PART VI : MOLECULAR LUMINESCENCE SPECTROSCOPY

(Recommendations 1985)

1. INTRODUCTION

This document does not aim to be completely self-contained since many of the terms and units needed for describing Molecular Luminescence Spectroscopy have already appeared in Parts I, II and III. However to facilitate reference, all terms important to Molecular Luminescence, together with their symbols and units – and these include many appearing in previous documents – are presented in the Tables.

In the past, the terms quantum yield and quantum efficiency have usually been considered interchangeable. It is now recommended that these terms should be used strictly as defined in Sec. 4.6.

In part VI, the use of photon quantities is presented for the first time in these series. Photon quantities are important in Molecular Luminescence Spectroscopy and although they have been in use for some years, no international organization has come forward with recommendations for symbols for these quantities. Where the measurement is primarily interested in the number of photons flowing in a beam of radiation, it is recommended that a subscript p be used on the corresponding energy or flux quantity (see Sec. 4.2 and Table VI.3).

2. DEFINITION OF LUMINESCENCE AND PARAMETERS USED IN ANALYSIS

2.1 Types of luminescence

The various types of molecular luminescence observed can be classified by (a) the mode of excitation to the excited state capable of emission and (b), the type of molecular excited state (Table VI.1). *Fluorescence* is the spin-allowed radiative transition while *phosphorescence* is the result of a spin-forbidden radiative transition.

TableVI.1. Classification of types of luminescence

(a)	excitation mode	luminescence type
	absorption of radiation (UV/VIS)	photoluminescence
	chemical reaction	chemiluminescence, bioluminescence
	thermally activated ion recombination	thermoluminescence
	injection of charge	electroluminescence
	high energy particles or radiation	radioluminescence
	friction	triboluminescence
	sound waves	sonoluminescence
(b)	excited state (assuming ground singlet state)	luminescence type
	first excited singlet state	fluorescence, delayed fluorescence
	lowest triplet state	phosphorescence

Fluorescence, *delayed fluorescence* and phosphorescence can also arise from excited states higher than the first and therefore the transition should be indicated by a subscript. However the quantum yield of radiative processes from higher excited states are generally several orders of magnitude lower than the quantum yields of emission from the first excited state. Therefore if no special indication is given, the quantum yields are those of the respective first excited states.

Three types of delayed fluorescence are known:

(i) *E-type delayed fluorescence*: The first excited singlet state becomes populated by a thermally activated radiationless transition from the first excited triplet state. Since in this case the population of the singlet and triplet states are in thermal equilibrium, the lifetimes of delayed fluorescence and the concomitant phosphorescence are equal.

(ii) *P-type delayed fluorescence:* The first excited singlet state is populated by interaction of two molecules in the triplet state (triplet-triplet annihilation) thus producing one molecule in the excited singlet state. In this biphotonic process the lifetime of delayed fluorescence is half the value of the concommitant phosphorescence.

(iii) *Recombination fluorescence:* The first excited singlet state becomes populated by recombination of radical ions with electrons or by recombination of radical ions of opposite charge.

Whereas delayed fluorescence rarely has analytical applications, fluorescence and phosphorescence are of practical importance in luminescence analysis. Absorption of light is the preferred mode of excitation while *chemiluminescence* (production of luminescence radiation by chemical reaction) as yet plays a minor role.

2.2 Aborption and deactivation processes

In principle *radiative* and *radiationless transitions* can be distinguished in molecules. The first occurs by absorption or emission of light quanta, and the latter is the result of the transformation of electronic excitation energy into vibrational/rotational energy.

In both radiative and radiationless transitions the principle applies that transitions between terms of the same multiplicity are spin-allowed while transitions between terms of different multiplicity are spin-forbidden (*spin conservation rule*).

The intercombination prohibition for transitions between terms of different multiplicity in molecules becomes more relaxed the more efficiently spin-orbit coupling (jj coupling) perturbs the wavefunctions of the pure states into wavefunctions of mixed spin states. As a result, spin-forbidden transitions can sometimes compete with spin-allowed transitions.

Generally, the transition probabilities of radiationless transitions are higher, the smaller the energy difference between the ground vibrational levels of the electronic states that are involved in the transition.

The definitions of the various radiative and raditionless transitions which occur in molecules are illustrated in the term scheme in Fig. VI.1.

