Detailed Picosecond Kerr-Gated Time-Resolved Resonance Raman Spectroscopy and Time-Resolved Emission Studies of Merocyanine 540 in Various Solvents

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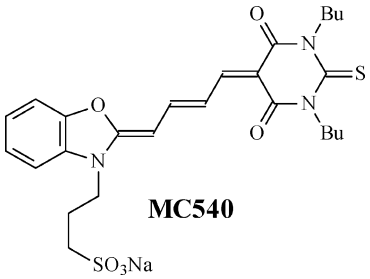
By using the unique fluorescence rejection method of Kerr-gating, detailed picosecond time-resolved resonance Raman experiments have been performed on the highly fluorescent photodynamic therapy dye, Merocyanine 540 (MC540). This has enabled collection of the first-singlet trans excited resonance Raman spectra of this dye in a range of protic and aprotic solvents of varying viscosity and polarity, as well as an organized reverse micelle. The detailed vibrational spectra support the idea that protic solvents form a H-bonded cluster around the oxygen groups of the thiobarbiturate group, which lock the group in position and hinder its rotation. In the reverse micelle containing hexane/AOT and a water pool ($\omega_o = 32$) the dye is orientated to permit the thiobarbiturate group to interact with water pool molecules. Using the Kerr-gate setup, time-resolved emission spectra of MC540 were also recorded in various solvents. The dye undergoes fast vibrational cooling (2−10 ps), which can be related to the solvent's thermal diffusivity. A second slower process (20−100 ps) also occurs, which is viscosity dependent and is associated with structural relaxation of the polymethine unit within the dye.

1. Introduction

The anionic and polymethine dye Merocyanine 540 (MC540) has found numerous applications in the biological field due to its ability to bind to biomembranes and proteins.[1] Although



MC540

originally developed as a potentiometric biological probe in the staining of various cellular membranes,[2] the discovery of MC540's phototoxicity has resulted in its use as a photosensitizer for the purging of leukemic cells from autologous remission bone marrow grafts,[3] preclinical sterilization of blood products,[4] and cell eradication of various cancer cell lines.[5]

Over the last twenty years numerous photophysical studies have been performed on MC540 and its derivatives to obtain a clear picture of what is occurring following photoexcitation.[6] Spectroscopic techniques including time-resolved transient absorption spectroscopy,[7] steady-state fluorescence anisotropy measurements,[8] and more recently fluorescence correlation spectroscopy[9] have afforded invaluable kinetic information on

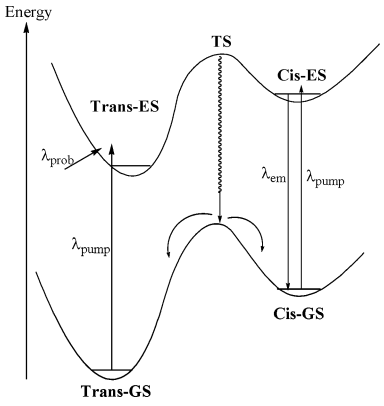


Figure 1. Simplified potential-energy diagram proposed to account for light-induced and thermal isomerization steps in MC540. Also shown is a basic view of how the time-resolved experiments were carried out.

lifetimes of excited states and rates of light-activated and thermal structural alterations that occur within the dye. It is now well established that the dye adopts an all-trans arrangement in the ground state[10] and, upon promotion to the first-excited singlet state, isomerizes via a twisted state (TS) to create an unstable isomer (Figure 1). There is some evidence to support that isomerization occurs at the first double bond,[11] though the central double bond is generally regarded as the site for twisting in creation of a cis isomer.[12] Vibrational spectra, however, are lacking for the ground and more importantly excited state of MC540, which could shed more light onto what structural alterations occur following excitation. Such information can play a key role in identifying how MC540 interacts with its surroundings, particularly in a more organized medium remi-

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niscent of a cell. Picosecond time-resolved resonance Raman (TR^3) spectroscopy is a powerful tool for probing the excited-state structure of molecules and has been successfully applied to probing solute−solvent interactions.[13] One problem with the technique is the need to have nonemissive samples, but a recent breakthrough using optical Kerr-gating to reject fluorescence has opened up the possibility of obtaining high-quality Raman data on emissive samples.[14,15] Hence, we now report the application of this spectroscopic tool to map out the excited-state structure of MC540 in a range of solvents.

