - Phosphorescence
      - High E state has long lifetime
      - Emission can be several seconds or longer after absorption

In either case emitted light has a larger $\lambda$ than absorbed light

*Chemiluminescence* - excited state obtained by chemical reaction

All three can be used analytically, but Fluorescence techniques are dominant

Are inherently very sensitive, 10-1000 times more sensitive than UV absorption

Also luminescent response tend to be linear over a very large range

Tend to have many matrix effects so have to be careful

Excellent when combined with separation (HPLC, capillary Electrophoresis, Etc) because these remove interfering matric components and can detect small amount that come through the column.

15A Theory

Fluorescence and Phosphorescence can be
  - simple or complex
  - solid, liquid or gas state

If emitted light same wavelength as absorbed light
  - resonance radiation
  - Resonance Fluorescence

More often emitted light is $>\lambda$ (longer $\nu$) than absorbed light
  - Shift in $\lambda$ (or $\nu$) called *Stokes shift*

15A-1 Excited states Producing Phosphorescence or Fluorescence

First need to review electron spin and singlet/triplet state
Electron Spin
Pauli exclusion - no 2 electrons can have same set of 4 quantum #
No more than 2 electrons can share the same orbital
(Have the first 3 Q#'s be the same)
TO share the same orbital 4th Q# must be different
4th Q# is electron spin. Can be either +1/2 or -1/2
So to share same orbital must have different spins

If all electrons paired - molecule has not net magnetic field when placed in a magnet - called *diamagnetic*

If some unpaired electrons - unpaired electrons interact with external magnetic field to create molecular magnetic field that is attracted to external field - called *paramagnetic*

Singlet/Triplet Excited States
Singlet state
Molecule with all electrons paired in orbitals
Called singlet because observe a single energy level, even when molecule exposed to external magnetic field

Doublet state
Molecule with 2 energy levels in a magnetic field
Why???
Occurs if have a SINGLE unpaired electron
(A free radical)
½ of unpaired electrons are in +1/2 state
Other ½ of unpaired electrons in -1/2 state
One interacts favorably with magnetic field, lowering E of state
Other interacts unfavorably with magnetic field, raising E of state

Excited states
Start with molecule in singlet state
When excited 1 electron of pair is moved to excited state
If two electrons are still have opposite spins-
is called singlet excited state
Molecule is diamagnetic
If two electrons have same spin-
Called triplet excited state
Molecule is paramagnetic
Called triplet because Observe 3 E levels (won’t explain why now)
Triplet excited state E < Singlet Excited state E
Singlet–Triplet transitions at same E level
Fairly probably
Happen quickly $10^{-5}$-$10^{-8}$s

Singlet–Triplet transitions to different E levels
Far less probable (forbidden transition)
Because less probable don’t occur frequently
Molecule get stuck, have longer lifetimes
$>10^{-5}$ s

Also means chance of Singlet–triplet absorption event very low
So most absorption is to a singlet excited state

Energy-Level Diagrams for Photoluminescent Molecules

Figure 15-1

Lowest horizontal line a ground state
Assume singlet
Call $S_0$

Several vibrationally excited state just above this
At RT all molecules should be in $S_0$ vibrationally ground state
Is this right? Assume a low end IR vibration
Low E end maybe 660 cm$^{-1}$ or 15μm
C=λν ; 3x10$^8$ = ν(15x10$^{-6}$)
ν=3x10$^8$/15x10$^{-6}$ = 2x10$^{13}$
E=νν
=6.626x10$^{-34}$ x 2x10$^{13}$
=1.3x10$^{-20}$
Boltzman

$$\frac{N_j}{N_0} = 1e^{-\frac{-E}{kT}}$$

$$\frac{X}{1} = e^{-3.44}$$

So only a few % are vibrationally excited!

