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Picosecond Spectral Relaxation of Curcumin Excited State in a Binary Solvent Mixture of Toluene and Methanol

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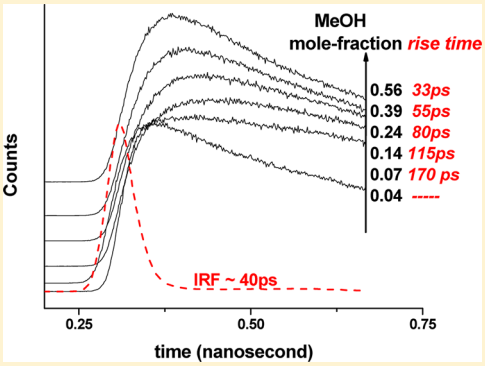
Supporting Information

ABSTRACT: Picosecond spectral relaxation of the excited state of curcumin in a binary solvent mixture of toluene and MeOH (or MeOH-d_4) is reported with an instrument time resolution of ~40 ps. With increasing mole fraction of MeOH (MeOH-d_4) the fluorescence intensity and lifetime of curcumin increase to a maximum at a MeOH (MeOH-d_4) mole fraction of 0.14 (0.40) and then decrease. In addition, fluorescence decays taken at the red edge of the emission spectrum started to show measurable rise times (170 to 30 ps), the magnitude of which decreased gradually with increasing alcohol mole fraction. This is attributed to the modulation of the nonradiative rates associated with the excited-state intermolecular H(D) bonding between the pigment and the polar protic solvent. As a consequence, the solvation times in the binary mixture were observed to slow down considerably (20−40 times) at certain solvent compositions compared to neat MeOH. The fact that three Gaussian components are needed to adequately represent the steady-state emission spectra and two isosbestic points are observed in the time-resolved area normalized emission (TRANE) spectra of the pigment suggests the existence of at least three species in the excited state. The observed results are rationalized with a scheme where ground state of the pigment exists in free and H-bonded (intermolecular) state. Optical excitation results in a mixture of these species in the excited state and the observed spectral relaxation correspond to the conversion of these two species in to a third species where dipolar solvation and intermolecular H-bonding have been optimized.

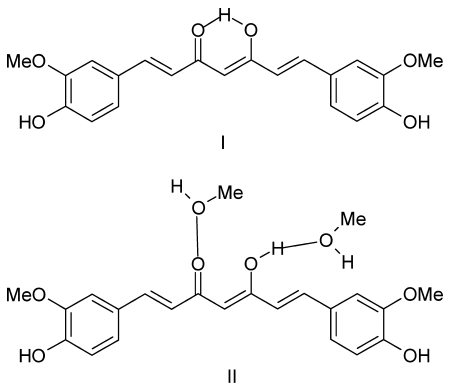


INTRODUCTION

Curcumin, which exhibits a variety of biological and photochemical properties,[1] is currently the subject of a large number of investigations. Several studies have focused on the photophysical properties of the excited states of curcumin[2−12] and have predicted about the occurrence of two fundamental photophysical processes in the excited state, namely the excited-state hydrogen atom (or proton) transfer (ESHT) and solvation.[3−12]

In a recent study by Palit and co-workers,[10] subpicosecond time-resolved fluorescence and absorption spectroscopic techniques are used to study the dynamics of the excited singlet (S_1) state of curcumin in a wide variety of neat solvents. In nonpolar solvents because of the presence of six-membered hydrogen-bonded chelate ring of the cis-enol form (Scheme 1) the ESHT process is expected to be primarily intramolecular in nature, and based on previous studies,[13−16] it should be completed within a few hundreds of femtoseconds. However, in polar protic solvents, like methanol (MeOH), there will be significant perturbation of intramolecular H bond as solvent molecules will compete to form intermolecular H bond with curcumin. As a result, specific solvent effects such as H bond reorganization between curcumin and solvent will affect the relaxation of the S_1 state of curcumin in these solvents, and indeed it has been observed.[10] In neat solvents this relaxation is typically completed within a few picoseconds. However, we

Scheme 1. Likely Structures of Curcumin in Non-Hydrogen-Bonding (top) and Hydrogen-Bonding (bottom) Solvents Showing the Intramolecular and Intermolecular Hydrogen Bonds[a]



[a]The bottom picture is oversimplified in the sense that only two solvent molecules are shown and the phenolic OH groups are excluded from hydrogen bonding with the solvent.

