

AGGREGATION OF ROSE BENGAL MOLECULES IN SOLUTION

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Summary

Absorption, emission and excitation spectral data support the thesis that rose bengal forms H-type aggregates in water and polar, protic solvents.

1. Background

Rose bengal is a xanthene "acid" dye which first appeared in Schultz's tables in 1881 [1 - 4] (acid dyes are those used to dye wool from an acidic bath). Its structure is shown in Fig. 1. The fundamental xanthene dyes fluorescein, eosin and erythrosin differ from rose bengal only in aromatic ring substitution. Fluorescein is substituted at positions 1 - 8 and 3' - 6' with hydrogens. Eosin has hydrogens at positions 3' - 6', but bromines at positions 2, 4, 5 and 7 while erythrosin is similar only with iodines at these positions [5]. The color and spectroscopy of the xanthene is almost entirely a function of xanthene ring substitution. Their acid/base indicator action derives from changes in the ionization state at C3. In a classic example of the heavy-atom effect, fluorescein which is unsubstituted fluoresces with very high quantum efficiency. Eosin, erythrosin and rose bengal intersystem cross to the triplet state after light absorption with a higher probability. Their

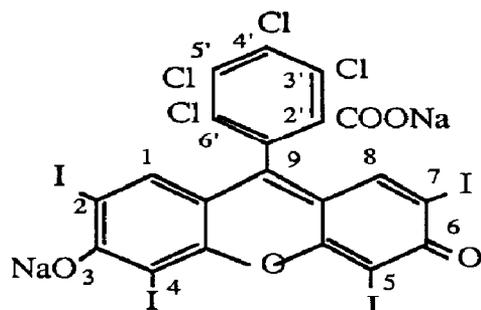


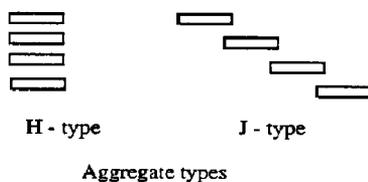
Fig. 1. The structure of rose bengal.

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excited state distribution shows a high population of triplet relative to singlet when compared with fluorescein, and hence they also show a lower quantum yield of fluorescence. It is the spectroscopic properties of the xanthenes which makes them of interest to the synthetic photoscientist. All of the spectroscopic properties and lifetimes depend on solvent and these have been discussed in detail in many other publications [6].

Because it is so strongly absorbing, rose bengal plays an active role in many areas of photochemistry. Its spectral properties are of interest because one can use them as a molecular probe to study chemical environment, for example in solution or within biological molecules.

Of most interest in the context of this study is the spectroscopy of rose bengal in concentrated solutions. On the basis of hydrophobic interactions many cationic dyes such as crystal violet and methylene blue give rise to much different absorption and emission spectra at high concentrations in water. The result, it is theorized, derives from the formation of aggregates the structure of which may be layers (H-type aggregates) or end-on slipped (J-type aggregates). The former are experimentally categorized because a blue shift derives at higher concentration. The latter give rise to a red shift.



Rose bengal is a bis anionic dye and it has been suggested that it also has a tendency to aggregate in solution. It does not follow Beer's law at concentrations above 10^{-5} M and the spectra are both of different shape and shifted at higher concentrations. Thus the aggregation phenomena of rose bengal and the other xanthenes have been previously studied in solution by means of absorption spectroscopy [7]. The absorption spectrum of rose bengal in dilute solution consists of two peaks separated by about 30 nm. In more concentrated solution the shorter wavelength of these peaks grows and shifts several nanometers toward the blue. We can now assign the longer-wavelength band exclusively to the monomer while the shorter-wavelength band derives from a combination of the monomer and the dimer respectively [8] and its size as well as its shape depend on concentration.

Because of its very high extinction coefficient, it is difficult to obtain an accurate absorption spectrum for rose bengal solutions at concentrations above 10^{-4} M. This greatly complicates the study of aggregation phenomena in cases where the spectra of the aggregates do not differ significantly from the spectrum of the monomer. This we have found to be the case with the xanthenes. As a result of spectral subtleness, aggregation phenomena of rose bengal have never been fully investigated, though both the formation of a dimer and multimers has been assumed to be possible by many workers.

