Suppression of the influence of stray light on quantitative measurement of I_D/I_M for weakly emitting dilute solutions of polymers

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Summary
     A simple method is described for suppression of the influence of
stray light on the quantitative measurement of the intensity of the
fluorescence from dilute solutions of weak emitters. The method
improves the reliability of the measurements of the fluorescence quantum
yield under these conditions. It is also useful in the evaluation of
the ratio of the intensities of excimer and monomer emission in systems
that form intramolecular excimers with a small overlap of the monomer
and excimer bands.

Introduction
     Fluorescence methods enjoy widespread use for study of the
conformations and dynamics of polymers in dilute solution because of the
high sensitivity of the method and the favorable time scale of the
fluorescence life time (1-5). In many of these applications, the system
under study contains a very low concentration of chromophores that have
a small quantum yield for fluorescence, Q, thereby making the observed
emission susceptible to distortion by a variety of artefacts. Potential
artefacts include Raman scattering (and perhaps intrinsic fluorescence)
from the solvent, Rayleigh scattering from the solution, fluorescence
from the cell itself, and any other source of stray light (3,6).
Accurate measurements require careful attention to the choice of
solvent, use of solvents and samples of highest purity, clean sample-
handling equipment, and instrumentation with excellent stray light
rejection. Even with the greatest of care, stray light, in the broadest
sense, is often a nonnegligible portion of the light that reaches the
detector in the measurements of weak emitters at low concentrations.
     This communication describes a simple method that can improve the
accuracy of quantitative measurements of the steady state fluorescence
intensity under conditions where some stray light persists after careful
attention to the purity of the solvents and samples and the cleanliness
of the cells. The method utilizes a series of measurements performed
with samples of differing concentrations. It is illustrated by
application to Q and measurements of the ratio of excimer to monomer
emission from dilute solutions of polystyrene (PS), several polyesters,
and model compounds for the polyesters.

Materials
     PS, dimethylterephthalate (DMT), dimethylisophthalate (DMI), and
dimethylphthalate (DMP) were purchased from Aldrich Chemical Company.
The polyesters were synthesized as described elsewhere (7). The

fluorescence measurements were performed with an SLM 8000C fluorometer equipped with a double monochromator in the excitation path.

Results and discussion

Fluorescence quantum yield

The fluorescence quantum yield, Q, is usually obtained by comparison of the emission from a sample with that from a standard, such as quinine sulfate in 0.1 N sulfuric acid, for which Q is 0.546 at 25°C when the excitation is in the range 220-340 nm (6,8-10). The fluorescence quantum yield can be written as

$$Q \sim A^{-1} \int I \, d\lambda \qquad (1)$$

where A denotes the absorbance of the fluorophore at the wavelength of excitation, and I is the intensity of the fluorescence at wavelength λ. The integral is performed over the emission band. Consequently Q is proportional to $d(\int I \, d\lambda)/dA$, the slope of the straight line obtained when $\int I \, d\lambda$ is plotted against A for samples of differing concentration, c. Using the subscript s to denote the properties of the standard,

$$Q = Q_s \, d(\int I \, d\lambda)/dA \, / \, [d(\int I \, d\lambda)/dA]_s \qquad (2)$$

Typical data for analysis via Equation 2 are depicted in Figure 1. The four sets of data are described by straight lines (there is no inner filter effect in this range of concentrations), but the straight lines do not extrapolate to the origin. Failure to extrapolate to the origin implies a small, constant interference that might arise from the solvent, cell, or any source of stray light. If Q were calculated from a single measurement for the sample, and a single measurement for the standard, according to

$$Q = Q_s \, [\int I \, d\lambda/(\int I \, d\lambda)_s](A_s/A) \qquad (3)$$

the result would depend upon the c selected for the evaluation of $\int I \, d\lambda$ and A. Each integrated emission spectrum would contain a contribution from the interference, and the impact of that contribution would depend on c. The influence of this interference is eliminated when all of the data is used for the calculation of Q from Equation (2). The values of Q obtained for the three samples of PS from the data in Figure 1, and for two additional samples of this polymer, are collected in Table 1. This Table also includes Q's for twelve other materials that are weaker emitters. The twelve additional Q's are 1-2 orders of magnitude smaller than the Q's for PS. The values of Q for these very weak emitters can be determined much more reliably when the analysis of the data proceeds via Equation 2 rather than via Equation 3.

