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Spectroscopic investigation of inner filter effects by phthalocyanine solutions

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Abstract

In this study we report a systematic investigation of phthalocyanine (Pc) optical properties, showing that the effect of re-absorption of fluorescence plays a critical role in the line-shape of Pc optical spectra. We demonstrate that a strong dependence of the photoluminescence parameters on the concentration of phthalocyanines solution accounts for the apparent experimental evidence of a fluorescent dimer band in the emission at high concentrations. Despite a model based on fluorescent aggregates seems to describe the photophysical behavior of the system, additional experiments, such as electronic absorption spectroscopy, and fluorescence spectroscopy in which the optical pathlength inside the solution is suitably modified, provide a correct explanation of the observed optical behavior. We demonstrate that the shapes of optical spectra are completely independent on phthalocyanine aggregation state and are due only to experimental artifacts, i.e. inner filter effects.

Keywords: Phthalocyanines; Fluorescence spectroscopy; Absorption spectroscopy; Inner filter effects; Aggregation state

1. Introduction

Phthalocyanines aggregation is a fairly well-known phenomenon and has been largely discussed in the literature [1–3]. Interactions between adjacent phthalocyanine (Pc) rings have been reported both in organic and aqueous phase, resulting in the coupling between the electronic states of two, or more, Pc units. The photophysical properties of Pcs are extensively affected by their state of aggregation: in particular, dimerization and aggregation result in a remarkable modification of the absorption and emission bands and may induce significant quenching of the usually strong Pc fluorescence [4]. The spectroscopic behavior of Pc dimers and higher aggregates is important for both the investigation of the fundamental chemical–physical properties of the compounds, and their industrial applications, where aggregation may induce large alterations in the features of a dye-marker [5], and for bio-medical research, as the excellent photodynamic activity of soluble Pcs [6] is often characterized by high local dye concentrations (up to 10^{-4} M) [7,8].

The photophysics of the aggregates strongly depends upon the relative geometry of the macrocycles, resulting in a wide range of spectroscopic behaviors. The phthalocyanine aggregates are characterized by substantial variations in the electronic absorption spectrum. Both blue- and red-shifted absorptions are allowed, depending on the interaction between the two adjacent Pcs. Generally, the aggregates described in the literature exhibit blue-shifted spectra. For these species, selection rules entail a large reduction in the fluorescence quantum yield, owing to the increased rate of internal conversion from the first excited singlet state, elicited by dimer formation [9]. According to the exciton coupling theory [10], the dimer conformation causes the individual monomeric singlet levels to split into pairs of new states. In this geometry, the HOMO–LUMO optical transition is allowed only to the upper dimer level (accounting for the blue-shift of the Q-band for most Pc dimers), whereas the transition from the lowest excited singlet level to the ground state is optically forbidden, so that most Pc dimers show no detectable fluorescence [11]. Experimental demonstrations of fluorescent Pc dimers are extremely rare, and various studies mostly describe examples of non-emitting aggregates [12,13]. Only very recently, fluorescence from dimers or higher order aggregates, formed in cooled solutions of mononuclear Pcs, was demonstrated for the first time [5].

It is worth noting that such photophysical issues are critical, since they originate experimental artifacts which make spectroscopic data often misleading. Photoluminescence

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(PL) by phthalocyanines dimers, in fact, has been invoked in the last years by several groups. Red-shifted emission as well as increased fluorescence decay time was reported from concentrated solutions of sulfonated aluminum phthalocyanines ($AlPcS_4$) and recognized as fluorescence from dimeric or aggregates species [14]. Only later Dhami et al. provided an alternative explanation for such observation, discussing the perturbation of the monomer emission by re-absorption of radiation by ground state species [9]. Additional evidence of fluorescent dimers in concentrated solutions of tetrasulfonated zinc phthalocyanine was proposed also by Kaneko et al. [15], though the correct description in terms of phthalocyanines ring protonation [16,17] was later given by Beeby et al. [18]. Hence, in view of the extensive use of phthalocyanine solutions in many research areas and applications, and of the controversial understanding of the interactions between adjacent Pcs, it seems very important to carefully characterize the photophysics of the aggregates.

