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PHTHALOCYANINE FLUORESCENCE AT HIGH CONCENTRATION: DIMERS OR REABSORPTION EFFECT?

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Abstract—For tetrasulfonated aluminum phthalocyanine ($AlPcS_4$), dimer formation is characterized in the absorption spectrum by a broadening of the Q-band and the appearance of a new band at the red edge of the spectrum. The high concentrations required to produce dimers, however, often leads to anomalous observations in fluorescence spectroscopy. In the present study, we have examined the photophysical characteristics of two dye systems; $AlPcS_4$ in a 66% ethanol/water mixture and disulfonated aluminum phthalocyanine in methanol. Using absorption spectroscopy, the formation of dimers is shown to be prevalent only in the case of $AlPcS_4$. The fluorescence emission spectra in both cases, however, exhibit similar spectral changes with increasing dye concentration. The measured fluorescence decay profiles for both dyes also show similar trends: They are monoexponential, invariant with emission wavelength and have decay times that increase with dye concentration. These distortions are sometimes incorrectly attributed to dimer fluorescence. We find no evidence for the existence of dimer fluorescence and demonstrate that these data can be readily explained, by taking into consideration the effects of reabsorption of fluorescence.

INTRODUCTION

The sulfonated aluminum phthalocyanines, $AlPcS_n$† (n = 0–4), are of considerable interest as they show excellent photodynamic activity.[1] Incorporation of sensitizers into biological membranes invariably results in high local concentrations (10^{-5}–10^{-4} *M*)[2,3] where dimers or higher aggregates may form. It is known, however, that phthalocyanine dimers and higher order aggregates are much more inactive than monomers as sensitizers.[4] In order to rationalize their photobiological effect it is important to characterize fully the photophysical properties of the monomeric and aggregated forms.

It is well established that porphyrin dimers and higher oligomers have reduced fluorescence quantum yields relative to the monomeric forms[5] but are still photobiologically active, playing an important role in the photodynamic action of hematoporphyrin derivative sensitizers.[6] Conversely, the phthalocyanine dimers and aggregates are generally believed to have facile deactivation pathways available to the excited electronic states and are believed to be essentially nonfluorescent and, because they do not lead to the triplet state, are not directly involved in photodynamic action.[5,7] For example, Wagner *et al.*[4] have shown that on varying the degree of sulfonation of gallium phthalocyanine there is a decrease in singlet oxygen production, which is consistent with an increase in dimerization. Ohno *et al.*[8] found that the model dimer compound, bis(phthalocyanato) tin IV, which forms face-to-face stacking dimers, was nonfluorescent. This lack of fluorescence from the dimer was attributed to rapid relaxation processes from a low-lying (lower than the lowest excited singlet state) dipole forbidden charge resonance state. Intense fluorescence from model μ-oxo bridged porphyrin dimers,[9–12] $(TPPSc)_2$, $(TPPAl)_2$ and $(TPPNb)_2$, has similarly been attributed to emission from the lowest excited singlet state, the charge resonance state in these species being of higher energy. Spikes and Bommer[13] have reported that the dimer of tetrasulfonated zinc phthalocyanine, $ZnPcS_4$, displays no fluorescence emission and, similarly, using fluorescence emission and thermal lensing techniques Negri *et al.*[14] have determined that the dimer of the diamide of zinc tetracarboxyphthalocyanine also does not fluoresce. These observations are believed to be due to an increase in the rate of internal conversion from the first excited singlet state, upon dimer formation, which will result in a reduction in the fluorescence and triplet quantum yields.

