# Role of the Medium on the C343 Inter/Intramolecular Hydrogen Bond Interactions. An Absorption, Emission, and $^1$HNMR Investigation of C343 in Benzene/*n*-Heptane Mixtures

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C343, a common molecular probe utilized in solvation dynamics experiments, was studied in homogeneous media. Absorption, emission, and $^1$HNMR spectroscopies were used to investigate the behavior of C343 in benzene and in benzene/*n*-heptane mixtures. We demonstrate the implications of the medium polarity, measured as the Kamlet−Taft polarity-polarizability ($\pi^*$) parameter, in the C343 inter/intramolecular hydrogen bond (H-bond) interactions and the role that this interaction plays in the dimerization process of the dye. In pure benzene, the dimer prevails because the intermolecular H-bond interaction is favored. On the other hand, as the *n*-heptane content increases the intramolecular H-bond is the strongest and the C343 monomer is favored. As the polarity of the medium decreases, the solvophobic interaction makes that C343 monomer species experiences a more complicated aggregation process beyond the simple monomer dimer equilibrium present in pure benzene. Thus, the addition of *n*-heptane to the mixture yields a C343 higher-order aggregates species. Thus, our work reveals the importance that the medium has on the behavior of a widespread dye used as chromophore for very different systems such as homogeneous and microheterogenous media. This is very important since the use of chromophores without understanding its chemistry can induce artifacts into the interpretation of solvation dynamics in heterogeneous environments, in particular, those provided by biological systems such as proteins. Considerable care in choosing and characterizing the system is required to analyze the results fully.

## Introduction

The 7-aminocoumarins, like Coumarin 343, C343 (Scheme 1), are strong chromophores that are used in a broad range of applications such as laser dyes[1] and probing the dynamics of condensed-phase environments like solutions and in more-complex environments like proteins and organized systems.[2−9] Basically, the properties that cause the 7-aminocoumarines to be effective for those purposes are (1) they are rigid, (2) they have strong radiative rates, and (3) they possess strong solvatochromism.[10]

It is already known that the absorption and emission spectroscopy of C343 depends strongly on its environment.[3,11−16] The similarities in the structure of C343 to other probes used in solvation dynamics, such as C153 and C102, has led to its use for aqueous environments where the former probes are insoluble.[17] Because the steady-state spectra shift with changing solvent dielectric constant, C343 spectral shifts have been used to measure dipolar relaxation.[12,16] It is known that the polarity word is not always properly defined and this can lead to various interpretation.[18] Nevertheless, solvent polarity and solute−solvent interactions are two of the most important factors that control the rates of chemical reaction.[18,19] The solvent effects on physical or chemical processes are frequently studied by empirical solvent parameters to determine the predominant interactions. One of the most useful approaches for elucidating and quantifying different solute−solvent interactions is the Kamlet−Taft solvatochromic comparison method (KTSCM).[20] This method quantifies solute−solvent interactions from absorption and emission bands showing that the solvatochromic behavior of molecular probes can depend not only on the polarity of the medium (nonspecific interactions) but also on specific interactions such as the H-bonding properties of the solvents. Thus, the nonspecific and specific solvents interactions are defined by the following empirical solvent parameters: the polarity-polarizability ($\pi^*$), the H-bond acceptor ability of the solvents ($\beta$), and the H-bond donor ability of the media ($\alpha$).[20]

We have demonstrated[21] that polarity ($\pi^*$) alone cannot account for the solvatochromic behavior of C343 and that specific interactions play an important role in the C343 spectroscopy. Thus, we found that the carboxylic acid group of C343 causes that the molecule displays greater sensitivity to the H-bond acceptance ability ($\beta$) than to the $\pi^*$ value of the medium. Also, this sensitivity increases in the excited state. This challenges the general interpretation of the solvatochromic behavior of the C343 that primarily reflects the polarity of the medium.[3,4,11,16,17]

The fact that C343 is more sensitive to the H-bond acceptor ability of the solvent upon excitation suggests that the intramolecular H-bond between the carboxylic and the ester group (see Scheme 1) weakens in the excited state. In a series of density functional theory (DFT) calculations, Cave et al.[10] have shown that in the ground state C343 forms an intramolecular H-bond between the hydrogen of the carboxyl group and the nearby carbonyl group while its excited state exists without such H-bond. Also, the C343 crystal structure shows that the carboxylic acid proton resides in close proximity to the ester carbonyl interacting through H-bonding.[22] Cave et al.[10] suggest that the ground-state dipole moments are quite different in the various C343 possible structures and thus should yield different solvent stabilization energies, but introducing in the calculations