In this frame, we report here a systematic investigation on zinc phthalocyanines (ZnPc) spectroscopy, showing that the effect of re-absorption of fluorescence plays a critical role in determining the modification of the Pc optical properties. In particular, we demonstrate that the strong dependence of the photoluminescence spectra on the phthalocyanines concentration ([Pc]) gives rise to spectral features which may be confused with fluorescent dimer bands in concentrated solutions of Pc. To this purpose, in the first part of the paper, we describe the optical behavior of ZnPc in terms of possible formation of emitting aggregates with increasing concentrations, and show that such a model well agrees with the experimental data. However, in the second part of the work, a set of properly designed experiments, primarily aimed at reducing the fluorescence optical pathlength inside the solution, provides a satisfactory explanation of the observed optical behavior in terms of re-absorption effects.

2. Materials and methods

All the starting materials were purchased from Aldrich Chemical Co. and used as received. Silica gel (Merck) was used for the chromatographic separations. Solvents were dried and distilled under N_2 atmosphere. The synthesis of the Zn(II) tetra-4-(2,4-di-*t*-amylphenoxy)-phthalocyanine (ZnPc) was performed according to established methods [19]. NMR, FTIR, UV-Vis and LC/MS spectra confirmed that the desired compound was obtained.

Fluorescence spectroscopy was always performed in $CHCl_3$ at room temperature (20 °C) by using a Xenon lamp as the source of excitation (2 nm bandwidth) and a 3 ml standard quarz cuvette (10 mm × 10 mm). Photoluminescence spectra (PL) of control samples, without phthalocyanines, were recorded and subtracted from the experimental samples to correct for background interference. The emitted light was observed at right angles to the excitation radiation (right angle geometry). For those experiments in which the optical pathlength of fluorescence inside the solution (d) was varied, the cuvette was mechanically translated along the direction perpendicular to the excitation, so that different regions of the Pc solution were irradiated.

Absorption spectra were collected at room temperature by a Varian Cary 100 Scan UV-Visible spectrophotometer. The spectra of the most concentrated solutions were obtained by replacing the standard, 10 mm pathlength quarz cuvette, with a 1 mm cuvette.

For statistical considerations, the experimental results are the average of five independent measurements. The data shown in the figures throughout the manuscript are the mean values obtained from the experimental data, and the error bars indicate the standard deviations.

3. Results and discussion

The photoluminescence spectra of the ZnPc at two different concentrations are presented in Fig. 1a, clearly showing that the concentration of the solution is a crucial parameter. Despite the similar line-shapes and the comparable intensities, a remarkable red-shift with increasing the concentration is evident, the first fluorescence spectrum (1.98×10^{-5} M) being characterized by a wavelength of emission maximum (λ_{max}) of 695.5 nm, whereas the second (2.20×10^{-4} M) having its maximum at 706 nm. Both these spectra, but especially the red-shifted one, are not typical of a metallo-Pc monomer, whose λ_{max} usually ranges from 685 to 695 nm [5]. Such a dependence on the concentration suggests the occurrence of some concentration-related effect affecting the photophysical properties of the system. It seems that different fluorescent species, dissimilarly correlated to the dye concentration, account for such an effect.

The results obtained over a wider range of Pc concentrations ($3.30 \times 10^{-4} \div 1.00 \times 10^{-7}$ M) are reported in Fig. 1b. At very low concentrations, the ZnPc luminescence spectra are characterized by a $\lambda_{max} \cong 690$ nm, in agreement with the spectral features of a monomeric metallo-Pc. However, as the concentration of phthalocyanine increases, the emission initially increases in intensity, exhibiting a slight shift in the emission maximum to longer wavelengths, while, at higher concentrations, the intensity appears to clearly diminish, the red-shift becoming more and more pronounced. The λ_{max} ranges from 689 nm, at the lowest concentration, up to 707.5 nm, at the highest concentration. Such a photophysical behavior may suggest that the variation of the dye concentration results in the enhancement or suppression of fluorescence from different emitting bands, possibly disclosing the formation of different Pc species in solution. In particular, the low-concentration-band, centered around 690 nm, seems to account for the presence of monomeric phthalocyanines in the solution, while the rise in intensity in the region around 705–710 nm, at higher concentrations, might be ascribed to a second emission band, related to aggregate species (probably dimers). A deeper insight in the