2. Experimental Section

Merocyanine 540 and aerosol-OT (AOT, dioctylsulfosuccinate sodium salt) were commercially purchased and purified by reported procedures. Water was purified by a Millipore filtration system, and all other solvents used in spectroscopic investigations were of HPLC grade.

Picosecond Kerr-gated time-resolved resonance Raman (ps-K-TR^3) experiments were carried out at the Lasers for Science Facility at the Rutherford Appleton Laboratory using a purpose-built picosecond laser system operating with an optical parametric amplifier (OPA) arrangement previously described elsewhere.[16] In a typical experiment a solution of MC540 (concentration ~ 2 mmol) was pumped through a fine jet (500 μm) that was excited with a 6 μJ 1 ps (fwhm) laser pulse (λ_{ex} = 550 nm) and probed at varying time delays using a 2 μJ 1 ps (fwhm) laser pulse (λ_{prob} = 400 or 600 nm). Scattered Raman light was collected and imaged into a CCD detector, and data were processed using in-house programs. Fully baseline corrected and time-delayed resonance Raman spectra were obtained by subtracting positive spectra from a −50 ps spectrum using the program "Raman".[17] Ground-state spectra were obtained by using the −50 ps spectrum and subtracting out solvent peaks. Fully corrected spectra were least-squares fitted to a series of Gaussian peaks from which peak maxima and half-widths were extracted.

The fluorescence-suppressed ps-K-TR^3 time-resolved emission spectra were recorded using a high-sensitivity optical Kerr-gate with a gate width of ~4 ps.[18] The device comprises two cross polarizers and a Kerr medium (CS_2) activated to propagate the light of interest by an intense linearly polarized gating pulse with polarization tilted by 45°. The interaction of the gating pulse with the Kerr medium induces a transient anisotropy. This transforms the polarization of the light, in this case the fluorescence and Raman scatter, propagating through the medium from linear to elliptical polarization. The propagation length through the Kerr medium or the strength of anisotropy can be chosen so that the medium acts as a $\lambda/2$ waveplate and the polarization of the Raman light is transformed back to being linearly polarized but rotated by 90° with respect to its original polarization direction. This light is then transmitted through the cross polarizer onto the spectrometer slit. In Raman applications, fluorescence emitted after the gate is closed is efficiently blocked by the cross polarizer. The fluorescence suppression ratio achievable is given by the fluorescence lifetime and the gate width and can be as high as ~10^5 for long-lived fluorescence species (>1 μs) and ~10^2−10^4 for lifetimes in the range ~1−100 ns. The specifications of our gate are transmittance in the open state of up to ~40%, rejection ratio of 10^5 and a usable spectral range of 300−700 nm. In time-resolved fluorescence measurements the probe pulse is blocked and the pump is delayed with respect to the Kerr shutter gating pulse to obtain temporal slices of fluorescence spectra at desired time delays.

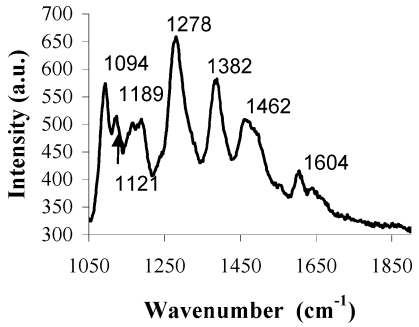

Figure 2. Ground-state resonance Raman spectrum recorded of MC540 in ethanol at 22 °C.

Collected fluorescence decay profiles were least-squares fitted to two exponentials (eq 1) written using a commercial package.

$$I_t - I_\infty = A \exp(-t/\tau_1) + B \exp(-t/\tau_2) \qquad (1)$$

The first-excited cis isomer fluorescence was measured using a slight adaptation of the previous experimental setup, but in which an OPA pulse (λ_{ex} = 532 nm, 5 μJ) with a minus 10 ns delay was used to create the long-lived (~10 ms) ground-state cis isomer. A second pulse (λ_{ex} = 600 nm, 2 μJ at −50 ps) was then used to create the excited cis isomer and its emission spectra recorded at different time delays using the optical Kerr-gating technique. To remove background ground-state emission, the experiment was repeated without the 10 ns delayed excitation pulse. Subtraction of the two emission profiles collected from these two experiments in the program "Raman" afforded the cis isomer emission spectrum. The total area of the spectra collected over different time delays was used to calculate the cis isomer fluorescence lifetime. A subtracted emission profile was least-squares fitted to three Gaussian peaks and exported to a commercial spreadsheet.

