Chapter 2

Theory of Ultraviolet Spectroscopy, Light Interaction with Matter

Contents

2.1 Theory of Spectroscopy: Light Interaction with Materials

Spectroscopy refers to the interaction of electromagnetic energy with matter, using the science of spectrophotometry [Greek: (*specere* = to look at) + (*photos* = light) + (*metron* = to measure)]. The science of measuring ultraviolet energy interaction with matter as absorption, reflection, and scattering is the subject of this chapter. The matter interacting with ultraviolet energy is comprised of liquids, gases, semi-solids, slurries, solid composites, films, and ground particles; and is defined as the qualitative or quantitative measurement of the interaction of electromagnetic radiation (emr), as ultraviolet energy, with the particles themselves and with the atoms or molecules within a defined volume of matter.

The emr interacts mainly as scattering, absorption, reflectance, and emission when "confronting" solid matter. A variety of spectrometer configurations are used to optimize the measurement of electromagnetic radiation as it interacts with matter. The subject of this chapter is a broad overview of the different ways light, as particles and waves, interacts with molecules. This chapter is intended to be a reference chapter for referring to the various characteristics and mathematical descriptions of basic light interactions with atoms and molecules. Note: some portions of this chapter are reproduced from J. Workman and A. Springsteen (Eds.), Applied Spectroscopy: A Compact Reference for Practitioners, Academic Press, Boston (1998). (Used with Permission).

2.1.1 *The Physics of Light Interaction with Solid Materials*

Light interacts with solid materials as scattering, absorption, transmission (transmittance), reflectance (both regular and diffuse reflection), and diffraction. The purpose of spectroscopy is to quantify or qualify these interactions by the use of a variety of photon producing and photon detection devices. Some of the physics of these interaction phenomena will be presented within this chapter. A general reference describing this theory in more detail is found in reference.[1]

Solids, as black body radiators, emit light and this emitted light can be characterized by its radiated power, spectral profile, and photon flux. The spectroscopy of solids is a vast field which would encompass many hundreds of volumes and thus the scope of this chapter is limited to the basic mathematical concepts and relationships related to the transmittance/transmission or reflectance/reflection interaction as molecular spectroscopy of liquids and solid materials.[2–8]

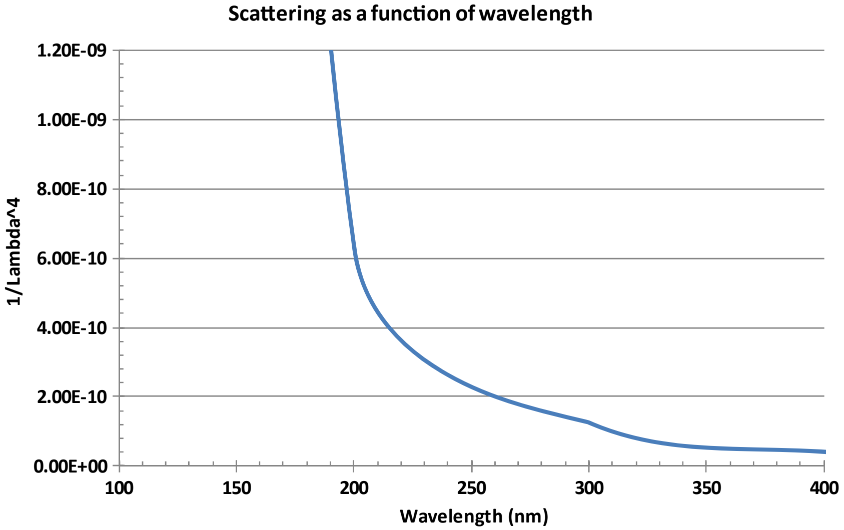

Fig. 2.1 Scattering as a function of wavelength to 400 (in nanometers).

2.1.1.1 *Light Scattering*

Light scattering as defined by Lord Rayleigh is elastic scattered light, and occurs when the intensity of the scattered energy (I_{RS}) is inversely proportional to the 4th power of the incident light wavelength (λ), as given by Equation 2.1 and shown in Figure 2.1.

$$I_{RS} \propto \frac{1}{\lambda^4} \tag{2.1}$$

The intensity ratio of Rayleigh scattered light incident onto a solid particle surface is given by Equation 2.2.

$$\frac{\bar{I}_S}{I_0} = \frac{1}{r^2}\left(\frac{2\pi}{\lambda}\right)^4 \alpha^2 \sin^2\theta \tag{2.2}$$

Where $\bar{I}_S$ = the scattered radiation intensity flux per unit time (seconds); I_0 = the incident beam radiation intensity (in energy counts); r = the average particle radius; λ = the wavelength of the incident radiation; α = the proportionality factor (if a surface is isotropic, the α is by definition identical for all angles of reflectance); and θ = the reflected light angle versus normal incidence.[9–13] Note from Equation 2.2 that the scattered radiation intensity is proportional to the inverse of the square of