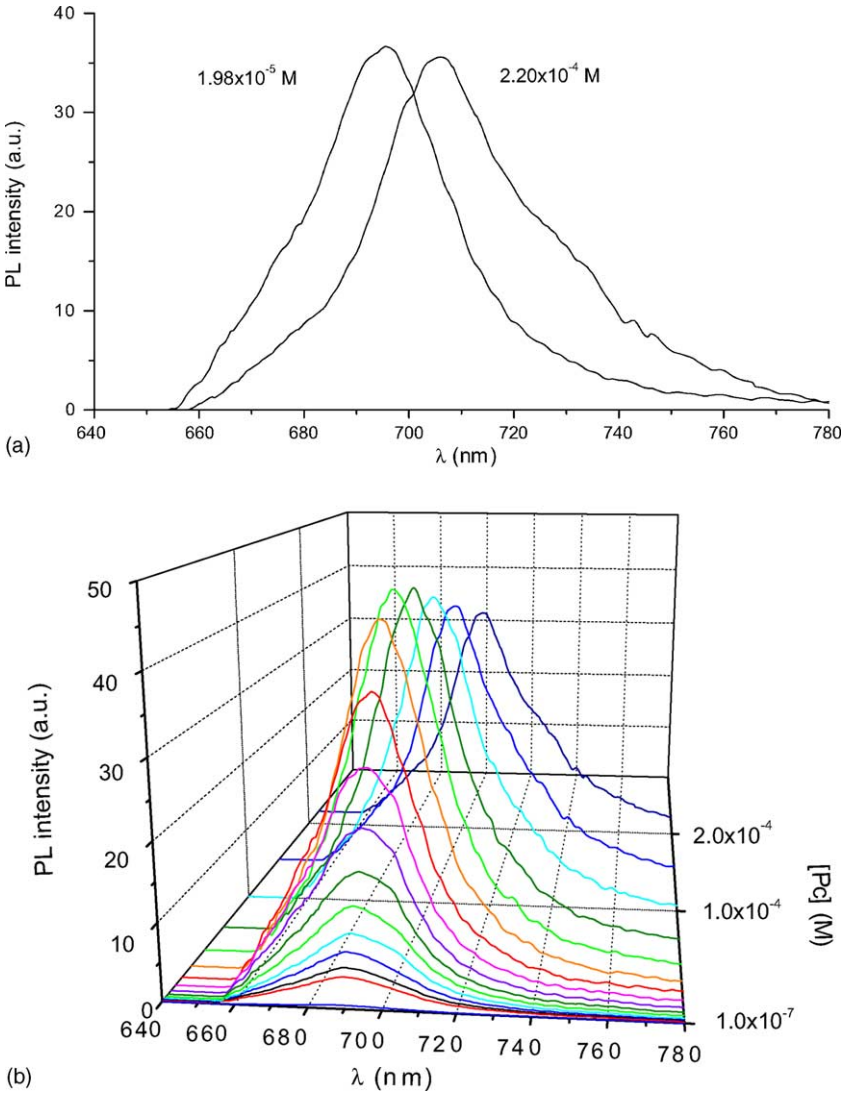

Fig. 1. (a) Photoluminescence (PL) spectra of ZnPc at two different concentrations, 1.98×10^{-5} and 2.20×10^{-4} M (λ_{exc} was 610 nm); (b) PL spectra in the whole range of Pc concentrations (from 3.30×10^{-4} to 1.00×10^{-7} M).

