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COMMISSION ON SPECTROCHEMICAL AND OTHER OPTICAL PROCEDURES FOR ANALYSIS*

NOMENCLATURE, SYMBOLS, UNITS AND THEIR USAGE IN SPECTROCHEMICAL ANALYSIS—VI MOLECULAR LUMINESCENCE SPECTROSCOPY

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Comments from the viewpoint of languages other than English are especially encouraged. These may have special significance regarding the publication in various countries of translations of the nomenclature eventually approved by IUPAC.

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NOMENCLATURE, SYMBOLS, UNITS AND THEIR USAGE IN SPECTROCHEMICAL ANALYSIS
PART VI : MOLECULAR LUMINESCENCE SPECTROSCOPY

CONTENTS

1. INTRODUCTION

Part VI is a sequel to previous documents in the series "Nomenclature, Symbols, Units and their Usage in Spectrochemical Analysis" issued by the Analytical Chemistry Division of IUPAC.

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 Section 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 Section 4.2 and Table 4.1).

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 2.1). Fluorescence is the spin-allowed radiative transition while phosphorescence is the result of a spin-forbidden radiative transition.

TABLE 2.1 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 concomitant 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) plays a minor role. The other excitation modes listed in Table 2.1 do not prove to be useful in analysis.

2.2 Absorption 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 radiationless transitions which occur in molecules are illustrated in the term scheme in Fig. 2.1.

2.2.1 Absorption. Singlet-singlet absorption results in the transition from the singlet ground state of the molecule into singlet excited states ($S_o \rightarrow S_n$) 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 ($T_1 \rightarrow T_n$) 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 ($S_o \rightarrow T_n$) 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 band 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 analysis. 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 systems 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): $S_n \dashrightarrow S_1$, $S_1 \dashrightarrow S_o$, $T_n \dashrightarrow T_1$ being distinguished.

Intrachromophoric radiationless transitions between states of different multiplicity are named intersystem crossing (ISC): $S_1 \dashrightarrow T_n$, $T_1 \dashrightarrow S_o$, $T_1 \dashrightarrow S_1$ 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 fluorescence Y_F and phosphorescence Yp are related to the radiative and radiationless rate constants as follows:

$$Y_F = \frac{k_{FM}}{k_{FM} + k_{TM} + k_{GM}}$$

$$Y_P = \frac{k_{TM}}{k_{FM} + k_{TM} + k_{GM}} \qquad \left(\frac{k_{PT}}{k_{PT} + k_{GT}}\right)$$

where the rate constants relate to the transitions as follows:

rate constant	transition
k_{FM}	fluorescence
k_{TM}	ISC ($S_1 \dashrightarrow T_n$)
k_{GT}	ISC ($T_1 \dashrightarrow S_o$)
k_{GM}	IC ($S_1 \dashrightarrow S_o$)
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_q 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 phosphorimetry).

(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 reverse 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 fluorimetry. 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. 2.1.

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.

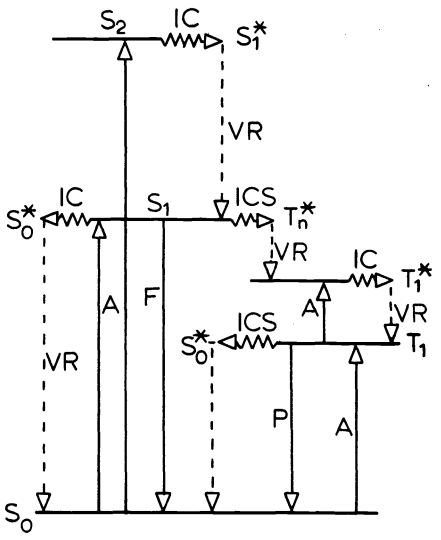

FIG. 2.1 Schematic diagram of radiative (solid vertical lines), radiationless (wavy horizontal lines), and vibrational relaxation (broken vertical lines) between electronic states in a π-electronic system.

States: S_0 = ground state, S_1 = first excited singlet state, S_2 = second excited singlet state, T_1 = lowest triplet state, and T_n = excited triplet states.

Transitions: A = absorption ($S_0 \rightarrow S_n$, $S_0 \rightarrow T_1$, $T_1 \rightarrow T_n$), IC = internal conversion ($S_n \dashrightarrow S_1$, $S_1 \dashrightarrow S_0$, $T_n \dashrightarrow T_1$), ISC = intersystem crossing ($S_1 \dashrightarrow T_n$, $T_1 \dashrightarrow S_0$), VR = vibrational relaxation, F = fluorescence ($S_1 \rightarrow S_0$), and P = phosphorescence ($T_1 \rightarrow S_0$)