2.2.1 Absorption.

Singlet-singlet absorption results in the transition from the singlet ground state of the molecule into singlet excited states (S_0 --> S_n) and leads to the UV/VIS absorption spectrum.

The analogous *triplet-triplet absorption* takes place with the transition from the lowest triplet state of the molecule to higher triplet states (T --> T_n) thus leading to the triplet-triplet absorption spectrum.

Singlet-triplet absorption takes place with the transition from the singlet ground state of the molecule to triplet states (S_0 --> T_n) and results in the singlet-triplet absorption spectrum.

Each absorption transition is characterized by the energy of the absorbed radiation, the oscillator strength and the polarization of the transition as well as the vibrational structure of the bad system. The oscillator strength depends on the multiplicities of the participating electronic states, their orbital character (π,π^* or n,π^* states) and on the symmetries of the initial and final states.

The knowledge of the UV/VIS absorption spectra of the compounds studied is of particular importance in luminescence anlysis. In this context it has to be taken into account that UV/VIS absorption spectra measured in a solid matrix at low temperatures are generally different from spectra measured in fluid solution at room temperature. Smaller half-widths of the bands and higher molar absorption coefficients of the absorption maxima are invariably observed in the solid matrix.

2.2.2 Radiationless transitions.

Intrachromophoric radiationless transitions take place within the term system of the molecule, *interchromophoric radiationless transitions* between the term system of two non-conjugated parts of the molecule, *intermolecular radiationless transitions* between two molecules of identical or different species.

Interchromophoric and intermolecular radiationless transitions are *electronic energy transfer processes.*

Intrachromophoric radiationless transitions between. states of the same multiplicity are named *internal conversion* (IC): S_n --> S , S --> S_0 , T_n --> T being distinguished.

Intrachromophoric radiationless transitions between states of different multiplicity are named *intersystem crossing* (ISC): S --> T_n , T --> S_0 , T --> S are known.

The following electronic energy transfer processes are known: singlet-singlet (spin-allowed), triplet-triplet (spin-allowed), singlet-triplet (spin-forbidden) and triplet-singlet transfer (spin-forbidden).

The most important property of radiationless transitions for analytical work is the transition probability because this determines the yield of luminescence . The quantum yields of luminescence Y_F and phosphorescence Y_P are related to the radiative and radiationless rate constants as follows:

$$Y_F = k_{FM}/(k_{FM} + k_{TM} + k_{GM})$$

$$Y_P = [k_{TM}/(k_{FM} + k_{TM} + k_{GM})]\ [\ k_{PT}/(k_{PT} + k_{GT})]$$

where the rate constants relate to the transitions as follows:

rate constant	transition
k_{FM}	fluorescence
k_{TM}	ISC (S --> T_n)
k_{GT}	ISC (T --> S_0)
k_{GM}	IC (S --> S_0)
k_{PT}	phosphorescence

Luminescence quenching is defined as the radiationless redistribution of the excitation energy via interaction (electronic energy or charge transfer) between the emitting species and the quencher. Quencher and emitter can be molecules of the same species (concentration quenching) or of different species. The deactivation of the primarily excited emitter can lead to the activation of the quencher followed by radiative deactivation (*sensitized luminescence*). In some cases concentration quenching is accompanied by the formation of a new bimolecular species which is capable of emission (*excimer*- and *exciplex-luminescence*).

In special cases luminescence quenching effects can be used to enhance sensitivity and/or selectivity in the luminescence analysis of mixtures:

(i) The observed rate constants *k* of fluorescence quenching by external heavy atom perturbers are often significantly different even in the case of closely related compounds, for example isomers.

(ii) The strong depopulation of the fluorescing singlet excited state by external heavy atom perturbers can lead to a large population of the phosphorescing triplet excited state (*enhanced phosphorescence analysis*).

(iii) In general, strong electron acceptors quench the fluorescence of alternant polycyclic aromatic hydrocarbons more efficiently than the fluorescence of the non-alternant systems and the revers effect takes place with strong electron donors as fluorescence quenchers.

The application of the effects mentioned in (i) and (iii) in luminescence analysis are examples of the technique of *quenched fluorescence analysis*. The use of the terms "enhancophosphorimetry" and "quenchofluorimetry" is not recommended.

2.2.3 Radiative transitions.

As to the definition of fluorescence, delayed fluorescence and phosphorescence see Section 2.2 and Fig. VI.1

Fluorescence radiation occurring at wavelengths longer than absorption, i.e. , the normal case, is said to be of the Stokes type. Fluorescence radiation occurring at shorter wavelengths than absorption is classified as the anti-Stokes type.