Recently Yoon *et al.*[15] reported red-shifted emission and increased fluorescence decay times compared to the monomer from concentrated solutions of $AlPcS_4$ and attributed this to fluorescence from dimeric or aggregated species. These results are unusual when compared to the existing literature on other phthalocyanine species discussed above and the findings of our own group in recent work with various sulfonated aluminum phthalocyanines.[16] An alternative explanation for these observations is the distortion of the monomer emission by reabsorption of radiation by ground state species. This is likely to occur at high concentrations of phthalocyanine solutions due to their large extinction coefficients and high degree of spectral overlap between absorption and emission spectra. Such effects are frequently overlooked although they have been discussed in the literature[17–19] and are commonly referred to as the inner filter or a reab-

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†*Abbreviations:* $AlPcS_n$, sulfonated aluminum phthalocyanine; PDT, photodynamic therapy.

sorption effect. Clearly these two models differ considerably and it is very important to distinguish which occurs. The presence of photoactive phthalocyanine aggregates would have important implications for photodynamic therapy (PDT) using these compounds. Using time-resolved and steady-state techniques it is possible to distinguish between the fluorescent aggregate and reabsorption models. Our data, obtained from aqueous/alcoholic $AlPcS_n$ solutions, are analyzed accordingly.

MATERIALS AND METHODS

Chemicals. Disulfonated aluminum phthalocyanine ($AlPcS_2$) was prepared according to the method of Ambroz *et al.*[20] and tetrasulfonated aluminum phthalocyanine ($AlPcS_4$) was prepared using the condensation method[21] and identified using elemental analysis. The purity of the samples was checked using reversed-phase HPLC.[22] Water was purified by distillation followed by processing in an ELGA UHQ. Methanol and ethanol (BDH, HPLC grade) were tested to ensure that no extraneous fluorescence was present and used without further purification.

Steady-state absorption and fluorescence measurements. Absorption spectra were recorded on a conventional UV/visible spectrophotometer (Perkin-Elmer Lambda 2). The spectra of the most concentrated solutions were obtained by replacing the standard, 10 mm pathlength quartz cuvette with a 1 mm cuvette. Corrected fluorescence emission spectra (excitation at 610 nm) were obtained with a Spex FluoroMax spectrofluorometer using a standard quartz cuvette (10 mm × 10 mm). The bandpass of both excitation and emission slits was 0.6 nm.

Time-resolved fluorescence measurements. Fluorescence decays were recorded using the technique of time-correlated single photon counting.[23] Excitation was provided using a cavity-dumped rhodamine 6G dye laser (Coherent 590 CD/7220), synchronously pumped by the second harmonic of a mode-locked Nd:YAG laser (Coherent Antares 76-s). This produced a 3.8 MHz pulse train of 8 ps pulses at a wavelength of 610 nm. Fluorescence was collected perpendicular to excitation and passed through a polarizer set at the magic angle. The detection system consisted of a 0.22 m double monochromator (Spex), photomultiplier tube (Hamamatsu R928), constant fraction discriminator (Ortec 584), time-to-amplitude converter (Ortec 547) and a multichannel pulse height analyzer (Canberra 35). This produced a typical instrument response function of 450 ps full width at half maximum.

All decays were collected to 20 000 counts in the channel of maximum intensity and analyzed by a nonlinear, least-squares, iterative reconvolution fitting procedure.[23] Plots of the weighted residuals, autocorrelation function, serial correlation coefficient and reduced chi-squared were used to judge the quality of the fit.

RESULTS

The normalized absorption spectra of $AlPcS_4$ in a 66% ethanol/water mixture and $AlPcS_2$ in methanol at various concentrations are shown in Fig. 1. These spectra have been normalized to the amount of phthalocyanine added to the solution.