2 different Electronically excited singlet state ($S_1 + S_2$)
Each also has vibrationally excited state
1 Electronically excited triplet state ($T_1$)
Also vibrationally excited upper levels
As said earlier this is usually lower E than $S_1$

Can excite molecule to any of the vibrations states above $S_1$
or $S_2$
\[ \lambda_1(S_0=S_1) > \lambda_2(S_0=S_2) \]
Low probability of excitation to $T_1$ so not shown

15A-2 Rates of Absorption and Emission
Absorption of a photon take about $10^{-14}$ to $10^{-15}$

Fluorescence emission much slower

Lifetime of excited state $\propto 1/A$
Big absorption, short lifetime
\[ \sim 10^3 - 10^5, \text{ lifetime } 10^{-7} - 10^{-9} \text{ sec} \]
Small absorption, long lifetime
\[ \sim < 10^3, \text{ lifetime } 10^{-5} - 10^{-6} \text{ sec} \]
Phosphorescence even longer $10^{-4}$ tp $10^{-1}$ sec

15A-3 Deactivation Process
Relaxation of $T_1$ ro $S_1$ state to $S_0$
If radiates $E$ - Phosphorescence or Fluorescence
Can also be non-radiative process

Usually non-radiative process is faster so will occur first
That is why not too many molecules have Phos or Fluor

Let's look at these non-radiative processes

Vibrational Relaxation
Molecules excited to upper vibrational states lose $E$ almost instantly
($10^{-12}$ sec) via molecular collisions to drop to lowest vibrational state

So Fluorescence and Phosphorescence is always from the lowest vibration state

But can drop to a vibrationally excited $S_0$ state, so still see broad emission bands

Net result Excitation $E$ > Emission $E$
Excitation $\lambda$ < Emission $\lambda$
In fact often look like mirror images (Figure 15-2)

Internal Conversion
Intermolecular processes where a molecule goes to a Lower Electronic state without emission of radiation
Process not well understood
Highly efficient
  So most molecules relax this way rather than F or P

More efficient when E levels overlap in vibrational states
  (Conversion of S₂ to S₁ in figure 15-1)
  SO fluorescence almost always from S₁ state, even if excited
  by S₂ radiation
  (Figure 15-2, can excite at either 240 or 350, will get same
   Emission)

Internal conversion form S₁ to S₀ also not well understood
  Sometime these state also overlap vibrationally so can do
  the fast vibration mode

  This is though occur in aliphatic compounds, thus
  aliphatics do not fluoresce

_Predissociation_ can occur
  S₁ does an Internal conversion to an S₀ that is at such a high
  vibrational level that a bond breaks

  Subtle difference between this and _dissociation_, where E
  directly excites molecule to a vibrationally excited state
  where the bond breaks

**External Conversion**
When you are deactivating an excited molecule, if the energy goes
  to the solvent or some other solute, this is called _External
Conversion or collisional quenching_

  Again details not well understood, but we can tell it happens
  Change solvent - change Fluorescence
  Make solvent cold and viscous so # collisions reduced-
  Molecule become more fluorescent

  This process probably involved in radiationless relaxation of S₁ and
  T₁ states

_Intersystem Crossing_
Process by which the spin of an excited electron is reversed
  Probability enhanced if vibrational level of two state overlap
  Most common in molecules containing heavy atom like I or Br
  These atoms have large spin/orbital interaction
Also helped by presence of paramagnetic species
Dissolved $O_2$ will increase phosphorescence, but decrease fluorescence

**Phosphorescence**

triplet $\rightarrow$ singlet conversion even rarer then converse
hence this state is very long lived ($10^{-4}$ to 10 sec)
Internal and external conversion compete
Phosphorescence usually only seen at low T, or highly viscous media, or molecules attached to solid media

15A-4 Variables that affect Fluorescence and Phosphorescence

**Quantum Yield or Quantum Efficiency**

Ratio of # of molecules that lumines/tot # of molecules
Can approach 1 for very fluorescent materials
0 for non-fluorescent material

Looking at previous E diagram can see that it is a competition between rate of several different mechanisms

$$\Phi = \frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d}$$

$k_f$ rate of fluorescence
$k_i$ intersystem crossing
$k_{ec}$ external conversion
$k_{ic}$ internal conversion
$k_{pd}$ predissociation
$k_d$ dissociation

Now can look at for structural implications
Fluorescence, predissociation and dissociation rate affected by molecular structure
Remaining affected by environment

**Transition types**

Exciting UV radiation for fluorescence usually $>250$ nm
Higher E (shorter $\lambda$) usually deactivates by predissociation or dissociation (high enough E to break a bond!)