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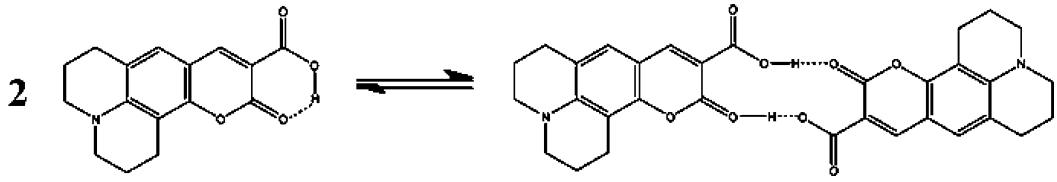
**SCHEME 1: C343 Monomer−Dimer Equilibrium**



**C343 Monomer** **C343 Dimer**

C343-solvent interactions neither reorders nor dramatically alters the energy difference between the two lowest-energy structures of C343: (i) the hydrogen is on the carboxyl group forming an intramolecular H-bond with the nearby carbonyl group and (ii) the H-bond is broken by rotating the hydroxyl bond torsional angle 180°. Also, Cave et al.[10] have calculated the C343 ground-state dipole moment and the value estimated was too high in comparison with the experimental value found in chloroform.[23] They have attributed the differences to the possibility that nonplanar conformations are adopted by the carboxyl group in solution. On the other hand, a close inspection of the work reveals that the ground-state dipole moment of the C343 is different depending on the ground-state conformation. Interesting is that the conformation with no intramolecular H-bond interaction has lower dipole moment value than the one with H-bond interaction. Moreover, the nonintramolecular H-bond conformation dipole moment value is very close to the experimental value obtained in chloroform.[23] Thus, it seems that the medium should have impact on the inter/intramolecular H-bond interaction.

On the other hand, other results[21] suggest that, due to its very low solubility in cyclohexane, C343 forms aggregates with nondefined stoichiometry in nonpolar solvents or solvents with low polarity and H-bond acceptance ability. Thus, it seems that the medium also has a remarkable impact on C343 aggregation process.

Frequently and because C343 has sensitivity to specific properties of the systems, it has been used to learn about the structure of different organized media such as reverse micelles, which are aggregates of surfactants formed in a nonpolar solvent.[2−6,21,24−27] The nonpolar organic pseudophase commonly used in reverse micelles are saturated hydrocarbons such as *n*-heptane and isooctane[24] where the C343 low solubility leads it to be located at micelle interfaces or interior avoiding problems of partitioning. On the other hand, aromatic solvents such as benzene and toluene can also be used to create the reverse micelles media where the C343 solubility is higher than in saturated hydrocarbons.[24] As it was previously discussed the C343 inter/intramolecular H-bond interactions make it a tricky molecule and it is interesting and necessary to clearly understand the behavior of C343 in the pure solvents before it would be used as molecular probe in such complex systems.

In summary, although C343 is a very interesting and exciting molecule used as chromophore, there are critical aspect of its behavior that are not well understood. For example, it seems to us that it is crucial to understand the role of the solvent on the C343 H-bond interaction. In this way, since the nonpolar organic pseudophases required to create reverse micelles systems have very low $\beta$ values parameter, the $\pi^*$ solvent parameters should be considered to understand the C343 solvatochromic behavior. Thus, it will be interesting to investigate the dye behavior changing the $\pi^*$ parameter value of the solvent.

In this work, we perform a detailed investigation about the effect that the medium has on the C343 inter/intramolecular H-bond interaction. Thus, we have used absorption, emission, and $^1$HNMR spectroscopies to investigate the behavior of C343 dissolved in benzene and in different benzene/*n*-heptane mixtures. The results show that C343 exhibits strong intermolecular H-bond interaction in pure benzene ($\pi^* = 0.59$)[28] and only the dimer species is detected (Scheme 1). On the other hand, as the *n*-heptane ($\pi^* = -0.08$)[28] content increases, the intramolecular H-bond interaction (Scheme 1) prevails and now, besides the dimer species, the C343 monomer species can also be detected. Moreover, as the *n*-heptane content in the mixture further increases, the C343 solubility decreases and, for this reason, C343 monomer species start to form higher-order aggregates beyond the dimer species present in pure benzene. These higher-order aggregate species were previously suggested for C343 dissolved in cyclohexane, solvent similar to *n*-heptane, based in the absence of a clear isosbestic point in the C343 absorption spectra at different dye concentrations.[21]

## Materials and Methods

Benzene and *n*-heptane (HPLC grade, Aldrich) and $d_6$-benzene (Aldrich) were used as received. Coumarin 343 (C343, Exciton) was used without further purification.

