## 5.7 NEPHELOMETRY AND TURBIDIMETRY

Much of the theory and equipment used in spectrophotometry applies with little modification to **nephelometry** and **turbidimetry**. These fields involve the **scattering** of light by nontransparent particles suspended in a liquid; examples of such particles include fine precipitates and colloidal suspensions. In *nephelometry*, we measure the amount of radiation *scattered* by the particles; in *turbidimetry*, we measure the amount of light *not scattered* by the particles. These processes are illustrated in Figures 5.47 and 5.48. The applications of nephelometry include the estimation of the clarity of drinking water, beverages, liquid pharmaceuticals, and other products where the transparency is important and in the determination of species that can be precipitated, such as calcium or barium by precipitation as the phosphate or sulfate insoluble salt. The quantity of calcium or barium

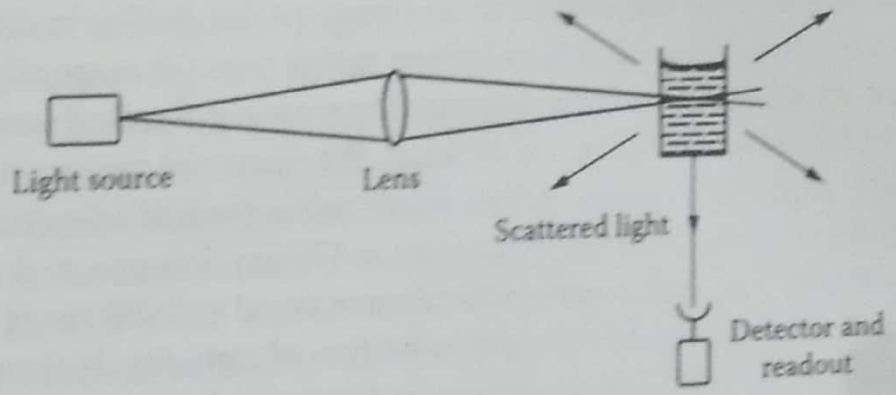


**Figure 5.47** Schematic optical system for nephelometry.

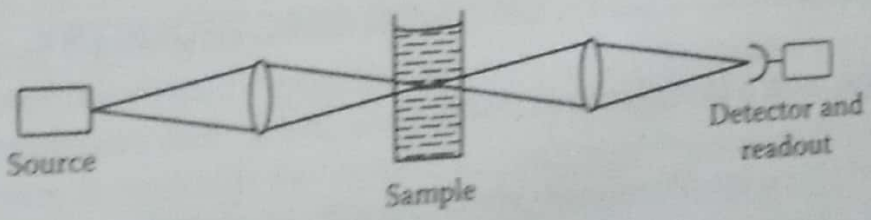


**Figure 5.48** Schematic optical system for turbidimetry.

present is measured by the amount of radiation scattered by the precipitated compound. From the intensity of scattered radiation, the original concentration of calcium or barium can be determined. Conversely, sulfate and phosphate can be determined by precipitation as the barium compound. Process analyzers using nephelometry or turbidimetry can be used to monitor the clarity of a plant stream or water treatment facility stream on a continuous basis.

When using nephelometry or turbidimetry for quantitative analysis, standard suspensions or standard turbid solutions are required for calibration. The precipitate or suspension standards must be prepared under rigidly controlled conditions. This is essential because the scattering of light depends on the *size*, *shape*, and *refractive index* (RI) of the *particles* involved, as well as on the concentration of particles. Some particles also absorb light, which will cause an error in the turbidity measurement. It is necessary for a given solution to produce the same number of particles of the same size and other properties listed for the degree of light scattering to be meaningful. Interferences include dirty sample cells and any colored or absorbing species that will remove light from the light path. Any absorbance of light will result in an erroneously high turbidity, just as turbidity results in an erroneously high absorbance.

The wavelength of the light scattered most efficiently depends on the physical size of the scattering particles. From this, it can be reasoned that the size of the scattering particle may be determined if the wavelength of scattered light is accurately known. This type of light scattering forms the basis for the measurement of polymer MWs from the size of polymer molecules.

For water analysis, the formulation of turbid standards is very difficult, so most water laboratories use a synthetic polymer suspension as a standard. The formazin polymer suspension is easy to make and more stable and reproducible than adding clay or other particles to water to prepare standards. Alternatively, suspensions of polymer beads of the appropriate size can be used as scattering standards. (See *Standard Methods for the Examination of Water and Wastewater* for details.)

In the determination of a given species by a precipitation reaction, it is critical to control the experimental conditions. Two identical samples of equal concentration of analyte will scatter light equally only if they form the same number and size distribution of particles when they are precipitated. This depends on many experimental conditions, including the sample temperature, the rate at which the precipitant and the sample are mixed, the degree of agitation or stirring, and the length of time the precipitates are allowed to stand before measurement. Procedures usually call for the use of a stopwatch to make all measurements at the same point in time, such as 60 s after the reagent was added. Interferences include other particles and colored or absorbing species. Sulfate in drinking water can be determined turbidimetrically by precipitation as barium sulfate over the range of 1–40 mg/L sulfate, with precision of about 2% RSD and accuracy, estimated by recovery of spiked samples, of about 90%.

Other working definitions of turbidity can be used. We discussed measuring the color of beer earlier, using the ASBC protocol. Turbidity in beer is defined by them as a function of the difference in the absorbances of decarbonated beer at 700 nm and at 430 nm (Johnson et al.). If the absorbance at 700 nm for a calculated 0.5 in. path length is less than or equal to 0.039 times the absorbance for the same path length at 430 nm, the beer is said to be free of turbidity. If the value at 700 nm is $>0.039 \times \text{Abs}_{430\,\text{nm}}$, the beer is turbid and would need to be clarified by centrifugation or filtration in order to get a true color measurement.