Ratios of the intensities of the fluorescence at two wavelengths

A simple extension of the above procedure may prove useful in the determination of the ratio of the intensities of the fluorescence at two wavelengths. One circumstance in which this ratio is important is the intramolecular formation of excimers. The simplest measurement of the extent of formation of the excimers is denoted by I_D/I_M, where I_D and I_M are the intensities of the fluorescence at wavelengths dominated by excimer and monomer emission, respectively. Illustrative results for
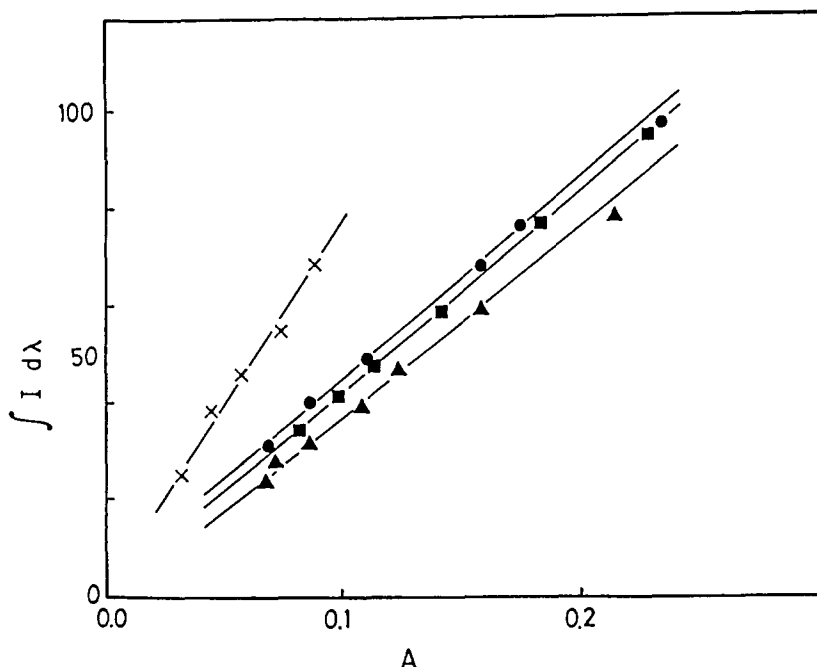
Figure 1. Integrated area of the fluorescence emission (in arbitrary units), as a function of A at 254 nm, for quinine sulfate (x) and for PS samples with M_W of 1940 (▲), 5480 (■) and 19400 (●). The solvent is 1.0 N sulfuric acid for quinine sulfate and 1,2-dichloroethane for PS.