concentration-dependent line-shape is obtained by deconvolving the fluorescence spectra by means of two Gaussians, representing the two different fluorescent species (monomers and dimers), and investigating the photophysical properties of the two individual bands in the entire range of examined Pc concentrations. An example of such analysis is reported in Fig. 2a. The deconvolution (minimization of the χ^2 criterion) exhibits a good fit for all the PL spectra, and results in the decomposition of the integrated Pc luminescence in two distinct bands having the two maxima (λ_{max1} and λ_{max2}) at 689 and 707.5 nm (monomer and dimer, respectively). This analysis reveals that the two bands have different dependence on the Pc concentration (Fig. 2b). The integrated emission of the dimer band increases with the concentration almost in the whole examined range (showing a significant contribution to phthalocyanine emission since 10^{-5} M). This band exhibits a clear quadratic dependence on [Pc] up to 3×10^{-5} M (fit quality: $R^2 = 0.9994$), while, at higher concentrations, the slope of the curve progressively reduces. The monomer band integrated intensity first increases with concentration (up to 3×10^{-5} M), and then reduces rapidly, consistent with the dominant dimer emission in highly concentrated solutions. The monomer photoluminescence shows only in the first part of the plot ($10^{-7} \div 10^{-5}$ M) a rapid increase of fluorescence emission versus concentration (quadratic dependence, $R^2 = 0.9923$), and then rapidly decreases to zero. This result is indeed more evident in Fig. 2c, where the two relative contributions have been normalized to the integrated Pc emission. As shown, there is a strong influence of the concentration upon the aggregation state of the phthalocyanines, indicating also that, for [Pc] > 5×10^{-5} M, dimers prevail. In conclusion, according to this description, the convolution of these two fluorescent bands accounts for the observed photoluminescence from Pc solutions.

Despite the above analysis seems to describe very well the photophysical behavior of the system, it is well known that

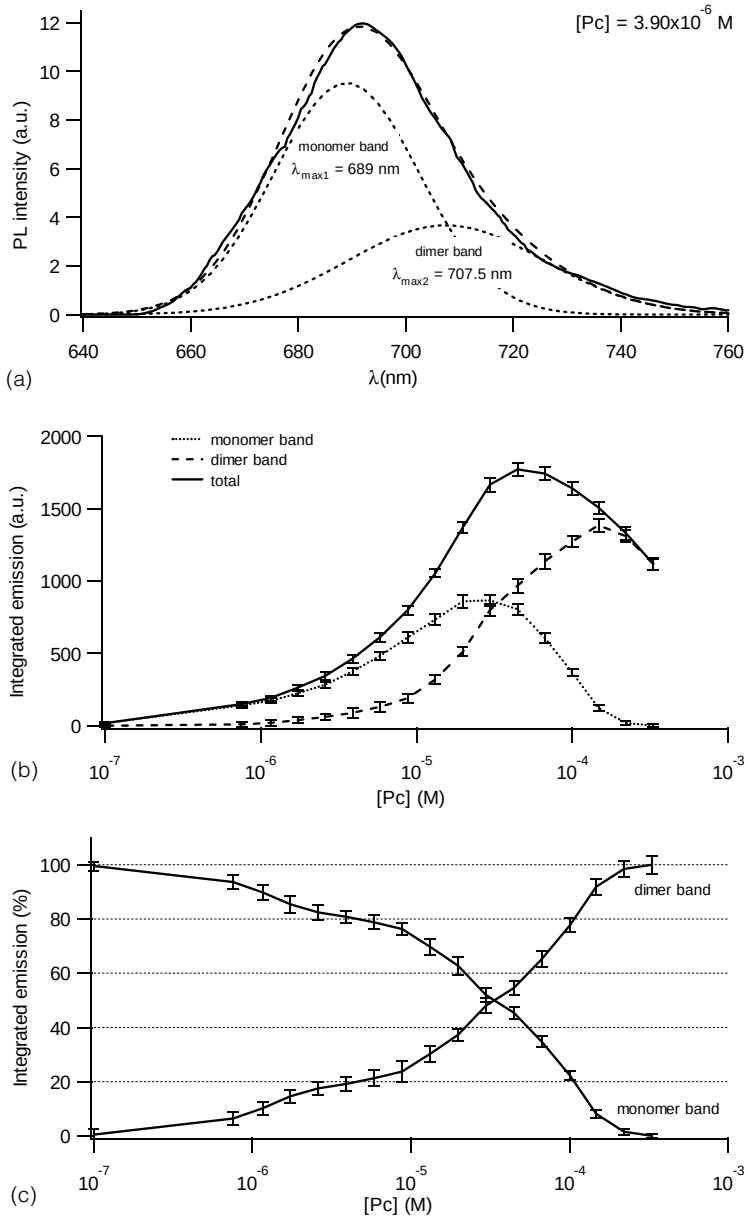

Fig. 2. (a) Example of the deconvolution analysis; (b) dependence of the fluorescence emission of the two single emitting species on Pc concentration; (c) relative contribution of the two bands.

fluorescent Pc dimers are extremely rare. In what follows, we indeed demonstrate that the conclusions drawn in Fig. 2 are incorrect and merely derive from experimental artifacts. The first hint that the dimer fluorescence is unlikely to occur is provided by electronic absorption spectroscopy.