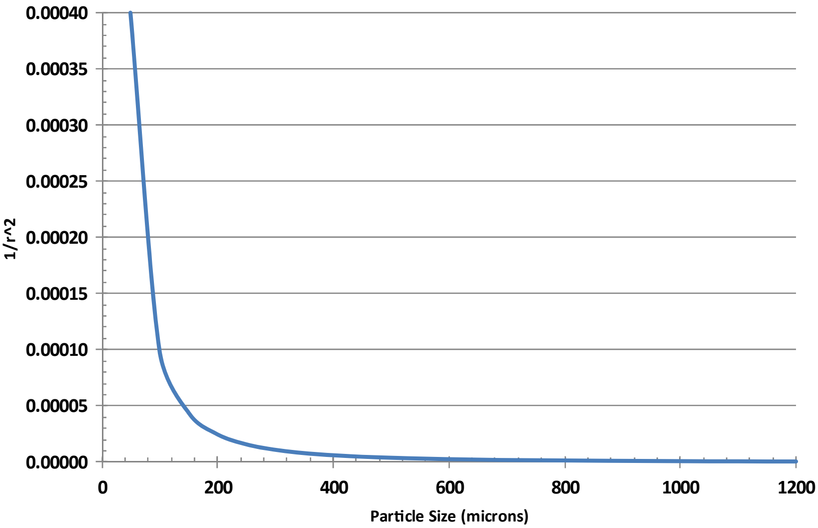

Fig. 2.2 Rayleigh scattering as a function of average particle size (radius) up to 1200 microns (1.2 millimeters).

the average particle size radius given as illustrated in Equation 2.3 and Figure 2.2.

$$I_s \propto \frac{1}{r^2} \tag{2.3}$$

2.1.1.2 *Absorption and Transmittance*

Light passing through an absorbing material is attenuated as a function of the absorption coefficient (a, or often denoted as ε) in units of $\text{Liter} \cdot \text{Mole}^{-1} \cdot \text{cm}^{-1}$ of the material, and the thickness (t) of the material in cm following the relationship[14–15] as:

$$I = I_0 e^{-at} \tag{2.4}$$

Furthermore, the fraction (in T units) of a collimated beam of light of wavelength (λ) in nm transmitted through a material of index of refraction (n) and a thickness (t) in cm is given by:

$$\mathrm{I_T} = \frac{\mathrm{I_0}}{1 + \frac{4\rho^2}{(1-\rho^2)} \sin^2\left(\frac{2\pi\,\mathrm{nt}}{\lambda}\right)} \tag{2.5}$$

where $\rho^2 = \mathrm{R}$ (the reflectance/reflection) given for normal incidence as:

$$R = \frac{(n-1)^2}{(n+1)^2} \tag{2.6}$$

2.1.1.3 *Reflectance*

The reflectance (R) of light at normal incidence passing through a refractive index (n_1) into a second material of refractive index (n_2) is given by the equation:

$$R = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} \tag{2.7}$$

For films or coatings of refractive index (n_f) deposited on glass with a refractive index of (n_g) as is the case with A-R (anti-reflective) coated optics, the reflection (R) from the coated surface is calculated using a simplified formula as:

$$R = \frac{(n_g - n_f^2)^2}{(n_g + n_f^2)^2} \tag{2.8}$$

The desired reflectivity may be computed using this equation.

2.1.1.4 *Optical Density*

The Absorbance (A or Au), or optical density (O.D.) of a material is defined by the mathematical relationship:

$$A = O.D. = -\log_{10}\left(\frac{I}{I_0}\right) = \frac{1}{2.303}\ln\left(\frac{I_0}{I}\right) \tag{2.9}$$

where I_0 = the intensity of the incident light beam, and I = the intensity of the transmitted beam after sample interaction. The O.D. of the material in which the light is passing through is given by the above expression and is a term used synonymously with absorbance.

2.1.1.5 *Opacity*

Is an expression that refers to the fraction of energy that is not transmitted by a sample, and is expressed using the equation:

$$\mathrm{O} = \frac{\mathrm{I_0}}{\mathrm{I}} \tag{2.10}$$

2.1.1.6 *Diffraction*

Diffraction can be loosely defined as the bending of light around objects.[16–18] The defining principle for diffraction is Christiaan Huygens' principle, which informs us that every point of a wave front can be considered a secondary point from which waves propagate in multiple directions. A single narrow slit can demonstrate the alternating light and dark patterns shown by diffraction.

The secondary waves propagated during diffraction have an intensity proportional to:

$$1 + \cos\frac{\theta}{2} \tag{2.11}$$

where θ is the propagation angle of the secondary wave in relationship to the original (or primary) wave. This relationship defines the intensity for waves propagated in the direction of the original wave as 1, and 0 intensity for the wave propagated at 90° to the original wave. The central bright band in a diffraction pattern is approximately 20 (or more) times brighter than the next (or adjacent) bright band in a typical diffraction pattern resulting from a narrow slit. The reader is referred to the index for additional information and treatment of the topic of diffraction and diffraction gratings.

2.1.1.7 *Emissivity*

The power (p) radiated or emitted by a black body radiator is given by the relationship:

$$p = kT^4 \tag{2.12}$$

where k is the Stefan-Boltzmann law proportionality constant equal to 5.67×10^{-12} Watts/($\text{cm}^2 \times {}^\circ\text{K}^4$), and T = °K = (°C + 273).

The spectral profile of this emitted radiation is given by Max Planck's hypothesis as shown in the following Equations 2.13 through 2.15.

$$E_p = h\nu \tag{2.13}$$

Where E_p = the photon energy (in joules) at a given frequency (ν, in units of sec.^{-1}), and where h = Planck's constant = 6.6256×10^{-3} joule-sec. (equivalent to 6.6256×10^{-27} erg-sec.). And since $\nu = \frac{c}{\lambda}$ it follows:

$$E_p = \frac{hc}{\lambda} \tag{2.14}$$

Thus, using this equation the energy of each photon at a particular wavelength is determined.