$\sigma^*$$\rightarrow$$\sigma$ transition rare in fluorescence
$\pi^*$$\rightarrow$$\pi$ or $\pi$$\rightarrow$$\pi$ more common
Also agrees with fact that in excited state molecule rapidly transforms to lowest excited state, hence a π* state not a σ* state

**Quantum Efficiency and Transition Type**
Empirically see fluorescence mostly from π-π* excitation rather than n-π*

Why?
π-π* 100-1000 x greater molar absorptivity than n-π*
Means greater probability for transition and shorter lifetime (k_f large) to excited state both in forward and backward direction

Thermodynamic suggests less overlap in π* S1 and T1 vibrational state, so intersystem crossing is lower and less change of competing phosphorescence

**Fluorescence and Structure**
Most intense fluorescence found in aromatics with low π-π* transitions
A few
Aliphatic and alicyclic carbonyl compounds
Highly conjugated double bonds
But most aromatics

Most unsubstituted aromatics fluoresce
Get better with more rings and higher degree of condensation
**Heterocyclic don’t fluoresce (See page 361)**
N heterocyclics, lowest E is an n-π*
That rapidly converts to triplet for phosphorescence
Fused rings usually do fluorescence
**Fused rings with N do fluoresce Examples page 362**
Substitutional an rings shift absorption and fluorescence peaks
Can also affect efficiency
Large halogens increase phosphorescence due to heavy atom effect, thereby decreasing fluorescence

Iodobenzene or nitrobenzene are prone to predissociation

Carbonyl’s or carboxylic acids inhibit fluorescence
Make n-π* transition E less so this predominates
And \( n-\pi^* \) have low fluorescence yields

**The effect of Structural Rigidity**
- Fluorescence increased by rigidity
- Fluorescent efficiency about 1.0
- Biphenyl efficiency about 2

*Structures on page 363*

Also observed for metal chelators

Don’t know reason for sure, but think when not-rigid internal conversion rate increases for more radiationless deactivation

**Temperature and Solvent Effects**
- Efficiency ↓ as \( T \uparrow \)
  - Probably more collisions so more deactivation by external conversion
- Efficiency ↓ as solvent heavy atoms ↓
  - As in internal heavy atoms, increases rate of triplet formation
  - Can be used to enhance phosphorescence

**Effect of pH on Fluorescence**
- If aromatic ring has acid or base functionality, Fluorescence is pH dependent

  Whichever form (acid or base) has more resonance forms has better fluorescence because has lower 1\(^{st}\) excited E level

**Effect of Dissolved \( O_2 \)**
- Most often because \( O_2 \) is paramagnetic, promotes intersystem crossing and enhanced phosphorescence, quenching fluorescence

  Can also be due to photooxidation

**Effect of Concentration of Fluorescence Intensity**
- Fluorescence depends on first absorbing light
  - Will be linear with concentration up until absorbance >.05

  Self quenching and self absorption can also cause non-linearity
  - Self quenching due to molecular collisions making radiationless E transfers

  Self absorption only occurs at wavelength where absorbance and fluorescence overlap
15A-5 Emission and Excitation Spectra (figure 15-3)

Excitation
- Look at all (or 1) luminescence
- Vary excitation E
- Since just UV excitation of molecules should look like absorption curve

Fluorescence
- Now set excitation at a single wavelength, and follow wavelength of Fluorescence
- Will often look like mirror E of excitation

Phosphorescence
- Now set excitation at a single wavelength, and follow wavelength of Phosphorescence a fraction of a sec after excitation light is turned off (Can't do on our machine)
- Should be an even longer wavelength than Fluorescence since even lower E

15B Instruments

Basic set up shown in figure 15-4
- This is essentially a double beam system since have 2 PM tubes and a differential amplifier. What is advantage of this set up? (Don't have to worry about lamp drift)
- Our machine is a single beam, so data is not as precise

Called a spectrofluorimeter or fluorimeter

Note use of 2 monochrometers
- 1 for excitation, 1 for emission
- Note geometry, read off light at 90° to excitation
  - Why?
    - Emission at all angles
    - At 90 you don't see any of the incoming light
    - Also minimizes scattering from solution and cell

  - Also mean you need a cell with 2 polished sides so more expensive

Machines vary in sophistication and price
- Can get designed with simple filters
- Or tunable monochrometers

True spectrofluorimeters have two monochrometers

If characterizing fluorescence characteristics to molecular/structural
parameters need to make measurements carefully, since detector response and lamp output will vary with $\lambda$.

