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Spectroscopic evidence of monomeric aluminium phthalocyanine tetrasulphonate in aqueous solutions

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Abstract

Aqueous solutions of aluminium phthalocyanine tetrasulphonate ($AlPcS_4$) were investigated by means of absorption and fluorescence spectroscopy. The absorption spectrum of $AlPcS_4$ is independent of concentration in a wide range (from 10^{-8} to 10^{-4} M). The fluorescence spectrum measured with a standard setup is strongly dependent on $AlPcS_4$ concentration, and the fluorescence maximum is gradually red-shifted with increasing concentration. Calculations that take into account reabsorption of fluorescence (inner-filter effect) fit the experimental observations at low concentrations (up to 10^{-6} M). Disagreement between the calculations and spectra recorded at higher concentrations (above 10^{-5} M) shows that the reabsorbed light may be reemitted as fluorescence. The influence of inner-filter effects on the spectral shape was demonstrated by the experiments where a fibre-optic front-face fluorescence setup was applied: Under such conditions the shape of the fluorescence spectra for a high concentration (10^{-3} M) coincided with that of a low concentration (10^{-8} M). In conclusion, the present spectroscopic results show that $AlPcS_4$ does not form aggregates and is a very stable compound in aqueous solutions.

Keywords: Absorption spectroscopy; Aggregation; Fluorescence spectroscopy; Inner-filter effect; Reabsorption

1. Introduction

Phthalocyanines are promising photosensitizers for photodynamic therapy (PDT) [1–3] since they can be activated by light at relatively long wavelengths [4,5]. Many phthalocyanines are rather lipophilic. However, addition of sulphate or other groups to the outer ring creates water-soluble derivatives. Thus, sulphonated phthalocyanines are the most efficient photosensitizers [5,6]. Sulphonated phthalocyanines were found to be taken up more efficiently than porphyrins into cells *in vitro* [7] and show better tumour localising properties and photosensitizing effects in tumour models [8,9]. However, sulphonated phthalocyanines, as well as other tetrapyrroles, tend to aggregate in aqueous media and in organic solvents [10]. Basically, dimers and higher oligomers of tetrapyrroles have reduced fluorescence quantum yields and photosensitizing activities as compared with monomers [11,12]. Aggregation may lead to photochemical inefficiency. Since water constitutes a major part of cells and tissues, the behaviour of photosensitizers in aqueous solutions is important to be elucidated in view of PDT. However, it seems that particularly tetrasulphonated aluminium phthalocyanine ($AlPcS_4$) has been overlooked as a potential photosensitizer for PDT for several years. In the present study $AlPcS_4$ was investigated for possible aggregation at different concentrations in vitro by means of absorption and fluorescence spectroscopy.

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2. Materials and methods

2.1. Chemicals

Aluminium phthalocyanine tetrasulphonate ($AlPcS_4$) was purchased from Frontier Scientific (former Porphyrin Products, Logan, UT). A stock solution of $AlPcS_4$ (10^{-3} M) was prepared in phosphate buffer saline (PBS, pH 7). Other concentrations were prepared by dilution of the stock solution in PBS. For a stability test, an $AlPcS_4$ solution of 10^{-5} M was stored in a closed plastic laboratory tube at room temperature (20–25 °C) for up to 110 days.

2.2. Absorption spectroscopy

Absorption spectra of $AlPcS_4$ were recorded with a Perkin–Elmer Lambda 15 UV/VIS spectrophotometer (Shelton, previously Norwalk, CT). Absorption was measured in a standard 10 mm quartz cuvette, except for the concentration of 10^{-4} M where a standard 1 mm quartz cuvette was used.

2.3. Fluorescence spectroscopy

Fluorescence emission spectra were recorded by means of a Perkin Elmer LS50B luminescence spectrometer (Shelton, previously Norwalk, CT) equipped with a R928 photomultiplier (Hamamatsu, Japan). Fluorescence spectra were acquired using a standard fluorescence detection setup (90 °C angle between excitation beam and emission registration using a 10 mm pathlength quartz cuvette in a standard cuvette holder) or by placing a fibre-optic probe (a Perkin–Elmer accessory consisting of two bundles of silica fibers joint in parallel at a measuring tip) on the surface of the 1 mm or 10 mm cuvette. Additionally, a thin layer of solution (for the case of 10^{-3} M) was used, which was achieved by placing a drop (0.05 ml) between two glass plates thus giving a solution layer of an approximate thickness 0.02 mm.