The absorption spectra were measured by using Shimadzu 2401 equipment at 25 °C unless otherwise indicated. A Spex fluoromax apparatus was employed for the fluorescent measurements. Corrected fluorescence spectra were obtained using the correction file provided by the manufacturer. The path length used in the absorption and emission experiments was 1 cm. Because the absorption and emission bands are broad the $\lambda_{max}$ was measured by taking the midpoint between the two positions of the spectrum where the absorbance is equal to $0.9 \times A_{max}$. Thus, the uncertainties in $\lambda_{max}$ are about 0.1 nm.

Fluorescence decay data were measured with the time correlated single photon counting technique (TCSPC) (Edinburgh Instrument FL-900) with a PicoQuant subnanosecond Pulsed LED PLS 370 (emitting at 370 nm) < 600 ps fwhm. Fluctuations in the pulse and intensity were corrected by making an alternate collection of scattering and sample emission. The quality of the fits was determined by the reduced $\chi^2$, and for the best fit $\chi^2$ must be around 1.0.[29] It must be noted that in any exponential fit (mono or bi) we have tried to perform multi- or monoexponential fitting but the statistic of the decays were not improved or became worse.

For the $^1$HNMR experiments, a Bruker 400 NMR spectrometer was used. The spectra were recorded at a digital resolution of 0.02 Hz/data point. The spectrometer probe temperature, 25 °C, was periodically monitored by measuring the chemical shift difference between the two singlets of a methanol reference sample. Chemical shifts were measured relative to internal TMS.

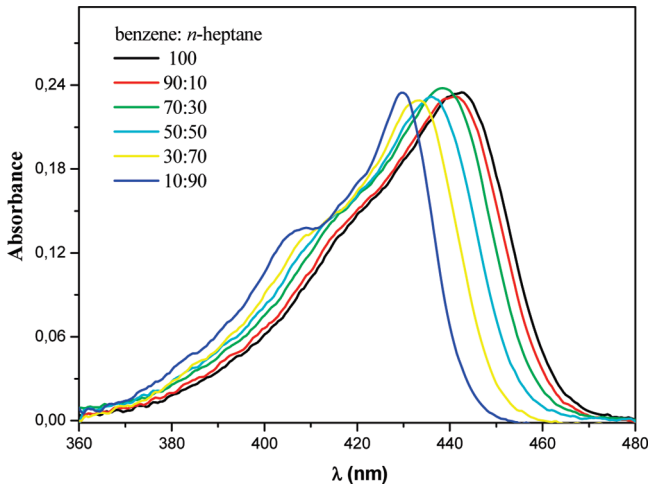



**Figure 1.** Absorption spectra of C343 in different benzene/*n*-heptane mixtures. [C343] = $6 \times 10^{-6}$ M.

## Results and Discussions

**Absorption and Emission Studies.** The absorption spectra of C343 were measured in different benzene/*n*-heptane mixtures and typical results are shown in Figure 1. In Table 1, the different $\pi^*$ values for the mixtures are gathered. As it can be observed, the absorption band in pure benzene shows a maxima at $\lambda = 442$ nm with a shoulder at $\lambda = 418$ nm. As the amount of *n*-heptane (solvent in which the dye is practically insoluble, $\pi^* = -0.08$, $\alpha = \beta = 0.00$)[28] increases and the polarity of the medium decreases (Table 1), the main band shifts hypsochromically to $\lambda = 430$ nm and the shoulder at 418 nm develops into a band that peaks at $\lambda = 408$ nm for the 10:90 benzene/*n*-heptane mixture ($\pi^* = 0.02$. See Table 1).