Since it is well established that some of the xanthene dyes are self-quenchers at elevated concentration, concentration changes also cause significant changes in the observed fluorescence spectra. Previous workers, however, have improperly attributed artifactual spectroscopic observations to the dimer [9]. In fact, with fluorescein, concentration quenching completely alters the fluorescence spectrum and dramatically alters the observed emission. With rose bengal, self-quenching of the singlet by the ground state of another molecule of rose bengal is also observed. Therefore, great care is needed in interpreting the fluorescence spectra in order to distinguish the effect of aggregation from those which derive from such concentration quenching.

In this study we have undertaken detailed studies of the absorption and emission spectra of rose bengal in protic solvents and in aqueous solution as a function of pH. We are able for the first time to clarify the effects of aggregation on the emission and absorption spectra in this anionic dye system.

2. Results

2.1. Fluorescence distortion and concentration quenching

The fluorescence and absorption data of rose bengal in methanol at various concentrations are shown in Table 1. For a dilute solution (5.5×10^{-6} M), the maximum peak wavelengths in the fluorescence *excitation* spectra correspond to those observed in the absorption spectrum. With increasing concentration of the dye in methanol, the peaks in the absorption spectrum do not shift and neither do new peaks appear. The fluorescence excitation spectrum, however, undergoes a gradual change as a function of concentration. At a concentration of 2.87×10^{-4} M, the excitation peaks move to 427 nm and 464 nm. These are remarkably different from the absorption spectrum at the same concentration. The maximum emission wavelength shifts from 575 nm in dilute solution to 594 nm in the more concentrated solution.

TABLE 1

Fluorescence and absorption spectra of rose bengal in methanol at various concentrations

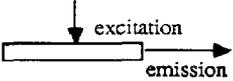
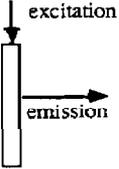
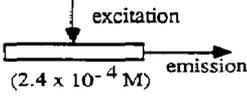
Concentration (M)	Excitation (nm)	Emission (nm)	Absorption (nm)
2.87×10^{-4}	427, 464	594	518, 557
4.88×10^{-5}	504, 528	584	518, 557
2.02×10^{-5}	518, 542	578	518, 557
1.23×10^{-5}	518, 550	575	518, 557
5.51×10^{-6}	518, 557	575	518, 557

Fluorescence spectra were taken in the right angle mode in a 1 cm square cell.

Using a rectangular cell (1 cm \times 0.2 cm) to observe the fluorescence, one can clarify the situation. This is shown in Table 2. When the path length of the incident radiation is 0.2 cm and that through which the emission is observed is 1 cm, the excitation spectrum of rose bengal solution at a concentration of 4.88×10^{-5} M is exactly the same as the absorption spectrum, or identical with the excitation spectrum at lower concentrations (5.51×10^{-6} M). However, the emission maximum is shifted toward longer wavelengths by 8 nm, compared with that at lower concentration. When the cell is turned 90° such that the incident path length was 1 cm and the path length of emission 0.2 cm, the excitation spectrum became quite different showing two peaks at 504 nm and 528 nm. The emission spectrum changes to an exact replica of that at lower concentration. These data provide unambiguous evidence for concentration quenching by the rose bengal solution [10 - 13].

TABLE 2

Fluorescence of rose bengal in methanol (4.88×10^{-5} M) as a function of path length

Measuring Mode	Excitation (nm)	Emission (nm)
1 	518,557	584
2 	504,528	576
3 	504,528	-----

2.2. Observations of the aggregation of rose bengal molecules in water

We obtained the authentic fluorescence spectra of rose bengal in water throughout the concentration range 10^{-3} - 10^{-6} M using front-face techniques. The wavelengths at which the two excitation peaks were observed always remained at the original position of the absorption maxima in water, i.e. 549 nm and 515 nm. However, the ratio of the intensities of the two peaks varied with concentration. This is definitively the result of the aggregation and deaggregation of rose bengal molecules in solution at higher concentration or lower concentration respectively. The composite spectra of aggregated rose bengal solution can be isolated into the spectrum of the monomer and of the dimer by means of a simple mathematical treatment [8].

The excitation spectrum of rose bengal consists of two peaks at 549 nm and 515 nm. The ratio I_1/I_2 (the ratio of I_1 , the intensity of peak at 549 nm, to I_2 , the intensity of peak at 515 nm) is related to the concentration of

monomer and dimer in the solution. I_1/I_2 drops as the concentration of rose bengal increases (see Figs. 2, 3, 4 and 5). This is because an equilibrium exists between monomer and dimer.