## 5.8 MOLECULAR EMISSION SPECTROMETRY

### 5.8.1 Fluorescence and Phosphorescence

Introduction:

If "black light" (UV light) illuminates certain paints or certain minerals in the dark, they give off visible light. These paints and minerals are said to *fluoresce*. An energy diagram of this phenomenon is shown in Figure 5.49. For fluorescence to occur, a molecule must absorb a photon

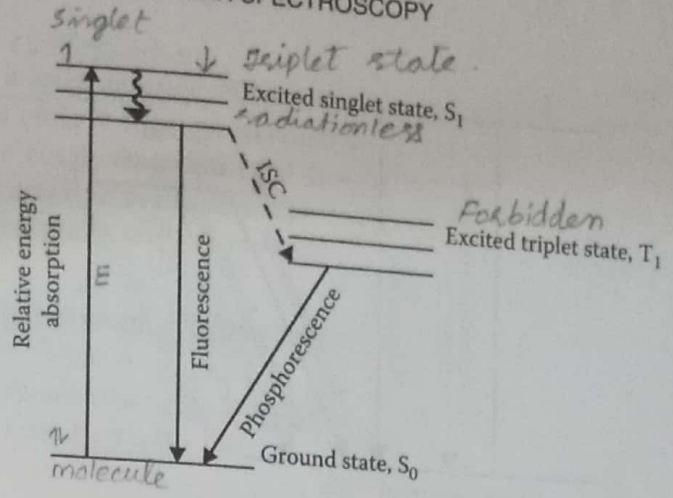


**Figure 5.49** Schematic diagram of the ground state, excited singlet state, and excited triplet state, of a molecule. The wavy line denotes a radiationless transition from a higher vibrational level in the excited singlet state to the lowest vibrational level in the excited singlet state. The dotted arrow marked to intersystem crossing (ISC) shows the radiationless intersystem crossing from the excited singlet state to the excited triplet state. The length of the solid arrows denotes the relative energy of the transitions: absorption > fluorescence > phosphorescence. This results in $\lambda_{phosphorescence} > \lambda_{fluorescence} > \lambda_{absorption}$.

and be promoted from its ground state to an excited vibrational state in a higher electronic state. There are actually *two* possible electronic transitions. Electrons possess the property of spin; we can think of this simplistically as the electron rotating either clockwise or counterclockwise. For two electrons to occupy the same orbital, their spins must be opposite to each other; we say that the spins are paired. If one electron is raised to the excited level without changing its spin, the electron in the excited level is still opposite in spin to the electron left behind in the valence level. This excited state of the molecule in which electron spins are paired is called a *singlet* state. If the electron spins are parallel (both spinning in the same direction as a result of the excited electron reversing its spin), the excited state is called a *triplet* state. Each "excited state" has both a singlet and corresponding triplet state. Singlet state energy levels are higher than the corresponding triplet state energies. Singlet states are designated $S_1$, $S_2$, $S_3$, and so on; triplet states are designated $T_1$, $T_2$, $T_3$, and so on. The ground state is a singlet state, $S_0$. Figure 5.49 shows a ground state with the first excited singlet and triplet states. Some vibrational sublevels of the excited states are also shown.

The molecule absorbs energy and an electron is promoted to one of the higher vibrational levels in the singlet state; this is a vibrationally excited electronic state. The vibrationally excited molecule will rapidly "relax" to the lowest *vibrational* level of the electronic excited state $S_1$. This relaxation or loss of energy is a radiationless process, shown by the wavy arrow. Energy decreases but no light is emitted. Now the molecule can return to the ground state by emitting a photon equal to the energy difference between the two levels. This is the process of *fluorescence*: excitation by photon absorption to a vibrationally excited state, followed by a rapid transition between two levels with the same spin state (singlet to singlet, in this case) that results in the emission of a photon. The emitted photon is of lower energy (longer wavelength) than the absorbed photon. The wavelength difference is due to the radiationless loss of vibrational energy, depicted by the wavy line in Figure 5.49. This type of fluorescence, emission of a longer wavelength than was absorbed, is what is usually seen in solutions; it is called *Stokes* fluorescence. The lifetime of the excited state is very short, on the order of 1–20 ns, so fluorescence is a virtually instantaneous emission of light following excitation. However, the lifetime of the fluorescent state is long enough that time-resolved spectra can be obtained with modern instrumentation. A molecule that exhibits fluorescence is called a *fluorophore*.

The transition from the singlet ground state to a triplet state is a forbidden transition. However, an excited singlet state can undergo a radiationless transition to the triplet state by reversing the spin of the excited electron. This is an energetically favorable process since the triplet state is at

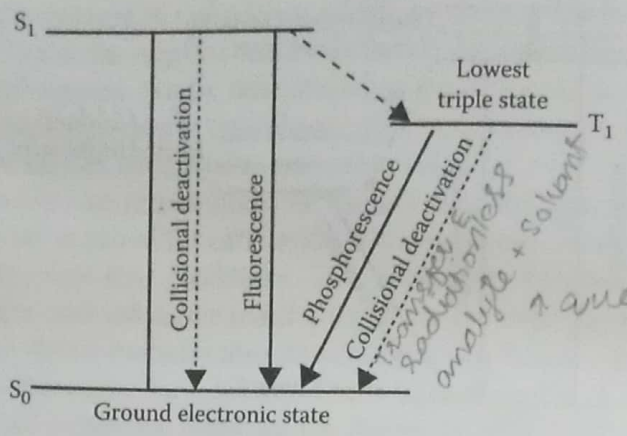


**Figure 5.50** Processes by which an excited molecule can relax to the ground state. (Adapted from Guilbault, G.G., ed., *Practical Fluorescence*, 2nd edn., Marcel Dekker, Inc., New York, 1990. With permission.)