Furthermore, since 1 Watt = $\frac{\text{joule}}{\text{sec.}}$, the number of photons (N_p) per second in an emission phenomenon is calculated as:

$$N_p = \frac{1}{E_p} \tag{2.15}$$

where N_p = the number of photons per second (as the photon flux). Thus emission light sources are generated by heating metal filaments or metal surfaces to obtain the appropriate emission spectral characteristics in power, spectral profile, and photon flux.[4,7,19] These equations may be used to compose the requirements for an emission source based on the temperature of the source and the photon flux. Equations 2.13 and 2.14 may be used to calculate the spectral energy output relative to frequency or wavelength for a black body radiator. More detail is discussed for various types of emission sources, and the reader is referred to the index for Planck's constant and emissivity. (See also Volume 2, Chapter 2.)

2.1.2 *Spectrophotometry of Solids*

2.1.2.1 *Photometers*

Photometers are spectrophotometers (spectrometers) characterized as instruments which use interference filters to select the wavelength from a broad energy source that is made incident to the sample specimen. Photometers may be simple with anywhere from 1 to 3 wavelengths or more complex with multiple wavelengths and complex filter systems (up to 20 or more individual filters). Several such designs are illustrated in the section on instrumentation. The reader is referred to the index for Photometers. The optical design for a simple photometer is shown in Figure 2.3.

2.1.2.2 *Dispersive Spectrometers*

Dispersive spectrometers (or spectrophotometers) rely on the use of a monochromator (or monochromators) to disperse specific frequencies of light from a broadly emitting light source incident to the sample specimen. There is much

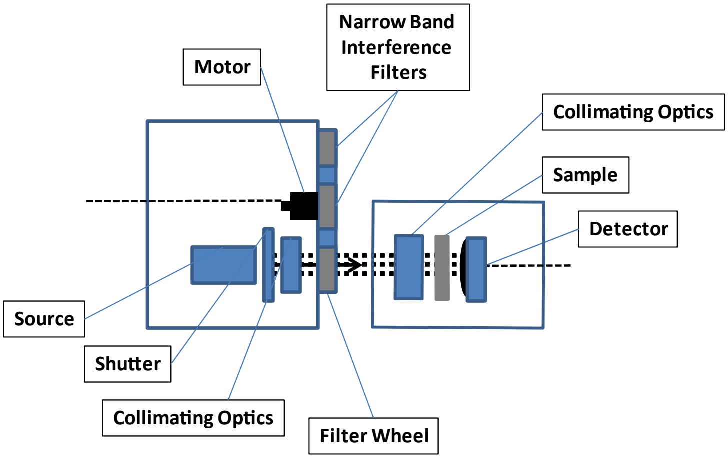

Fig. 2.3 Illustration of a simple filter photometer optical design.

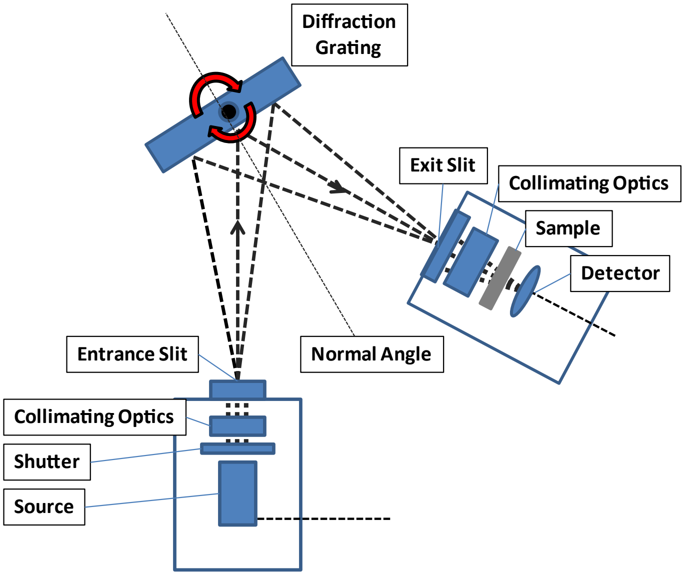

Fig. 2.4 Simple single monochromator optical design.

more information on this type of instrument found in the index under dispersive instruments or monochromators. A simple, single monochromator optical design is shown in Figure 2.4.

2.1.2.3 *The Integrating Sphere*

The purpose of an integrating sphere detector system is to provide a collection device for reflected, divergent, and scattered light from a sample. Whenever it is desirable to capture the total reflected light from a sample, the integrating sphere is preferred for its light gathering abilities.

