10.3.6.3 Measurement and use of luminescence parameters in analysis

10.3.6.3.1 Classification of luminescence parameters

The luminescence property of an analyte as measured by the appropriate instrument will often be distorted by instrumental and sample effects, and the property would be referred to as the *measured luminescence parameter*. Corrected parameters are those derived by correcting the measured parameters for instrumental artifacts, for post-filter effects and other sample effects. Table 10.14 lists the luminescence parameters and the symbols used.

TABLE 10.14 Classification and symbols for luminescence parameters

Name	Emission spectrum	Excitation spectrum	Lifetime	Quantum yield	Degree of anisotropy	Polarization spectrum	
						emission	excitation
Measured	E_m	X_m	τ_m	Y_m	r_m	E_{pm}	X_{pm}
Corrected	E_c	X_c	τ_c	Y_c	r_c	E_{pc}	X_{pc}

10.3.6.3.2 Emission spectra

The *measured emission spectrum* of a sample is the spectrum as obtained from the instrument. The *corrected emission spectrum* is obtained after correcting for instrumental and sample effects and is usually represented by a graph of ϕ_λ may be transformed to other quantities as follows:

wavelength scale (nm),

$\phi_{p,\lambda} = d\phi_p / d\lambda = \phi_\lambda / hc$ (N_p per nm)

energy scale (cm^{-1}),

$\phi = d\phi / d = \phi_\lambda \lambda^2$ (W per cm^{-1})

$\phi_{p,} = d\phi_p / d = \phi_\lambda \lambda^3 / hc$ (N_p per cm^{-1})

where N_p is photons per second.

The shape of the emission spectrum depends on the quantity plotted; $\phi_{p,\lambda}$ or $\phi_{p,}$ are preferred since they may be used to calculate *quantum yields of luminescence*.

10.3.6.3.3 Excitation spectra

The spectrum observed by measuring the variation of the luminescence flux from an analyte as a function of the excitation wavelength is termed a *measured (fluorescence,*

phosphorescence) excitation spectrum. A *corrected excitation spectrum* is obtained if the photon flux incident on the sample is held constant.

10.3.6.3.4 Excitation-emission spectra

The three-dimensional spectrum generated by scanning the emission spectrum at incremental steps of excitation wavelength (x axis = emission wavelength, y axis = excitation wavelength, z axis = emission flux) is called a (*fluorescence, phosphorescence) excitation-emission spectrum* (or *EES*). (Such spectra are commonly represented as contour diagrams or as isometric projections.)

A *synchronously excited (fluorescence, phosphorescence) spectrum* obtained by varying both the excitation and emission wavelengths simultaneously is a two-dimensional spectrum which corresponds to the curve where a plane, parallel to the z-axis, intersects the EES.

10.3.6.3.5 Lifetime of luminescence

The *lifetime of luminescence* is defined as the time required for the luminescence intensity to decay from some initial value to e^{-1} of that value (e = 2.718 ...). Lifetimes can be measured by *phase fluorimetry (phosphorimetry)* where the phase shift between the sinusoidally modulated exciting light and the emitted light is measured. *Flash fluorimetry (phosphorimetry)* is the term used when decay times of luminescence are measured using a pulsed source of radiation. It is often necessary to separate the signal due to the light flash from the luminescence emission signal by a deconvolution technique in order to obtain the correct decay curve for emission. Decay times corrected for this effect are termed *corrected decay times of fluorescence* or *phosphorescence*.

10.3.6.3.6 Quantum yields

The quantum yield of luminescence of a species is the ratio of the number of photons emitted to the number of photons absorbed by the sample. The *measured quantum yield of luminescence (fluorescence or phosphorescence*) is the result of measurement made with a fluorescence (phosphorescence) spectrometer when no corrections are made for instrumental response or for sample effects. The *corrected quantum yield of luminescence* is obtained when the measured quantum yield is corrected for instrumental response, pre- and post-filter effects and refractive index effects. The *energy yield of luminescence* of a species is defined as the ratio of the energy emitted as luminescence to the energy absorbed by the species. Quantum yields of fluorescence (phosphorescence) of an analyte are often reduced due to quenching by other species in the analyte solution. *Quenching processes* generally follow the *Stern-Volmer law*:

$$(Y_0/Y) - 1 = k_0 c_Q \tau_0$$

where Y_0 = luminescence yield in the absence of quencher Q
Y = luminescence yield with quencher of concentration c_Q
k_Q = rate constant for quenching
τ_0 = luminescence lifetime in the absence of quencher Q

The *quantum efficiency of luminescence* is defined as the fraction of the molecules in a particular excited state which emit luminescence (fluorescence or phosphorescence), in contrast to quantum yield which applies to the system as a whole.

10.3.6.3.7 Linear Polarization of Luminescence

Polarization of emission is not of great importance in molecular luminescence spectroscopy unless the solvent used is viscous or solid. The relations between the *degree of polarization*, *P*, the *degree of depolarization*, *D*, and the *degree of anisotropy*, *r*, (See Table 10.16) are:

$$P = 3r / (2 + r)$$
$$D = (1 - r) / (1 + 2r)$$

The *corrected luminescence excitation polarization spectrum* of an analyte is obtained when the polarization is measured as a function of the excitation wavelength which should be specified.

The *corrected luminescence emission polarization spectrum* is the (fluorescence, phosphorescence) spectrum observed when polarization is measured as a function of emission wavelength using a fixed and specified excitation wavelength.

10.3.6.3.8 Quantitative analysis

In *fluorescence analysis*, the *blank measure* is predominantly due to scattering of the exciting radiation, especially *Raman scattering*. Fluorescence from the solvent and sample cuvette as well as light scattering in the spectrometer can also be important. In *phosphorescence analysis*, the blank measure is due to phosphorescent impurities in the solvent and sample cuvette. Other methods of luminescence analysis would include *chemiluminescence analysis*, where a reaction produces luminescence radiation. A blank measure must also be made for this method. Figure 10.16 illustrates different types of luminescence spectrometer.