Figure 2 shows the C343 emission spectra in different benzene/*n*-heptane ratios at $\lambda_{exc} = 372$ nm. As it can be observed in pure benzene the emission band peaks at $\lambda = 462$ nm independently of the excitation wavelength (not shown). As the *n*-heptane content increases, the band shifts hypsochromically to $\lambda = 440$ nm and its intensity increases. Also, a shoulder emerges at $\lambda = 463$ nm at 10:90 benzene/*n*-heptane mixture.

To explain our results, we will briefly review some of the data previously found for C343 in cyclohexane[21] ($\pi^* = 0.00$),[28] solvent that we will consider similar to *n*-heptane at least in the empirical parameters value. Previous spectroscopic studies performed in cyclohexane reveals that due to the low solubility of C343 the dye forms aggregates with no defined stoichiometry.[21] It seems that nonspecific interactions (solvophobic interaction) play a key role to make that C343 aggregation process goes beyond the simple monomer−dimer equilibrium yielding higher-order aggregates. This conclusion was based in the absence of a clear isosbestic point in the C343 absorption spectra varying the dye concentration. The spectroscopic data showed that in cyclohexane the monomer species band absorbs at 405 nm and emits at 435 nm, while the higher-order aggregates species absorption band peaks at 425 nm and emits at 460 nm with low emission quantum yield in comparison with the monomer emission quantum yield.[21] Because C343 absorption spectrum appeared at lower energy relative to the monomer, we concluded that C343 forms J-aggregates,[30,31] that is, aggregates with dye molecules arranged head-to-tail in a slanted stack.[30,31] Emission at lower energy relative to the monomer with lower emission quantum yield has also been reported for J-aggregates.[32−35]

Here in this work, we will discuss first the results in pure benzene ($\pi^* = 0.59$, $\alpha = 0.00$, and $\beta = 0.10$).[28] The C343 solubility in this solvent is measurably higher than in cyclohexane ($\pi^* = 0.00$, $\beta = 0.00$)[28] and *n*-heptane because the solvophobic interaction diminishes as consequence of the higher medium polarity. Moreover, neither in benzene nor in *n*-heptane can the C343 COOH group (Scheme 1) interact with the solvents through H bond interaction due to the very low $\beta$ parameter value. The fact that the emission spectrum is excitation wavelength independent suggests that we can only detect one C343 species in benzene.[36] As the C343 absorption and emission maxima values in benzene (Figure 1 and 2) do not match either the monomer nor the higher-order aggregate (in cyclohexane) absorption and emission maxima values,[21] we assume that the major C343 species in benzene is a dimer species because the inter H-bond association through the COOH group (See Scheme 1) is favored.

To understand the effect that the medium polarity (changed with the *n*-heptane addition) has on the C343 interactions with the environment, the following has to be taken into account: the monomer−dimer equilibrium (Scheme 1) shifts to the dimer species if the intermolecular H-bond interaction is stronger than the intramolecular one, while the opposite stands if the intramolecular H-bond interaction is the strongest. Thus, as the *n*-heptane content increases the changes shown in Figure 1 and 2 can be explained considering that the following C343 species are present: (i) C343 monomer species ($\lambda_{abs} = 408$ nm and $\lambda_{em} = 440$ nm); (ii) C343 dimer species ($\lambda_{abs} = 430$ nm and $\lambda_{em} = 463$ nm) and (iii) the C343 higher-order aggregate species ($\lambda_{abs} = 425$ nm and $\lambda_{em} = 460$ nm). Thus, as the polarity of the medium decreases the intramolecular H-bond interaction prevails and the equilibrium shift to the monomer species (Scheme 1) which, because the low solubility in the medium forms higher-order aggregates as it was discussed.

Because of the complexity of the C343 behavior in this solvent mixture and because all of the C343 species can be present showing absorption and emission intensity at any of the *n*-heptane content, it is not surprising that Figure 1 shows no dramatic absorbance changes. On the other hand, it is well-known that monomer species has very high emission quantum yield values in comparison with the aggregated species ones.[36,37] Thus, it is not unexpected that Figure 2 shows a remarkable increases in the emission intensity where the C343 monomer species emits ($\lambda_{em} = 440$ nm), as the *n*-heptane content increases.