The following characteristic parameters of radiative transitions are the most important in luminescence analysis:

(i) the luminescence spectrum

(ii) the luminescence quantum yield

(iii) the luminescence lifetime

(see Sections 4.1, 4.3 and 4.4).

Phosphorescence quantum yields sufficient for analytical applications are generally obtained only if bimolecular radiationless deactivation of the phosphorescing triplet state is avoided by carrying out the measurements in a solid matrix (at low or room temperature) or by carrying out the measurements with the substance in the adsorbed state (at low or room temperature). Room temperature phosphorescence in liquid solution can be applied to analysis provided the solution is efficiently deoxygenated.

If the luminescence lifetimes of the different species of a mixture to be analyzed differ sufficiently time-resolved luminescent measurements can be used for analytical purposes.

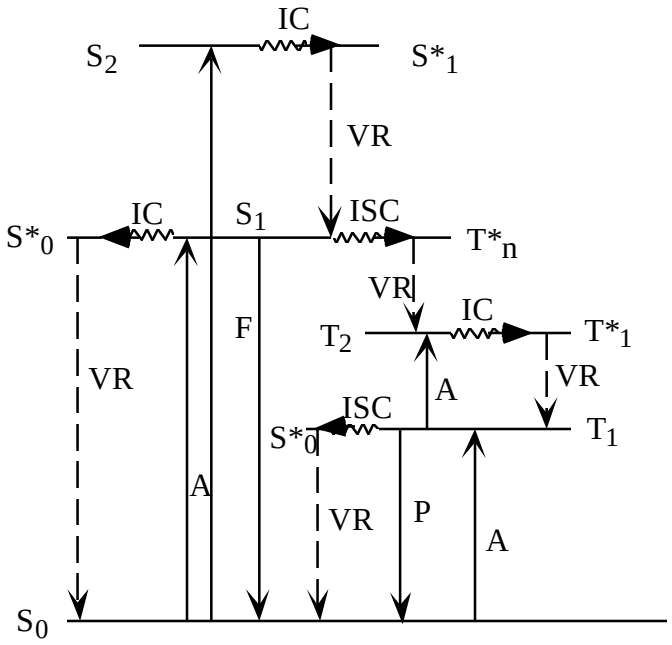

Figure VI.1. Schematic diagram of radiative (solid vertical lines), radiationless (wavy horizontal lines), and vibrational relaxation (broken vertical lines) between electronic states in a p-electronic system. States: S_0 = ground state, S_1 = first excited singlet state, S_2 = second excited singlet state, etc. Transitions: A = absorption (S_0 --> S_n , S_0 --> T_1 , T_1 --> T_n), IC = internal conversion (S_n --> S_1 , S --> S_0 , T_n --> T), ISC = intersystem crossing (S_1 --> T_n , T_1 --> S_0), VR = vibrational relaxation, F = fluorescence (S_1 --> S_0), and P = phosphorescence (T_1 --> S_0).

2.2.4 Matrix effects.

The following matrix effects are important in luminescence analysis:

(i) Acid/base interaction - Addition of acid or base to the solution of a fluorescing or phosphorescing compound which contains functional groups with dissociatable protons or lone or non-bonded electron pairs can lead to spectral shifts by protonation.

In some cases, aromatic molecules having non-bonded electron pairs fail to fluoresce in non-activating solvents because the lowest excited singlet state is of the n,π* type which usually favours intersystem crossing. The addition of small amounts of acid results in protonation with the non-bonded pairs often raising the energy of the n,π* to such a degree that the lowest π,π* state becomes the lowest excited singlet state, making fluorescence likely .

(ii) Shpol'skii spectra - In so-called Shpol'skii matrices, especially alkanes, in which the dimensions of the dissolved and the solvent molecule are similar, fluorescence (phosphorescence) spectra at low temperature are often characterized by a very large number of bands with very small half-widths. Such spectra are useful for the identification of compounds.

(iii) External heavy atom effects - If compounds with elements which have a large Z-number (heavy atoms) are present in the matrix there can be generally observed a decrease of fluorescence quantum yield and fluorescence lifetime, an increase of phosphoresc~nce quantum yield, a decrease of phosphorescence lifetime and in some cases characteristic changes of the vibrational structure and relati ve intensity distribution of the phosphorescence spectrum. These external heavy atom spin-orbit coupling effects are useful to enhance sensitivity andtor selectivity in luminescence analysis (see Section 2.2.2) .