The integrating sphere consists of a hollow sphere or hemisphere coated with a highly Lambertian (diffusely reflecting) surface. An important characteristic of an ideal sphere is that the intensity of the reflected energy at any part of the sphere surface is proportional to the total energy entering the sphere. This relationship is ideally independent of the specific point of entry to the sphere and is independent of the incident angle of the energy into the sphere. The main considerations in the use of an integrating sphere include sphere throughput, sphere efficiency, average reflectance at the sphere wall, and sphere error.[10]

The throughput (τ) of an integrating sphere, sometimes referred to as another measure of sphere efficiency, is given by the following expression. Note that τ should be maximized to provide the maximum throughput.

$$\tau = \frac{A_e R_w}{A_S} \left(\frac{1}{\left[1 - R_w \left(\frac{1 - A_{T-e}}{A_S}\right)\right]} \right) \tag{2.16}$$

Where A_e = the area of the sphere exit port (to the detector), given by πr^2; R_w = the reflectivity of the sphere wall (e.g., 1.00 = 100%); A_S = the total area of the sphere (e.g., $4\pi r^2$); and A_{T-e} = the total area of all sphere ports minus the area of the exit port(s). Sphere efficiency (E_S) is given by the relationship:

$$E_S = \frac{A_{ep}}{(1 - \bar{R}) A_S} \tag{2.17}$$

where A_{ep} = the area of the exit port (as πr^2); A_S = the total sphere area (as $4\pi r^2$); and $\bar{R}$ = the average reflectance of the sphere wall including the entrance and exit ports and the sample. The $\bar{R}$ is determined using the relationship:

$$\bar{R} = \frac{R_S A_\sigma + R_w \left(4\pi r^2 - \sum_{i=1}^{n} A_n\right)}{4\pi r^2} \tag{2.18}$$

where R_S = the reflectivity of the sample (e.g., 1.00 = 100%); A_σ = spherical area of the sample port (as πr^2); R_w = the reflectivity of the sphere wall coating (1.00 = 100%); and r = the radius of the sphere. The expression $\sum_{i=1}^{n} A_n$ is the sum of the spherical area of all ports on the sphere, where n = the number of ports; and πr^2 is the spherical area of each port, where r = the radius of the port.

For any integrating sphere, the sphere error (E_S) due to the variance in sphere efficiencies between the sample and the standard when both are measured at the identical port, is given by:

$$E_S = \frac{\varepsilon_s}{\varepsilon_r} - 1 = -\frac{(R_r - R_s)\left(\frac{A_\sigma}{A_S}\right)}{1 - R_w\left(\frac{d}{A_S}\right) - R_\sigma\left(\frac{A_e}{A_S}\right)} \tag{2.19}$$

where ε_s = the sphere efficiency when measuring a sample; ε_r = the sphere efficiency when measuring the reference reflectance standard material; R_r = the reflectance of the reference standard material (e.g. 1.00 = 100%); R_σ = the reflectance of the sample; A_S = the total sphere area; R_w = the reflectance of the sphere wall coating; and $d = A_S - A_{ent} - A_{exit} - A_\sigma$. Where A_{ent}, A_{exit}, and A_σ are the areas of the entrance, exit, and sample ports as πr^2, respectively.

In addition to the above expressions, the total power received at the detector ($\mathrm{P_d}$) as a function of input power ($\mathrm{P_i}$) is given using the expression:[10,13]

$$\mathrm{P_d} = \frac{E_S A_d}{\pi\left(\frac{d_{exit}^2}{4} + \delta^2\right)}\mathrm{P_i} \qquad (2.20)$$

where $\mathrm{E_S}$ = the sphere efficiency (as calculated above); $\mathrm{A_d}$ = the detector window area; $\mathrm{d_{exit}}$ = the exit port diameter; and δ = the distance from the exit port entrance to the detector window. There is a specific section on the "integrating sphere" in this set of volumes and the reader is referred to the index for additional details. A basic design of an integrating sphere for optical measurements is shown in Figure 2.5.

2.1.3 *Total Transmittance/Absorption and Reflectance/Absorption Spectroscopy*

The total transmittance (or transmission) of light through solids or liquids is given by the following Beer-Lambert law relationship. Note: for reflectance spectroscopy,

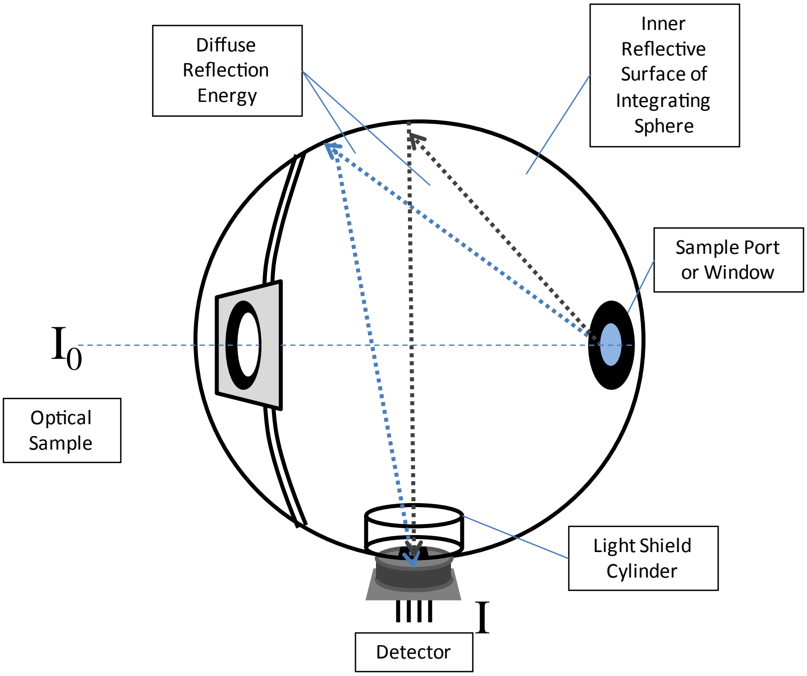

Fig. 2.5 Basic integrating sphere design.