The luminescence signal was calibrated with a LS-1-CAL calibrated light source (Ocean Optics, Inc., Dunedin, FL). Thus, fluorescence emission spectra are corrected for the spectral sensitivity of the instrument. The setup of 0.02 mm layer did not influence fluorescence spectra since glass is transparent for wavelengths above 320 nm (spectrum not shown).

3. Results

The shape of the absorption spectra of $AlPcS_4$ as recorded in PBS solutions were independent of the concentration in the range 10^{-8}–10^{-4} M and the data obey the Beer–Lambert law (Fig. 1(a)). The absorption

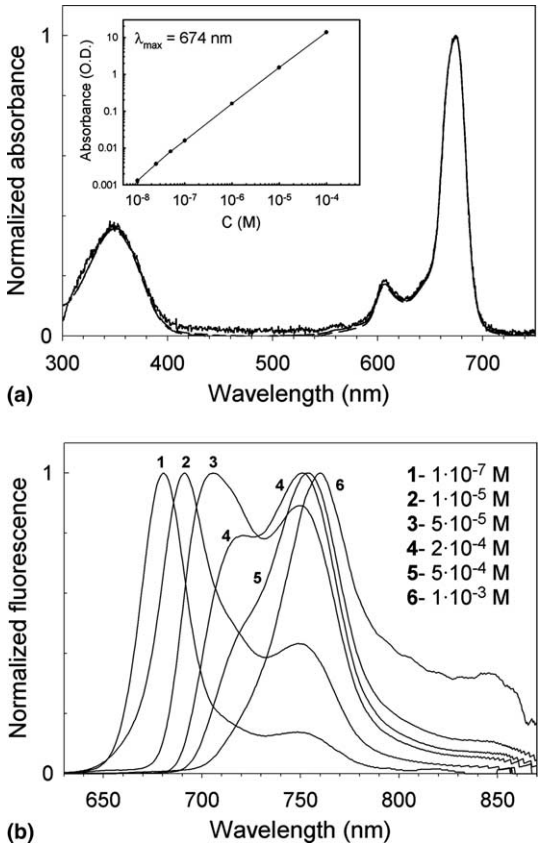

Fig. 1. (a) Absorption spectra measured for different $AlPcS_4$ concentrations. The absorption spectra represent the concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. The noisy spectrum represents the lowest concentration. An inset in the panel a shows the absorbance at 674 nm (pathlength 10 mm). A cuvette with a pathlength of 1 mm was used in the case of 10^{-4} M and the absorbance is multiplied by a factor of 10 for this point. (b) Fluorescence spectra (excitation 400 nm) measured for different $AlPcS_4$ concentrations. The fluorescence spectrum for 10^{-8} M is not shown since it is identical with that for 10^{-7} M.

spectrum for 10^{-3} M was not recorded since it was not possible to make a 0.02 mm layer in a reproducible way.

The main fluorescence maximum gradually shifted from 680 nm for the lowest concentration studied (10^{-8} M) to around 710 nm for 10^{-4} M (Fig. 1(b)). A relative growth of the second emission band, with a peak at 750–760 nm, is clearly visible. This peak dominates at high concentrations in the range 2×10^{-4}–10^{-3} M.

Furthermore, fluorescence spectra were recorded using the fibre-optic probe at the surface of cuvettes of different pathlength and compared with the fluorescence spectrum of a diluted sample (10^{-8} M) measured with a standard fluorescence setup. The spectral shape of the fluorescence spectra was dependent on the sample thickness for high concentrations (10^{-5}–10^{-3} M), as shown for 10^{-3} M (Fig. 2, curves 2–5). Subsequently, for a certain setup the shape of the fluorescence spectra for the low (10^{-8} M) and high (10^{-3} M) concentrations becomes practically similar (Fig. 2, curves 1 and 2).

