27 Molecular Fluorescence Spectroscopy

27A Theory of Molecular Fluorescence

Fluorescence: an analytically important emission process in which atoms or molecules are excited by the absorption of electromagnetic radiation.

Phosphorescence:

The excited species then relax to the ground state, giving up their excess energy as photons.

1. Sensitivity: $10^1 \sim 10^3 >$ absorption spectroscopy

2. Large linear ranges
   - Excitation wavelength
   - Fluorescence wavelength

27A-1 Relaxation Processes (Fig. 27-1)

Fig. 27-1 Energy-level diagram shows some of the processes that occur during (a) absorption of incident radiation, (b) nonradiative relaxation, and (c) fluorescence emission by a molecular species. Absorption typically occurs in $10^{-15}$ s, whereas vibrational relaxation occurs in the $10^{-11}$ to $10^{-10}$ s time scale. Internal conversion between different electronic states is also very rapid ($10^{-12}$ s), whereas the lifetime of fluorescence is typically $10^{-10}$ to $10^{-5}$ s.

a. Nonradiative relaxation

1. Vibrational deactiviation, or relaxation:
   - collisions between excited molecules and molecules of the solvent $\rightarrow$ temp. of the medium ↑

Fig. 27-2 Fluorescence spectra for 1 ppm anthracene in alcohol:
(a) excitation spectrum; (b) emission spectrum.
2. Internal conversion:
   between the lowest vibrational level of an excited electronic state and the
   upper vibrational level of another electronic state

b. Fluorescece emission
   electronically excited molecules relax to any of the several vibrational states of
   the ground electronic state.

Resonance Lines and the Stokes Shift
   Resonance fluorescence has an identical wavelength to the radiation that caused
   the fluorescence.
   **Stokes-shifted** fluorescence is **longer** in wavelength than the radiation that caused
   the fluorescence.

Relationship Between Excitation Spectra and Fluorescence Spectra
   energy differences between vibrational states \( \cong \) both ground and
   excited states
   absorption or excitation spectrum and the fluorescence spectrum for a
   compound \( \rightarrow \) mirror images (Fig. 27-2)

27A-2 Fluorescent Species
   **quantum yield** \( \Phi \) of molecular fluorescence: ratio of the number of molecules
   that fluoresce to the total number of excited molecules
   (or the ratio of photons emitted to photons absorbed)

   \[
   \text{Quantum yield} = \Phi_F = \frac{k_F}{k_F + k_{nr}} \quad k_F: \text{the first order rate constant for fluorescent}
   \quad \text{relaxation}
   \quad k_{nr}: \text{the rate constant for radiationless relaxation}
   \]

Fluorescence and Structure
   aromatic rings, certain aliphatic and alicyclic carbonyl compounds, highly
   conjugated double-bonded structures
   pyridine, furan, thiophene and
   pyrrole do not exhibit molecular fluorescence
   fused-ring structures
   exhibit fluorescence
Table 27-1 Effect of substitution on the fluorescence of Benzene derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>RIF*</th>
<th>Compound</th>
<th>RIF*</th>
<th>Compound</th>
<th>RIF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>10</td>
<td>Fluorobenzene</td>
<td>10</td>
<td>Phenol</td>
<td>18</td>
</tr>
<tr>
<td>Toluene</td>
<td>17</td>
<td>Chlorobenzene</td>
<td>7</td>
<td>Anisole</td>
<td>20</td>
</tr>
<tr>
<td>Propylbenzene</td>
<td>17</td>
<td>Bromobenzene</td>
<td>5</td>
<td>Aniline</td>
<td>20</td>
</tr>
<tr>
<td>Phenolate ion</td>
<td>10</td>
<td>Iodobenzene</td>
<td>0</td>
<td>Benzonitrile</td>
<td>20</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>3</td>
<td>Anilinium ion</td>
<td>0</td>
<td>Nitrobenzene</td>
<td>0</td>
</tr>
</tbody>
</table>

* Relative Intensity of Fluorescence

The Effect of Structural Rigidity

structural rigidity ↑ → rate of nonradiative relaxation ↓ → fluorescent ↑

ex1: bridging methylene group in fluorine
ex2: fluorescing dyes are adsorbed on a solid surface
ex3: organic cheleting agents complex with a metal ion

\[
F \text{ of 8-hydroxyquinoline} \ll F \text{ of Zinc complex}
\]

Temperature and Solvent Effects

\[\text{Temp.} \uparrow (\text{collision} \uparrow) \text{ or solvent viscosity} \downarrow \rightarrow \text{probability of collisional relaxation} \uparrow \rightarrow \text{quantum efficiency of fluorescence} \downarrow\]

27 B Effect of Concentration on Fluorescence Intensity

\[F = K'(P_0 - P) \rightarrow F = K' P_0 c\]

\[F = K' P_0 \left[2.3\varepsilon bc - \frac{(-2.3\varepsilon bc)^2}{2!} - \frac{(-2.3\varepsilon bc)^3}{3!} - \ldots \right]\]

when \(\varepsilon bc = A < 0.05 \rightarrow F = 2.3K'\varepsilon bcP_0 \rightarrow F = Kc\)

a plot of \(F\) vs \(c\) → linear at low conc.