We also explored C343 dimerization process using time-resolved emission spectroscopy (TCSPC). Please note the higher-order aggregates emission lifetime was previously characterized.[21] TCSPC measurements were performed for C343 in benzene/*n*-heptane mixtures at one excitation wavelength and at the two different emission wavelengths that correspond to the monomer ($\lambda_{em} = 440$ nm) and the dimer species ($\lambda = 470$ nm) as given in Table 1. At high benzene content, where the dimer dominates the absorption spectrum, the fluorescence decay fits well to a single exponential decay with a time constant corresponding to the fluorescence lifetime of the dimer $\tau_1 = 3.60 \pm 0.04$ ns. Probing at the highest *n*-heptane content with excitation into the high energy feature where the contribution from the monomer should be higher, the fluorescence exhibits a biexponential decay with a $\tau_1$ corresponding to the dimer, and a second lifetime, $\tau_2 = 1.90 \pm 0.02$ ns, that we assign to the monomer species. Also, note that the main contribution to the emission decays is always from the dimer species, even at the highest *n*-heptane content monitored. These results agree with literature reports of aggregate lifetimes that are longer than the monomer.[21,34,37]

**TABLE 1: Lifetimes for Coumarin 343 (C343) in Different Benzene/*n*-Heptane Ratios**

| | | $\lambda_{exc}$ = 370 nm | | | |
|---|---|---|---|---|---|
| benzene/*n*-heptane ratios | $\pi^*$ | $\lambda_{em}$ = 440 nm $\tau$/ns | $\chi^2$ | $\lambda_{em}$ = 470 nm $\tau$/ns | $\chi^2$ |
| 100 | 0.59[a] | 3.60 ± 0.04 | 1.0 | 3.60 ± 0.04 | 1.0 |
| 90:10 | 0.55[b] | 3.20 ± 0.06 | 1.2 | 3.20 ± 0.05 | 1.2 |
| 10:90 | 0.02[b] | 3.20 ± 0.02 (80%)<br>1.90 ± 0.02 (20%) | 1.1 | 3.12 ± 0.02 (85%)<br>1.90 ± 0.02 (15%) | 1.2 |

[a] From reference 28. [b] $\pi^* = X_{hp}\pi^*_{hp} + X_{bz}\pi^*_{bz}$ where $X_{hp}$ and $X_{bz}$ are the molar fraction of *n*-heptane and benzene, respectively.

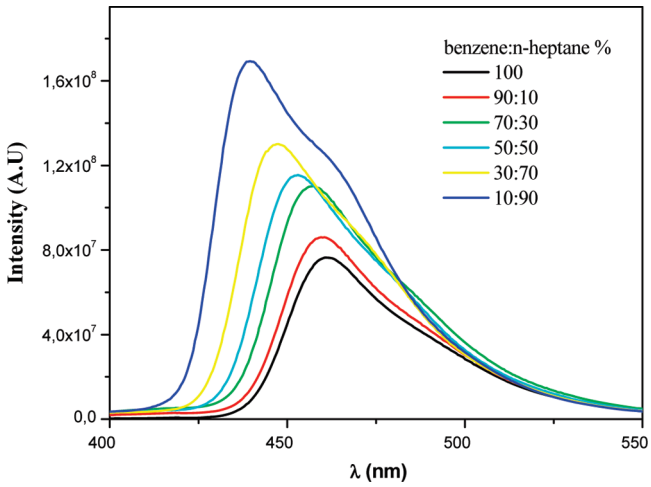



**Figure 2.** Emission spectra of C343 in different benzene/*n*-heptane mixtures. [C343] = 6 × $10^{-6}$ M. $\lambda_{exc}$ = 372 nm.