For solutions with pH values of 6.05 (Fig. 2), 7.0 (Fig. 3), 7.9 (Fig. 4), and 11.0 (Fig. 5), similar results were obtained. Thus one can rule out

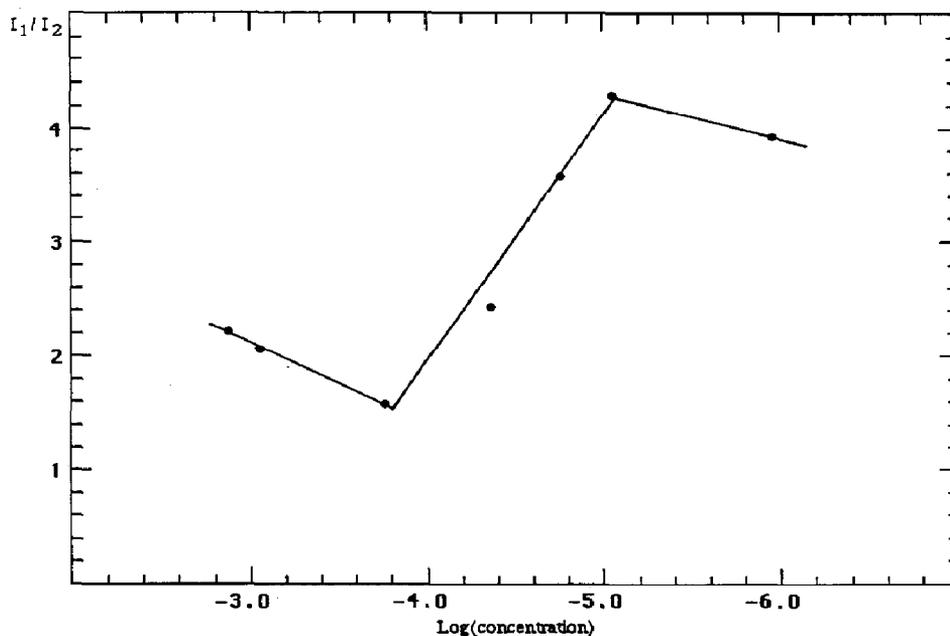


Fig. 2. Ratio of peak intensities I_1/I_2 as a function of concentration of rose bengal in water (pH 6.05).

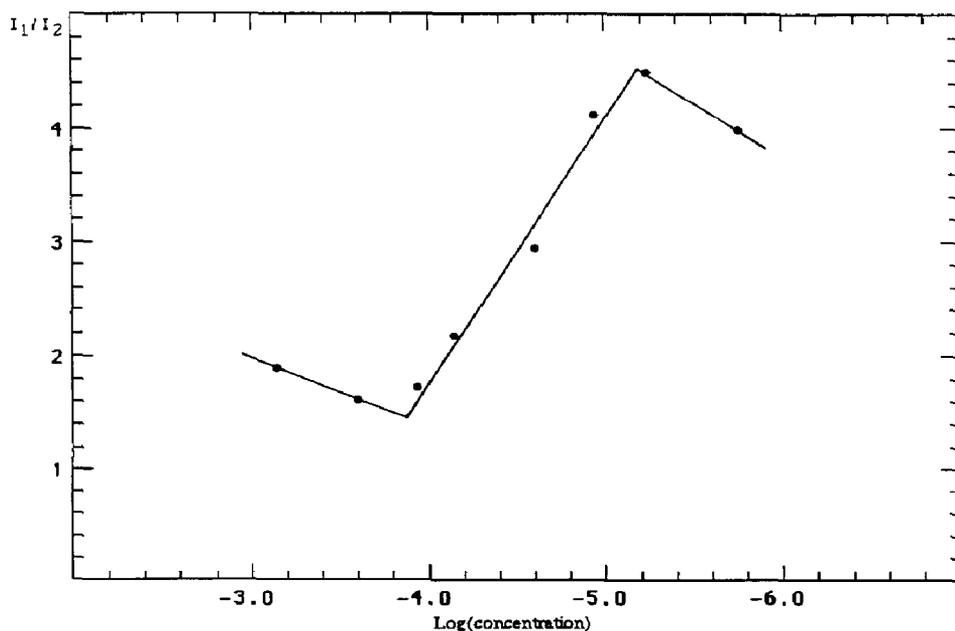


Fig. 3. Ratio of peak intensities I_1/I_2 as a function of concentration of rose bengal in water (pH 7.0).