the R term can be substituted for the T term in the following relationships:

$$\mathrm{I_t = I_0 e^{-ecl}} \quad \text{or} \quad \ln\frac{\mathrm{I_0}}{\mathrm{I_t}} = \mathrm{ecl} \quad \text{or} \quad \mathrm{I_t = I_0 \times 10^{-ecl}} \tag{2.21}$$

where $\mathrm{I_t}$ = the intensity of the transmitted or reflected light at a specific wavelength; $\mathrm{I_0}$ = the incident beam intensity at that wavelength; e = the molar extinction coefficient (absorptivity as e, or a or ε) at the wavelength of $\mathrm{I_t}$ and $\mathrm{I_0}$; c = the concentration of the absorbing molecules; and l = the thickness of the material. Note: for the above nomenclature; ecl = the total Absorption. This law is most often seen in other equation forms as:

$$\frac{1}{\mathrm{T}} = 10^{\mathrm{A}} \tag{2.22}$$

and by taking log 10 of each side of the equation we have:

$$\log\frac{1}{\mathrm{T}} = \mathrm{A} \tag{2.23}$$

Absorbance is related to the concentration of the absorbing molecules as A = ecl. And it follows that:

$$A = -\log_{10}\left(\frac{I}{I_0}\right) = \log_{10}\left(\frac{I_0}{I}\right) \tag{2.24}$$

$$-A = \log_{10}\left(\frac{I}{I_0}\right) \tag{2.25}$$

$$\frac{I}{I_0} = 10^{-A} \Rightarrow \frac{I_0}{I} = \frac{1}{10^{-A}} \tag{2.26}$$

$$\frac{I}{I_0} = T \tag{2.27}$$

$$\%\mathrm{T} = \mathrm{T} \times 100 \tag{2.28}$$

This relationship between Absorbance (A) and the ratio of I/Io is shown in Figure 2.6.

Transmission spectroscopy is most useful with clear materials, such as thin films, crystalline plates, and clear liquids. It is not particularly useful for opaque or turbid materials, or highly scattering materials.[10,13,20,21]

The measurement should not be used when intermolecular interactions vary with concentration of absorbing molecules; or with solvent type or concentration; or when refractive index changes drastically with concentration. Turbidity will markedly reduce the value of transmission measurements as will significant color changes in the material with dilution or analyte concentration. This straight

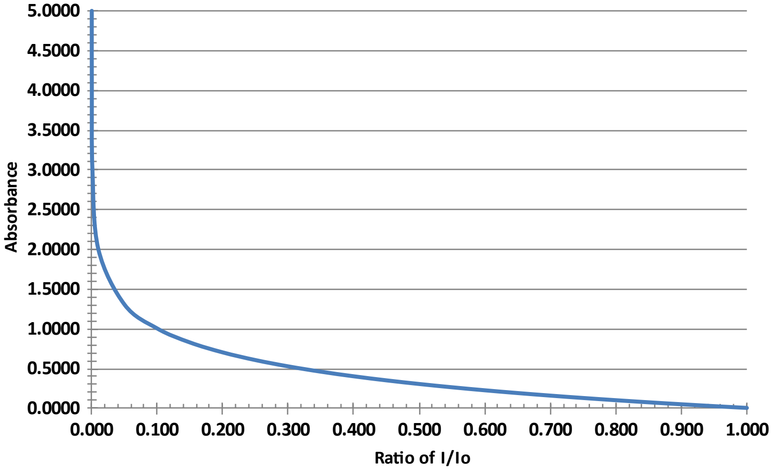

Fig. 2.6 Illustration of relationship between absorbance and I/Io from 0.00001 to 1.0.

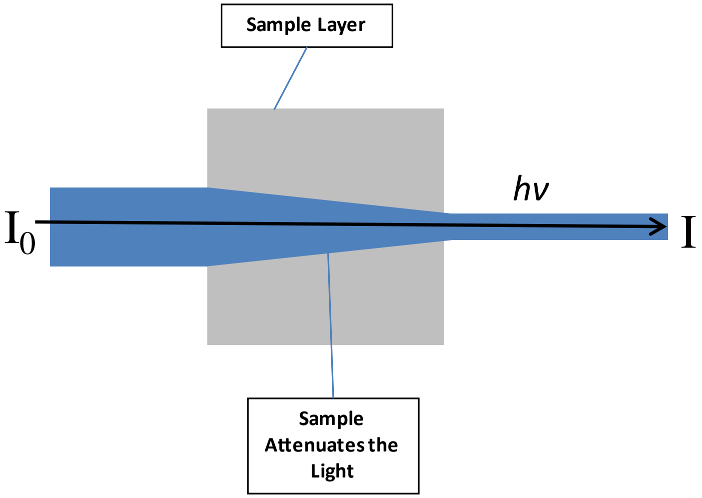

Fig. 2.7 Direct transmittance measurement geometry for solid samples.

through measurement geometry is referred to as direct transmission/transmittance (Figure 2.7).