For simple concentration measurements, don’t fuss about these details just set excitation and emission $\lambda$, s and set in some standards and go.

Section 15B-1 is focused on these simpler type instruments

**15B-1 Components of Fluorometers and spectrofluorometers**

**Sources**
Size of Fluorescence signal directly proportional to amount of light, so brighter the light, the better the fluorescence

Tungsten, H$_2$ or D$_2$ lamps not good enough

If a Filter monochrometer use a H$_2$ vapor lamp  
Lines at 254,302,313,546,578,691,773 nm  
Use filter to select for line you want  
With that kind of spread there is usually one close enough to work.

If Selectable monochrometer use high pressure Xenon Arc  
(Ours is a 75W, I can bring in a 450W from the CD machine)

Some have lamp on capacitor and discharge at a constant frequency  
Even brighter, + can use ac signal processing  
Also maybe time resolved?

Can use lasers, but fussier, and limited $\lambda$ so need a good reason, usually some specialized application

**Filter and Monochrometers**
Nothing special here, use whatever filter or monochrometer works

**Transducers**
Since usually a very low signal, most machines use a Photomultiplier tube for best S/N

**Cell and Cell Compartments**
Both cylindrical and square cells are used (ours is square)  
A bit more spendy because have 4 polished sides and must match in 2 directions  
Also since chasing low light levels, need to make scattered light a minimum in detector with added baffles
15B-2 Instrument Designs

**Fluorometers or filter photometers**
- Simple, rugged, cheap (like machine used in Shane’s lab for DNA determination) usually tuned to 1 or two λ
- Can be single or double beam
- < $5000

**Spectrofluorimeters**
- Obtain excitation, emission spectra or both
- Usually going to be double beam (but ours is single)

Note when comparing spectra obtained - Each instrument will give different spectra because has different components absorbing light. Only in the most modern and expensive instrument will you find correction factor to give a ‘true’ corrected output spectra

**Spectrofluorimeters based on array transducers**
- Use of array detectors to get entire spectrum is recent development

**Neat trick shown in figure 15-8**
- Get excitation and emission in 1 experiment!

**Fiber Optic**
- Run light through fiberoptic tubes, nothing really new, just can be useful for some specialized applications

**Phosphorimeters**
- Same basic design, but need to be able to turn light off and detector on as various short intervals to differentiate between fluorescence and phosphorescence
- Can do electronically or mechanically
- Most phosphorescence done at liquid N₂ temp so also include in sample holder

15B-3 Instrument Standardization
- Because source, detector, and other variable change from day-to-day
- Need to calibrate machine daily

One standard that can be used is 10⁻⁵ M Quinine Sulfate

15C Applications and Methods
- Usually used at lower conc than absorbance
Among most sensitive available

Usually 10 to 1000 times more sensitive

On flip side precision and accuracy aren't as good usually by factor of 2-5, with phosphorescence being worse than fluorescence

**15C-1 Fluorometric determination of Inorganic Species**

2 methods
- Direct - have a fluorescent chelator that measures
- Indirect - look at quenching action of material you are quantitating

**Cations that Form Fluorescing Chelates**

Works OK for non-transition metals nicely complementary because non-transition metals are usually colorless so are harder to do by absorption

Usually doesn’t work as well for transition metals
1. Transition metal often paramagnetic (so transition to Triplet and phosphorescence interferes)
2. Usually have many closely spaced E levels so more internal conversion to quench fluorescence as well

**Fluorometric Reagents**

- 4 shown page 372
- We have a few, might try some day

Will Chelate this makes rings rigid, so then fluoresce.