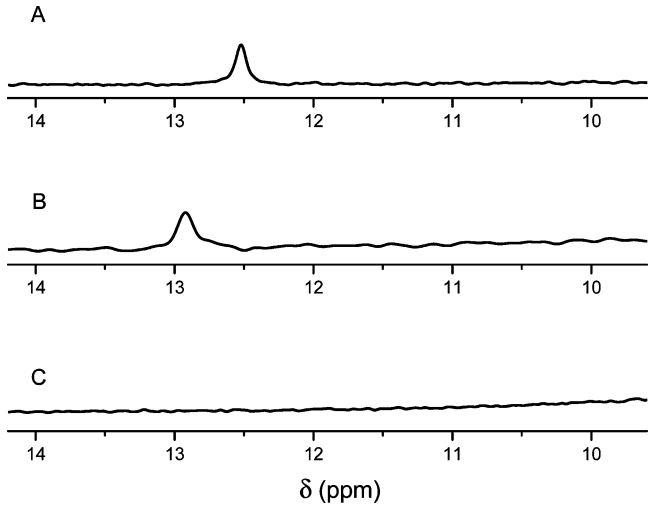



**Figure 3.** [1]HNMR signal of the COOH group of C343 in (A) chloroform; (B) benzene; (C) benzene/*n*-heptane 10:90.

**[1]HNMR Studies.** To gain more insight about the C343 inter/intramolecular H- bond competition and to understand how the solvent polarity affect these interactions, we measure the C343 [1]HNMR spectra in different solvents and in different benzene/*n*-heptane mixtures. Figure 3A−C shows the [1]HNMR spectra obtained for C343 in chloroform, benzene, and in 10:90 benzene/*n*-heptane mixture, respectively, in the region of the COOH resonance signal. As it can be observed, the COOH signal appear in chloroform and in benzene but is absent in the benzene/*n*-heptane mixture. It is known that H-bonding shifts the resonance signal of a proton to lower field. In addition, for carboxylic acids the favored H-bonded dimeric association makes that the carboxylic proton displays a broad resonance signal in the range of $\delta$ = 10.0 to 13.0.[38] On the other hand, intramolecular H-bonds, especially those defining a six-membered ring (C343 monomer species), generally display a very low-field proton resonance.[38,39] Table 2 shows the COOH chemical shift as the *n*-heptane content increases in the benzene/*n*-heptane mixture. As it can be seen, there is a downfield shifting of the signal as the polarity of the medium decreases. The same conclusion is obtained comparing the C343 [1]HNMR spectra in chloroform and benzene (Figure 3).

**TABLE 2: [1]HNMR Chemical Shifts for the COOH Group of C343 in Benzene/*n*-Heptane Mixtures[a]**

| benzene/*n*-heptane | $\delta$/ppm |
|---|---|
| 100:0 | 12.6 |
| 90:10 | 12.6 |
| 70:30 | 12.8 |
| 50:50 | 12.9 |
| 30:70 | 13.1 |
| 10:90 | ND |

[a] ND = not detected.

From the data shown in Table 2 it can be deduced that the equilibrium shown in Scheme 1 shifts toward the monomer as the *n*-heptane content increases, because the intramolecular H-bond prevails over the intermolecular H-bond. Interesting, in the 10:90 benzene/*n*-heptane mixture the carboxylic proton signal is not detected in our equipment. As it was previously discussed, when the *n*-heptane content is high C343 higher-order aggregates species are present due to the solvophobic interaction. This aggregation process probably makes the COOH NMR signal so broad that it cannot be detected in our equipment.

In summary, we demonstrate the importance of the medium polarity in the C343 inters/intramolecular H-bond interaction and the role that this interaction plays in the dimerization process of the dye. In pure benzene, the dimer prevails because the inter H-bond interaction is favored. On the other hand, as the polarity of the medium decreases the intramolecular H-bond is the strongest interaction and the C343 monomer is favored.

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## References and Notes

(1) Schäfer, E. P. *Dye Lasers*, 3rd ed.; Springer-Verlag: Berlin, 1990.
(2) Riter, R. E.; Undiks, E. P.; Kimmel, J. R.; Levinger, N. E. *J. Phys. Chem B* **1998**, *102*, 7931.
(3) Riter, E. R.; Kimmel, J. R.; Undiks, E. P.; Levinger, N. E. *J. Phys. Chem. B* **1997**, *101*, 8292.
(4) Riter, R. E.; Undiks, E. P.; Levinger, N. E. *J. Am. Chem. Soc.* **1998**, *120*, 6062.