Molecular modeling calculations were performed using the commercial package Titan at the semiempirical level.[19]

3. Results

3.1. Ground-State and Time-Resolved Resonance Raman Spectra of MC540. The role played by the local solvent environment on the photophysical properties of molecules is key to understanding both their physical and chemical processes. Time-resolved absorption, fluorescence, and vibrational spectroscopy have been at the forefront of probing such solvent effects. Time-resolved resonance Raman (TR^3) has more recently been used to provide mode-specific details of solvent-induced structural alterations for various probe molecules. One major drawback of the resonance Raman (rR) technique is the need for low fluorescent samples because Raman signals are "lost" in the background noise. To obtain ground- and excited-state rR spectra of MC540, the technique of ps-K-TR^3 spectroscopy was employed. This unique spectroscopic tool permits both rejection of fluorescence from a sample and collection of high-quality temporally resolved rR data. The high fluorescence from MC540 has to date hampered collection of the excited-state Raman spectrum of the dye. The ground-state (GS) spectrum has, however, been reported in water and methanol along with some band assignments. For a comparison with the previous GS all-trans rR spectrum, the corresponding Raman spectrum of MC540 in ethanol was obtained following excitation of the sample (λ_{ex} = 550 nm) and probing (λ_{prob} = 600 nm) and is shown in Figure 2. Initially, it was expected that this probe wavelength would permit collection of temporally resolved rR spectra of both the trans and cis isomers of MC540.[20]

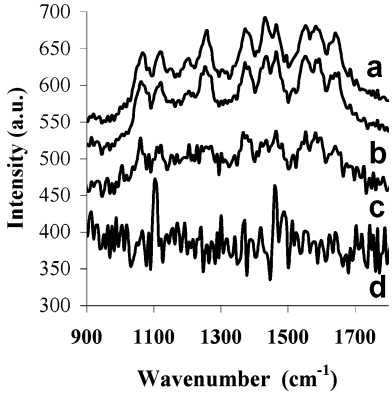

Figure 3. Time-resolved resonance Raman spectra recorded of MC540 in ethanol (concentration ~2 mmol) following excitation with a 1 ps laser pulse at $\lambda_{exc} = 550$ nm and $\lambda_{prob} = 400$ nm. Time delays shown are 10 ps (a), 50 ps (b), 500 ps (c), and 2000 ps (d).

TABLE 1: Collection of Observed Resonance Raman Bands for the First-Singlet Trans Excited State of MC540 in Ethanol at 22 °C

Raman shift (cm^{-1})[a]	band assignment
1641	C═O (thiobarbiturate)
1583	C═C
1554	C═C
1466	C═N
1431	C═N
1382	CH_3 deformation
1259	benzoxazole
1199	C—H
1115	C—H
1067	C—H deformation

[a] Values are ±15 cm^{-1}.

However, owing to multiphoton events it was only possible to acquire the GS Raman spectrum of the dye.[21]

As illustrated in Figure 2, the rR spectrum is dominated by a number of well-resolved bands and is comparable to that reported by Ehrenberg and Pevzner,[22] although with broader bandwidth associated with the lower resolution of the picosecond system. The bands at 1094 and 1189 cm^{-1} are assigned to aromatic in-plane C—H deformations, whereas the strong band at 1278 cm^{-1} is associated with the benzoxazole unit. The band at 1382 cm^{-1}, though not assigned, is characteristic of cyanine dyes and is presumably associated with the methyl group of the thiobarbiturate terminal.[23] The broad band at 1462 cm^{-1} is allocated to the C—N bond of the benzoxazole group. Although weak, the 1604 cm^{-1} signal is most likely due to a C═C stretching mode of the conjugated methine subunit.

The TR^3 spectra of MC540 in ethanol were collected using $\lambda_{ex} = 550$ nm and probing to the blue end of absorption profile ($\lambda_{prob} = 400$ nm). At this wavelength a transient absorption profile is located, which is associated with the first-excited singlet state. The rR spectra of MC540 in ethanol at varying time delays are illustrated in Figure 3. It should be noted that the Raman spectrum of the GS is rather weak at this probe wavelength because it is well off-resonance with the ground-state electronic absorption band. In ethanol the lifetime of MC540 has been determined by single-photon-counting experiments to be 410 ps,[24] and so the disappearance of the Raman bands over some 2 ns is consistent with this result.