2.1.3.1 *Diffuse Transmittance*

Diffuse Transmittance (DT) is defined as the total transmitted light passing through (and interacting with) a non-infinite thickness of a diffusely reflecting, or slightly

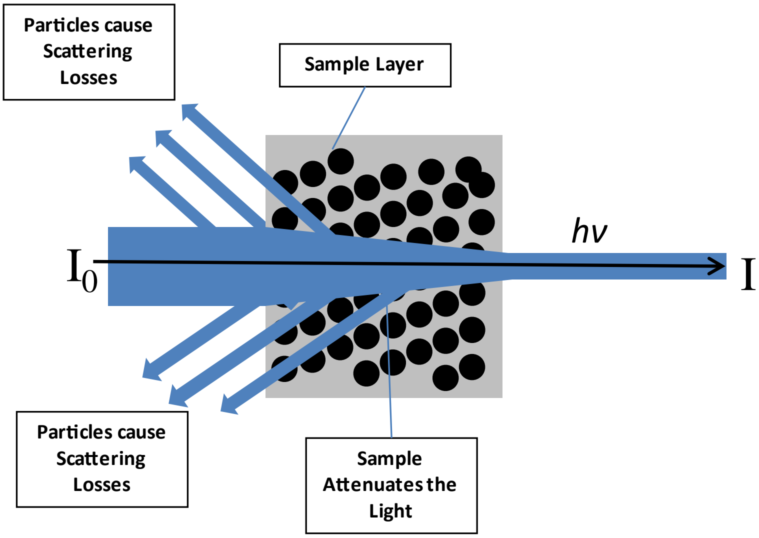

Fig. 2.8 Diffuse transmittance measurement geometry for solid samples.

energy scattering medium; this medium being comprised of multiple diffusely reflective surfaces, like powders or pellets (Figure 2.8).

2.1.3.2 *Specular (Regular) Reflectance*

Specular, external (or regular) reflectance (R_r) is described by the use of the Fresnel Equation as:

$$R_r = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} \tag{2.29}$$

where n_2 and n_1 are the refractive indices of two materials, n_2 being the material for which regular reflectance is measured and n_1 being the surrounding medium. For a material in air, this relationship can be expressed as:

$$R_r = \frac{(n_m - 1.0003)^2 + n_m^2\varepsilon^2}{(n_m + 1.0003)^2 + n_m^2\varepsilon^2} \tag{2.30}$$

where n_m = the refractive index of the material, and ε = the absorption (or extinction) coefficient of the material.

Applications for external (or regular) reflectance include surface measurements for metals or semiconductors. The technique is used to measure the dielectric function of solids; for characterization of thin films; and to relate the reflectivity of a material surface to its electronic and/or surface structure. Thin film thickness (t, in units of wavelength) is measured using this technique combined with the mathematical relationship:

$$t = \frac{f}{2\sqrt{\left(n_m^2 - \sin^2 \alpha\right)} \times d} \tag{2.31}$$

where f = the number of complete interference fringes between initial and final fringe interval (peak to peak); d = the wavelength difference for the fringe count interval; and α = the angle of incidence.

The refractive index of a thin film (n_t) is calculated using:

$$n_t = \sqrt{\left[\frac{(\sin^2 \alpha_1 d_1^2 - \sin^2 \alpha_2 d_2^2)}{(d_1^2 - d_2^2)}\right]} \tag{2.32}$$

where α_1 = the first angle of incidence; d_1 = the spacing between the fringes of the interference pattern created using incident light at angle α_1; α_2 = the second angle of incidence used to create a second interference pattern; and d_2 = the spacing between fringes of the second interference pattern created by light at angle of incidence α_2 (peak to peak).

2.1.4 *Fluorescence*

2.1.4.1 *Introduction*

Photons with energies in the ultraviolet (190 nm to 360 nm or 180 nm to 350 nm for some fluorometers) to the blue-green visible (350 nm to 500 nm) regions of the spectrum will excite an electronic transition in molecules that fluoresce (i.e., fluorophores). Fluorescence is an electronic transition from a ground state to the excited state and with the emission of a photon to return to the ground state. Once excited the molecule relaxes (Stokes shift) to the ground state while emitting a photon within a femtosecond (10^{-15} seconds) to picosecond (10^{-12} seconds) timeframe. The Stokes shift indicates a lower energy of the fluorescence photons than the energy of the excitation photons. The fluorescence typically has a lifetime (or duration) of nanoseconds (10^{-9} seconds) per transition. Figures 2.9 and 2.10 show Jablonski diagrams for the quantum energy transitions for infrared, Rayleigh and Raman scattering, and fluorescence (Figure 2.9); and of fluorescence excitation, radiative energy decay pathways, with photon emission. Note that Ex denotes the energy scale, and T1 is the lowest energy triplet state (Figure 2.10).[22–23]

A fluorescence spectrophotometer normally has an excitation monochromator, that defines the excitation energy, and an emission monochromator that provides a full spectrum of the fluorescence emission. Figure 2.11 demonstrates the schematic