(iv) Paramagnetic compounds - Paramagnetic substances which are present in the matrix enhance spin-orbit coupling in the luminescing compound. Therefore in general they cause luminescence effects of the same kind as observed with heavy atom perturbers (see above).

3. INSTRUMENTAL PARAMETERS

The instrument used to measure luminescence emission spectra is termed a luminescence (fluorescence, phosphorescence) spectrometer. (see Part III).

3.1 Excitation source

Generally in luminescence spectroscopy a high flux of radiation (the excitation source) is needed for the excitation or the analyte and metal vapour of gas discharge lamps are commonly used. For a discussion of various radiation sources, see Part V of this series.

Flash lamps i.e., lamps which contain an inert gas which can be rapidly pulsed, or lasers which give a short output pulse, are useful for determining short luminescence decay times.

3. 2 Optical systems

The selection of radiation of the required wavelength from the excitation source for excitiing the analyte may be achieved with filters or with an excitation monochromator using entrance and exit slits to give the required spectral band width (see Part I 5).

Luminescence radiation of the required wavelength is selected from the sample by all emission monochromator. Where a single beam of radiation is used for excitation and a single beam of luminescence radiation is taken from the sample, the instrument would be termed a single-beam (luminescence spectrometer. Double-beam spectrometers are used for improving stability and for the direct measurement of excitation spectra. Double-(spectral) beam spectrometers are used where two sampled sre to be excited at two different wavelengths. A double-(synchronous) beam spectrometer is a luminescence spectrometer in which both the excitation and emission monochromators scan the excitation and emission spectra simultaneously, usually with a fixed wavelength difference between excitation and emission. Examples of the four types of luminescence spectrometers are shown on Fig. VI.2.

3.3 Photodetectors

The photomultiplier tube using single photon counting or current measurement, is the most satisfactory detector for measuring luminescence emission. Other detectors are often used in luminescence spectroscopy for monitoring the energy or photons in the excitation beam and for calibrating procedures. Theremopiles (series connected thermocouples attached to a blackened collector surface), bolometers (thin blackened collector with a high temperature coefficient of resistance) and pyroelectric detectors (based on the temperature dependence of ferroelectricity in some crystals) are detectors which produce an electrical signal proportional to the energv flux on the collector surface.

Ouantum counters produce an electrical signal proportional to the photon flux absorbed in a fluorescent solution. Chemical actinometers are detectors in which the amount of a chemical product formed is proportional to the numbers of photons absorbed. Silicon photodiodes may he used either in the photovoltaic or photoconductive modes for measuring radiation fluxes and, although less sensitive than photomultipliers, their gain stability is very good.

Image devices (Vidicons, photodiode arrays, etc.) are sometimes used in luminescence spectrometry especially for fast acquisition of data.

Where photodetectors are switched on (or off) usually in a repetitive manner employing electronic switches, they are termed gated photodetectors.