a lower energy level than the singlet state. This radiationless transition, shown schematically in Figures 5.49 and 5.50, is called *ISC*. The molecule can relax to the ground state from the triplet state by emission of a photon. This is the process of *phosphorescence*: excitation by absorption of light to an excited singlet state and then an ISC to the triplet state, followed by emission of a photon due to a triplet–singlet transition. The photon associated with phosphorescence is even lower energy (longer wavelength) than the fluorescence photon, as seen from the relative energy levels in Figures 5.49 and 5.50. Because the triplet–singlet transition is forbidden, the lifetime of the triplet excited state is long, up to 10 s in some cases. The sample will "glow" for some time after the excitation light source is removed. "Glow in the dark" paint is an example of phosphorescent material.

Fluorescence and phosphorescence are both types of luminescence. They are specifically types of *photoluminescence*, meaning that the excitation is achieved by absorption of light. There are other types of luminescence. If the excitation of a molecule and emission of light occurs as a result of chemical energy from a chemical reaction, the luminescence is called *chemiluminescence*. The "glow sticks" popular at rock concerts and Halloween are an example of chemiluminescence. The light emitted by a firefly is an example of *bioluminescence*. *Electroluminescence* is induced by current. Both photoluminescence and electroluminescence are exhibited by semiconductor quantum dots and solid-state sources of single photons, made from materials such as InAs.

As shown in Figure 5.50, there are other ways for molecules to return to the ground state. Excited molecules may collide with other molecules; it is possible for energy to be transferred during this collision. The molecule returns to the ground state but does not emit radiation. This is called collisional deactivation or *quenching*. Quenching occurs in solution by collision of the excited analyte molecule with the more numerous solvent molecules. Quenching in fluorescence is often a serious problem, but with care, it can be minimized. Quenching by collision with the solvent molecules can be reduced by decreasing the temperature, thus reducing the number of collisions per unit time. The same result can be achieved by increasing the viscosity—for example, by adding glycerine. Dissolved oxygen is a strong quenching agent. It can be removed by bubbling nitrogen through the sample. Phosphorescence is very susceptible to quenching; the molecule in a triplet state has an extended lifetime in the excited state, so it is quite likely that it will collide with some other molecule and lose its energy of excitation without emitting a photon. Phosphorescence is almost never seen in solution at room temperature because of collisional deactivation. Low temperatures must be used and the analyte must be constrained from collision. This can be done for fluorescence and phosphorescence by converting the sample into a gel (highly viscous state) or glass, or by adsorption

controlling collisions:
of the analyte onto a solid substrate. "Organized" solvents such as surfactant micelles have been used successfully to observe room temperature phosphorescence and to greatly enhance fluorescence by reducing or eliminating collisional deactivation. Even with the appropriate experimental care, only a small fraction of available analyte molecules will actually fluoresce or phosphoresce, since radiationless transitions are very probable.

### 5.8.2 Relationship between Fluorescence Intensity and Concentration

The intensity of fluorescence $F$ is proportional to the amount of light absorbed by the analyte molecule. We know from Beer's law that

$$\frac{I_1}{I_0} = e^{-abc} \tag{5.4}$$

so, subtracting each side of the equation from 1 gives

$$1 - \frac{I_1}{I_0} = 1 - e^{-abc} \tag{5.5}$$

We multiply each side by $I_0$:

$$I_0 - I_1 = I_0(1 - e^{-abc}) \tag{5.6}$$

Since, $I_0 - I_1$ = amount of light absorbed, the fluorescence intensity, $F$, may be defined as

$$F = (I_0 - I_1)\Phi \tag{5.7}$$

where $\Phi$ is the quantum efficiency or quantum yield. The quantum yield, $\Phi$, is the fraction of excited molecules that relax to the ground state by fluorescence. The higher the value of $\Phi$, the higher the fluorescence intensity observed from a molecule. A nonfluorescent molecule has $\Phi = 0$.

Therefore, fluorescence intensity is equal to

$$F = I_0(1 - e^{-abc})\Phi \tag{5.8}$$

From Equation 5.8, it can be seen that fluorescence intensity is related to the concentration of the analyte, the quantum efficiency, the intensity of the incident (source) radiation, and the absorptivity of the analyte. $\Phi$ is a property of the molecule, as is the absorptivity, $a$. A table of typical values of $\Phi$ for fluorescent molecules is given in Table 5.13. The absorptivity of the compound is related to the fluorescence intensity (Equation 5.8). Molecules like saturated hydrocarbons that do not absorb in the UV/VIS region do not fluoresce.

The fluorescence intensity is directly proportional to the intensity of the source radiation, $I_0$. In theory, the fluorescence intensity will increase as the light source intensity increases, so very intense light sources such as lasers, mercury arc lamps, or xenon arc lamps are frequently used. There is a practical limit to the intensity of the source because some organic molecules are susceptible to photodecomposition.