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show in this report that in a binary mixture of toluene and MeOH the relaxation of curcumin excited state depends upon the solvent composition. For certain solvent compositions it can even be detected by fluorescence spectroscopy with 40 ps time resolution and showing an insignificant isotope effect.

MATERIALS AND METHODS

Curcumin (Sigma product no. C7727) was further purified by TLC according to a reported method.[18] Deuterated methanol (MeOH-d_4) from Across Chemicals (99 atom % D; product no. A 0305352) was used as received. For the experiments on curcumin in MeOH-d_4, curcumin was allowed to equilibrate in the deuterated solvent for 48 h (as reported earlier[9]) to ensure complete exchange of the enolic hydrogen with deuterium.

Steady-state absorption spectra were obtained on GBC UV–vis spectrophotometer with 1 nm resolution. Steady-state fluorescence spectra were obtained on a Spex Fluorolog fluorimeter with a 4 nm band-pass and corrected for lamp spectral intensity and detector response. For all spectroscopic measurements the optical density of curcumin (1 cm optical path) were kept typically ~0.2 at the maximum, which corresponds to micromolar concentrations of the drug.

The laser system used for the time-resolved experiments consisted of a Coherent Mira 900F femtosecond laser system, the output of which was pulse picked (Coherent 9200 pulse picker) at a rate of 3.8 MHz and then frequency doubled in an ultrafast harmonic generation system (Inrad 5-050). The fluorescence decays were recorded using a time-correlated single photon counting (TCSPC) system from Edinburgh Instruments (Lifespec-Red). This system equipped with a Hamamatsu MCP-PMT has an instrument response function (IRF) of ~40 ps. The acquisition time window had a width of ~2.5 ns with 2048 channels, corresponding to 1.22 ps per channel. All the curcumin solutions were excited by vertically polarized light at 420 nm, and the emission was detected at magic angle over the whole band after rejecting the excitation light by an appropriate filter. To obtain the time-dependent spectra in toluene–MeOH and toluene–MeOH-d_4 mixtures, fluorescence decays were taken at 10 nm intervals with a resolution of 4 nm, having 10K counts in the peak channel. The fluorescence traces were fitted to a single- or multiexponential function after deconvolution by the iterative reconvolution method using FAST software supplied by the manufacturer of the TCSPC system.

RESULTS

In a binary mixture of toluene and MeOH (MeOH-d_4) the steady-state fluorescence spectra of the pigment shows an interesting behavior (Figure 1). The vibrational structure present in the fluorescence spectra of the pigment in toluene starts to change even at very low mole fractions (up to ~0.01) of MeOH (MeOH-d_4). After that the spectral shape starts to get broad accompanied by a red-shift and an increase in fluorescence intensity until MeOH (MeOH-d_4) mole fraction of 0.14 (0.40). The fluorescence intensity then drops down significantly as the mole fraction of the polar protic solvent approaches unity. The variation in fluorescence lifetime follows a similar pattern (Table 2 and Figure S1, Supporting Information) Corresponding changes in the absorption spectra of the pigment however were relatively insignificant (Figure S2). In order to find out which properties of the solvent (polarity, H-bonding ability) are responsible for this kind of