The absorption spectrum of a phthalocyanine is characterized by the transition from the ground state, $^1S_0$, to a singlet excited state, either ^1Q or ^1B. According to the literature, its absorption spectrum is a sensitive probe for the presence or absence of coupling between two (or more) phthalocyanine units [20], since dimers can be directly detected by the presence of visible absorbance peaks, Q_d (in the range 625 ÷ 635 nm), blue-shifted in relation to the Q(0,0) band of the monomer (centered around 680–682 nm) [4,21–23].

As shown in Fig. 3a, the Zn phthalocyanine species exhibits identical absorption spectra throughout the concentration range $3.3 \times 10^{-4} \div 10^{-7}$ M, revealing no presence of aggregation states in $CHCl_3$ and at room temperature. The absorption spectrum is that typical of a monomeric Pc. Even at the highest dye concentrations, there is no experimental evidence of a decrease of the monomeric Q-band absorption and simultaneous growth of any new peak in the 625–635 nm region. Since a completely uncoupled Pc shows the same electronic spectrum of its mononuclear analogue [11,18], we conclude that, in the experimental conditions reported here, no formation of phthalocyanine aggregates occurs. This clearly casts doubts on the previous interpretation of our PL spectra based on the fluorescent dimers. The

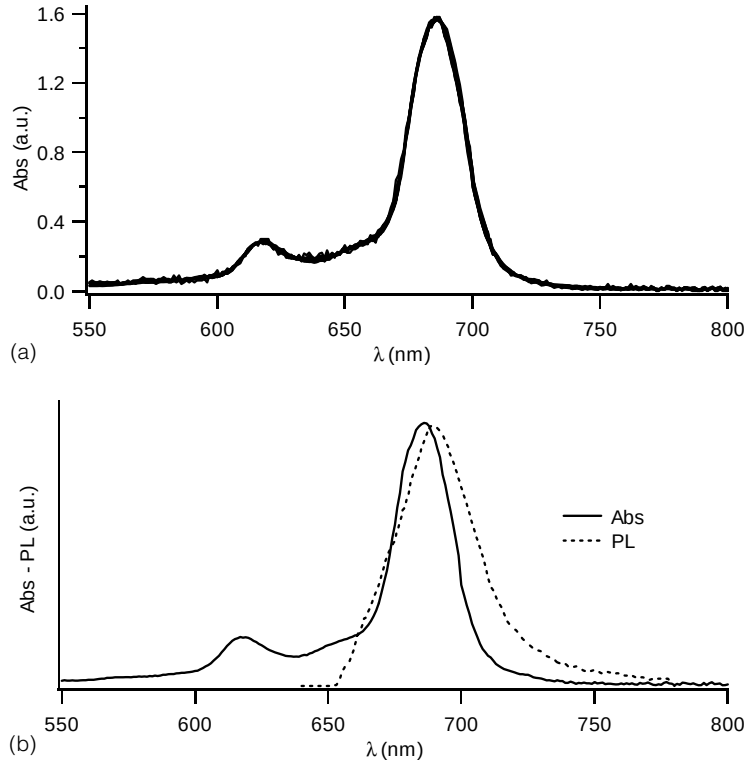

Fig. 3. (a) ZnPc absorption spectra in the concentration range from 3.30×10^{-4} to 1.00×10^{-7} M (the spectra have been normalized to their maximum intensities); (b) overlap between the absorption and the emission spectra.

emission spectra of the ZnPc are progressively more affected by the increase of concentration, in the form of a shift in the emission maximum and a change in the intensity of the emission (Fig. 1b), but this experimental evidence is completely independent on phthalocyanine aggregation state.