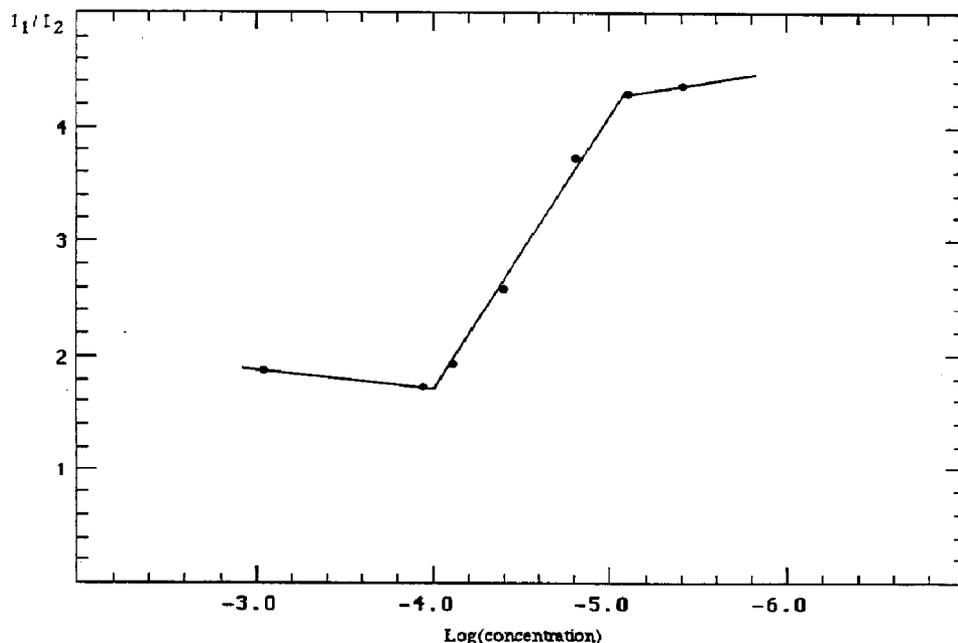


Fig. 4. Ratio of peak intensities I_1/I_2 as a function of concentration of rose bengal in water (pH 7.9).

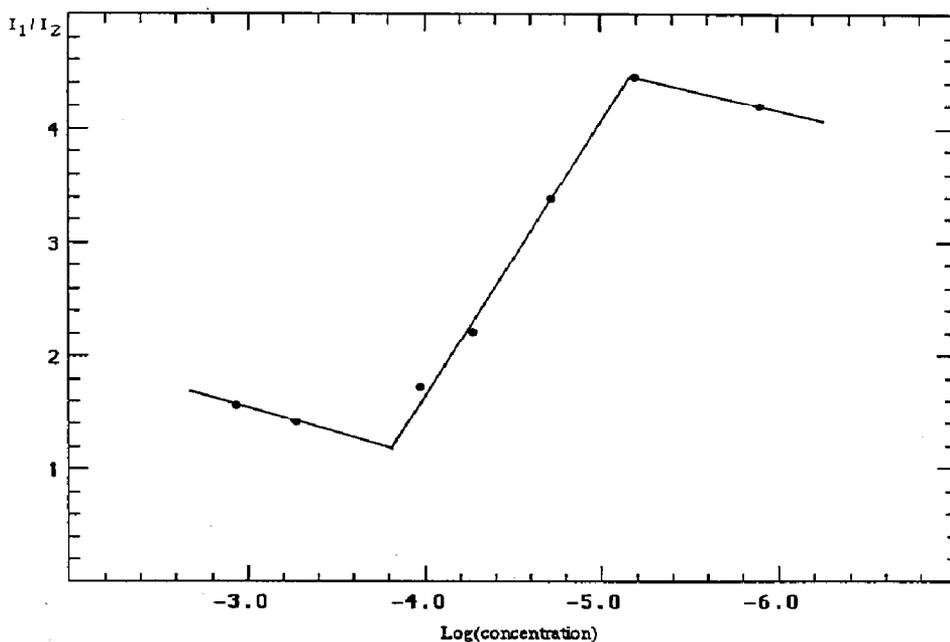


Fig. 5. Ratio of peak intensities I_1/I_2 as a function of concentration of rose bengal in water (pH 11).

contributions from zwitterion species or other kinds of isomers from individual rose bengal molecules to the spectrum [6] for these would clearly change in the pH range 6 - 11.

