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# Acid Effect on Photobase Properties of Curcumin

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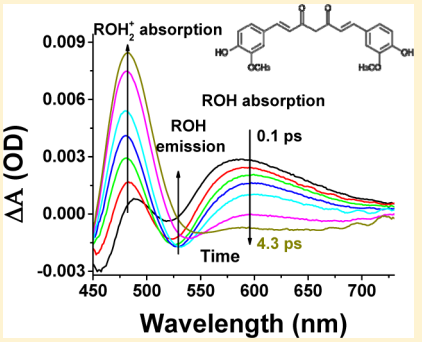
Supporting Information

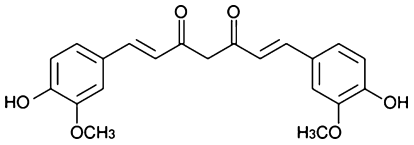
**ABSTRACT:** Femtosecond UV−vis pump−probe spectroscopy was employed to study the acid effect on curcumin in the excited state. Curcumin in solutions of weak acids was found to be a photobase forming a protonated curcumin within a few tens of picoseconds from the time of excitation. The excited-state protonation reaction is also observed in the steady-state emission spectrum as a new red emission band with a maximum at 620 nm in the presence of weak acids. The transient pump−probe spectrum consists of four spectral bands, two emission bands, and two absorption bands. We assign a transient absorption band at ∼600 nm and an emission band at ∼540 nm to the neutral ROH form of curcumin. An absorption band at ∼500 nm and an emission band at 620 nm are assigned to the protonated $ROH_2^+$ form of curcumin.




## INTRODUCTION

Numerous nutritional and medicinal virtues of curcumin (shown in Scheme 1), attributed to it in India by traditional cuisine and folk medicine have been earning scientific recognition in recent years.[1−4] These are in addition to its valuable uses as a dye for fabric and as a food-coloring agent.

**Scheme 1. Molecular Structure of Curcumin**




The renewed interest in curcumin has allowed research to further unfold its equally intriguing and no less complex photophysical and photochemical properties.[1,5−12] The steady-state spectra of curcumin are highly solvent-dependent,[13,14] with absorption and emission maxima ranging from 408 to 430 nm and from 460 to 560 nm, respectively.

Ultrafast spectroscopic techniques by several groups have also helped to reveal both protic and polar factors in the solubility of curcumin. For curcumin in alcohols, some have suggested[15−17] the occurrence of excited-state intramolecular hydrogen transfer (ESIHT), after detecting two decay components in the excited-state kinetics with short decay times and a pronounced isotope effect. Others suggested that curcumin has a strong intramolecular charge-transfer character, which is due to the planar structure of its cis-enol conformer.[18] They argued that hydrogen-bond reorganization governs the solvent dynamics of curcumin, their results showing good correlation with the solvation-dynamics data acquired by Maroncelli and co-workers[19] on coumarin dyes in the same alcohols. High concentrations of mild bases such as sodium and potassium acetate in methanol and ethanol solutions were found[20] to have a notable effect on both the time-resolved and the steady-state emissions, which are composed of two decay components attributed to proton transfer. Thus it was established that curcumin is a photoacid.

We have recently[21] reported on the photobasic properties of curcumin. Following on studies by Saini and Das,[22,23] a full account of all of the Kamlet−Taft[24] solvent parameters was set as the framework for the fluorescence-upconversion investigations. Addition of 1 M HCl acid to methanol was found to have a profound effect on the absorption and emission of curcumin. In trifluoroethanol, a new band at 620 nm appeared and was attributed to a protonated form of curcumin designated as $ROH_2^+$. For the absorption spectrum of curcumin in hydrogen-bond-donating solvents, a new band at 530 nm appeared at high acid concentrations.

In the current work, femtosecond pump−probe, spectroscopy is used to further explore the excited-state protonation reaction of curcumin in the presence of the weak organic acids, acetic acid ($pK_a$ = 4.7), and trifluoroethanol ($pK_a$ = 12.4). The existence of four spectral bands in the transient pump−probe spectrum is reported: at short times emission is dominated by a 530 nm band and absorption by one at 600 nm. Both are assigned to the two bands of the ROH, the neutral form of curcumin. However, at longer times, an increase is seen in two new bands in the transient pump−probe spectra. These are attributed to the excited-state protonated, $ROH_2^+$, form of curcumin, with absorption peaking at 500 nm and emission at 620 nm. These bands grow stronger with the addition of formic acid. We explain the large dependence of the curcumin