Fig. 27-7 Calibration curve for the spectrofluorometric determination of tryptophan in soluble proteins from the lens of a mammalian eye.
When c increasing to Abs. > 0.05 (or the transmittance < 90%), linearity is lost and F lies below an extrapolation of the straight-line plot.

**self-quenching effect**: analyte molecules absorb the fluorescence produced by other analyte molecules.

**27C Fluorescence Instruments**

*Fluorometer*: employs filter for wavelength selection

*Spectrofluorometer*: employ a filter to limit the excitation radiation and a *grating monochromator* to disperse the fluorescent radiation from the sample.

![Typical fluorescence instruments](image)

**Fig. 27-8** Typical fluorescence instruments.

**8-hydroxquinoline**  
(Reagent for Al, Be and other metal ions)

**Alizarin garnet R**  
(reagent for Al, F⁻)

**Fig. 27-9** Some fluorometric chelating agents for metal cations. Alizarin garnet R can detect Al³⁺ at levels as low as 0.007 μg/mL. Detection of F⁻ with alizarin garnet R is based on quenching of the fluorescence of the Al³⁺ complex. Flavanol can detect Sn⁴⁺ at the 0.1 μg/mL level.

**27D Applications of Fluorescence Methods**

10¹~10³ more sensitive than absorption method

1. ↑ power of the excitation beam,
2. amplifying the detector signal

Beer's law:  
\[ c = k \log \left( \frac{P_0}{P} \right), \quad k = \frac{1}{ab}, \]

\[ P_0 \uparrow \rightarrow P \uparrow, \quad \rightarrow \text{no effect on sensitivity} \]
27D-1 Methods for Inorganic Species

**Direct methods:** reaction of the analyte with a chelating agent to form a complex that fluoresces

**Indirect method:** diminution or *quenching* of fluoresces of a reagent as a result of its reaction with the analyte

Fluorometric reagents for the determination of cations are aromatic compounds with two or more donor functional groups

### Table 27-2 Selected Fluorometric Methods for Inorganic Species

<table>
<thead>
<tr>
<th>Ion</th>
<th>Reagent</th>
<th>Wavelength, nm</th>
<th>Sensitivity, μg/mL</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>Alizarin garnet R</td>
<td>470</td>
<td>500</td>
<td>10.007</td>
</tr>
<tr>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Al complex of Alizarin garnet R (quenching)</td>
<td>470</td>
<td>500</td>
<td>0.001</td>
</tr>
<tr>
<td>B&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>Benzoin</td>
<td>370</td>
<td>450</td>
<td>0.04</td>
</tr>
<tr>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>2-(o-Hydroxy-phenyl )-benzoxazole</td>
<td>365</td>
<td>Blue</td>
<td>2</td>
</tr>
<tr>
<td>Li&lt;sup&gt;+&lt;/sup&gt;</td>
<td>8-Hydroxyquinoline</td>
<td>370</td>
<td>580</td>
<td>0.2</td>
</tr>
<tr>
<td>Sn&lt;sup&gt;4+&lt;/sup&gt;</td>
<td>Flavanol</td>
<td>400</td>
<td>470</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Benzoin</td>
<td>-</td>
<td>Green</td>
<td>10</td>
</tr>
</tbody>
</table>

27D-2 Methods for Organic and Biochemical Species

More than 100 entries: adenine, anthranilic acid, aromatic polycyclic hydrocarbons, cysteine, guanidine, indole, naphthols, certain nerve gases, proteins, salicylic acid, skatole, tryptophan, uric acid and warfarin.

Medicinal agents: adrenaline, alkylmorphine, chloroquin, digitalis principles, lysergic acid diethylamide (LSD), penicillin, phenobarbital, procaine, reserpine and steroids.

Plant products: chlorophyll, ergot alkaloids, rauwolfia serpentian alkaloids, flavonoids and rotenone.

Food products, pharmaceuticals, clinical samples and natural products.