Conversely, these finding can be explained by the re-absorption of the fluorescence induced by ground state species, owing to the high extent of spectral overlap between absorption and luminescence spectra (Fig. 3b). Such large overlap is a peculiar feature of the photophysics of most phthalocyanine, due to the extremely small Stokes shift of their fluorescence [9]. For a definite molecule, moreover, the strength of re-absorption will depend primarily on the concentration of the dye solution, as well as on the mean optical pathlength of the fluorescence inside the solution itself [9,24]. At very low concentrations ($\sim 10^{-7}$), the perturbations of the photoluminescence spectra are obviously quite weak. When re-absorption phenomena become significant, the shift of the emission maximum (λ_{max}) with increasing concentration is remarkable, producing an apparently new band on the red edge of the monomeric emission, which might be erroneously ascribed to fluorescence from aggregate species.

In most spectroscopic studies, and also in the results previously reported here, fluorescence is usually observed from the center of a standard (10 mm × 10 mm) cuvette. Based on the geometry of the cuvette in such photoluminescence experiments (right angle geometry), the detection system collects the emission after an optical pathlength of 5 mm through the solution, with the consequence that the inner filter effects can significantly affect the measured spectra.

In general, when such re-absorption problems are known to alter the accuracy of an experiment, a minimization of such an effect can be obtained by employing a triangular cross-section cuvette, in fixed 90° detection systems [9,25], or a backscattering approach, in order to reduce the optical pathlength of the fluorescence in the solution. In both cases, the measured fluorescence spectra are expected to display a monotonic increase in intensity with increasing concentration, without any significant shift of the λ_{max}. These techniques, however, do not guarantee a complete absence of artifacts in the collected spectra, since the inner filter effects, though minimized, keep their strong dependence on the concentration (at high concentrations, the dependence upon pathlength becomes extremely critical). In addition, the use of these geometries presents some difficulties. First, in the case of low dye concentrations, it may be difficult to predict the effects of perturbation, as the excitation light can penetrate in the bulk of the solution. Second, the evaluation of the mean optical pathlength of luminescence, which is a crucial parameter of the experiment, is a quite complicated problem.

In this frame, we have performed a series of experiments designed to continuously increase the fluorescence optical pathlength inside the solution (d), by mechanically translating the irradiated region of the Pc solution. The usual

right angle geometry was maintained and all the other experimental parameters were left unchanged. By means of these experiments, we have carried out a systematic investigation of zinc phthalocyanine optical properties, analyzing, with four different d values (1.0, 2.5, 5.0 and 7.5 mm), the same Pc concentration range of the previous experiments ($3.3\times10^{-4}\div10^{-7}$ M). We have thus analyzed the four different trends (one for each d value) of the shifts of the emission maxima (λ_{max}) and the variations of the integrated emissions versus Pc concentration. Our analysis clearly shows that the effect of re-absorption of fluorescence plays a critical role and demonstrates that no fluorescent dimeric species forms in our experiments.

As expected (Fig. 4a), there is a similar dependence of the red-shift on Pc concentration, for the individual optical pathlengths of fluorescence (the curve for $d = 5.0$ mm refers to the PL spectra already shown in Fig. 1b). Nevertheless, this analysis points out the different degree of perturbation of phthalocyanine concentration on the emission spectra. In particular, in the low concentration range ($10^{-7}\div10^{-6}$ M), the emission spectra are marginally affected by re-absorption, whereas, at high values, a very large red-shift is induced (up to 21 nm), for the $d = 7.5$ mm geometry. Interestingly, also in the case of $d = 1$ mm, despite the poor influence showed at low concentrations, a significant red-shift is elicited in the higher [Pc] range ($\Delta\lambda_{max}$ up to 12 nm), indicating that a strong re-absorption by ground state molecules can occur also under this condition. A good confirmation of these results is provided by the analysis of the integrated emission as a function of Pc concentration (Fig. 4b). A strong dependence of the collected fluorescence on the geometries of the experiments was in fact recorded, as well as a response to phthalocyanine concentration very similar to the red-shift effect. It is noteworthy to observe the characteristic shapes of the curves obtained in Fig. 4b. Two counteracting effects are responsible for such optical behaviors. The emission intensity is proportional to the concentration of the fluorescent species, whereas, on the other hand, the probability that an emitted photon is re-absorbed by a ground state molecule (thus not reaching the detector) is also strictly related to the Pc concentration. The combination of these two competitive effects accounts for the overall spectral response of phthalocyanines. As revealed by Fig. 4b, each curve is roughly characterized by two main regions in which an effect prevails on the other. In more detail, at low concentrations, the rise of fluorescence intensity, due to the increasing number of emitting molecules, is dominant, while, at high [Pc], re-absorption of luminescence become so intense that leads