(5) Willard, D. M.; Riter, R. E.; Levinger, N. E. *J. Am. Chem. Soc.* **1998**, *120*, 4151.
(6) Singh, P. K.; Kumbhakar, M.; Pal, H.; Nath, S. *J. Phys. Chem. B* **2009**, *113*, 1353.
(7) Denat, F.; Diaz-Fernandez, Y. A.; Pasotti, L.; Sok, N.; Pallavicini, P. *Chem.—Eur. J.* **2009**, *16*, 1289.
(8) Singh, P. K.; Kumbhakar, M.; Ganguly, R.; Aswal, V. K.; Pal, H.; Nath, S. *J. Phys. Chem. B* **2010**, *114*, 3818.
(9) Riter, R. E.; Willard, D. M.; Levinger, N. E. *J. Phys. Chem. B* **1998**, *102*, 2705.
(10) Cave, J. R.; Castner, E. W., Jr. *J. Phys. Chem. A* **2002**, *106*, 12117.
(11) Drexhage, K. H.; Eikson, G. R.; Hawks, G. H.; Reynolds, G. A. *Opt. Commun.* **1975**, *15*, 399.
(12) Pant, D.; Levinger, N. E. *Langmuir* **2000**, *16*, 10123.
(13) Corbeil, E. M.; Levinger, N. E. *Langmuir* **2003**, *19*, 7264.
(14) Pant, D.; Girault, H. H. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3457.
(15) Pant, D.; Le Guennec, M.; Illien, B.; Girault, H. H. *Phys. Chem. Chem. Phys.* **2004**, *6*, 3140.
(16) Yamasaki, T.; Kajimoto, O.; Hara, K. *J. Photochem. Photobiol. A* **2003**, *156*, 145.
(17) Jimenez, R.; Fleming, G. R.; Kumar, P. V.; Marroncelli, M. *Nature* **1994**, *369*, 471.
(18) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 3rd ed.; V.C.H: Germany, 2003.
(19) Dutt, G. B.; Ghanty, T. K. *J. Phys. Chem. B* **2003**, *107*, 3257.
(20) Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R. W. J. *J. Org. Chem.* **1983**, *48*, 2877.
(21) Correa, N. M.; Levinger, N. E. *J. Phys. Chem. B* **2006**, *110*, 13050.
(22) Honda, T.; Fujii, I.; Hirayama, N.; Aoyama, N.; Miike, A. *Acta Crystallogr., Sect. C* **1996**, *C52*, 679.
(23) Moylan, C. R. *J. Phys. Chem.* **1994**, *98*, 13513.
(24) Yamasaki, T.; Kajimoto, O.; Hara, K. *J. Photochem. Photobiol. A* **2003**, *156*, 145.
(25) Imai, A. U. M.; Suzuki, I. *Colloid Surf., A.* **2008**, *324*, 79.
(26) Satpati, A. K.; Kumbhakar, M.; Nath, S.; Pal, H. *ChemPhysChem* **2009**, *10*, 2966.
(27) Silber, J. J.; Biasutti, M. A.; Abuin, E.; Lissi, E. *Adv. Colloid Interface Sci.* **1999**, *82*, 189.
(28) Marcus, Y. *Chem. Soc. Rev.* **1993**, 409.
(29) O'Connor, D. V.; Phillips, D. *Time-Correlated Single Photon Counting*; Academic Press: New York, 1983; Chapter 6.
(30) Kasha, M.; Rawls, H. R.; El-Bayoumi, M. *Pure Appl. Chem.* **1965**, *11*, 371.
(31) McRae, E. G.; Kasha, M. *Physical Processes in Radiation Biology*; Academic Press: New York, 1964; p 23.
(32) Wilkinson, F.; Worrall, D. R.; Vieira Ferreira, L. F. *Spectrochim. Acta* **1992**, *48 A* (2), 135.
(33) Ban, T.; Kasatani, K.; Kawasaki, M.; Sato, H. *Photochem. Photobiol.* **1983**, *37* (2), 131.
(34) Falcone, R. D.; Correa, N. M.; Biasutti, M. A.; Silber, J. J. *Langmuir* **2002**, *18*, 2039.
(35) Togashi, D. M.; Costa, S. M. B.; Sobral, A. J. F. N.; Gonsalves, A. M. d'A. R. *J. Phys. Chem. B* **2004**, *108*, 11344.
(36) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, 2006.
(37) Barltrop, J. A.; Coyle, J. D. *Principles of Photochemistry*; Wiley & Sons: New York, 1978; Chapter 2.
(38) Silverstein, R. M.; Webster, F. X.; Kiemle, D. J. *Spectrometric Identification of Organic Compounds*, 7th ed.; J. Wiley: New York, 2005.
(39) Jansma, A.; Zhang, Q.; Li, B.; Ding, Q.; Uno, R.; Bursulaya, B.; Liu, Y.; Furet, P.; Gray, S. N.; Geierstanger, B. H. *J. Med. Chem.* **2007**, *50*, 5875.

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