**15C-2 Fluorometric Determination of Organic Species**

100's-to 1000's of methods for organics and biologicals. Too many to count

**15C-3 Phosphorimetric Methods**

Fluorescence compounds and Phosphorescent compounds tend to be complementary. Can do one or the other, but not both

Phosphorescence not widely used
- Need to cool to liquid N\textsubscript{2} temp
- Less precise

Some newer techniques make more applicable, but still not a main-line technique
15C-4 Application for use in Chromatography
(We have one of these on the HPLC)
Important method - No details

15C-5 Lifetime measurements
Originally done only for phosphorescence because slower and easier to measure

Now can study luminescence down to $10^{-5}$ to $10^{-8}$ sec
Using lasers with $70$ to $100 \times 10^{-12}$ (pico) second light pulses

With this kind of equipment can light interchange between chromophores on a protein. Work a person at Ohio State used to find distance between atoms on a large molecule like a protein

Actually pretty hard to do, since light doesn't cut cleanly on and off (see figure 15-10) A lot of math, a lot of convolution of functions, a lot of fitting of curves

15D Chemiluminescence

Relatively new field

Not that many chemical species are known

But is growing field

15D-1 The Chemiluminescence Phenomena
Chemical reaction yields an electronically excited molecule that then goes to a ground state emitting $E$

In Biological systems called bioluminescence
firefly, jelly fish, bacteria, protozoa, crustacea
(In several of the higher animals is actually a bacteria colony living within the higher organism in a ?commensal? Relationship

First discovered over 100 years ago with some simple compounds

$A + B \rightarrow C^* + D$
$C^* \rightarrow C + h\nu$

Most reaction are a lot more complicated

Have a chemiluminescence quantum yield, $\Phi_{CL}$ photon emitted / excited
molecule

\[ \Phi_{CL} = \# \text{ of exited state/molecules reacted} \times (\text{excitation quantum yield } \Phi_{ex}) \]

\[ \times \# \text{ of photons / excited state (emission quantum yield } \Phi_{EM} \]

\[ I_C = \Phi_C \frac{dC}{dt} = \Phi_E \Phi_{EM} \frac{dC}{dt} \]

Chemiluminescence quantum yield, \( \Phi_{CL} \) usually 0.01 to 0.2

15D-2 Measurement of Chemiluminescence

A reaction vessel and a phototube to measure
No monochrometer needed because only 1 \( \lambda \) emitted
(But if you are synthesizing a new molecule you would need a monochrometer to find the \( \lambda \) of your new reaction)

Time curve line figure 15-11 is typical
Rise due to mixing of components
Exponential decay as reaction uses up starting materials

Use either peak height or integrated signal for quantitative measurements

15D-3 Analytical Applications

Usually a high sensitivity technique on order of ppb

Gases

Methods for NO in air
\[ \text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2 \]

\( \text{O}_3 \) in air (\( \text{O}_3 \) reacts with a dye)

S compounds in air
\[ 4\text{H}_2 + 2\text{SO}_2 \rightarrow \text{S}_2^* + 4\text{H}_2\text{O} \]

Inorganics

\( \text{O}_2, \text{H}_2\text{O}_2 \) and other strong oxidizing agents

React with

\[
\begin{array}{c}
\text{O} \\
\text{C=NN-N-R} \\
\text{H}
\end{array}
\]
Oxidation product is chemiluminescent

Rxn of Luminol is typical

\[
\begin{align*}
\text{NH}_2 & \quad \text{O} \\
\text{NH} & \quad \text{NH} \\
\text{O} & \quad \text{NH}
\end{align*}
\] + 2OH- + 2O2 →

\[
\begin{align*}
\text{NH}_2 & \quad \text{COO}^- \\
\text{COO}^- & \quad \text{NH}
\end{align*}
\] + hv

**Analysis of Organics/Biochemicals**

Link a biochemical reaction to one that generates H₂O₂ then detect using above luminol reaction

Uric acid + O₂ → Allantoin + H₂O₂
(Requires enzyme uricase)

Sucrose + H₂O → αGlu + Fru (requires invertase)
αGlu → βGlu (requires mutarotase)
βGlu + O₂ → Gluconic Acid + H₂O₂ (glucose oxidase)

Can have enzyme dissolved in solution
Can have enzymes immobilized on membrane as a sensor!