The Raman spectra at short time delays are dominated by a number of characteristic bands that can be assigned by comparison to the GS spectrum (Table 1). In contrast to the GS spectrum the region from 1500 to 1650 cm^{-1} is better resolved and affords enhanced information on vibrations associated with the polymethine region and thiobarbiturate group. By comparison with IR spectra of thiobarbituric acid analogues[25] the strong band at 1641 cm^{-1} is assigned to the C═O stretch and is a prominent band in Raman spectra recorded for MC540 in a series of protic linear alcohols. In contrast, this band is greatly diminished or absent in the TR^3 spectra of MC540 in aprotic solvents such as DMSO (Figure 4).

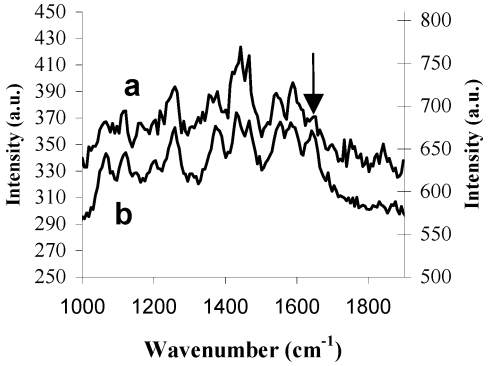

Figure 4. Time-resolved resonance Raman spectra recorded 10 ps after excitation of MC540 with a 1 ps laser pulse at $\lambda_{exc} = 550$ nm and $\lambda_{prob} = 400$ nm in DMSO (a) and EtOH (b). The arrow indicates the position of the diminished peak.

The larger band intensity in protic solvents is a consequence of changes in electron distribution in the vicinity of the C═O bond. This is most likely brought about through H-bonding in protic solvents, but the precise nature is not known. Undoubtedly, the lone pair of electrons on the carbonyl oxygen would be a preferred site for H-bonding. This would cause a corresponding redistribution of the electrons within the thiobarbiturate group. This change would also be expected to be accompanied by a small shift in the Raman bands associated with the carbonyl group. For example, the C═O σ-bond would be weakened and this mode expected to shift to a slightly lower wavenumber but this is below our spectral resolution (~15 cm^{-1}) as we do not observe any shift.[26]

3.2. MC540 in an AOT Microemulsion. In a nonpolar environment specific surfactants will aggregate to form a reverse micelle in which the polar headgroups point inward. These reverse micelles are able to confine water molecules in the hydrophobic pocket to create a microemulsion, which is an ideal model system for biological water molecules. One such microemulsion is created by dissolving dioctyl sulfosuccinate (AOT) in *n*-heptane, for which the water pool is ca. $2\omega_o$, where ω_o is the ratio of the number of water and AOT molecules present.[27] In a recent study by Bhattacharyya et al.[28] it has been shown that MC540 in an AOT microemulsion exists in a relatively nonpolar environment, and that nonradiative decay is greatly retarded. It was concluded that polarity was the major factor for retarding isomerization rather than the local microviscosity. A more detailed investigation of the structure and environmental influence of the microemulsion on MC540 was investigated, and TR^3 spectra in AOT ($\omega_o = 32$) were recorded. Illustrated in Figure 5 is the excited-state rR spectrum of MC540. The spectrum consists of a number of well-resolved resonances and is comparable to that obtained in protic solvents. The C═O stretch is once again prominent and located at 1635 cm^{-1}.

3.3. Time-Resolved Fluorescence Profiles of MC540 in Various Solvents. It is well documented that the fluorescence profile of MC540 in a range of solvents mirrors closely its absorption spectrum.[6] The Stokes' shift is also relatively small (~600 cm^{-1}), indicating that no large-scale structural change occurs upon formation of the all-trans excited state. We have undertaken a comprehensive investigation of the first-excited