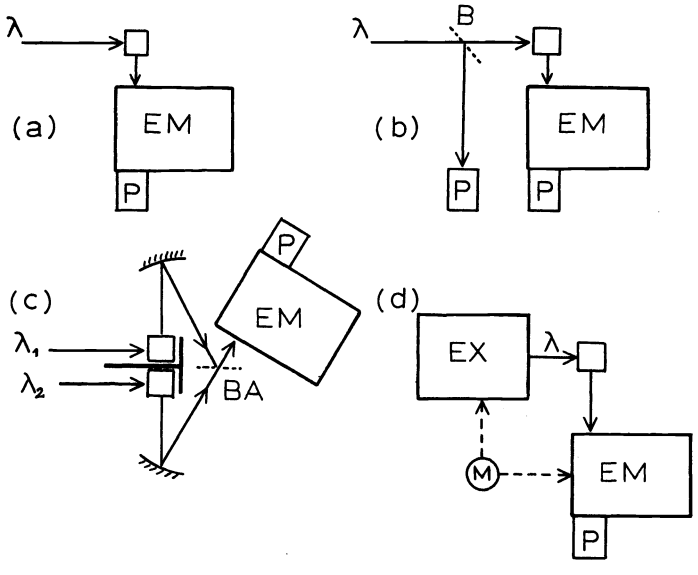

FIG. 3.1 Examples of types of luminescence spectrometers.

(a) single-beam, (b) double-beam, (c) double-(spectral) beam, (d) double-(synchronous) beam.

λ = excitation beam, S = sample cell, BS = beam splitter, BA = beam alternator, EM = emission monochromator, EX = excitation monochromator, P = photodetector, and M = wavelength drive

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.

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 phosphorescence quantum yield, a decrease of phosphorescence lifetime and in some cases characteristic changes of the vibrational structure and relative intensity distribution of the phosphorescence spectrum. These external heavy atom spin-orbit coupling effects are useful to enhance sensitivity and/or 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 Nomenclature, Symbols, Units and their Usage in Spectrochemical Analysis, Part III).

3.1 Excitation source

Generally in luminescence spectroscopy a high flux of radiation (the excitation source) is needed for the excitation of the analyte and metal vapour or 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 exciting 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, Section 5).

Luminescence radiation of the required wavelength is selected from the sample by an 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 samples are to be excited by 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. 3.1.

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. Thermopiles (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 energy flux on the collector surface.

Quantum 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 be 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.

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 devices used to separate phosphorescence from fluorescence. Wavelength modulation is used when the derivative $(d\Phi\lambda/d\lambda)$ of the luminescence spectrum is required. Modulation of plane polarised radiation may be achieved by, for example, rotating a plane polarizer in the optical beam.

3.5 Polarizers

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

TABLE 3.1 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	" " "
spectral bandwith of monochromator (if the excitation monochromator is of concern, replace m with ex and if the emission monochromator is of concern, replace with em)	λ_m	nm	Wavelength may be replaced by wavenumber or frequency
10% (or 1%) bandwidth of spectral filter	$\lambda_{0.1}$ (or $\lambda_{0.01}$)		See Part III
spectral radiant flux of source at wavelength λ	Φ^s_λ	W nm^{-1}	See Part III and Table 4.1

Terms	Symbols	Practical units	Notes
transmittance of excitation monochromator to non-polarized radiation at wavelength λ (if the emission monochromator is of concern, replace ex by em)	$\tau_{ex}(\lambda)$	1	See Part III and Table 4.1
optical conductance	G	m^2 sr	See Part I, Section 5.3.2
photodetector response at wavelength λ	$\gamma(\lambda)$	A W^{-1}	
solid angle over which radiation is absorbed in the cell	Ω_A	sr	
solid angle over which luminescence is measured	$\Omega_{F(P,DF)}$	sr	F denotes fluorescence, P phosphorescence, DF delayed fluorescence
degree of modulation (m = ratio of ac component to dc component)	$m_{F(P,DF)}$	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	θ	degrees	
delay time between termination of exciting radiation and measurement of fluorescence (phosphorescence, 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 = $t_E + t_D + t_O + t'_D$)	t_C	s	

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 Section 5). Table 4.1 lists the luminescence parameters and the symbols used.

TABLE 4.1 Terms, symbols and units relating to radiant energy and its interaction with matter

Terms	Symbols	Practical units	Notes
(radiant) energy	Q	J	See Part I
	Q_p	photons	
spectral (radiant) energy	$Q_\lambda = dQ/d\lambda$	J nm^{-1}	See Part III
	$Q_{p,\lambda} = dQ_p/d\lambda$	number of photons nm^{-1}	(photon quantity)