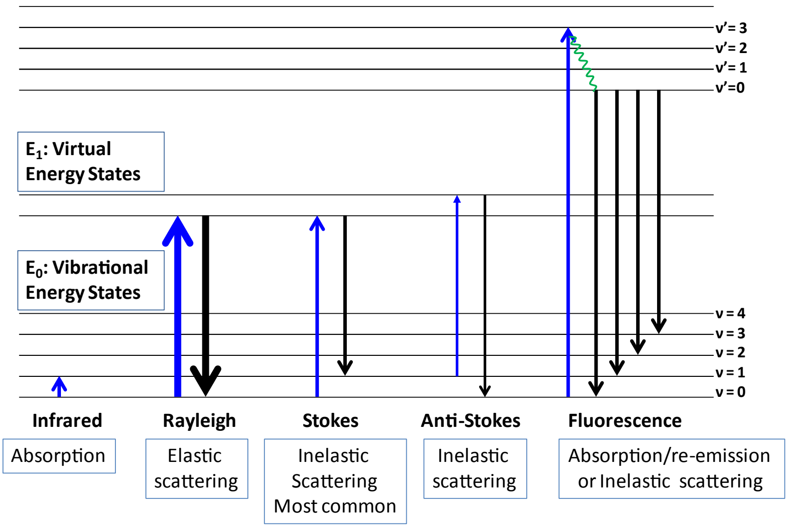

Fig. 2.9 Illustration of Jablonski diagram for the quantum energy transitions for infrared, Rayleigh scattering, Raman scattering, and fluorescence. Thickness of lines indicates the relative energy levels involved for each transition.

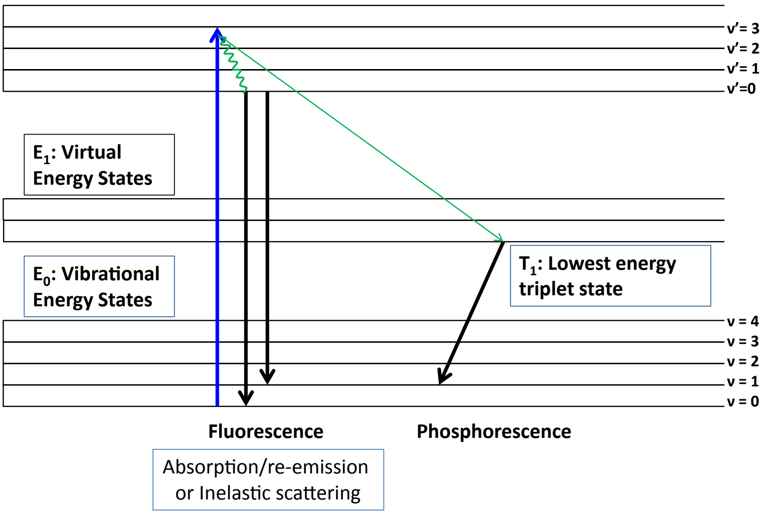

Fig. 2.10 Illustration of Jablonski diagram for the quantum energy transitions for fluorescence and phosphorescence. Shown are the radiative energy level pathways, with photon emission pathways. Note that E_0 and E_1 denote the energy levels, and T1 is the lowest energy triplet state illustrating phosphorescence. Thickness of lines indicates the relative energy levels involved for each transition.

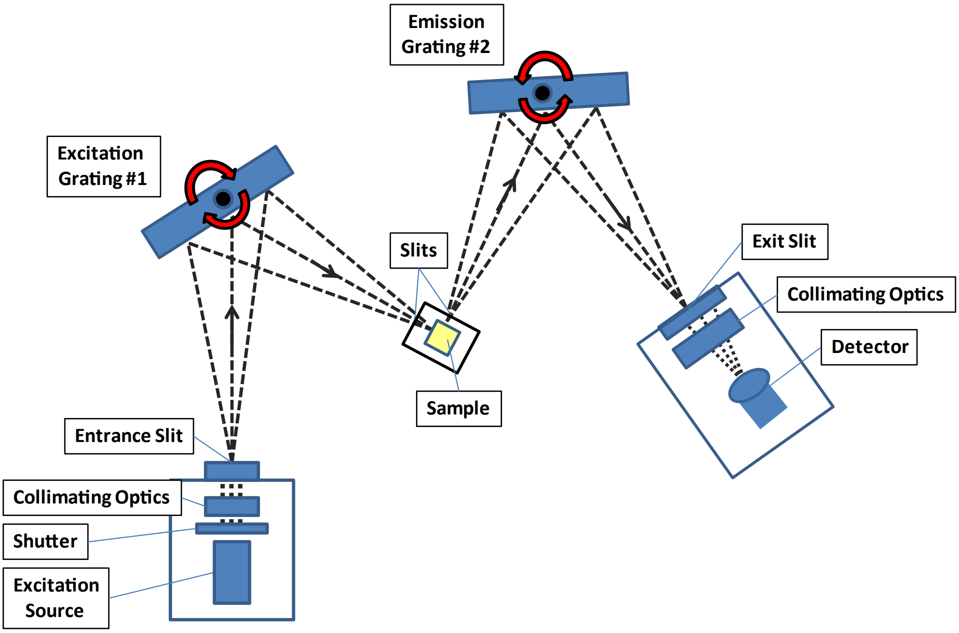

Fig. 2.11 Basic optical schematic illustration of a dual monochromator fluorometer: one excitation monochromator and one emission monochromator. Phosphorescence may also be measured by excitation and then shutting off the source lamp for longer-term phosphorescence emission measurement.

of a fluorometer (or fluorescence spectrophotometer). The overlapping excitation and emission spectra are illustrated in Figure 2.12 for a scanning fluorometer. Note that either the excitation or emission monochromators may be set to a fixed wavelength with the other monochromator set to scan for a given wavelength range. Table 2.1 lists the potential scanning sequences for a research grade fluorometer.