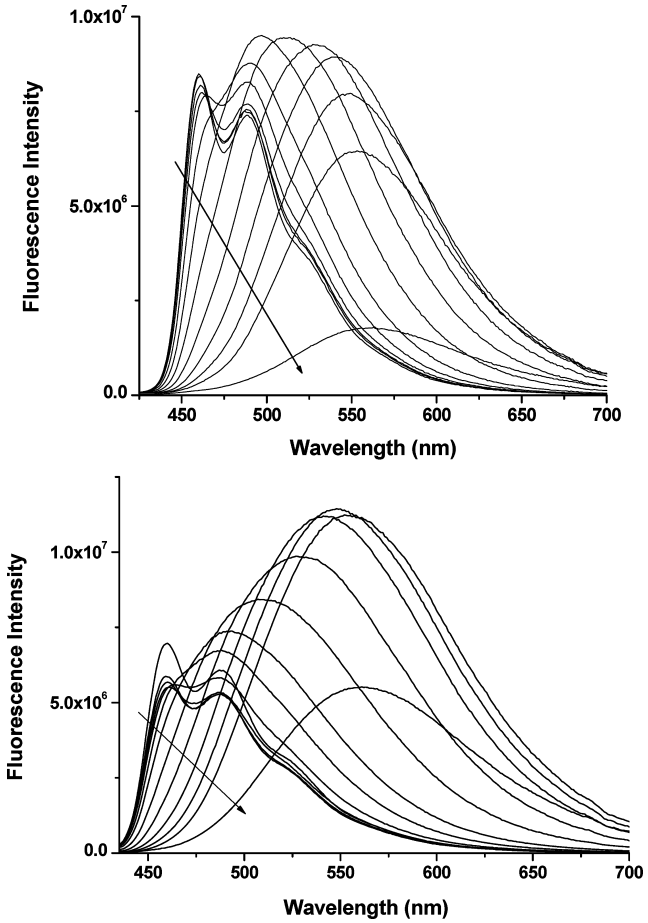

Figure 1. Evolution of curcumin fluorescence (λ_{ex} = 420 nm) in binary solvent mixtures of toluene–MeOH (top) and toluene–MeOH-d_4 (bottom). The arrows indicate increase in the MeOH/MeOH-d_4 mole fraction (0, 0.0013, 0.0025, 0.0050, 0.0100, 0.0200, 0.0400, 0.0700, 0.1400, 0.2400, 0.3900, 0.5600, and 1.0).

changes in the fluorescence of the pigment, we have replaced MeOH with chloroform ($CHCl_3$) and acetonitrile (MeCN). In the Kamlet–Taft scale, while the polarity of MeCN and $CHCl_3$ are comparable to that of MeOH, the former is primarily a H-bond accepting and the latter a H-bond donating solvent (Table 1). When MeOH is replaced by $CHCl_3$, the

Table 1. Kamlet–Taft Parameters of the Solvents Used in This Study

solvent	π^*	α (donating)	β (accepting)
toluene	0.54	0.00	0.11
MeOH	0.60	0.93	0.62
$CHCl_3$	0.58	0.44	0
MeCN	0.75	0.19	0.62

fluorescence properties of the pigment correlated with the mole fraction of $CHCl_3$; however, when MeOH is replaced with MeCN, the fluorescence properties does not exactly correlate with the mole fraction of MeCN; instead, it resembles the pattern seen with MeOH (Figures S3 and S4).

The relative changes of the photophysical parameters (fluorescence quantum yield, average fluorescence lifetime, radiative and nonradiative rates) of the pigment in the four solvent mixtures with varying mole fractions of the polar

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solvent are given in Figure 2. The trends are clearly different for the alcohol and nonalcohol solvents. While the nonradiative

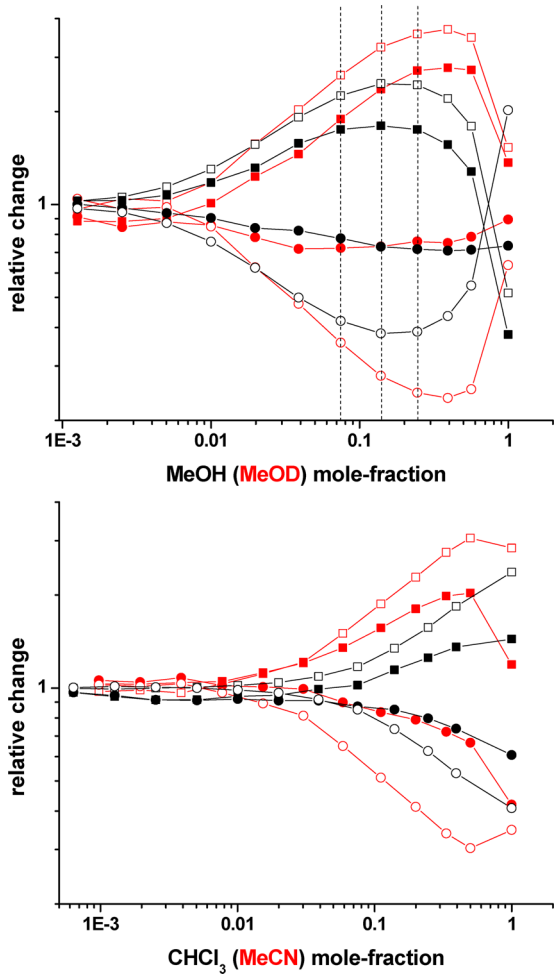

Figure 2. (top) Relative changes in the emission quantum yield (solid squares), average lifetime (hollow squares), radiative (solid circles), and nonradiative (hollow circles) rates of curcumin with increasing mole fractions of MeOH (black) and MeOH-d_4 (red). The vertical dashed lines indicate alcohol mole fractions where time dependent spectra were constructed. (bottom) Relative changes in the emission quantum yield (solid squares), average lifetime (hollow squares), radiative (solid circles), and nonradiative (hollow circles) rates of curcumin with increasing mole fractions of $CHCl_3$ (black) and MeCN (red). For clarity, the parameters were normalized with respect to that in toluene, and the *Y*-axis is represented in the logarithmic scale.