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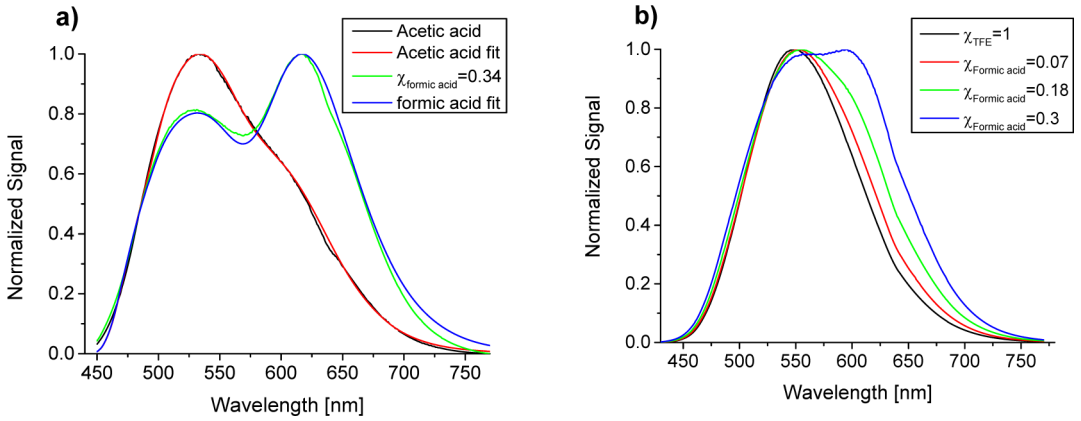


**Figure 1.** Normalized steady-state (time-integrated) emission spectra of curcumin in (a) mixtures of acetic acid and formic acid and (b) mixtures of trifluoroethanol (TFE) and formic acid.

excited-state lifetime on the formic acid concentration by suggesting that a substantial part of the nonradiative rate is controlled by the deprotonation reaction of the excited curcumin $ROH_2^{+}$* form.

## EXPERIMENTAL SECTION

Curcumin (of 81% purity) was purchased from Sigma-Aldrich. All measurements were carried out with fresh solutions of curcumin at the desired concentration and solvent. Acetic acid, trifluoroethanol and other chemicals used in this study were of analytical grade and were purchased from Aldrich and Merck. Multichannel transient-absorption measurements were conducted with the use of a pump−probe system (Helios, Ultrafast Systems) in a cuvette with a 2 mm optical path, and the data were analyzed with commercial software (Surface Xplorer, Ultrafast Systems). The samples were pumped at a frequency of 500 Hz with 80 fs pulses generated by an optical parametric amplifier (TOPAS, Light Conversion) tuned to 415 nm. To avoid degradation, the samples were continuously stirred inside the cuvette. The temporal chirp of the white-light continuum probe was measured in $D_2O$ under identical experimental conditions and the data were corrected for temporal chirp with the use of a built-in algorithm. The steady-state emission and absorption spectra were recorded by a Horiba Jobin Yvon FluoroMax-3 spectrofluorometer and a Cary 5000 spectrometer.

## RESULTS

Figure 1 (panels a and b) show the normalized steady-state (time-integrated) emission spectra of curcumin in mixtures of acetic acid and formic acid and in mixtures of trifluoroethanol (TFE) and formic acid.

In both formic acid mixtures, the spectra consist of two emission bands. The relative intensity of the long-wavelength band in the normalized spectrum increases with an increase in the mole ratio of formic acid.

We assign the short-wavelength emission band with a maximum at ∼540 nm in acetic acid and 550 nm in TFE to the ROH form of curcumin and the red band at 620 nm to protonated curcumin, $ROH_2^{+}$. The absorption band of curcumin in these solvent mixtures, up to $\chi_{formic} = 0.4$, is almost unaffected by the presence of formic acid and, therefore, we are exciting the same ground-state (ROH) form of curcumin. At higher mole fractions of formic acid, the protonation also occurs in the ground state. The absorption of the $ROH_2^{+}$ is at 530 nm, whereas the ROH absorption peak in these solvents is at about 410 nm. Figure 1a also shows a computed fit to the steady-state emission. The fitting procedure is explained in Discussion. We used the spectral-fit function of the steady-state emission spectrum for the analysis of the transient spectra that we obtained by means of the pump−probe spectroscopy technique. Table S1 of the Supporting Information provides the fitting parameters of the steady-state spectrum of curcumin in acetic acid and in a mixture of acetic/formic acids, $\chi_{formic} = 0.34$.

Previous studies[20,21,25,26] have revealed that curcumin UV−vis spectroscopy in both the ground-state and the excited-state is sensitive to the presence of acid or base in the solution.

In the current study, we focus our attention on the effect of acid on the photochemistry of excited-state curcumin in two protic solvents. Acetic acid is a weak protic acid with a $pK_a$ of 4.7. The Kamlet−Taft[24] solvent parameters of acetic acid are $\pi = 0.64$, $\beta = 0$, and $\alpha = 1.12$. The solvent parameters of TFE are $\pi = 0.73$, $\beta = 0$, and $\alpha = 1.5$, and its $pK_a$ is about 12.4. TFE and acetic acid cannot accept a proton but can transfer a proton to curcumin and other bases.