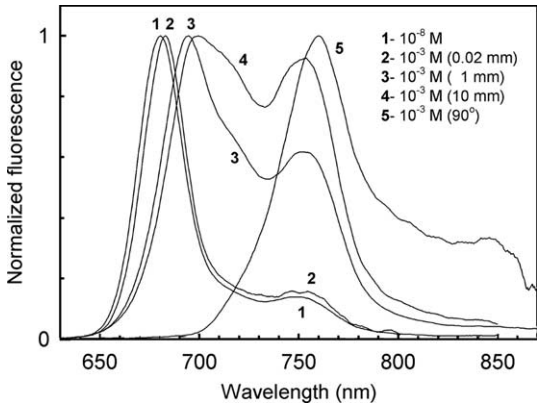

Fig. 2. Fluorescence emission spectra of $AlPcS_4$ at a low concentration (10^{-8} M, curve 1, standard cuvette holder) and a high concentration (10^{-3} M, curves 2–4, measured with the fibre-optic probe, curve 5 – standard cuvette holder).

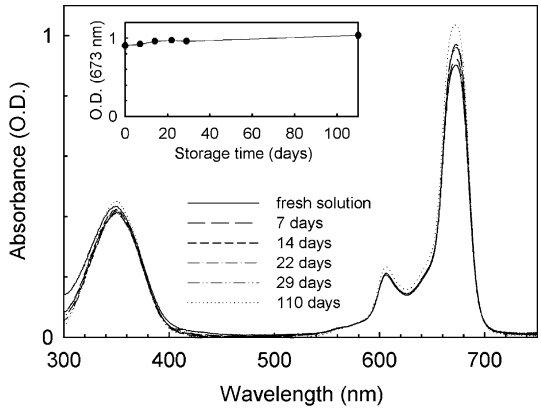

Fig. 4. Stability of $AlPcS_4$ solution (10^{-5} M) stored at room temperature (20–25 °C). Absorption spectra were measured in a quartz cuvette (pathlength 5 mm). Inset shows a peak value at 673 nm.

To a first approximation, distortion of a fluorescence spectrum caused by the inner-filter effect can be predicted using the Beer–Lambert law

$$F = F_0 \cdot 10^{-D(\lambda)} = F_0 \cdot 10^{-k \cdot A(\lambda)}, \quad (1)$$

where F_0 is a fluorescence spectrum measured under dilute conditions (in our calculations the spectrum at a concentration of 10^{-7} M was used), $D(\lambda)$ is the optical density, $A(\lambda)$ is the experimentally measured absorption spectrum of $AlPcS_4$ (in our calculations the spectrum measured for the concentration of 10^{-5} M was used), and k is a geometrical factor. Calculated spectra providing the best fit to the experimental results are shown in Fig. 3.

Aqueous solutions of $AlPcS_4$ are extremely stable yielding the same absorption spectra over a long time of storage (Fig. 4). A slight increase of the absorbance may be caused by evaporation of the solvent.

4. Discussion

Tetrapyrrolic compounds at concentrations (10^{-6}–10^{-3} M) that accumulate in cells tend to form aggregates in aqueous environment [10]. Specific effects of