rates pass through a minimum, the radiative rates remain more or less unchanged for the alcohol solvents. Figure 2 further shows that the observed trends are different for MeOH and MeOH-d_4. The maxima (or minima) of the photophysical parameters of the pigment shifts to a higher mole fraction (0.40 compared to 0.14) when the deuterated solvent is used.

We observe distinct rise times when decays are taken at the red edge of the emission spectrum (Figure 3) of the pigment, at certain MeOH (or MeOH-d_4) mole fractions. For example, at an alcohol mole fraction of 0.04 no rise time is observed, but increasing the mole fraction to 0.07 results in the appearance of a ~170 ps rise time. Further, the magnitude of the observed rise times depends upon the mole fraction of the alcohol solvent. As the alcohol solvent mole fraction is increased, the magnitude of the rise time decreases and at 0.57 mole fractions this becomes

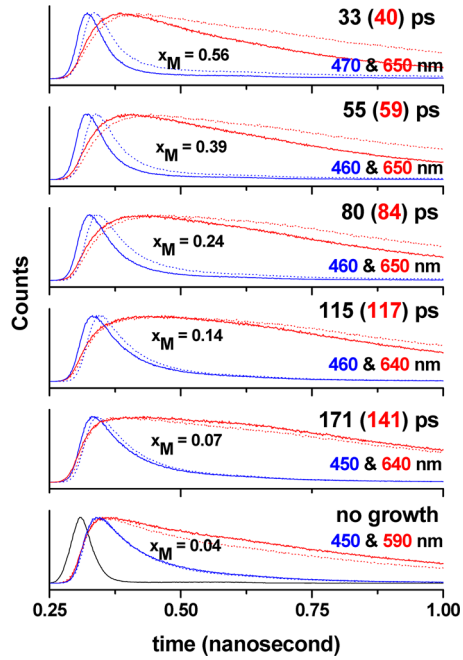

Figure 3. Fluorescence decays taken at the blue (blue line) and red (red line) edges of curcumin fluorescence spectra at different MeOH (solid lines) and MeOH-d_4 (dotted lines) mole fractions. The rise times obtained after fitting (black: MeOH; red: MeOH-d_4) are also shown against each mole fraction (x_M). For comparison, the instrument response function is shown at the bottom panel.

~30 ps. Unlike photophysical parameters (Figure 2), the observed rise times did not show any significant isotope effect. When MeOH is replaced by $CHCl_3$ and MeCN, no rise times could be observed, suggesting that spectral relaxation is too fast to be detected by ~40 ps time resolution in these solvent mixtures. In general, the signature of excited-state solvation is manifested as a growth in the kinetics at the red edge of emission spectrum. The dynamics of solvation are quantified by the decay of the solvent response function $C(t)$:

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)}$$

where $\nu(0)$, $\nu(t)$, and $\nu(\infty)$ denote the peak frequency of the fluorescence spectra at time zero, t, and infinity. The "zero time" emission spectrum has been approximated using the emission spectrum of Curcumin in a nonpolar solvent, *n*-hexane, according to an earlier method.[19] Determination of $\nu(\infty)$and $\nu(t)$ (peak frequencies of the steady-state and time-resolved fluorescence spectra) were carried out according to an earlier method[20] using steady-state emission spectrum and fitting parameters from wavelength resolved decay traces. Owing to the limited time resolution of our setup, we have constructed $C(t)$ at three different alcohol mole fractionS of 0.07, 0.14, and 0.24, where the observed rise times are of the order of 100 ps. The decays of $C(t)$ at these mole fractions are shown in Figure 4. The decays can be satisfactorily fitted with a single-exponential function with solvation times ranging from 100 to 200 ps (~20−40 times slower that observed in neat MeOH or MeOH-d_4[9,10]). A ~3-fold increase in alcohol mole fraction results in a ~2-fold decrease in solvation time. It is important to note here that owing to our limited time resolution we are missing the initial (10−30%) solvation dynamics.