In the case of $AlPcS_4$, on increasing concentration, the intensity of the Q-band peak, at 670 nm, decreases and a broad shoulder at the red edge of this absorption is seen to grow in, which can be attributed to dimer or higher order aggregate absorption.[5] The spectra for $AlPcS_2$ show no evidence of aggregate formation, except at the highest concentration (6.37×10^{-5} mol dm^{-3} of the dye). Therefore, in this instance, the ordinate corresponds to the extinction coefficient, with a value of 1.8×10^5 dm^3 mol^{-1} cm^{-1} at 670 nm, in agreement with the literature value.[24,25] For $AlPcS_4$ this is

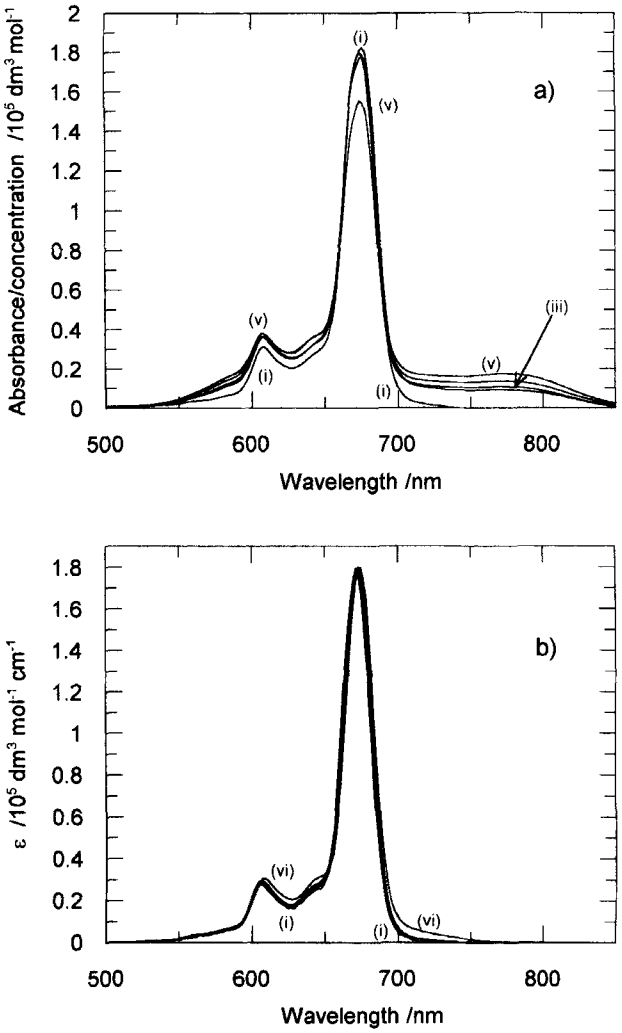

Figure 1. Absorption spectra of (a) $AlPcS_4$ in 66% (vol/vol) EtOH/H_2O at concentrations of (i) 0.11, (ii) 1.1, (iii) 5.6, (iv) 21.8 and (v) 110 × 10^{-6} mol dm^{-3}; and (b) $AlPcS_2$ in MeOH at concentrations of (i) 0.2, (ii) 0.6, (iii) 3.1, (iv) 6.3, (v) 12.5 and (vi) 63.7 × 10^{-6} mol dm^{-3}.

not the case, because the phthalocyanine does not exist in purely monomeric form.

The fluorescence spectra of both systems, with excitation at 610 nm, are shown in Fig. 2. As the concentration of dye increases, the emission spectrum initially increases in intensity with a shift in the emission maximum to longer wavelengths. At very high concentrations, the emission appears to diminish in intensity and to have two emission maxima. This can be seen more clearly for $AlPcS_4$ in the inset of Fig. 2a.

The $AlPcS_4$ spectra normalized to the emission maximum are shown in Fig. 3a. The shift in the emission maximum from 681 nm, at the lowest concentration, to 705 nm at the highest concentration is clearly visible. From this figure, the corresponding growth in intensity at 750 nm appears to be a second emission band.