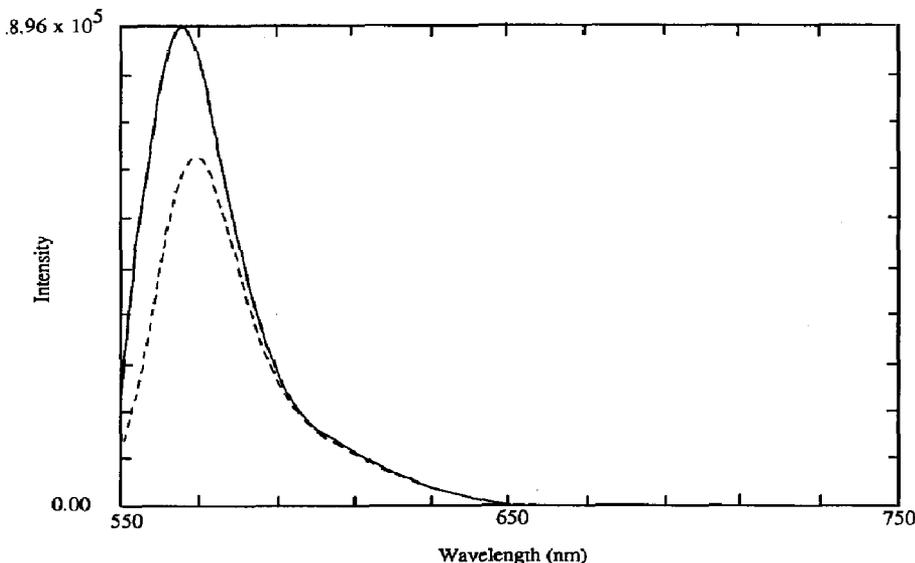
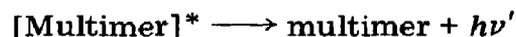


Fig. 6. Fluorescent emissions of rose bengal in methanol (5.35×10^{-4} M): —, excited at 549 nm, maximum 565 nm; ---, excited at 510 nm, maximum 570 nm.

The plot of I_1/I_2 can be divided into three regions. In region 1 ($C \geq 1.5 \times 10^{-4}$ M), the ratio I_1/I_2 increases as the concentration increases. However, the absolute intensity for each of the peaks goes down. The wavelengths of excitation into and emission from the dimer also show a small shift (from 515 nm to 510 nm; from 565 nm to 570 nm, Fig. 6). We attribute these wavelengths to the absorption of the multimer and emission from the multimer. Thus, the maximum absorption wavelength of the dimer is at about 510 nm, while its emission occurs at 570 nm. There is strong evidence that some higher level aggregation (trimer or multimer) begins to form in very high concentration ranges and as it does the equilibrium between monomer and dimer is disturbed. Plotting the ratio of the two peaks in the absorption spectrum of rose bengal *vs.* concentration, we obtain Fig. 7. In contrast to the ratio of I_1/I_2 as observed from the fluorescence spectrum which increases with the concentration, an inflection point is observed in the absorption spectrum around 1.5×10^{-4} M. Beyond this concentration the ratio of A_1/A_2 decreases. This indicates that the fluorescence quantum yield of the multimers is less than that of either dimer or monomer. The process resulting can be written as follows:



In region 2 (C between 1.5×10^{-4} and 6×10^{-6} M) of the A_1/A_2 *vs.* concentration plots, the absorption peaks show no shift. However, the ratio I_1/I_2 derived from the fluorescence spectrum rises as the concentration decreases. Exciting either peak 1 (549 nm) or peak 2 (515 nm) always