When the term $abc$ is $<0.05$, which can be achieved at low concentrations of analyte, the fluorescence intensity can be expressed as

$$F = I_0 abc\Phi \tag{5.9}$$

**Table 5.13 Fluorescence Quantum Yields, Φ**

| Compound | Solvent | Φ |
|---|---|---|
| 9-Aminoacridine | Ethanol | 0.99 |
| Anthracene | Hexane | 0.33 |
| 9,10-Dichloroanthracene | Hexane | 0.54 |
| Fluorene | Ethanol | 0.53 |
| Fluorescein | 0.1 N NaOH | 0.92 |
| Naphthalene | Hexane | 0.10 |
| 1-Dimethylaminonaphthalene-4-sulfonate | Water | 0.48 |
| Phenol | Water | 0.22 |
| Rhodamine B | Ethanol | 0.97 |
| Sodium salicylate | Water | 0.28 |
| Sodium sulfanilate | Water | 0.07 |
| Uranyl acetate | Water | 0.04 |

*Source:* Guilbault, G.G., ed., *Practical Fluorescence*, 2nd edn., Marcel Dekker, Inc., New York, 1990. With permission.
*Note:* Solutions are $10^{-3}$ M, temperatures 21°C–25°C.

That is, $F$, total fluorescence, $= KI_0c$, where $k$ is a proportionality constant. At low concentrations, a plot of $F$ versus concentration should be linear. But only a portion of the total fluorescence is monitored or measured; therefore,

$$F' = Fk' \tag{5.10}$$

where $F'$ is the measured fluorescence and

$$F' = k'I_0c \tag{5.11}$$

where $k'$ is another proportionality constant.

A plot of $F$ versus $c$ is shown in Figure 5.51. It is linear at low concentrations. The linear working range for fluorescence is about five orders of magnitude, from $10^{-9}$ to $10^{-4}$ M.

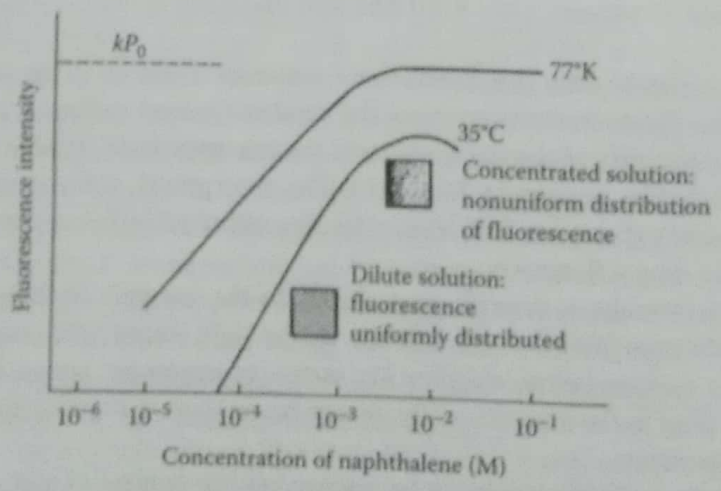


**Figure 5.51** Dependence of fluorescence on the concentration of the fluorescing molecule. (From Guilbault, G.G., ed., *Practical Fluorescence*, 2nd edn., Marcel Dekker, Inc., New York, 1990. With permission.)

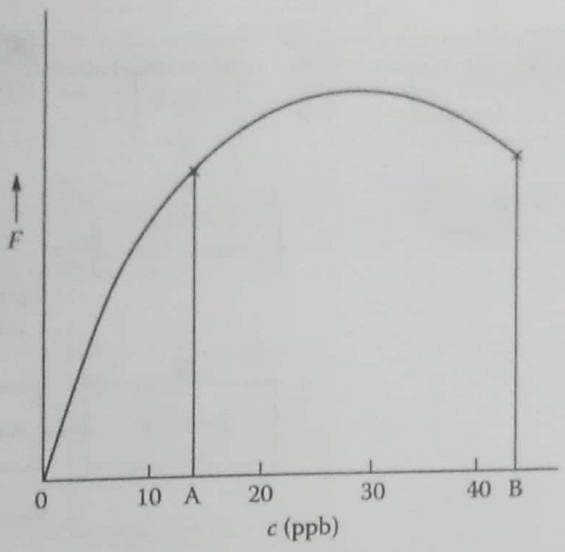


**Figure 5.52** Fluorescence intensity at high concentrations of analyte. Note the reversal of fluorescence at high concentration. Concentrations A and B give the same fluorescence intensity and could not be distinguished from a single measurement.

At higher concentrations, the relationship between $F$ and $c$ deviates from linearity. The plot of $F$ versus $c$ rolls over as seen in Figure 5.52. It can be seen that at higher concentrations, the fluorescence intensity actually decreases because the molecules in the outer part of the sample absorb the fluorescence generated by those in the inner part of the sample. This is called the "inner cell" effect or self-quenching. In practice, it is necessary to recognize and correct for this effect. It is impossible to tell directly if the fluorescence measured corresponds to concentration A or concentration B as shown in Figure 5.52. Both concentrations would give the same fluorescence intensity. Diluting the sample slightly can solve the dilemma. If the original concentration were A, then the fluorescence intensity would sharply decrease on dilution. On the other hand, if the concentration were B, then the fluorescence should increase on slight dilution of the sample.

## 5.9 INSTRUMENTATION FOR LUMINESCENCE MEASUREMENTS

A schematic diagram of a spectrofluorometer is shown in Figure 5.53.