With the exception of the very highest concentration, no aggregate absorption is observed for $AlPcS_2$, and the emission spectra can be normalized to the concentration of the dye and these are shown in Fig. 3b. In these instances the emis-

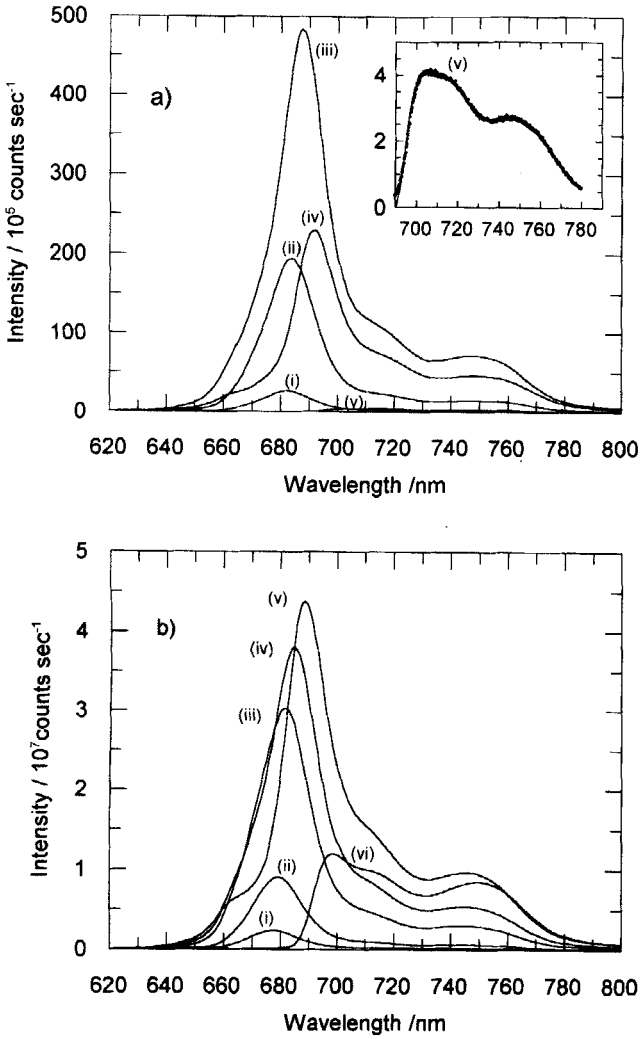

Figure 2. Corrected fluorescence emission spectra of (a) $AlPcS_4$ in 66% (vol/vol) $EtOH/H_2O$ at concentrations of (i) 0.11, (ii) 1.1, (iii) 5.6, (iv) 21.8 and (v) 110 × 10^{-6} mol dm^{-3} (inset shows spectrum at highest concentration and corresponding simulated spectrum using Eq. 1); and (b) $AlPcS_2$ in MeOH at concentrations of (i) 0.2, (ii) 0.6, (iii) 3.1, (iv) 6.3, (v) 12.5 and (vi) 63.7 × 10^{-6} mol dm^{-3}.