TABLE 1
Q's evaluated by two methods at 25°C in 1,2-dichloroethane

| Fluorophore	Q, Eq. 2	Q, A=0.05
PS (M_W = 1940)	0.022 ± 0.002	0.026
PS (M_W = 5480)	0.025 ± 0.002	0.030
PS (M_W = 9000)	0.024 ± 0.002	0.025
PS (M_W = 19400)	0.026 ± 0.002	0.021
PS (M_W = 79000)	0.029 ± 0.002	0.030
DMT	0.0019 ± 0.0003	0.001
polyethylene terephthalate	0.0029 ± 0.0002	0.0084
polydiethylene glycol terephthalate	0.0057 ± 0.0002	0.011
polytriethylene glycol terephthalate	0.0044 ± 0.0001	0.0010
DMI	0.0006 ± 0.0002	0.0009
polyethylene isophthalate	0.0014 ± 0.0001	0.0010
polydiethylene glycol isophthalate	0.0022 ± 0.0002	0.004
polytriethylene glycol isophthalate	0.0015 ± 0.0002	0.0009
DMP	0.0006 ± 0.0002	0.0
polyethylene phthalate	0.0031 ± 0.0004	0.0017
polydiethylene glycol phthalate	0.0011 ± 0.0001	0.0018
polytriethylene glycol phthalate	0.0018 ± 0.0002	0.0029
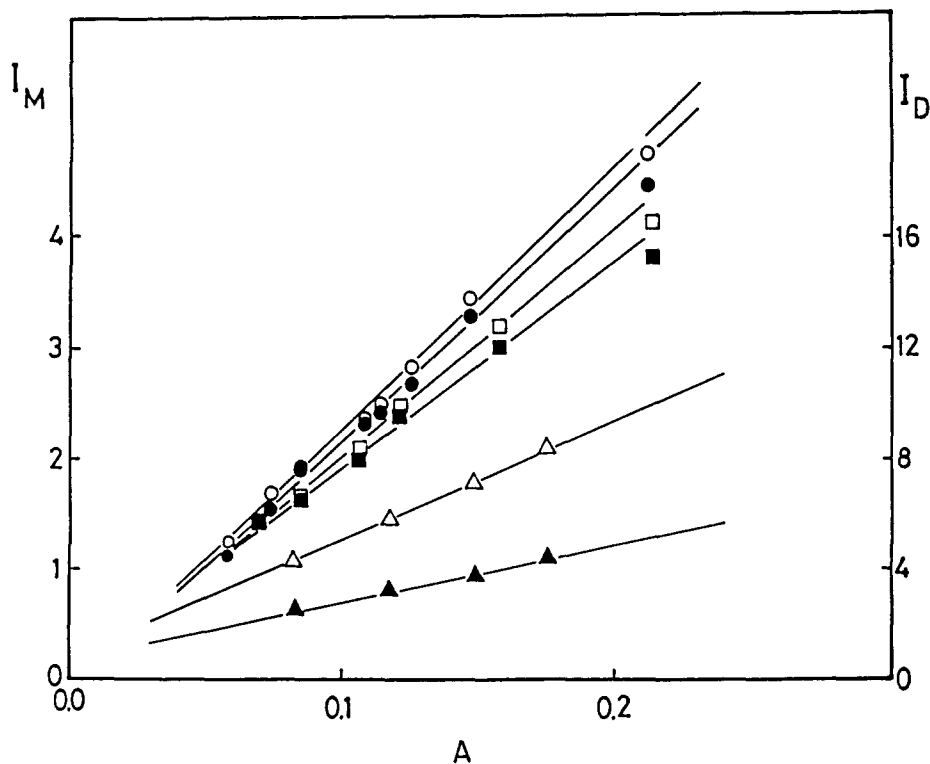
Figure 2. Intensity of the fluorescence (arbitrary units) at 290 nm (open symbols) and 335 nm (filled symbols) for PS samples with M_w of 517 ($\triangle$), 1940 ($\square$) and 9000 (o), as a function of A at 254 nm. The solvent is 1,2-dichloroethane, the temperature is 25°C, and excitation is at 254 nm.

PS are depicted in Figure 2. The ratios of the slopes for the fluorescence intensities for these three fractions at 290 nm and 335 nm, and also for eight additional fractions, are depicted as a function of molecular weight by the circles in Figure 3. The solid vertical lines denote the range for I_D/I_M that would have been obtained if the data at each concentration were analyzed independently. Values at the lower end of each vertical line are those measured at the higher c. Presumably the apparent I_D/I_M decreases with increasing c because stray light makes a larger contribution to I_M than I_D, since the former intensity is measured at lower wavelength. The increases in I_D/I_M with M_w at low M_w, and the approach to a constant value as M_w increases, have been observed previously with PS (2,5).
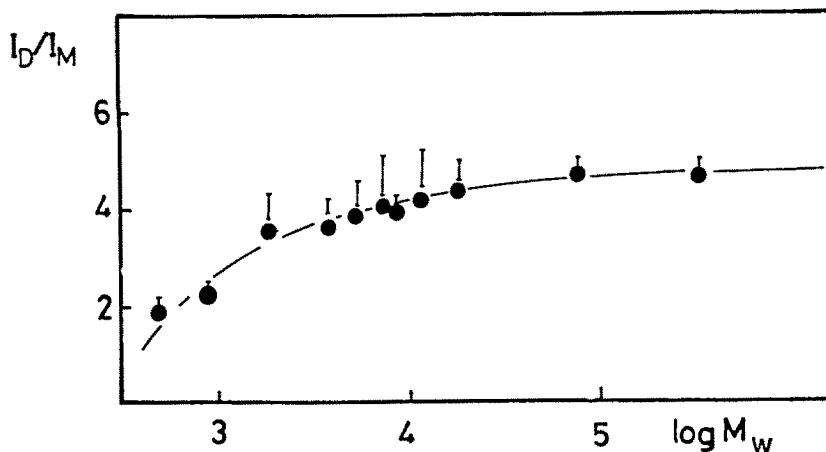
Figure 3. Variation of I_D/I_M with M_W for PS in 1,2-dichloroethane at 25°C. Vertical lines denote the range of the experimental results for $0.05 < A < 0.2$ at 254 nm, and the circles denote the values obtained from the ratios of slopes.