### 5.9.1 Wavelength Selection Devices

Monochromator:

Two monochromators are used: the primary or excitation monochromator and the secondary or fluorescence monochromator. These are generally grating monochromators, although filters can be used for specific analyses. The excitation monochromator selects the desired narrow band of wavelengths that can be absorbed by the sample. The sample emits light in all directions. The second monochromator is placed at 90° to the incident light beam. The second monochromator is set to pass the fluorescence wavelength to the detector. The 90° orientation of the second monochromator is required to avoid the detector "seeing" the intense incident light, thus eliminating the background caused by the light source. Unlike absorption spectrophotometry, the measurement is not of the small difference between two signals but of a signal with essentially no background. This is one reason for the high sensitivity and high linearity of fluorescence. Most fluorescence instruments are single-beam instruments. This means that changes in the source intensity will result in changes in the fluorescence intensity. To compensate for changes in the source intensity, some instruments split off part of the source output, attenuate it, and send it to a second detector. The signals from the two detectors are used to correct for drift or fluctuations in the source.

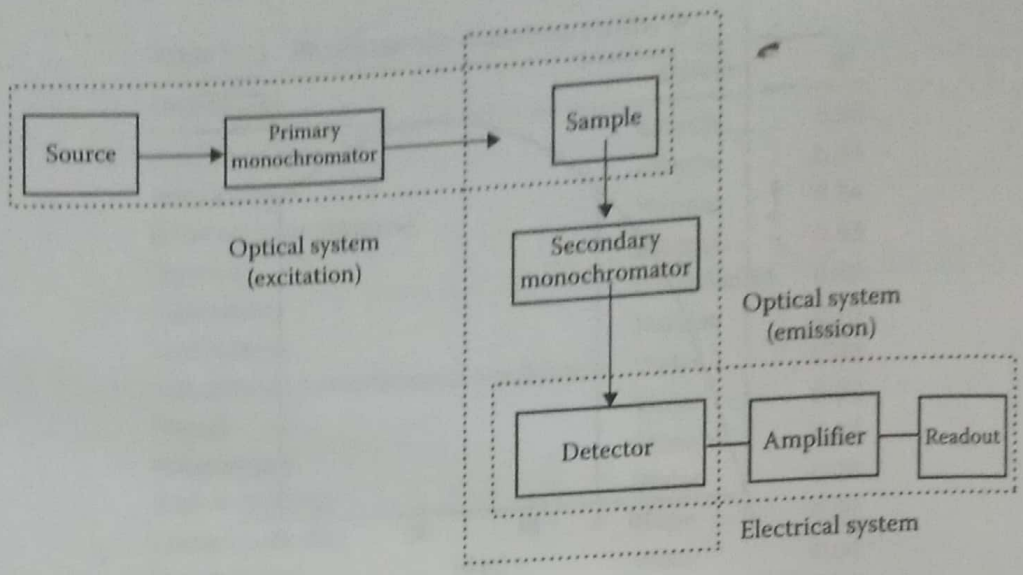


**Figure 5.53** Block diagram of the optical components of a typical fluorometer. (From Guilbault, G.G., ed., *Practical Fluorescence*, 2nd edn., Marcel Dekker, Inc., New York, 1990. With permission.)

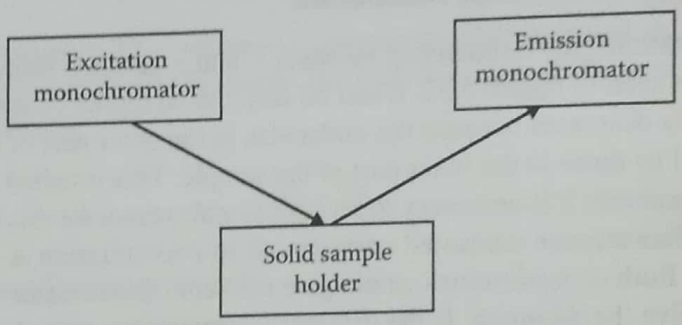


**Figure 5.54** Front-surface fluorescence geometry for a solid sample. (From Froelich, P.M. and Guilbault, G.G., In *Practical Fluorescence*, 2nd edn., Guilbault, G.G., ed., Marcel Dekker, Inc., New York, 1990. With permission.)

90° Geometrical Orientation; Front surface Geometry

The 90° geometry is the most common orientation for measuring fluorescence and works very well for solution samples that do not absorb strongly. Other angles are used in specific applications. For strongly absorbing solutions or for solid samples such as thin-layer chromatography plates, fluorescence is measured from the same face of the sample illuminated by the source. This is called front-surface geometry. It is shown schematically for a solid sample in Figure 5.54.

### 5.9.2 Radiation Sources

$I_f \propto I_{source}$

The fluorescence intensity is directly proportional to the intensity of the light source. Therefore, intense sources are preferred. Excitation wavelengths are in the UV and visible regions of the spectrum, so some of the same sources used in UV/VIS absorption spectrometry are used for fluorescence. The optical materials will of course be the same—quartz for the UV and glass for the visible region.

Mercury or xenon arc lamps are used. A schematic of a xenon arc lamp is given in Figure 5.55. The quartz envelope is filled with xenon gas, and an electrical discharge through the gas causes excitation and emission of light. This lamp emits a continuum from 200 nm into the IR. The emission spectrum of a xenon arc lamp is shown in Figure 5.56. Current fluorescence spectrometers use xenon flash lamps. Mercury lamps under high pressure can be used to provide a continuum, but low-pressure Hg lamps, which emit a line spectrum, are often used with filter fluorometers. The spectrum of a low-pressure Hg lamp is presented in Figure 5.57.