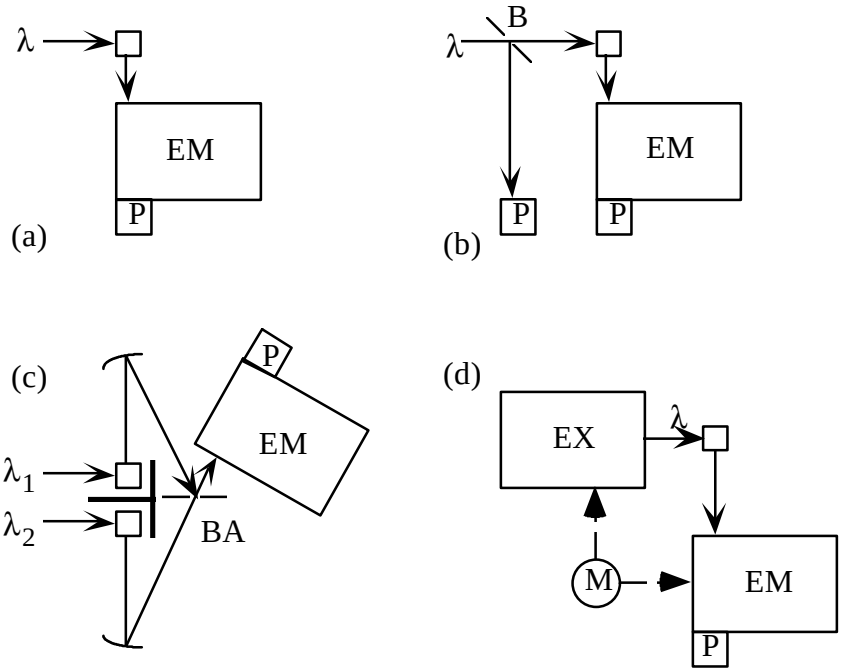

Figure VI.2. Examples of types of luminescence spectrometers: (a) single-beam, (b) double-beam, (c) double-(spectral) beam, (d) double-(synchronous) beam. λ = excitation beam, S = sample cell, B = beam splitter, BA = beam alternator, EM =- emission monoshromator, EX = excitation monochromator, P = photodetector, and M = wavelength drive.

3.4 Modulation of the optical signal

The optical beam can be modulated by mechanical or electronic means to give an intensity modulated beam. Often amplitude or frequency modulation is used in addition, for ease in signal processing. Gated photodetectors (Section 3.3) are frequently used in conjunction with modulated light to improve the signal/noise ratio, to separate fluorescence from phosphorescence or to measure luminescence decay times.

Phosphoroscopes are mechanical de~rices used to separate phosphorescence from fluorescence. Wavelength modulation is used when the derivative (dF_λ/dl) of the luminescence spectrum is required. Modulation of linear polarized radiation may be achieved by, for example, rotating a linear polarizer in the optical beam.

3.5 Polarizers

A linear polarizer is an optical device which allows the transmission of radiation of which the electric vector is restricted to one plane resulting in linearly polarized radiation.

4. MEASUREMENT AND USE OF LUMINESCENCE PARAMETERS IN ANALYSIS

4.1 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 artefacts, for post-filter effects and other sample effects (see Sec. 5). Table VI.3 lists the luminescence parameters and the symbols used.

Table VI.2. Terms, symbols and units for the excitation and detection of the analytical signal

Terms	Symbols	Practical Units	Notes
entrance (exit) slit width of monochromator	s	mm	See Part I
entrance (exit) slit height of monochromator	h	mm	See Part I
spectral bandwidth of monochromator (if the excitation monochromator is of concern, replace m with ex and if the emission monochramator is of concern, replace with em)	Δl_{m}	nm	Wavelength may be replaced by wavenumber or frequency
10% (or 1%) bandwidth of spectral filter	$\Delta l_{0.1}$ or $\Delta l_{0.01}$		See Part III
spectral radiant flux of source at wavelength	F_l^{s}	W nm^{-1}	See Part III and Table VI.1
transmittance of excitation monochromator to non-polarized radiation at wavelength l (if the emission monochromator is of concern replace ex by em)	$t_{\mathrm{ex}}(l)$	1	See Part III and Table VI.1
optical conductance	G	m^2 sr	See Part I 5.3.2
photodetector response at wavelength l	$\gamma(l)$	A W^{-1}	
solid angle over which radiation is absorbed in the cell	W_{a}	sr	
solid angle over which luminescence is measured	$W_{\mathrm{F(P,DF)}}$		F denotes fluorescence, P phosphorescence, DF delayed fluorescence
degree of modulation (m = ratio of ac component to dc component)	$m_{\mathrm{F(P,DF)}}$	1	For the exciting radiation use subscript ex
phase of ac modulated fluorescence or phosphorescence or delayed fluorescence with respect to the modulated exciting radiation	Q	degrees	
delay time between termination of exciting radiation and measurement of fluorescence (phophorescence, delayed fluorescence)	t_{D}	s	
excitation time (source "on time" per cycle)	t_{E}	s	
observation time (detector "on time" per cycle)	t_{o}	s	
cycle time (sum of the time for excitation and observation including delay times)	t_{c}	s	$= t_{\mathrm{E}} + t_{\mathrm{D}} + t_{\mathrm{o}} + t_{\mathrm{D}}'$

Table VI.3. Terms, symbols and units relating to radiant energy and its interaction with matter.