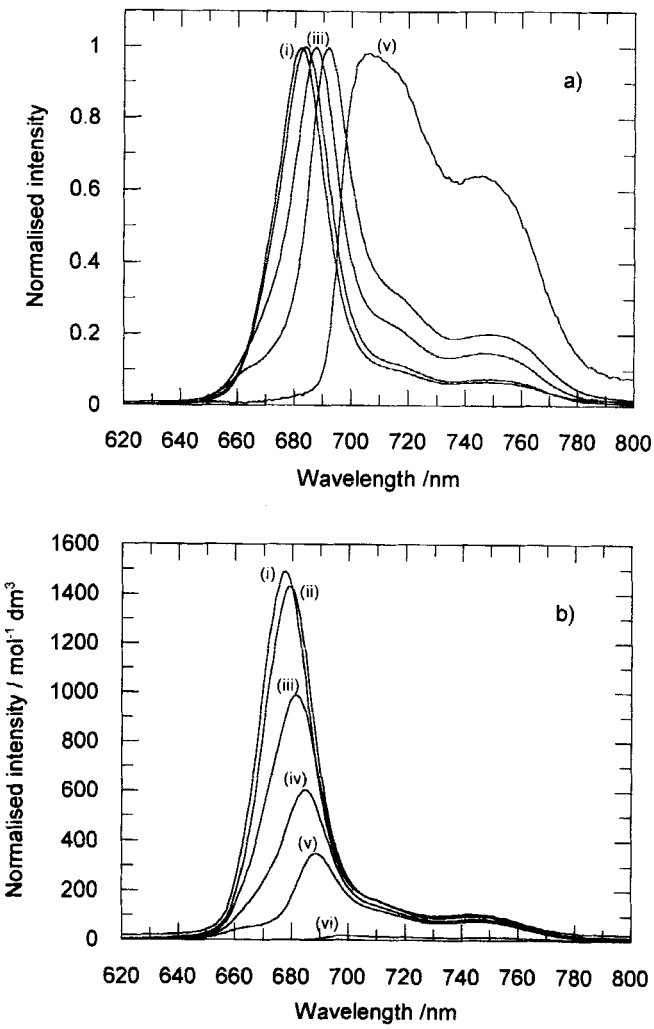

Figure 3. (a): Spectra shown in Fig. 2a normalized to unity at the maximum intensity. (b): Spectra shown in Fig. 2b normalized to concentration of phthalocyanine.

sion maxima also shift from 680 nm, at low concentration, to 697 nm, at high concentration. However, unlike the normalization procedure used for the $AlPcS_4$ spectra in Fig. 3a, the intensity of the shoulder at 750 nm shows only a modest decrease. For $AlPcS_4$, this simple normalization procedure of the emission spectra is not valid because the concentration of the emitting species is not known.

Fluorescence decay curves were measured for both species at the concentrations indicated above, with excitation at 610 nm and emission recorded at 15 nm intervals across the emission profile. Without exception, all the fluorescence decays could be well described by a monoexponential decay function yielding a single fluorescence decay time for each concentration, which did not vary with the emission wavelength. The data are plotted in Fig. 4 as a function of dye concentration. For both $AlPcS_4$ and $AlPcS_2$, the fluorescence decay time increases with increasing concentration approaching an asymptotic value at high concentration.

DISCUSSION

The Stokes shift for most phthalocyanines is small (220 cm^{-1}) and thus the overlap of the absorption and emission spectra is large. This effect is further accentuated when dimers are formed, as the red-shifted absorption occurs beneath the emission spectrum. In the present study the observed emission originates from the center of a conventional 1 cm cuvette and traverses 0.5 cm of the bulk solution prior to detection.

Under normal circumstances, when reabsorption is suspected, efforts are made to use an experimental arrangement that minimizes the effect.[26] The most common method, when using a fixed 90° detection system, is to use front face illumination by employing a triangular cross-section cuvette, such that the incidence angle of the excitation beam is *ca* 30° to the front face of the cuvette. There are both advantages and disadvantages to this approach: The excitation beam is not attenuated by the solution and thus more light reaches the region that is viewed by the optics of the detection system. If such an arrangement was used here, the measured fluorescence spectra would increase in intensity with increasing dye concentration and not show an initial rise followed by a

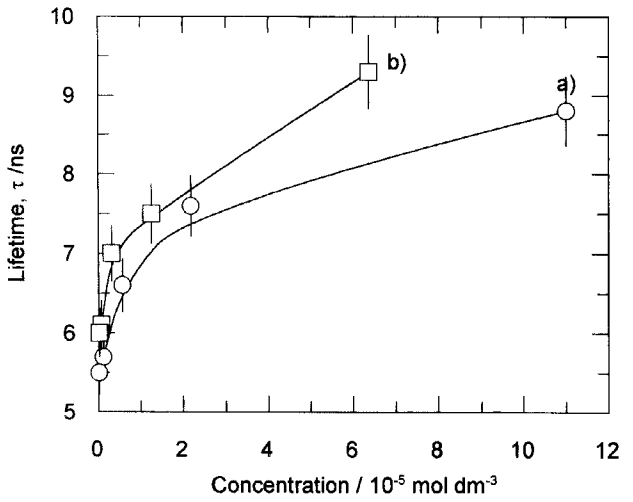

Figure 4. Variation of fluorescence decay time with concentration for (a) $AlPcS_4$ in 66% (vol/vol) $EtOH/H_2O$ and (b) $AlPcS_2$ in MeOH.