Lasers:

Because of their high intensity, laser light sources are ideal sources for fluorescence. The laser must exhibit a wide range of emission wavelengths, so tunable dye lasers have been the only choice until recently. The dye lasers are generally pumped by an Nd:YAG laser. Nd stands for neodymium and YAG is yttrium aluminum garnet. These pumped dye laser systems are very expensive and complicated to operate. They have much greater intensity output than lamps and so enable lower detection limits to be achieved. Recent advances in solid-state lasers have made small, less expensive visible wavelength lasers available. Solid-state UV lasers are available but do not have the required intensity for use as a fluorescence source.

### 5.9.3 Detectors

PMT, CCD, PDA:

The most common detector is the PMT. The operation of a PMT was described earlier in this chapter. Because the signal is small due to the low concentrations of analyte used, the PMT is often cooled to subambient temperature to reduce noise. The limitation of the PMT is that it is a single wavelength detector. This requires that the spectrum be scanned. As we have discussed, scanning takes time and is not suitable for transient signals such as those from a chromatographic column. Diode array detectors are now available to collect the entire spectrum at once instead of scanning. The CCD, a 2D array detector, is another alternative to scanning in fluorescence spectrometry. Both PDA and CCD detectors can be used with LC or CE systems for separation and detection of fluorescent compounds or "tagged" compounds in mixtures. LC and CE are discussed in Chapter 13.

### 5.9.4 Sample Cells

The most common cell for solutions is a 1 cm rectangular quartz or glass cuvette with four optical windows. For extremely small volumes, fiber-optic probes, microvolume cells, and flow cells are available. Gas cells and special sample compartments for solid samples are commercially available. Microplate readers are available for most instruments, permitting analysis of up to 384 samples.

### 5.9.5 Spectrometer Systems

Spectrometer systems for luminescence measurements are available from the major instrument companies, including Agilent, Horiba, PerkinElmer, Shimadzu, and Thermo Fisher Scientific. Only a few examples will be discussed; the company websites provide information, applications, notes, and photos of their instrumentation. Luminescence spectrometers range from high-end research instruments to dedicated application instruments.

For example, the Agilent Cary Eclipse spectrophotometer is a versatile instrument that allows fluorescence, phosphorescence, chemiluminescence, bioluminescence, and time-resolved phosphorescence measurements. It uses a xenon flash lamp, red-sensitive PMT, captures a data point every 12.5 ms, and can scan at 24,000 nm/min from the UV to 900 nm. Sample volumes can range from <0.5 mL to large, odd-sized samples using fiber-optic probe technology and accommodate a 384-well microplate reader. On the other end of the spectrum, there are instruments like the Thermo Fisher Scientific Quantech® Life Science UV Filter fluorometer, featuring a phosphor-coated Hg lamp for excitation in the 270–315 nm range, a red-sensitive PMT, and suited for nucleic acid and intrinsic protein fluorescence measurements. Versions of this instrument are available with a quartz-halogen lamp and extended spectral range. Also from Thermo Fisher Scientific is a fluorescence version of the NanoDrop, the NanoDrop 3300 Fluorospectrometer, which uses UV, blue, and white LED excitation sources covering a range from 400 to 750 nm, a 2048-element linear silicon CCD array detector, and uses as little as 1 μL of sample.

## 5.10 ANALYTICAL APPLICATIONS OF LUMINESCENCE

Fluorescence occurs in molecules that have low-energy $\pi \rightarrow \pi^*$ transitions; such molecules are primarily aromatic hydrocarbons and polycyclic aromatic compounds. Examples include those in Table 5.13, as well as compounds like indole and quinoline. Molecules with rigid structures exhibit fluorescence; the rigidity evidently decreases the probability of a radiationless deactivation. Some organic molecules increase their fluorescence intensity on complexation with a metal ion. The resulting complex structure is more rigid than the isolated organic molecule in solution. Molecules that fluoresce can be measured directly; the number of such molecules is estimated to be between 2000 and 3000 from the published literature. There are several compounds that exhibit strong fluorescence; these can be used to derivatize, complex or "tag" nonfluorescent species, thereby extending the range of fluorescence measurements considerably. Other analytes are very efficient at quenching the fluorescence of a fluorophore; there are quantitative methods based on fluorescence quenching.

A fluorometric analysis results in the collection of two spectra: the excitation spectrum and the emission spectrum. The excitation spectrum should be the same as the absorption spectrum obtained spectrophotometrically. Differences may be seen due to instrumental factors, but these are normally small, as seen in Figure 5.58, which shows the absorption and excitation spectra for Alizarin garnet R, a fluorometric reagent for aluminum ion and fluoride ion. The longest wavelength absorption maximum in the excitation spectrum is chosen as the excitation wavelength; this is where the first monochromator is set to excite the sample. It would seem reasonable to choose the wavelength that provides the most intense fluorescence as the excitation wavelength, but often short wavelengths from the high-intensity sources used can cause a compound to decompose. The emission spectrum is collected by the second monochromator. The emission or fluorescence spectrum for Alizarin garnet R is shown in Figure 5.58. Similar excitation and emission spectra are shown in Figure 5.59 for quinine and anthracene. Note, especially for anthracene, that the fluorescence