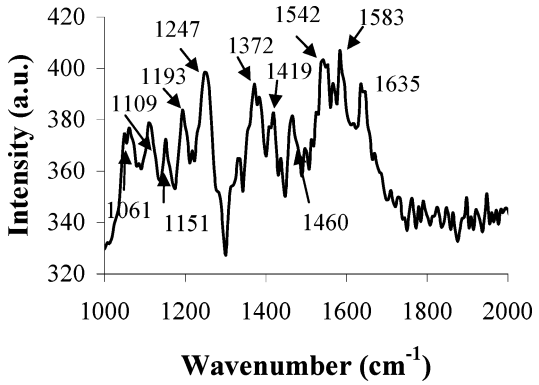

Figure 5. Time-resolved resonance Raman spectrum recorded 10 ps after excitation of MC540 with a 1 ps laser pulse at $\lambda_{exc} = 550$ nm and $\lambda_{prob} = 400$ nm in a reverse AOT micelle ($\omega_o = 32$). Note: the sharp dip in the spectrum between 1247 and 1372 cm^{-1} is an artifact caused by difficulty in subtraction of solvent peaks.

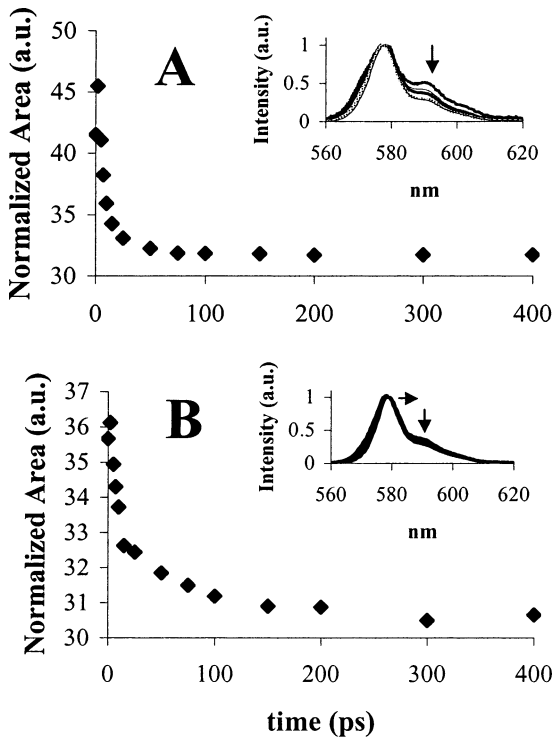

Figure 6. Decay kinetic profiles showing change in normalized emission area versus time for MC540 in (a) EtOH and (b) *n*-decanol. Insets depict normalized emission spectra with the arrow(s) indicating changes over time.

trans-state dynamics of MC540 by mapping changes in the fluorescence profile over the first several picoseconds immediately following photoexcitation. The fluorescence profiles generally display changes in spectral intensity and bandwidth and, in viscous solvents, display an alteration in peak position to lower energy. The most dominant modification is the reduction in intensity of the low-energy minor shoulder, which is associated with Franck−Condon vibrational splitting.[29] Illustrated in Figure 6 are the normalized fluorescence profiles of MC540 in ethanol and decanol along with their respective decay kinetics relating to the change in integrated normalized area. The use of the integrated peak area of the normalized emission profile is an adaptation of that reported by Gustafson and Buntinx[30] because this eliminates long-lived population decay. In most solvents in which measurements were carried out there is a fast (2−10 ps) and slow component (20−100 ps) to the decay kinetics profile (Table 2).

TABLE 2: Calculated Lifetimes for Vibrational Cooling and Skeletal Structural Reorganization of MC540 and Corresponding Solvent Parameters

solvent	τ_1 (ps)[l]	τ_2 (ps)[l]	η (cP)[m]	$10^8\lambda$ (m^2 s^{-1})
MeOH[a]	4.5	40.9	0.59	9.98
EtOH[b]	5.8	40.8	1.20	8.78
n-PrOH[c]	6.4	48.9	2.26	8.02
n-BuOH[d]	6.6	59.0	2.95	7.96
n-HexOH[e]	6.8	57.3	4.15	7.85
n-OctOH[f]	6.4	89.2	6.88	8.32
n-DecOH[g]	6.5	100.8	10.52	9.82
acetone	5.2		0.304	9.37
CH_3CN[h]	2.8	16.3	0.39	10.75
BuCN[i]	7.3	34.7	0.58	
DMF[j]	6.9		0.924	9.42
DMSO[k]	4.8	35.9	1.996	

[a] Methanol. [b] Ethanol. [c] *n*-Propanol. [d] *n*-Butanol. [e] *n*-Hexanol. [f] *n*-Octanol. [g] *n*-Decanol. [h] Acetonitrile. [i] Butyronitrile. [j] *N,N′*-Dimethylformamide. [k] Dimethyl sulfoxide. [l] Measured at 22 °C error ±10%. [m] Parameters described in text.