A procedure to separate the contributions of the monomer and excimer bands when there is overlap requires emission spectra at different c for the polymer and model compound (ethyl benzene or toluene for PS). The experimental excimer band intensity I^e_D(excimer) is composed of the sum of two intensities, pure excimer intensity I^o_D(excimer) and the monomer intensity at the excimer wavelength I^o_M(excimer). The last can be obtained as a fraction X of the experimental monomer band at the monomer wavelength I^e_M(monomer). The fraction X is calculated by the ratio of the experimental intensities at the excimer and monomer wavelengths, respectively, for the model compound. The experimental monomer intensity I^e_M(monomer), consists of contributions from the pure monomer at the monomer band I^o_M(monomer) and the pure excimer band at the same wavelength I^o_D(monomer). Neglecting the contribution of the pure excimer band at the monomer wavelength, and assuming that the only possibility of excimer formation is by intramolecular interaction,

$$d[I^o_D(\text{excimer})/I^o_M(\text{monomer})]/dc = 0 \qquad (4)$$

$$[dI^o_D(\text{excimer})/dc]/[dI^o_M(\text{monomer})/dc]=I^o_D(\text{excimer})/I^o_M(\text{monomer}) \qquad (5)$$

and the corrected excimer to monomer ratio can be obtained as

$$\frac{I^o_D(\text{excimer})}{I^o_M(\text{monomer})} = \frac{dI^e_D(\text{excimer})/dc - X \quad [dI^e_M(\text{monomer})/dc]}{dI^e_M(\text{monomer})/ dc} \qquad (6)$$

This method is particularly useful in the case of an overlap of the excimer and monomer bands in samples with very low intensity.

Examples are provided by polyesters from terephthalic acid and several glycols (7). As is shown in Table 2, values of excimer to monomer ratios differ very little if 390 nm (no monomer contribution, by using ratios of slopes directly) or 370 nm (discounting monomer contribution using equation 5) are used for measurement of the intensities of the excimer bands, the monomer band being measured at 324 nm. Additional results (11) in Table 2 show the ratio I_D/I_M for polyesters from isophthalic acid and several glycols, obtained by ratio of slopes. In this system there is very little overlap of the monomer and excimer bands, and both methods give the same results. This situation is also encountered in PS because I_D/I_M is large. In contrast, the extremely weak excimer emission from the polyesters from phthalic acid is best analyzed via Equation (6).

TABLE 2

I_D/I_M evaluated (A) directly from the ratio of slopes and (B) with the correction in Equation (6).

Sample	λ, I_D	λ, I_E	A	B
Toluene	335 nm	290 nm	0.05	0.00
PS (M_w — 1940)	335 nm	290 nm	3.5	3.5
PS (M_w — 5480)	335 nm	290 nm	3.7	3.7
PS (M_w — 9000)	335 nm	290 nm	3.8	3.5
PS (M_w — 19400)	335 nm	290 nm	4.3	4.2
PS (M_w — 79000)	335 nm	290 nm	4.6	4.6
DMT	390 nm	324 nm	0.00	0.00
	370 nm	324 nm	0.16	0.00
polyethylene terephthalate	390 nm	324 nm	0.18	0.18
	370 nm	324 nm	0.44	0.28
polydiethylene glycol terephthalate	390 nm	324 nm	1.7	1.6
	370 nm	324 nm	1.8	1.6
polytriethylene glycol terephthalate	390 nm	324 nm	0.66	0.66
	370 nm	324 nm	0.75	0.60
DMI	345 nm	310 nm	0.0	0.0
polyethylene isophthalate	345 nm	310 nm	0.37	0.37
polydiethylene glycol isophthalate	345 nm	310 nm	4.2	4.2
polytriethylene glycol isophthalate	345 nm	310 nm	1.3	1.3
DMP	397 nm	342 nm	0.40	0
polyethylene phthalate	397 nm	342 nm	0.46	0
polydiethylene glycol phthalate	397 nm	342 nm	0.39	0
polytriethylene glycol phthalate	397 nm	342 nm	0.33	0

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