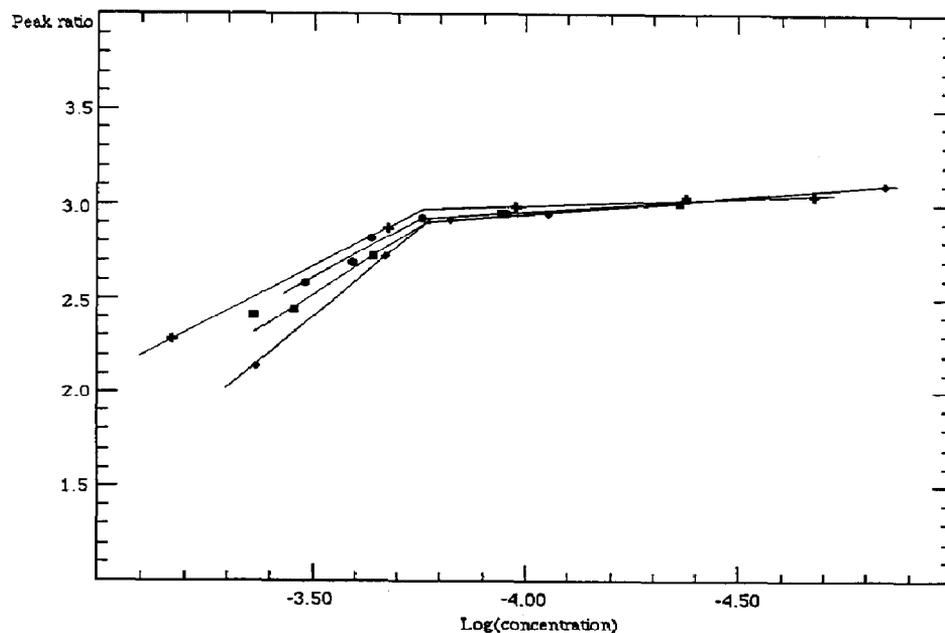
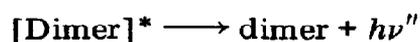
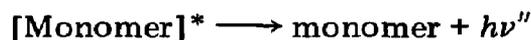


Fig. 7. Plot of absorption peak intensities ratio vs. concentration of rose bengal in water: ■, pH 11.9; ●, pH 8.0; +, pH 7.0; ◆, pH 6.05.

produces the same emission peak at 565 nm. This process can be modeled as follows:



For solutions having different pH values, we compute the inflection points from the I_1/I_2 values. These values and the values of the slopes do not differ significantly as a function of pH. Therefore it seems clear that pH has no effect on the aggregation of rose bengal in the pH region 6 - 11 (Table 3).

In concentration region 3 (below 6×10^{-6} M) of the I_1/I_2 plot, that is, the very low concentration region, surprisingly the I_1/I_2 ratio goes down. The

TABLE 3

Inflection points and slopes of aggregation plots

pH value	6.05	7.0	7.9	11.0
Slope	1.98×10^4	1.97×10^4	1.76×10^4	2.4×10^4
Inflection point	8.32×10^{-6}	7.41×10^{-6}	7.59×10^{-6}	9.55×10^{-6}
Concentration (M)	1.35×10^{-4}	1.48×10^{-4}	1.55×10^{-4}	9.77×10^{-5}

intensity of the peak at shorter wavelength becomes stronger. This is a false datum introduced by the front-face fluorescence spectrum observation method for very dilute solutions. At moderate concentrations, one irradiates and monitors the molecules on the exact front surface. The spectrum shows a true equilibrium of monomer and dimer in the front layer. When the concentration of the solution is very low, there are enough monomer molecules in the front layer to absorb incident light, but there are too few dimer molecules to absorb all the incident radiation at its wavelength (515 nm). Hence the 515 nm incident radiation will go into deeper layers of the solution, where it reaches dimers. The result is that the dimer concentration seems to be increased. Thus even front-face observation techniques should be used cautiously when studying the effect of concentration on dye emission spectra.

Based on the information above, we conclude that rose bengal aggregates are of the H type and that they begin to form in solutions of water or methanol at concentrations above 2.0×10^{-6} M. H-type aggregates are layered aggregates and they absorb at shorter wavelengths while emitting at longer wavelengths than the monomer. The rose bengal dimer absorbs at about 515 nm while fluorescing at about 570 nm. Above concentrations of 10^{-4} M the dimers accumulate even more rose bengal molecules into higher aggregates. These affect the excitation and emission spectra.

We have also observed the formation of aggregate structures from rose bengal polymers in dilute hydrocarbon solvents. The driving force for their formation is photochemical, however, and these are still under investigation [14].

3. Experimental details

Rose bengal (92%) in methanol (spectrophotometric grade) and water treated with a Nanopure device were used in all experiments. Absorption spectra were obtained using a Varian Cary 219 instrument in 1 cm, 0.1 cm and 0.01 cm cells. Fluorescence spectra were measured with a SPEX Fluorog. The slit width for the excitation spectrometer was set at 1 mm, which yields a bandpass of 1.8 nm, while the slit width for the emission spectrometer was set at 5 mm, which yields a bandpass of 9 nm. The acquisition mode 7 was used to correct the spectrum for the light source. Phosphate solutions were used throughout. The pH values of the solutions were measured with an Orion Research Ionalyzer/501 meter. For solutions having pH value 7.9, no buffer was used. Rose bengal dissolved in pure water gives a pH value of 7.9.

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