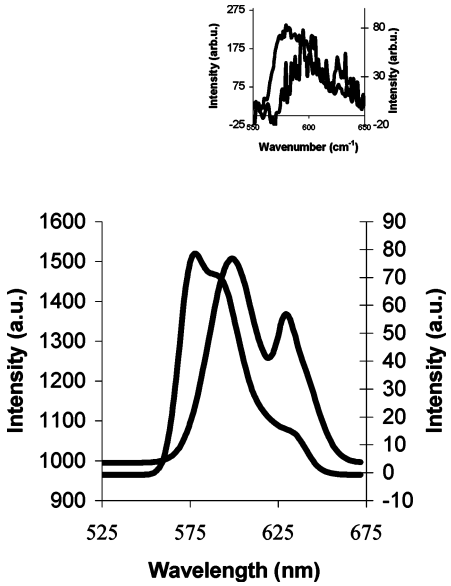

Figure 7. Time-resolved emission spectrum of trans-MC540 (a) and cis-MC540 (b) in MeOH at 22 °C. Note: the appearance of two peaks in the cis isomer spectrum is an artifact due to over subtraction problems and fitting of the spectrum.

Fluorescence profile alterations and decay kinetics were also obtained for MC540 in the AOT reverse micelle ($\omega_o = 32$). The fluorescence maximum shifts to lower energy and exhibits peak narrowing analogous to that reported for the dye in a viscous solvent such as decanol. The slow process lifetime (τ_2) was found to be 69 (±5) ps.

3.4. Emission Spectrum of Cis MC540. The cis isomers of thiacarbocyanine dyes are believed to be nonfluorescent at room temperature and upon photoexcitation are deactivated through internal conversion.[31] Hence, the cis form of MC540 has been regarded as nonfluorescent in most studies. The failure to collect rR spectrum of the cis isomer led us to attempt to see if emission from the cis isomer was possible using our sensitive apparatus. The weak emission spectrum of the excited cis isomer of MC540 was successfully collected in methanol along with its trans isomer emission profile. Illustrated in Figure 7 are the least-squares Gaussian band shape fitted spectra.[32] The cis isomer emission maximum is located at ca. 598 nm and is red-shifted (∼20 nm) with respect to the trans isomer spectrum. This observation is consistent with the absorption spectra recorded

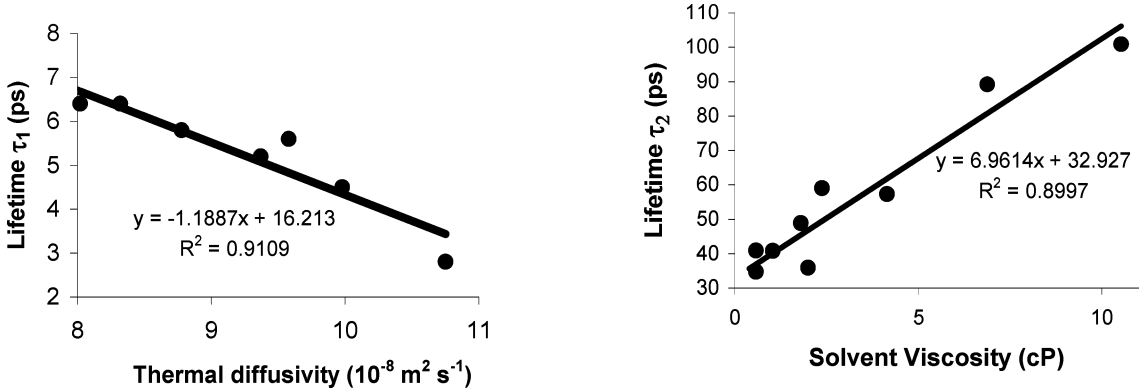

Figure 8. Plots of (a) change in lifetime (τ_1) versus thermal diffusivity (κ) and (b) change in lifetime (τ_2) versus solvent viscosity (η) for MC540 at 22 °C. Insets depict the best-fit equation to the data and the goodness-of-fit.

of the two isomers in methanol, for which the maxima are separated by ca. 10 nm.[7] Although the cis isomer emission is weak, the lifetime of the excited-state isomer in methanol was calculated to be on the order of 35 ps.