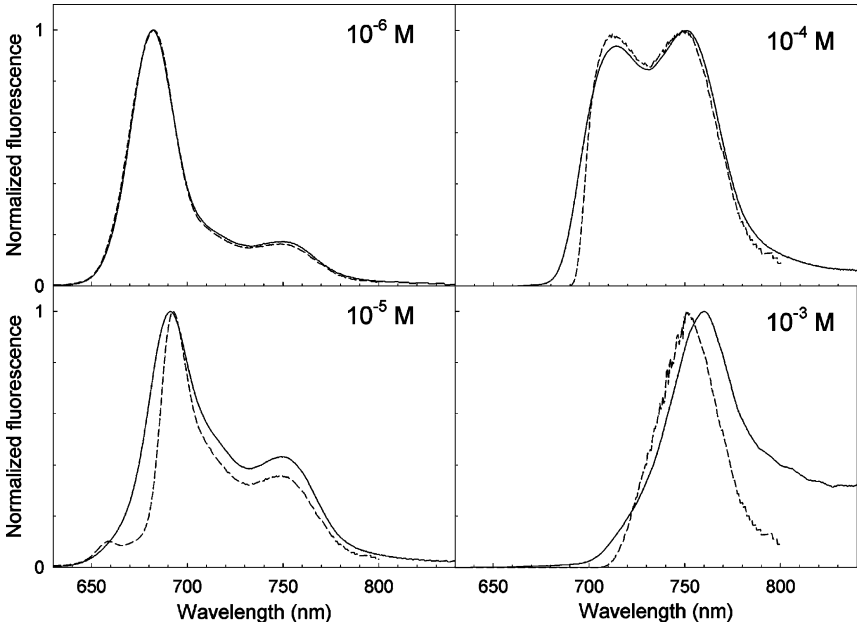

Fig. 3. Measured (solid lines, from Fig. 1(b)) and calculated (dashed lines, from Eq. (1)) fluorescence spectra of $AlPcS_4$ at various concentrations taking into account the inner-filter effect. The geometrical constant used in the calculations is $k(10^{-6}\ \mathrm{M}) = 0.1$, $k(10^{-5}\ \mathrm{M}) = 1.3$, $k(10^{-4}\ \mathrm{M}) = 15$ and $k(10^{-3}\ \mathrm{M}) = 200$.

dimerization of phthalocyanines are described in a number of studies [13–15]. Aggregates are usually photodynamically inactive and do not fluoresce [11,12,16]. It has been shown that the fluorescence emission of carboxylated zinc-phthalocyanine originates exclusively from the monomeric species, i.e., dimers do not fluoresce [17]. The fluorescence excitation spectrum, which closely resembles action spectrum for cell inactivation, corresponds to the absorption spectrum of phthalocyanine monomers and it was suggested that aggregates of $AlPcS_4$ have a low photobiological activity in cells [18,19].

Under certain conditions fluorescent aggregates of some phthalocyanines, particularly of disulphonated ones, may be formed [15,16,20]. Contradicting reports have been published concerning aggregation of tetrasulphonated phthalocyanines. Yoon et al. [21] attributed a red-shift of absorption and emission spectra of phthalocyanines at high concentrations to the formation of dimers. Later, Dhami et al. [22] stated that this may be wrong and that the data may be explained by reabsorption of fluorescence by ground state molecules of phthalocyanine. Our work agrees with this conclusion of Dhami et al. who used ethanol and water as solvents. Since alcohols are known to favour monomerization of tetrapyrroles [23], we studied aqueous solutions of $AlPcS_4$. In our work a clear spectroscopic evidence of non-aggregated $AlPcS_4$ is presented. Phthalocyanines have large extinction coefficients and a high degree of spectral overlap between absorption and fluorescence spectra (Fig. 1). Calculations that take into account the effect of reabsorption of fluorescence fit, to a first approximation, with the experimental observations at low concentrations (Fig. 3). The disagreement between the calculations and the recorded spectra at higher concentrations (above 10^{-5} M) indicate that the reabsorbed light is reemitted as fluorescence (Fig. 3). Moreover, we provide clear spectroscopic evidence that fluorescence at a very high concentration (10^{-3} M) is practically similar to that of a very low concentration (10^{-8} M) by choosing a proper fluorescence registration setup (Fig. 2). In contrast with the findings of Dhami et al., who reported an increase of absorption at around 700–800 nm at high concentrations, we show that the absorption spectra of $AlPcS_4$ are similar for all concentrations studied (Fig. 1(a)). This shows that no aggregation products absorb in the visible range.

In conclusion, the presented results provide spectroscopic evidence that $AlPcS_4$ does not form aggregates in the visible spectral range and is a very stable compound over prolonged storage period in aqueous solutions. Since it is difficult to unambiguously extrapolate results obtained in aqueous solutions to cellular environment directly, further studies in cells in vitro and in tissues in vivo are of interest.

5. Abbreviations

$AlPcS_4$	aluminium phthalocyanine tetrasulphonate
O.D.	optical density
PBS	phosphate buffer saline
PDT	photodynamic therapy

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