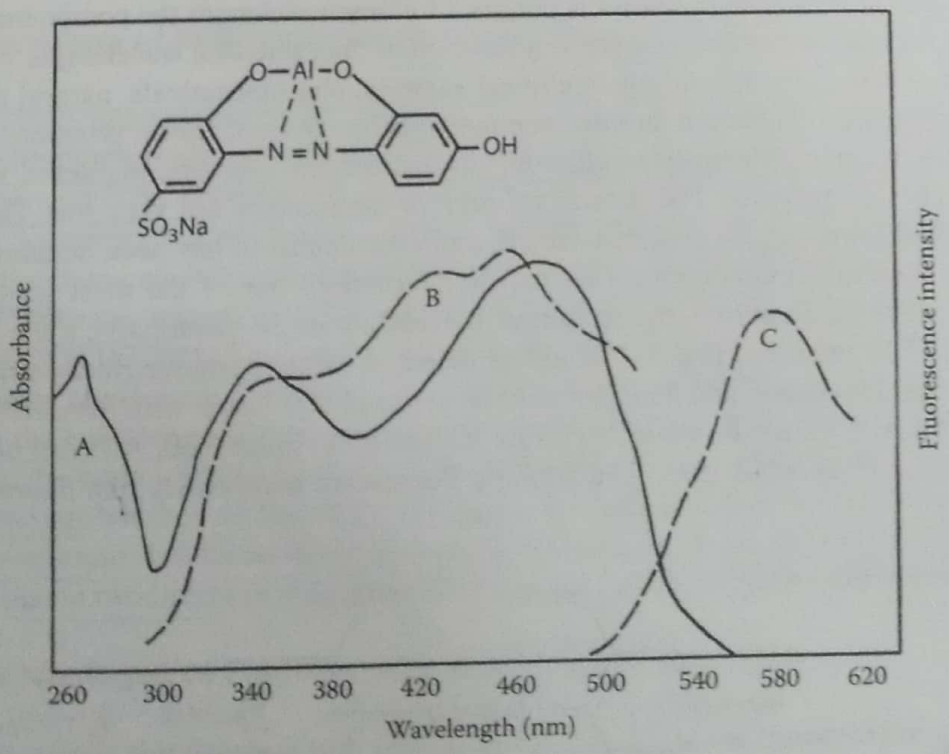


**Figure 5.58** Absorption and fluorescence spectra of the aluminum complex with acid Alizarin garnet R (0.008%): Curve A, the absorption spectrum; Curve B, the fluorescence excitation spectrum; and Curve C, the fluorescence emission spectrum. (From Guilbault, G.G., ed., *Practical Fluorescence*, 2nd edn., Marcel Dekker, Inc., New York, 1990. With permission.)

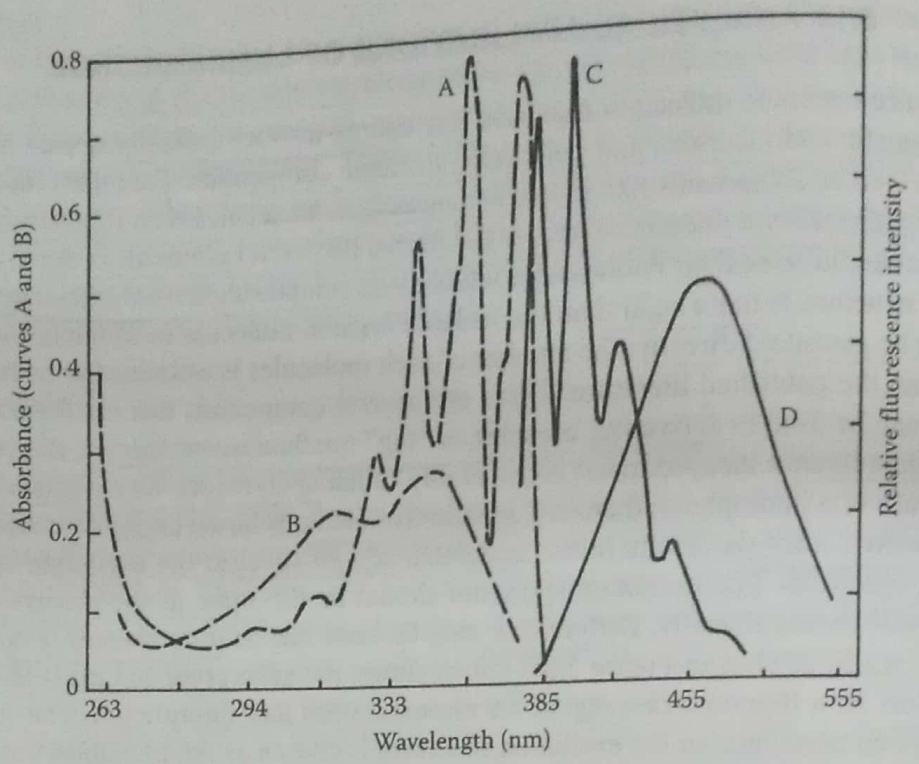


**Figure 5.59** Excitation and fluorescence spectra of anthracene and quinine: Curve A, anthracene excitation; Curve B, quinine excitation; Curve C, anthracene fluorescence; and Curve D, quinine fluorescence. (From Guilbault, G.G., ed., *Practical Fluorescence*, 2nd edn., Marcel Dekker, Inc., New York, 1990. With permission.)

(emission) spectrum is almost a mirror image of the excitation spectrum. The shape of the emission spectrum and wavelength of the fluorescence maximum do not depend on the excitation wavelength. The same fluorescence spectrum is obtained for any wavelength the compound can absorb. However, the intensity of the fluorescence is a function of the excitation wavelength.