drop (see Fig. 2a). It is not guaranteed that this approach eliminates the distortion to the profiles of the emission spectra and the lengthening of the measured decay times, although these effects would be minimized. Difficulties in using front face illumination to minimize reabsorption occur when the optical density of the solution at the excitation wavelength does not result in complete absorption of the excitation light at the surface, and penetration into the bulk of the solution occurs. Thus, for solution concentrations that are not sufficiently high to result in complete attenuation of the excitation light, the influence of reabsorption, although minimized, still occurs. Our reasoning for choosing a 1 cm pathlength cuvette is two-fold. For the concentration range that we have used, front face illumination does not result in complete attenuation of the excitation light, and thus it is difficult to predict the effects of distortion. Secondly, our concentration range matches that of Yoon *et al.*,[15] where a 1 cm cuvette was used, thus enabling an alternative interpretation of their data.

The extent of reabsorption will depend on: (1) the overlap between the absorption spectrum, $\epsilon(\nu)$, and the molecular fluorescence spectrum, $F(\nu)$; (2) the solution concentration, c; (3) the specimen thickness, l, through which the fluorescence photons have to escape. The distorted emission spectrum expected after reabsorption, $F'(\nu)$, can therefore be predicted, to a first approximation, using the following relationship,

$$F'(\nu) = F(\nu)[10^{-\epsilon(\nu)cl}]. \quad (1)$$

The molecular fluorescence spectrum was approximated by a spectrum measured under dilute conditions (1×10^{-7} mol dm^{-3}). The term $-\epsilon(\nu)cl$ in Eq. 1 can be considered to be proportional to the measured absorption spectrum. Hence the emission spectra distorted by reabsorption can be simulated. The measured and simulated spectra of 1.1×10^{-4} *M* $AlPcS_4$ in 66% ethanol/water can be seen in the inset of Fig. 2. The agreement is exceptional and assumes an effective pathlength l, through which the emission must travel, of 0.45 $\pm$ 0.02 cm. The remaining spectra have also been simulated using the same technique and are shown in Fig. 5a. These spectra have been normalized to the emission maximum and