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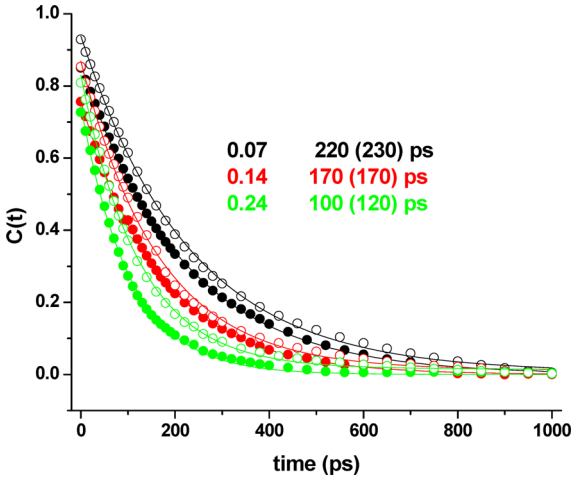

Figure 4. Decay of solvation response function $C(t)$ at different MeOH (solid) and MeOH-d_4 (hollow) mole fractions (black: 0.07; red: 0.14; green: 0.24). The raw data are given as points, and the fits are given as lines. The time constants given in parentheses correspond to those in MeOH-d_4. The errors in time constants are estimated to be ~10%.

Excited electronic state solvation can be classified into three categories: polar solvation, which results from the interaction of solvent dipoles with the solute charge distribution; nonpolar solvation, which results from repulsive and dispersion forces; and "specific" interactions, which most often result from hydrogen bonding. Assuming the latter to play a significant role in the relaxation process, we have attempted to investigate the possibility of the existence of different species in the excited state.

An attempt to resolve the emission spectra of the pigment into different Gaussian components is shown in Figure S5. For the three different alcohol mole fractions (0.07, 0.14, and 0.24) the emission spectra need at least three different Gaussian components, whereas in neat alcohol the spectra can be fitted to two components. The corresponding fitting parameters are provided in Table 2 of the Supporting Information. These results suggest that at least three different species may be present in the excited state of the pigment in the binary solvent mixtures. The presence of different fluorescent species in the excited state can also be confirmed by the existence of isosbestic points in the time-resolved area normalized emission (TRANE) spectra.[15]

The TRANE spectra of the pigment corresponding to MeOH (MeOH-d_4) mole fraction of 0.07, 0.14, and 0.24 at different times (0−1000 ps) constructed from the raw data are shown in Figure 5. The TRANE spectra of the pigment show the existence of two isosbestic points: one at initial time (0−100 ps) followed by a continuous shift of the spectra and finally another one at later times. The existence of two isosbestic points indicates that at least three species are involved in the observed spectral relaxation. In addition, the individual components (obtained from the Gaussian fits) of the corresponding steady-state spectrum (Figure S5) provide a rough idea about the spectral relaxation of the individual species. By plotting the time-dependent change of the intensity of the high- and low-energy peaks of the TRANE spectra, the kinetics of the corresponding species (say, I and III) may be obtained. Figure 6 shows the decay and growth characteristics of species I and III (positions at ~20 400 and ~18 518 cm^{-1}, respectively) for the three different MeOH (MeOH-d_4) mole fractions. All the kinetic traces can be fitted satisfactorily to a single-exponential function. Initially, when the alcohol mole fraction is smaller, the decay of the species I is significantly slower (~2 times) compared to the formation of species III, and as the mole fraction increases both the time constants decreases and becomes comparable.

DISCUSSION

As reported earlier,[3] the absorption and the fluorescence spectra of curcumin were observed to exhibit significant solvent effect. Compared to toluene, in all the polar solvents used in this study the vibronic structure in absorption and emission spectra are lost, suggesting significant solute−solvent interaction in the ground and excited state (Figure S5). The various photophysical parameters of curcumin in these solvents are provided in Table 1 of the Supporting Information. Although photoexcitation of curcumin to the S_1 state leads to a large change in dipole moment, ($\Delta\mu \approx 6.1$ D),[3] the larger solvatochromic red shift observed in MeOH (and MeOH-d_4) has been explained by the effect of both polarity and hydrogen-bonding ability of the solvent on the excited state of curcumin. The low quantum yield and the low lifetime of the pigment in nonpolar and polar protic solvents have been attributed to radiationless decay channel associated with the fully solvated ESPT state of the pigment.[3] In particular, the very short fluorescence lifetime of the pigment in nonpolar solvents such as toluene or hexane is attributed to very efficient intramolecular ESPT process.[10] In an earlier report,[21] the ground state pK_a values for the phenolic protons are estimated to be 10.5 and 10 and that for the enolic proton was estimated to be 8.4. The excited-state pK_a values are not yet measured, but they can be significantly lower if curcumin is a strong photoacid. Huppert and co-workers[12] studied the effect of a mild base, acetate ion, on the fluorescence properties of curcumin in methanol and ethanol. They propose two ESPT between the pigment and acetate ion: one involving the phenolic proton and another one involving the enolic proton. Considering the ground state pK_a values, it is therefore likely that ESPT between the pigment and methanol preferably involves only the enolic proton of the pigment.