Terms	Symbols	Practical Units	Notes
(radiant) energy	Q Q_p	J photons	see Part I
spectral (radiant) energy	$Q_l = dQ/dl$ $Q_{p,l} = dQ_p/dl$	J nm^{-1} photons nm^{-1}	see Part III (photon quantity)
radiance	B, L	W m^{-2} sr^{-1}	
(radiant) energy density	u, w	J m^{-3}	
radiant intensity	I	W sr^{-1}	
radiant intensity at time t = 0	$I(0)$	W sr^{-1}	
radiant intensity at time t after termination of exciation	$I(t)$	W sr^{-1}	
(radiant) energy flux	$F = dQ/dt$ $F_p = dQ_p/dt$	W photons s^{-1}	 (photon quantity)
spectral (radiant) energy flux	$F_l = dF/dl$ $F_{p,l} = dF_p/dl$	W nm^{-1} photons s^{-1} nm^{-1}	 (photon quantity)
radiant flux incident on (absorbing) medium	F_0	W	
radiant flux transmitted by (absorbing) medium	F_τ	W	
radiant flux reflectd by sample	F_r	W	
radiant flux absorbed by medium	F_a	W	
transmittance	$t = F_\tau / F_0$	1	
reflectance	$r = F_r / F_0$	1	
absorptance or absortivity	$a = F_a / F_0$	1	
internal transmittance	t_i	1	transmittance of medium itself disregarding boundary effects
internal absorptance	a_i	1	
internal absorptance	$A = -\log t_i$	1	

Table VI.3. Terms, symbols and units relating to radiant energy and its interaction with matter (cont).

Terms	Symbols	Practical Units	Notes
(linear) absorption coefficient	K	cm^{-1}	
molar absorption coefficient	e	$l\ mol^{-1}\ cm^{-1}$	also called molar lineic absorbance
A value at the wavelength peak (l_0)	$A(l_0)$	1	
integrated molar absorption coefficient	$\int e(l)dl$	$l\ mol^{-1}\ cm^{-1}$	
absorption path length	b, l	cm	
molar concentration of absorber	c_m	mol, l^{-1}	additional subscript can be used to denote species
wavelength at band peak	l_0	nm	
wavenumber at band peak	s_0, u_0	cm^{-1}	
wavelength of fluorescence (phosphorescence, delayed fluorescence)	$l_{F(P,DF)}$	nm	can be replaced by wavenumber or frequency
quantum yield of fluorescence (phosphorescence, delayed fluorescence)	$Y_{F(P,DF)}$	1	Y conforms with Part III and is recommended over previously used symbols
energy yield of fluorescence (phosphorescence, delayed fluorescence)	$Y_{eF(P,DF)}$	1	
quanturn efficiency of fluorescence (phosphorescence)	$h_{F(P)}$	1	see Section 4.6
lifetime of fluorescence (phosphorescence, delayed fluorescence)	$t_{F(P,DF)}$	s	see Section 4.5
dissociation constant (acid-base) of molecule in first excited singlet state ($=c_{H^+}c_{A^-}{}^*/c_{HA}{}^*$ in equilibrium at temperature T)	K^*_{aS}	$mol\ l^{-1}$	
dissociation constant (acid-base) of molecule in lowest triplet state ($=c_{H^+}c_{A^-}{}^*/c_{HA}{}^*$ in equilibrium at temperature T)	K^*_{aT}	$mol\ l^{-1}$	

Table VI.3. Terms, symbols and units relating to radiant energy and its interaction with matter (cont).

Terms	Symbols	Practical Units	Notes
radiant intensities of the beam resolved into directions parallel and perpendicular to the direction of polarization of the exciting radiation	$F_{//}$, $F_{\perp}$		
degree of polarization	$P = (F_{//} - F_{\perp})/(F_{//} + F_{\perp})$		
degree of polarization (corrected for depolarizing factors)	P_0	1	
degree of depolarization or dichroic emission ratio	$D = F_{\perp} / F_{//}$	1	
degree of anisotropy	$r = (F_{//} - F_{\perp})/(F_{//} + 2F_{\perp})$		

4.2 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 F_l (see Table VI.3) against wavelength. F_l may be transformed to other quantities as follows:

wavelength scale (nm);

$$F_{p,l} = \mathrm{d}F_p/\mathrm{d}l = F_l \; l/hc \qquad (N_p \text{ per nm})$$

energy scale (cm^{-1});

$$F_{\tilde{u}} = \mathrm{d}F/\mathrm{d}\tilde{u} = F_l \; l^2 \qquad (W \text{ per } cm^{-1})$$

$$F_{p,\tilde{u}} = \mathrm{d}F_p/\mathrm{d}\tilde{u} = F_l \; l^3/hc \qquad (N_p \text{ per } cm^{-1})$$

where N_p is photons per second.