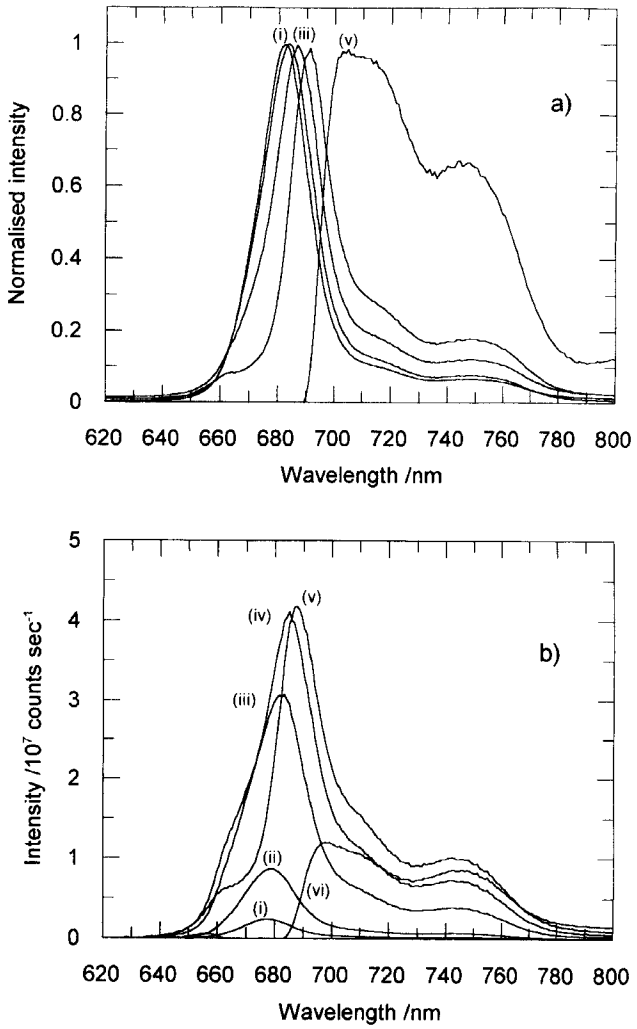

Figure 5. Spectra simulated using Eq. 1 for (a) $AlPcS_4$ in 66% (vol/vol) $EtOH/H_2O$ using effective pathlengths, l, of (i) 0.0, (ii) 0.73, (iii) 0.48, (iv) 0.34 and (v) 0.45 ($\pm$0.02) cm (data are normalized to unity at the maximum intensity for comparison with experimental data shown in Fig. 3a); and (b) $AlPcS_2$ in MeOH using effective pathlengths, l, of (i) 0.0, (ii) 0.75, (iii) 0.71, (iv) 0.57, (v) 0.49 and (vi) 0.31 ($\pm$0.02) cm (for comparison with experimental data shown in Fig. 2b).

can be compared with the measured spectra shown in Fig. 3a. A similar procedure was employed for $AlPcS_2$ in methanol. The simulated spectra are shown in Fig. 5b and can be compared with the experimentally measured spectra shown in Fig. 2b. The predicted value of the effective pathlength would be 0.5 cm based on the geometry of the cuvette in the fluorometer. The actual values required for a good fit, as listed in the respective figure legends, are seen to differ from this ideal value. From Eq. 1, it can be seen that the dependence upon pathlength is more critical at high concentration than at low concentration due to the power dependence of $F'(\nu)$ on l. In addition, this calculation neglects the effects of re-emission and the finite collection angle. In all cases, however, the measured spectra can be simulated using this simple procedure.

When the emission spectra are normalized to unity, as shown in Fig. 3a for $AlPcS_4$, the shift in the emission max-

Table 1. Experimental and decay times simulated using Eqs. 2–4

Concentration (10^{-6} M)	Experimental τ (ns) (±5%)	Simulated τ (ns)
a) $AlPcS_4$ in 66% (vol/vol) $EtOH/H_2O$, using a fluorescence quantum efficiency, q, of 0.4 and an effective pathlength, l, of 0.5 cm		
0.1	5.5	5.5
1.1	5.7	5.8
5.6	6.6	6.5
21.8	7.6	7.8
110.0	8.8	8.9
b) $AlPcS_2$ in MeOH, using a fluorescence quantum efficiency, q, of 0.56 and an effective pathlength, l, of 0.5 cm		
0.2	6.0	6.0
0.6	6.1	6.2
3.1	7.0	7.0
6.3	7.3	7.8
12.5	7.5	8.8
63.7	9.3	10.8

imum with increasing concentration is apparent. In addition, it *appears* that a new spectral feature grows in intensity on the red edge of the emission at 750 nm; this has been reported to originate from aggregate fluorescence.[15] It is important to note that the $AlPcS_2$ system, which shows no aggregation, still exhibits the same spectral shifts and apparent growth of a new species at 750 nm. The simulated spectra over the same concentration range indicate clearly that the spectral changes are due to reabsorption and *not* to aggregate emission. In addition, direct excitation into the supposed aggregate absorption band at 725 nm, yielded no detectable emission.