Terms	Symbols	Practical units	Notes
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 excitation	I(t)	$W\ sr^{-1}$	
(radiant) energy flux	$\Phi = dQ/dt$	W	
	$\Phi_p = dQ_p/dt$	number of photons s^{-1}	(photon quantity)
spectral (radiant) energy flux	$\Phi_\lambda = d\Phi/d\lambda$	$W\ nm^{-1}$	
	$\Phi_{p,\lambda} = d\Phi_p/d\lambda$	number of photons $s^{-1}\ nm^{-1}$	(photon quantity)
radiant flux incident on (absorbing) medium	Φ_o	W	
radiant flux transmitted by (absorbing) medium	Φ_τ	W	
radiant flux reflected by sample	Φ_r	W	
radiant flux absorbed by medium	Φ_a	W	
transmittance	$\tau = \Phi_t/\Phi_o$	1	
reflectance	$\rho = \Phi_r/\Phi_o$	1	
absorptance or absorptivity	$\alpha = \Phi_a/\Phi_o$	1	
internal transmittance	τ_i	1	Transmittance of medium itself disregarding boundary effects
internal absorptance	α_i	1	
internal absorbance	$A = -\log \tau_i$	1	
(linear) absorption coefficient	K	cm^{-1}	
molar absorption coefficient	ϵ	$mol^{-1}\ cm^{-1}$	Also called molar lineic absorbance
A value at the wavelength peak (λ_o)	$A(\lambda_o)$	1	
integrated molar absorption coefficient	$\int \epsilon(\lambda)\ d\lambda$	$mol^{-1}\ cm^{-1}$	
absorption path length	l, b	cm	
molar concentration of absorber	c_m	$mol\ \ell^{-1}$	An additional subscript can be used to denote the species

Terms	Symbols	Practical units	Notes
wavelength at band peak	λ_o	nm	
wavenumber at band peak	σ_o, $\tilde{\nu}_o$	cm^{-1}	
wavelength of fluorescence (phosphorescence, delayed fluorescence)	$\lambda_{F(P,DF)}$	nm	λ can be replaced by $\tilde{\nu}$ (wavenumber) or ν (frequency)
quantum yield of fluorescence (phosphorescence, delayed fluorescence)	$Y_{F(P,DF)}$	1	The symbol Y conforms with Part III and is recommended over previously used symbols
energy yield of fluorescence (phosphorescence, delayed fluorescence)	$Y_{eF(P,DF)}$	1	
quantum efficiency of fluorescence (phosphorescence)	$\eta_{F(P)}$	1	See Section 4.6
lifetime of fluorescence (phosphorescence, delayed fluorescence)	$\tau_{F(P,DF)}$	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}	
radiant intensities of the beam resolved into directions parallel and perpendicular to the direction of polarization of the exciting radiation	I_{11}, $I_\perp$		
degree of polarization	$P = (I_{11}-I_\perp)/(I_{11}+I_\perp)$		
degree of polarization (corrected for depolarizing factors)	P_o	1	
degree of depolarization or dichroic emission ratio	$D = I_\perp/I_{11}$	1	
degree of anisotropy	$r = (I_{11}-I_\perp)/(I_{11}+2I_\perp)$		

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 Φ_λ (see Table 4.1) against wavelength. Φ_λ may be transformed to other quantities as follows:

wavelength scale (nm);

$$\Phi_{p,\lambda} = d\Phi_p/d\lambda = \Phi_\lambda\lambda/hc \qquad (N_p \text{ per nm})$$

energy scale (cm^{-1});

$$\Phi_{\tilde{\nu}} = d\Phi/d\tilde{\nu} = \Phi_\lambda\lambda^2/hc \qquad (W \text{ per } cm^{-1})$$

$$\Phi_{p,\tilde{\nu}} = d\Phi_p/d\tilde{\nu} = \Phi_\lambda\lambda^3/h^2c^2 \qquad (N_p \text{ per } cm^{-1})$$

where N_p is photons per second.

The shape of the emission spectrum depends on the quantity plotted. $\Phi_{p,\lambda}$ or $\Phi_{p,\tilde{\nu}}$ 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).

These 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 Lifetimes 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 luminescence emission signal by a deconvolution technique in order to obtain the correct decay curve for emission. Decay times corrected for this erfect 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:

$$\frac{Y_o}{Y} - 1 = k_Q \, c_Q \, \tau_o$$

where Y_o = luminescence yield in the absence of quencher Q

Y = luminescence yield with quencher of concentration c_Q

k_Q = rate constant for quenching

τ_o = 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.

Note 1: Such spectra are commonly represented in two dimensions as an isometric display.

4.7 Plane 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 4.1) are:

$$P = \frac{3r}{2 + r}$$

$$D = \frac{1 - r}{1 + 2r}$$

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 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.

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, α/β where α = angle of incidence of the exciting beam on the plane surface of the sample, and β = 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 ($\alpha<90°$, $\beta<180°$).

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 impurity 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 if the solution is highly diluted.

5.3 Refraction effects

The luminescence flux emitted from the interior of a rectangular sample reaching a photodetector place 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 yield 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 4.2 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	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}