The shape of the emission spectrum depends on the quantity plotted. $F_{p,l}$ or $F_{p,\tilde{u}}$ are preferred since they may be used to calculate quantum yields of luminescence.

4.3 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. If the solution is sufficiently dilute that the fraction of the exciting radiation absorbed is proportional to the absorption coefficient of the analyte, and if the quantum yield is independent of the exciting wavelength, the corrected excitation spectrum will be identical in shape to the absorption spectrum.

4.4 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) (Note [1]).

The spectra are particularly useful for investigating samples containing more than one emitting species. Corrected EES are obtained if (a) the emission is corrected for instrumental response with wavelength, and (b) the exciting radiation flux in photons s^{-1} is held constant for all excitation wavelengths.

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.

4.5 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 necessay 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.

4.6 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 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 analytic solution. Quenching processes generally follow the Stern-Volmer law:

$$(Y_0/Y) - 1 = k_Q\, c_Q\, t_Q$$

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

t_Q = luminescence lifetime in the absence of quencher Q

The quantum effiency 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.

---

[1] Such spectra are commonly represented as contour diagrams or as isometric projections.

4.7 Linear polarization of luminescence

Polarization of emission is not of great importance in molecular luminescence spectroscopy unless the solvent used is viscous or solid . Measurement of polarization is usually made at right angles to the direction of propagation of the exciting radiation and must take account of the polarization effects of all optical components in the instrument. The relations between the degree of polarization *P*, the degree of depolarization *D*, and the degree of anisotropy *r*, (for definitions see Table VI.3) 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. Since this spectrum may depend on the emission wavelength monitored, this wavelength should be specified. The polarization is usually given as *r* or *P*.

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

4.8 Quantitative analysis

The analytical procedure used in luminescence spectrometry is similar to that described in Part III, Section 4, of this series of documents.

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 scatt~ering 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 chemilumillescence analysis, where a reaction produces luminescence radiation. A blank measure rmust also be made for this method .

The evaluation and assessment of the analytical result has been dealt with in previous documents (Parts I, II and III) .

5. FACTORS AFFECTING LUMINESCENCE DATA

5.1 Geometric arrangement of sample

The luminescence measured may depend on the directions of the exciting and emitting beams with respect to the sample. The angles relating to excitation and emission directions can be expressed by two figures, a, b where a = angle of incidence of the exciting beam on the plane surface of the sample, and b = angle between the exciting direction and observation direction. Front surface geometry is defined as a system where excitation and observation are from the same face of the sample ($a < 90°$, $b < 180°$).

5.2 Pre-filter, post-filter and self-absorption effects

The pre-filter effect arises when the luminescence detector does not see a portion of the luminescent volume where the excitation beam enters the sample. Thus the exciting beam flux is reduced by absorption by the analyte and interfering impurities before it enters the volume observed by the detection system.

The post-filter effect arises when the exciting beam does not fill the cell completely and luminescence is absorbed by the analyte and interfering impurities in the non-illuminated region facing the detector.

The self-absorption effect is the reabsorption of luminescence by the analyte and interfering impurities within the excitation volume.

All three effects are minimized if front surface geometry is used and/or the solution is highly diluted.

5.3 Refraction effects

The luminescence flux emitted from the interior of a rectangular sample reaching a photodetector placed at some distance from the sample is decreased by a factor of approximately n^2 (where n is the refractive index of the medium) compared with a medium whose refractive index is 1.0. Such effects are termed refraction effects.

5.4 Solvent and temperature effects

The type of solvent and its temperature can effect the luminescence yleld from an analyte as a result of quenching, exciplex formation, aggregation, etc. Temperature effect is the term used for changes in the luminescence parameters caused by changes in temperature while solvent effects are changes caused by altering the solvent or the solvent properties (see also Section 2.2.4).

Table IV.4. Classification and symbols for luminescence parameters

Name	Emission spectrum	Excitation spectrum	Lifetime	Quantum yield	Degree of anisotropy	Polarization spectrum emission	Polarization spectrum excitation
measured	E_m	X_m	t_m	Y_m	r_m	E_{pm}	X_{pm}
corrected	E_c	X_c	t_c	Y_c	r_c	E_{pc}	X_{pc}