Fluorometry is used in the analysis of clinical samples, pharmaceuticals, natural products, and environmental samples. There are fluorescence methods for steroids, lipids, proteins, amino acids, enzymes, drugs, inorganic electrolytes, chlorophylls, natural and synthetic pigments, vitamins, and many other types of analytes. The detection limits in fluorometry are very low. Detection limits of $10^{-9}$ M and lower can be obtained. Single-molecule detection has been demonstrated under *extremely* well-controlled conditions. This makes fluorometry one of the most sensitive analytical methods available. Therefore, the technique is widely used in quantitative trace analysis. For example, Table 5.10 indicated that $Al^{3+}$ could be detected spectrophotometrically using aluminon at about 0.04 ppm in solution and fluoride could be detected at 0.2 ppm with SPADNS. Using fluorometry and Alizarin garnet R, whose structure is shown in Figure 5.60, $Al^{3+}$ can be determined at 0.007 ppm and $F^-$ at 0.001 ppm. The strongly fluorescent compounds like fluorescein can be

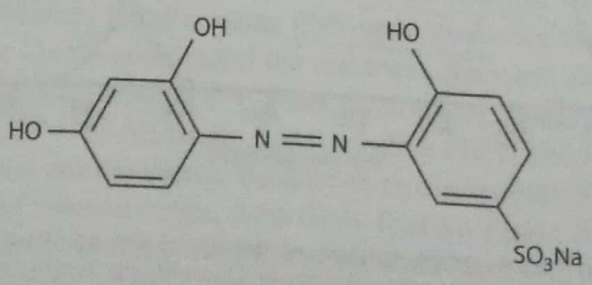


**Figure 5.60** Structure of Alizarin garnet R.

detected at part per trillion levels (ng/mL in solution), so use of such a compound as a "tag" can result in a very sensitive analytical method for many analytes.

The analysis of solid samples, especially using fiber-optic probes, has opened up new applications for fluorescence spectroscopy. Some of these include the examination of rocks and minerals to identify the presence of oil in shale, evaluation of gemstone quality and origin, and the identification of minerals at potential mining sites. Materials characterization of solar cell materials, semiconductors, organic LEDs, and polymers by fluorescence is another emerging area of study. Solid samples give much sharper fluorescence peaks than liquid samples due to the elimination of nonradiative relaxation paths. Therefore, high-resolution spectrometers are needed. Solar cell researchers need the ability to adjust the position of the sample in the excitation beam to mimic the movement of the sun, and fiber-optic probes eliminate the need to cut up large samples to fit into sample compartments. For example, stalactites often fluoresce due to the presence of humic and fulvic acids that precipitate along with the calcite. Use of a fiber-optic probe allows for nondestructive analysis of such samples in order to understand the environmental conditions under which they formed (McGarry and Baker).

Many materials benefit from analysis at cryogenic temperatures. At 77 K, enhanced fluorescence and phosphorescence are seen, and low-temperature studies are used for elucidation of the mechanisms of photochemical reactions, characterizing bandgap changes in semiconductors and other applications.

An interesting application of the use of chemiluminescence is the measurement of a ruthenium complex upon reaction with codeine (Purcell and Barnett). A 1 mM solution of tris(2,2′-bipyridyl) Ruthenium(II) in 0.05 M $H_2SO_4$ was oxidized to the $3^+$ state and then mixed with 1 mM codeine. The codeine is oxidized and the Ru reduced to the $2^+$ state, producing an excited-state intermediate that returns to the ground state by emission of a photon centered at about 610 nm. In this case, the excited state is produced by a chemical reaction; the emission of light is due to chemiluminescence. No light source was required for excitation. The same $Ru^{2+}$ complex can also be excited using an external light source; the same emission spectrum results, but this would be due to phosphorescence (Girardi et al.)

### 5.10.1 Advantages of Fluorescence and Phosphorescence

The advantages of fluorescence and phosphorescence for analyses of molecules include extremely high sensitivity, high specificity, and a large linear dynamic range. The sensitivity is a result of the direct measurement of the fluorescence or phosphorescence signal against a zero background signal, as described. Specificity is a result of two factors: First, not all molecules fluoresce; therefore, many molecules are eliminated from consideration. Second, two wavelengths, excitation and emission, are used in fluorometry instead of one in spectrophotometry. It is not likely that two different compounds will emit at the same wavelength, even if they absorb the same wavelength and vice versa. If the fluorescing compounds have more than one excitation or fluorescent wavelength, the difference in either the emission spectrum or the excitation spectrum can be used to measure mixtures of compounds in the same solution. In Figure 5.59, for example, the excitation spectra of quinine and anthracene overlap, but they do not emit at the same wavelengths, so the two compounds could be measured in a mixture. The linear dynamic range in fluorometry is six to seven orders of magnitude compared to one to two orders of magnitude that can be achieved in spectrophotometry.

### 5.10.2 Disadvantages of Fluorescence and Phosphorescence

Other compounds that fluoresce may need to be removed from the system if the spectra overlap. This can be done, for example, by column chromatography. Peaks may appear in the fluorescence spectrum, which are due to other emission and scattering processes; Rayleigh, Tyndall, and Raman scattering may be seen because of the high intensity of the light source used. Peaks due to fluorescent impurities may occur.

Self - quenching: Factors controlling:

Reversal of fluorescence intensity or self-quenching at high concentrations is a problem in quantitative analysis but can be eliminated by successive dilutions. Quenching by impurities can also occur and can cause significant problems in analysis. Changes in pH can frequently change structure, as we saw with phenolphthalein in Figure 5.44, and thereby change fluorescence intensity; pH must therefore be controlled. Temperature and viscosity need to be controlled as well for reproducible results. Photo decomposion & reactions.

Photochemical decomposition or photochemical reaction may be induced by the intense light sources used. In general, the approach of using the longest excitation wavelength possible and the shortest measurement time possible will minimize this problem.