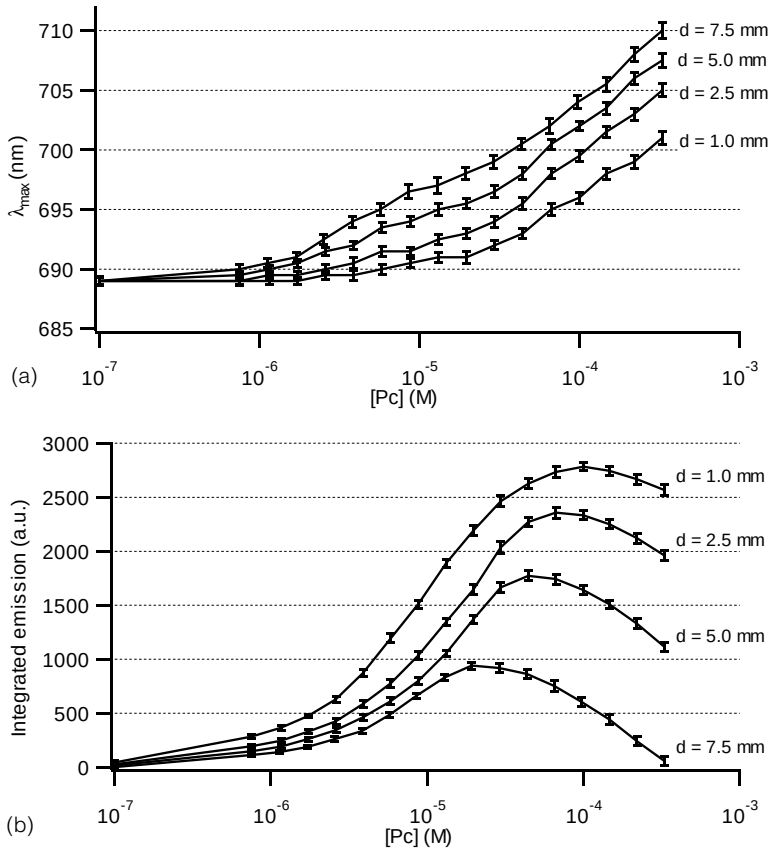

Fig. 4. (a) Analysis of the shift of the emission maximum (λ_{max}) for the four different optical pathlengths (d) with increasing Pc concentration; (b) variations of the integrated emissions (the assessment of the mean optical pathlengths d here utilized, however, neglects the effects of re-emission, although this approximation would be reasonable only in the case of low Pc concentrations).

to a marked reduction of detectable fluorescence. As expected, this complex dependence is strongly affected by the optical pathlength (d), such parameter directly acting on the shape and the intensity of the fluorescence spectra.

Obviously, owing to the large overlap between absorption and emission bands, also the excitation spectra (PLE) of zinc phthalocyanine are expected to be analogously distorted by the inner filter effects, thus providing, in some cases, distorted information about the photophysics of such compounds. Hence, if we collect such spectra ($\lambda_{em} = 710$ nm) in the four different configurations, extending the investigation throughout the large [Pc] range ($3.30 \times 10^{-4} \div 1.00 \times 10^{-7}$), we can observe the correct excitation spectrum only in the case of low Pc concentrations. The results of these experiments are reported in Fig. 5a and b (for $d = 7.5$ and 1.0 mm, respectively), where the PLE intensity have been normalized to the first peak (at 617 nm), in order to better highlight the distortions induced by the high Pc concentrations. The PLE spectra obtained with low [Pc] are typical of monomeric phthalocyanine, showing that there are no aggregates (no Q_d peak is rising in the PLE) and accurately matching the absorption profile of monomeric Pc. It is interesting to remark that also in this case we find a significant perturbation of the observed spectra at high [Pc], and that such perturbation is strongly related to the value of the optical pathlengths (d). These effects are analyzed more deeply in Fig. 5c, in which the ratios between the two peaks (I_{679}/I_{617}) are reported versus phthalocyanine concentration for the different geometries. As shown, the extent of the PLE distortion increases, with a certain continuity, with increasing [Pc], accounting for the shift of the emission band, specifically regulated by Pc concentration, but also for the different degrees of attenuation of the two excitation wavelengths (617 and 679 nm) at the various phthalocyanine concentrations. Moreover, additional PLE experiments performed at low concentrations by scanning different emission regions ($\lambda_{em} = 680$, 710 and 730 nm) yielded the identical excitation spectrum (monomeric), thus providing the definitive proof of the complete absence of any fluorescence band from aggregate species.