2.1.4.2 *Fluorescence and Phosphorescence*

Fluorescence is the emission of light by a fluorescent material (i.e., fluorophore) that has absorbed higher energy electromagnetic radiation. As mentioned, the emitted or fluorescence energy has lower energy, or longer wavelength, than the excitation radiation. Ultraviolet excitation energy used in fluorescence (typically 180 to 380 nm) provides the most dramatic forms of fluorescence and the emitted energy is often in the visible region (lower energy than the ultraviolet region).

The excitation and emission process of a fluorophore may be written as Equation 2.33.

$$E_0 + h\nu_{ex} \rightarrow E_1 \rightarrow E_0 + h\nu_{em} + heat \tag{2.33}$$

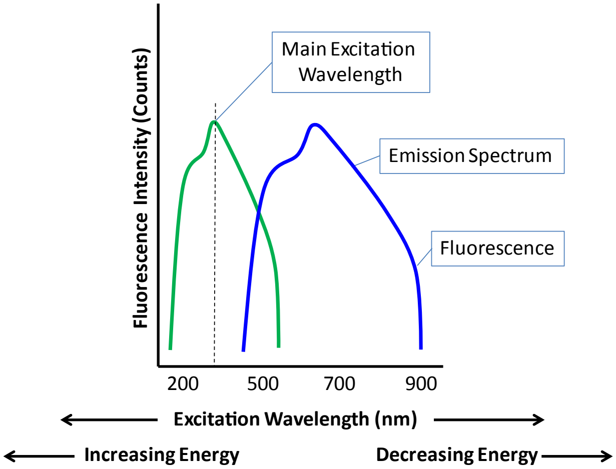

Fig. 2.12 Representation of scanning ranges for a dual monochromator fluorometer. The excitation monochromator may scan from the ultraviolet to the visible range and the emission monochromator may also scan from the ultraviolet to the visible range. The fluorescence is of lower energy (longer wavelength) than the excitation wavelength for typical fluorescence.

Table 2.1 Fluorometer scanning functions.

Excitation wavelength(s)	Emission wavelength(s)	Useful for determining
Scanning	Scanning	Locate EX and EM Wavelengths
Fixed	Scanning	EM Spectrum
Scanning	Fixed	Max EX Wavelength
Fixed	Fixed	Quantitative Analysis

This is symbolic when a lower energy state (E_0) is excited to a higher energy state (E_1) due to excitation energy ($h\nu_{ex}$). The resultant higher energy state remains for approximately a nanosecond (10^{-9} seconds) lifetime and re-emits fluorescence energy in the form of a fluorescent emission photon at lower energy ($h\nu_{em}$). Some energy in the form of heat or infrared is often emitted as well. The term $h\nu$ is used here to represent photon energy as h = Planck's constant and ν = (Gr: nu) = frequency of light. The energy states are E_0, sometimes referred to as S_0 representing the ground state of the fluorophore, and E_1 (or S_1) is the first excited state (referring to the electronic state of the molecule). Heat may also dissipate into a solvent material during the energy state relaxation process (this is referred to as *non-radiative* relaxation energy).

A second type of *non-radiative* relaxation occurs when the excitation energy is dissipated to a triplet state, which then causes a phosphorescence relaxation process. Fluorescence quenching may occur when the energy that would result in fluorescence is absorbed by a second molecule, preventing a fluorescence response or actually decreasing the fluorescence following excitation. Resonance fluorescence may occur when intense excitation energy causes the absorption of 2 photons where the emitted radiation is both at shorter and longer wavelengths than the excitation radiation, this is sometimes referred to as a Raman effect.

2.1.4.3 *Quantum Yield*

The fluorescence quantum yield (Q) gives the efficiency of the fluorescence process. It is defined as the ratio of the number of photons emitted to the number of photons absorbed, as shown in Equation 2.34.

$$Q = \frac{p_{em}}{p_{ab}} \tag{2.34}$$

Where the efficiency is equal to the number of photons emitted (P_{em}) ratioed to the number of photons absorbed (P_{ab}). A highly efficient fluorophore will demonstrate a Q ratio of 0.10 to 0.15; the maximum theoretical Q efficiency is 1.0. Fluorescence quantum yields are measured by comparison to a standard material fluorescence. The quinine salt *quinine sulfate* in a sulfuric acid solution is a common fluorescence standard (see Chapter 8).

2.1.4.4 *Fluorescence Lifetime*

The first-order fluorescence lifetime is the average time the molecule stays in its excited state prior to emitting a lower energy photon. The concentration of the excited state $[E_1]$ at any time is given as Equation (2.35), demonstrating an example of exponential decay.

$$[\mathrm{E1}] = [\mathrm{E1}]_0 e^{-\Gamma \mathrm{t}} \tag{2.35}$$

Where [E1] is the concentration of excited state molecules at some time t; $[\mathrm{E1}]_0$ is the initial concentration of molecules at time 0; and Γ is the decay rate equal to the inverse of the fluorescence lifetime. For commonly used fluorescent compounds, typical excited state decay times for photon emissions will range from 0.5 to 20 or more nanoseconds.

2.1.4.5 *Phosphorescence*

Phosphorescence is a form of photoluminescence with a slower time scale of re-emission than fluorescence. Phosphorescence is associated with "forbidden" energy state transitions that occur very slowly. This process may emit radiation at a low energy rate for several hours after absorption of excitation photons. It may be measured using most spectrofluorometers (Figure 2.11).

References

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