4. Discussion

Following from previous photophysical studies on MC540 and its derivatives a clearer picture of how this dye's excited state behaves is beginning to emerge. These time-resolved resonance Raman and emission studies have unfolded more information about what happens to the dye following initial excitation and which group interacts the most strongly with the local environment.

4.1. Vibrational and Conformational Cooling. Upon photoexcitation of a molecule in a solvent, the excess vibrational energy is first redistributed and is then followed by the dissipation of excess energy to the surrounding solvent. Probing of this vibrational cooling has been extensively studied using *trans*-stilbene by Iwata and Hamaguchi,[33−35] who have found an adequate correlation between vibrational cooling and the thermal diffusivity of the solvent (κ)[36] (eq 2). Here, λ is the

$$\kappa = \frac{\lambda}{c\rho} \tag{2}$$

thermal conductivity, c is the heat capacity, and ρ is the density. In the model, the temperature decrease in the solute molecule is offset by an energy release to the nearest-solvent molecules. Following this initial transfer of energy the outer-sphere solvent molecules take away the excess energy.

Using this methodology, we have measured the fast relaxation process (τ_1) for MC540 in a range of solvents and results show a good linear relationship to κ (Figure 8). Using the proposed energy dissipation model, it is reasonable that excess energy within MC540 is immediately dispersed among nearest-neighbor solvent molecules. This energy is then distributed to outer-sphere solvent molecules via normal heat conduction. As observed for *trans*-stilbene, the dissipation of energy is reliant not on the solute−solvent interaction but on heat migration in the bulk solvent.[35]

In viscous solvents such as decanol, the lifetime of the second component in the decay profile is very pronounced and so is more in line with a structural reorganization.[37] Indeed, there is a good correlation between the lifetime (τ_2) and the bulk solvent viscosity (η),[38] as depicted in Figure 8.[39] It is noted that both protic and aprotic solvents are included in the plot. The linear dependence of τ_2 on viscosity is in accordance with the Debye−Stokes−Einstein[40] expression, which assumes hydrodynamic friction, and so we can imply that

$$\tau_2 = \frac{\eta V}{k_B T}$$

where V is the volume of the rotor, k_B is Boltzmann's constant, and T is temperature (K). The slope of the graph from Figure 8 is 7(±1) ps/cP, which gives a rotor volume of only ca. 29 Å^3.[41] The total volume of MC540 calculated at the semi-empirical level is around 548 Å^3, whereas the thiobarbiturate and benzoxazole groups are ca. 200 and 300 Å^3, respectively. In view of this, the central linker volume is ∼48 Å^3, which supports that reorientation dynamics involves skeletal motion of at least two methine groups. This result is perhaps not too surprising because the polymethine is the most flexible part of the dye and is an ideal location to dissipate excess energy.

Using the lifetime value τ_2 for MC540 in the AOT reverse micelle ($\omega_o = 32$), an estimate of the local viscosity is about 5 cP. Microviscosities of micelles have been reported in the range 15−200 cP,[42] and so this value seems relatively low. However, this finding does support the proposal put forward by Bhattacharyya et al. that polarity, rather than viscosity, is the factor that controls isomerization of the dye in the micelle.[28]

4.2. Environmental Effects. In the ground state, MC540 exists in equilibrium with its zwitterionic (dipolar) form in which there is a shift of electron density away from the tertiary nitrogen of the benzoxazole to a C═O group of the thiobarbiturate terminal. The solvatochromic behavior of MC540 is not particularly pronounced and does not display large negative solvatochromism characteristic of highly polar cyanine dyes.[43] As a result, it appears that the dipolar form contributes less toward the overall structure than to the corresponding nonpolar form. Hence, measurement of the actual dipole moment of the ground and excited state of MC540 has been difficult and it is not completely clear if there is a decrease or increase in dipole moment upon formation of the excited state. Recent experiments by Čunderlíková and Šikurová[44] support that molecular charge distribution is altered upon excitation, so that a less polar structure is obtained and any specific hydrogen bonding would decrease. The rR experiments carried out on the first-excited trans isomer indicates that the H-bonding site as the C═O group(s) of the thiobarbiturate terminal. Hence, a H-bonded solvent cluster would effectively anchor this group so that any rotation would involve the benzoxazole unit and either the central or first double bond. This is in agreement with a previous study[10] demonstrating that altering the alkyl chain length on the nitrogen atoms of the thiobarbiturate group had little affect on the ground-