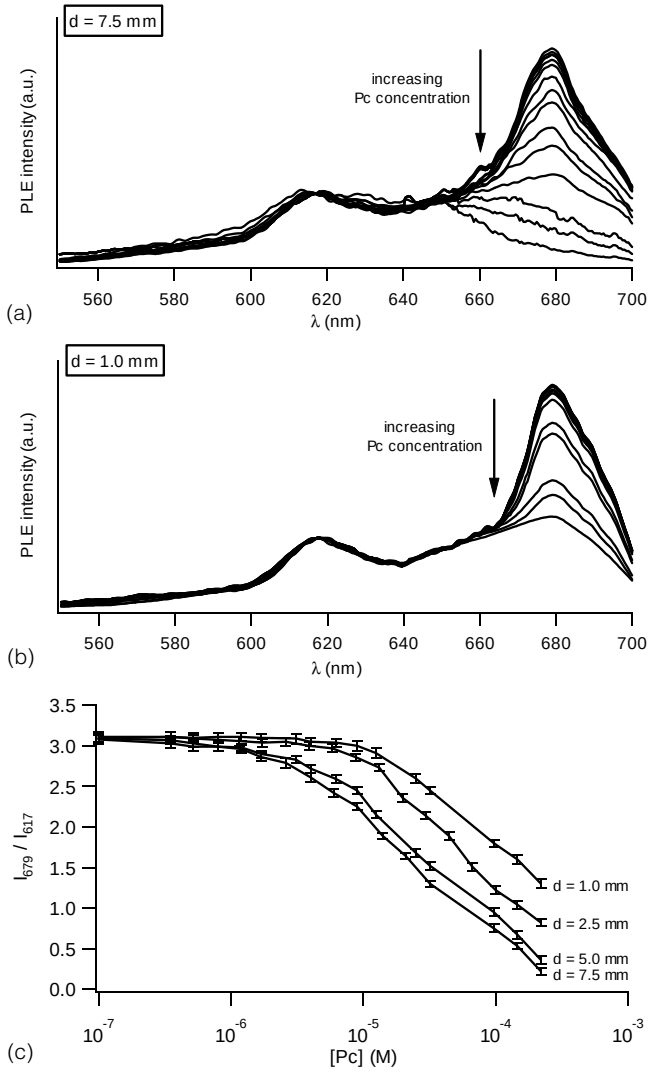

Fig. 5. (a) and (b) Excitation spectra (PLE) of ZnPc ($\lambda_{em} = 710$ nm) in the concentration range from 3.30×10^{-4} to 1.00×10^{-7} M, for $d = 7.5$ and 1.0 mm, respectively (the spectra have been normalized to the maximum intensity of the first peak, at 617 nm); (c) analysis of the distortions of the PLE spectra vs. Pc concentration, for the four different d values.

In conclusion, our systematic investigation on phthalocyanine spectroscopy demonstrates that all the perturbations induced on the measured photophysical parameters, in the high [Pc] range, can be explained solely as re-absorption effects, any speculation about fluorescent dimers being completely unfounded. In our opinion, this problem seems an essential point to be clarified, in consideration of the importance of the optical properties of phthalocyanine solutions in many applications in which high concentrations are involved, and of the controversial understanding of the interactions between adjacent Pcs.

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