The fluorescence properties of the pigment in binary solvent mixtures containing MeOH or MeOH-d_4 are significantly different than mixtures containing $CHCl_3$ or MeCN (Figure 2 and Figure S3) which arises primarily due to the modulation of the nonradiative rates of the pigment. Compared to $CHCl_3$ and MeCN, intermolecular H(D)-bonding is expected to be stronger in MeOH (MeOH-d_4). Since the ESPT process in curcumin has been predicted to be nonradiative in nature, the significant changes observed in the nonradiative rates shows that excited-state intermolecular H-bonding between the pigment and the polar protic solvent MeOH (or MeOH-d_4) is significantly perturbed in the presence of toluene. Comparing the Kamlet−Taft parameters of the polar solvents (Table 1), it can be argued that both H(D)-bond accepting and H(D)-bond donating properties are responsible for this behavior. In neat MeOH (or MeOH-d_4), this intermolecular H (D) bond is likely to involve one Curcumin molecule with "several" MeOH (or MeOH-d_4) molecules consisting of a H (D) bonding network. A simplified picture involving only two solvent molecules is shown in Scheme 1. The decay of the excited state is expected to depend upon the "strength" of this network. In the Kamlet−Taft scale, the polarity of toluene is comparable to

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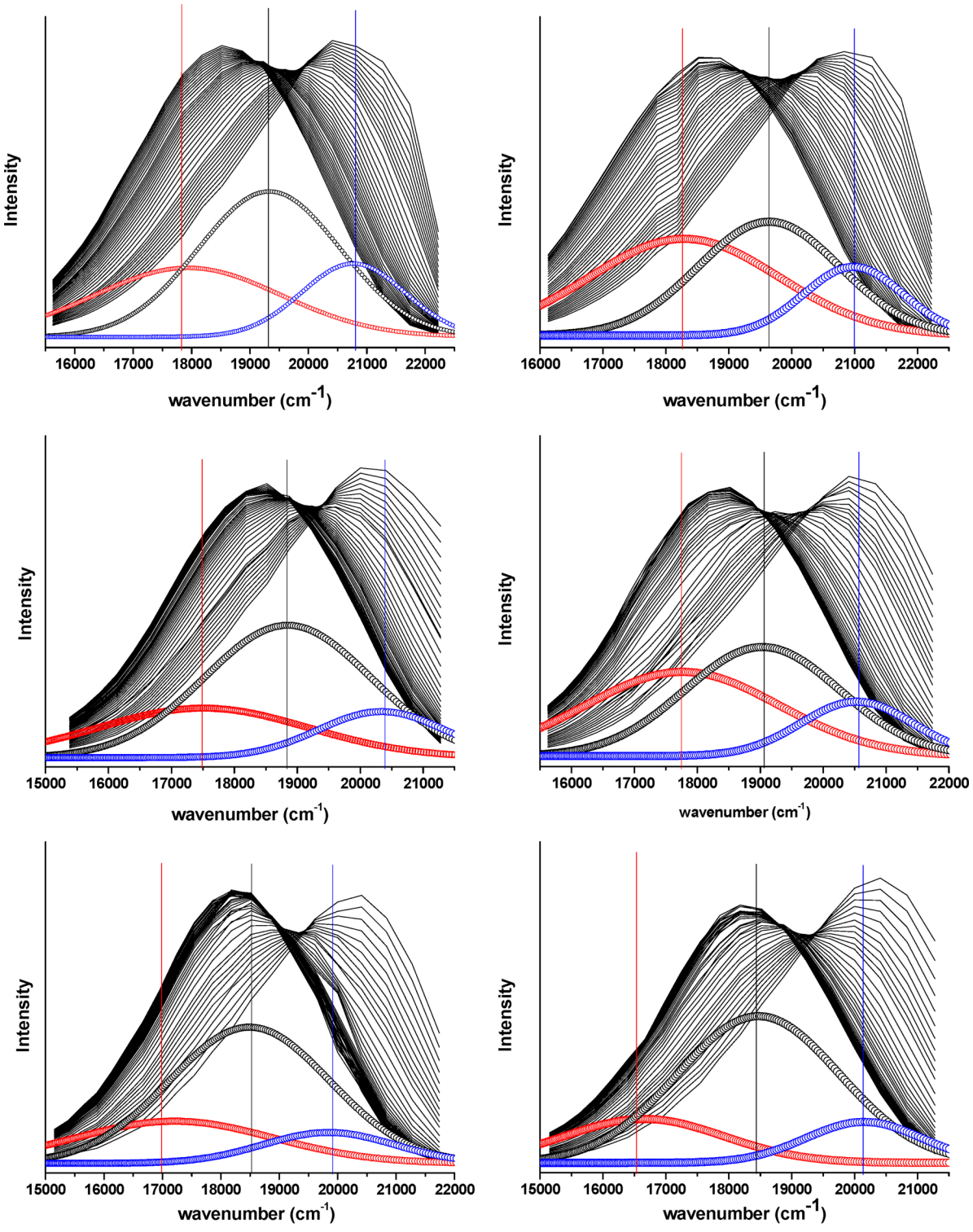