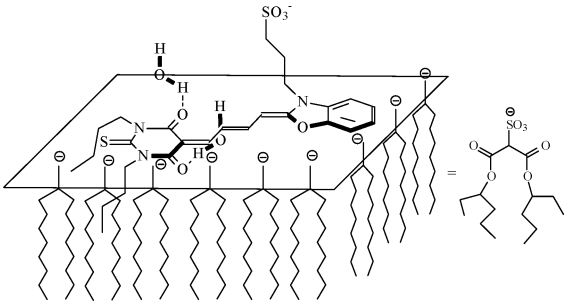

Figure 9. Proposed location of MC540 in the reverse AOT micelle ($\omega_o = 32$) showing interacting water molecules. The picture is not to scale.

state cis to trans isomerization rate. It is not clear at this stage whether H-bonding increases or decreases upon photoexcitation because the ground-state rR spectrum could not be obtained at the probe wavelength used in the excited-state structure measurements.

The similarity of the rR spectrum of MC540 in the reverse micelle and decanol suggests that the dye is in similar environments. Miller and Lelkes[45] proposed that in a phospholipid membrane the dye is located with the polar sulfonate group toward the more polar outer surface and the polymethine core orientated parallel to the surface but in a uniform nonpolar environment. The thiobarbiturate is surface bound with the alkyl chains embedded in the hydrocarbon core. Within the large water pool, reverse micelle MC540 presumably adopts a similar arrangement, with water molecules of the water pool interacting with the C═O groups of the barbiturate terminal (Figure 9).

5. Conclusions

The detailed time-resolved photophysical measurements that we have managed to carry out have afforded a better insight into the excited-state structural behavior of MC540. In particular, alterations in the fluorescence profiles of MC540 over relatively fast time scales have permitted us to delve into subtle vibrational and structural cooling effects. As observed in related polyene molecules, photoexcitation of all-trans MC540 produces a highly energetic molecule that dissipates heat via fast vibrational cooling to the surrounding solvent molecules. The vibrationally cooled yet still energetic all-trans structure then undergoes fast structural reorientation/relaxation, which involves twisting of at least two of the methine groups in the molecular backbone. This latter observation is consistent with the proposed idea that a twisted state is produced in MC540, from which deactivation occurs to repopulate the ground-state trans isomer and the ground-state cis isomer (Figure 1).

To be an effective photodynamic agent, the wasteful energy loss processes in MC540 that compete with triplet formation need to be curtailed; isomerization has been identified for some time now as the major contributor. Identification of factors that influence isomerization of MC540 are hence important for the design of new improved photodynamic agents based on this dye. From our studies the amidic groups of the thiobarbiturate terminal of MC540 have been identified as the preferred site for its interaction with the local environment. Hence when considering isomerization within MC540, one has to now consider a second solvent sphere that is asymmetric around the molecule. For instance, H-bonded solvents will tend to cluster at the thiobarbiturate moiety and effectively anchor the unit, and is a plausible reason isomerization of the dye seems to involve rotation around the benzoxazole group. In aprotics the solvent molecules are presumably more evenly distributed around MC540. At this stage the strength of the H-bonding at the amidic oxygen sites is still unclear and will require the use of a more sensitive technique to probe the noncovalent interaction. Future studies are in progress to use picosecond infrared absorption transient excitation (PIRATE)[46] to delve more deeply into how MC540 interacts with its local surroundings. It is expected these structure−reactivity experiments will complement our synthetic work on MC540 derivatives, in which the benzoxazole group is functionalized with H-bonding sites so that both ends of the molecule become anchored in protic solvents.

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(21) Although it was possible to collect what appeared to be a time-resolved rR spectrum of the first-excited trans state, it did in fact mirror too closely the ground-state trans spectrum obtained using the negative time delay. The spectrum collected at long time delays (~2 ns), which was first identified as the ground-state cis isomer, turned out to be the inverse mirror image of the ground-state trans isomer. It is known that the cis isomer is photolabile and so the spectrum is likely to be the result of over subtraction of the negative time delay data.

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