The phenomenon of re-emission, neglected by the above procedure, can be investigated by observing the time-resolved emission properties. The observed fluorescence decay times τ' can be modelled using the expression[17]

$$\tau' = \frac{\tau}{1 - aq} \tag{2}$$

where τ is the molecular fluorescence decay time obtained from a dilute solution, a is the probability of reabsorption of an emitted photon and q is the molecular fluorescence quantum efficiency. The product aq can be calculated from Eq. 3.[17]

$$aq = \int_0^\infty F(\nu)[1 - 10^{-\epsilon(\nu)cl}]\, d\nu \tag{3}$$

where $F(\nu)$ is normalized by the relation

$$\int_0^\infty F(\nu)\, d\nu = q. \tag{4}$$

Although the expression in Eq. 2 approximates to emission from the center of a sphere, it allows a reasonable prediction of a fluorescence decay time distorted by the reabsorption effect to be made. More advanced methods are available to evaluate[19] or eliminate[18] the influence of reabsorption when measuring fluorescence decay profiles.[18,19] Sakai *et al.*[19] have shown that the effect of reabsorption increases the measured fluorescence decay times and at very high concentrations it produces nonexponential decays. The concentration range used here did not exhibit any measurable nonlinearity in the fluorescence decay profiles. In conclusion, both simple[17] and more advanced treatments[19] of the effects of reabsorption predict an increase in the measured decay time with concentration.

Using the fluorescence spectrum recorded under dilute conditions, as the molecular fluorescence spectrum, $F(\nu)$, and assuming fluorescence quantum yields of 0.40 and 0.56 for $AlPcS_4$ in aqueous ethanol and $AlPcS_2$ in methanol,[27] respectively, the measured fluorescence decay times were simulated. These data are tabulated in Table 1. All decays are seen to be single exponential, as judged by the stringent fitting criteria discussed in the Materials and Methods and invariant with emission wavelength. Furthermore, an excellent correlation between the measured fluorescence decay time and that calculated using Eq. 2 is observed. If the emission peak at high concentration at 750 nm was due to dimer emission, the fluorescence decay profiles would be expected to be at least biexponential, with the relative yields of at least two decay times varying with the emission wavelength at which the decay was measured; this is not observed. This evidence, combined with the fluorescence spectral data, indicates that the effects of concentration on the measured photophysical characteristics can be explained *solely* by a reabsorption effect.

CONCLUSIONS

The steady-state emission spectra of the sulfonated aluminum phthalocyanines, $AlPcS_4$ and $AlPcS_2$, show similar distortions with increasing concentration. For both dyes the fluorescence decay curves, as a function of concentration, are monoexponential and invariant with emission wavelength. In the case of $AlPcS_4$ in 66% ethanol/water, the absorption spectra show clear evidence of dimer or aggregate formation in the form of an absorption at lower energy than the normal monomer. With the exception of the very highest concentration, there is no evidence for aggregation of $AlPcS_2$ in methanol. This observation alone suggests that the fluorescence data cannot be interpreted in terms of dimer or aggregate fluorescence. Furthermore, direct excitation of the dimer absorption band of $AlPcS_4$ at 725 nm yields no fluorescence that can be attributed to a dimer species. We conclude that there is no fluorescence from dimers of $AlPcS_4$ under the experimental conditions used here.

Using a simple model of fluorescence reabsorption we have successfully interpreted both the steady-state and time-resolved fluorescence data. The distortions in the emission spectra are a result of the high degree of spectral overlap between the absorption and emission spectra. With increasing dye concentration the effect manifests itself as an apparent shift in the emission maximum and a relative increase in the intensity of the emission shoulder at 750 nm. This is independent of the dye studied, with both the aggregating and nonaggregating systems exhibiting the same effect. Similarly, both systems exhibit the same trend in the fluorescence decay data, with the decay times increasing with increasing dye concentration, as predicted by theory.[17,19]

To conclude, our results do not support the argument of aggregate fluorescence, as suggested by Yoon *et al.*[15] Conversely, they can be rationalized fully using a simple model

based on a reabsorption effect. In view of the importance of the photophysics of soluble phthalocyanines in the field of PDT, where high dye concentrations occur, we feel that this is a very important distinction to be made. Moreover, this distinction should be emphasized in order to avoid future misinterpretation.

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