Figure 5. TRANE spectra (from 0 to 1000 ps, constructed from raw data) of curcumin at different MeOH (left panel) and MeOH-d_4 (right panel) mole fractions: top, 0.075; middle, 0.14; bottom, 0.24. The individual components (hollow circles, red, black, and blue) of the corresponding steady-state spectra (obtained after Gaussian fit; Table 2 and Figure S5, Supporting Information) are also shown.

methanol, but its H-bonding ability (both donating and accepting) is much less (Table 1). Therefore, we propose that the observed fluorescence properties of curcumin in toluene−alcohol mixtures (which also show a significant isotope effect) depend upon the modulation of the intermolecular H (D) bonding network (between MeOH or MeOH-d_4 molecules) by toluene.

In addition to ESPT, solvation is another major photophysical process of the pigment in the excited state.[9,10,12] The average solvation time of curcumin in neat methanol was earlier observed to be ∼4−7 ps.[9,10] The slow rise times (and consequently slow solvation times) of the pigment observed only in toluene−alcohol mixture and their dependence on alcohol mole fraction (Figure 3) imply that specific H(D)-

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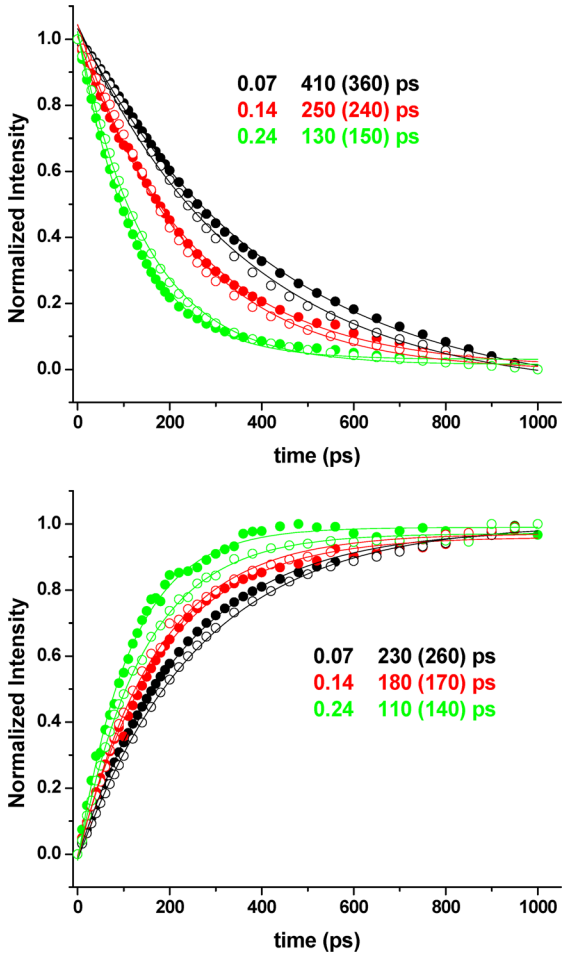

Figure 6. Decay (top) and rise (bottom) kinetics of the high (~20 408 cm^{-1}) and low energy (~18 518 cm^{-1}) peaks of the TRANE spectra shown for the three mole fractions of MeOH (solid) and MeOH-d_4 (hollow). The time constants in parentheses correspond to those for the deuterated solvent. The errors in time constants are estimated to be 10−20%. The initial (t = 0 ps) and final (t = 1000 ps) intensities are normalized between unity and zero (or zero and unity).

bonding interactions between excited curcumin and MeOH (MeOH-d_4) is getting affected in the presence of toluene. As discussed earlier, the H-bonding properties of toluene are significantly poor compared to MeOH (MeOH-d_4). As a result, the intermolecular H-bonding between the alcohol molecules in the alcohol−toluene solvent mixture is expected to get disturbed, resulting in a diminished H-bonding property of the alcohol. This, in turn, will affect the specific H(D)-bonding interactions between the pigment and the alcohol solvent resulting in lengthening of the reorganization process of the intermolecular H(D) bond, and as a result, the solvation time of the excited state of the pigment gets slowed down significantly. The role of toluene thus seems to be critical in controlling the strength and reorganization of the H-bonding network between the pigment and the alcohol solvent in the excited state. At this point it is relevant to examine the absorption spectra of curcumin to ascertain whether intermolecular H(D) bond is present in the ground state of the pigment. The vibronic features in the absorption spectra (Figure S2) start to smear out at MeOH (MeOH-d_4) mole fractions of 0.4 (0.07) and above which implies that the ground state of the pigment is likely to be heterogeneous, the degree of heterogeneity being dependent on MeOH (MeOH-d_4) mole fraction. However, the emission spectrum (Figure 1) starts to lose the vibronic features much earlier (0.02 for MeOH and 0.01 for MeOH-d_4). This indicates that specific H(D) bonding interaction between curcumin and the alcohol solvent is definitely stronger in the excited state. The driving force behind this is obviously the electron localization around the keto and enolic oxygen (due to a large change in the dipole moment) of the pigment after photoexcitation. However, another important factor is the local concentration of the alcohol solvent around the solute which will change with its mole fraction.

This, in turn, is expected to affect events like (i) breakage of the intramolecular H(D)-bond, (ii) formation of the intermolecular H(D)-bond with the alcohol solvent, and (iii) solvation of the excited state, in addition to other events after photoexcitation of the pigment. The existence of at least three species in the excited state is supported by the fact that three Gaussian components are needed to adequately represent the steady-state emission spectra (Figure S6), and two isosbestic points are observed in the TRANE spectra of the pigment (Figure 5). Based upon the results obtained so far, the likely sequence of events after photoexcitation of the pigment can be summarized as follows:

Photoexcitation of curcumin in toluene−MeOH (MeOH-d_4) solvent mixtures leads to the formation of both species I and II (Scheme 1) in the excited state. The relative population of I and II will depend on the alcohol mole fraction; increasing the alcohol mole fraction is likely to increase the population of species II. Species I and II gets converted into another species III which is fully solvated and intermolecular H-bonding between III and MeOH (MeOH-d_4) has been optimized. There are possibly several events in between species I, II, and III which are discussed earlier. Solvent relaxation and H-bond reorganization are expected to be the significant ones, but the order in which they act and how they act still remains unclear. It is important to note that the probability of species I and II decaying directly to the ground state cannot be ruled out. Assuming the high- and low-energy peak (obtained from TRANE spectra, Figure 6) represents species I and III, respectively, the observed kinetics can be explained by the fact that with increasing alcohol mole fraction breakage of the intermolecular H(D) bond and formation and reorganization of the intermolecular H(D) bond in the excited state are expected to be faster. It is interesting to note that no significant isotope effect was observed in the spectral relaxation, except that observed in the fluorescence properties (Figure 2) with changing solvent composition.

■ CONCLUSION

In conclusion, the results reported here shows that ESHT reactions of curcumin excited state in binary solvent mixtures of toluene−methanol depends critically upon solvent composition which can be attributed to the modulation of the hydrogen-bonding network. For further understanding, the effect of structural variation in the solvents (benzene or xylene replacing toluene and long chain alcohols replacing methanol) on the excited-state ESHT reaction needs to be investigated, and currently these are being investigated. In addition, early time dynamics which can be investigated by the femtosecond fluorescence up-conversion technique is expected to provide a more complete picture.

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■ ASSOCIATED CONTENT

Supporting Information

Tables and figures containing various photophysical parameters of curcumin in different solvent mixtures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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