**Time-Resolved Measurements by Pump−Probe Spectroscopy.** Pump−probe spectroscopy with ∼120 fs full-width at half-maximum instrument response was used to study further the effect of acid on curcumin photochemistry. Figure 2 (panels a−d) shows the pump−probe transient spectra of curcumin in acetic acid at several points in the time domain between 0 and 820 ps.

As seen in the figure, the spectra consist of positive and negative contributions. The positive part of the spectrum is attributed to an absorption from $S_1 \rightarrow S_n$ of the ROH form at early times after excitation, whereas at long times, the absorption is assigned to the $ROH_2^{+}$ form of curcumin.

Figure 2b shows the spectra at the time window of 0.2−8 ps. Over this time window, the main contribution to the spectrum is positive. Two absorption peaks are observed, one at short wavelengths (∼500 nm) and the second at 600 nm. These spectra also include an emission spectrum with a peak at ∼540 nm, but its intensity is weaker than that of the absorption. We assign this emission to the ROH form of curcumin, and it is the main emission component in the steady-state emission spectrum shown in Figure 1a.

In Discussion, we provide an analysis of the transient spectra. We fit the transient spectra by two methods. The first is by global analysis, and the second is by spectral fit of individual transient spectra at several times. The second fitting procedure is carried out under the assumption that the spectrum consists


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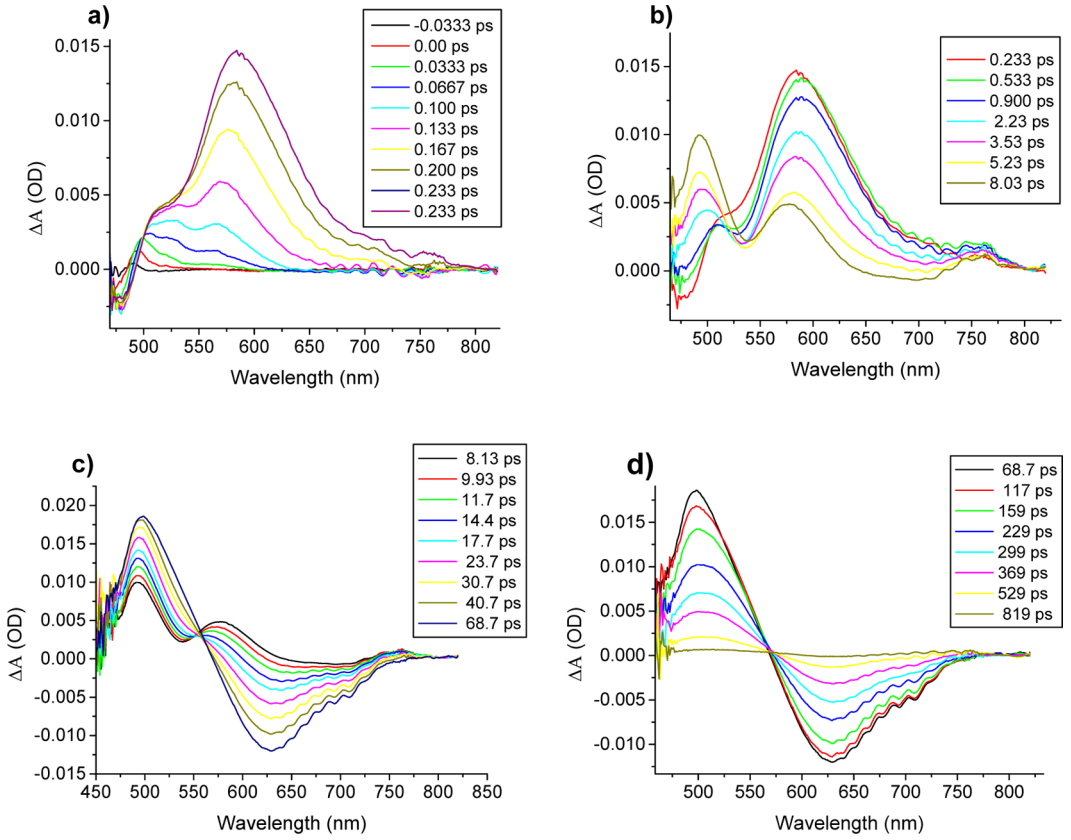


**Figure 2.** Pump–probe transient spectra of curcumin in acetic acid at several points in the time domain between 0 and 500 ps.

of two emission bands, that of the ROH and that of the $ROH_2^+$ forms of curcumin, and two absorption bands of the $S_1 \rightarrow S_n$ transitions of ROH and of $ROH_2^+$. We obtain reasonable fitting results with the use of both methods. We conclude from the fittings that the photochemical reaction taking place is

$$ROH + AH \xrightarrow{\vec{k}} ROH_2^+ + A^-$$

Curcumin is a photobase capable of accepting a proton from weak acidic solvents like acetic acid ($pK_a$ = 4.7) and TFE ($pK_a$ = 12.4). It can also react with an excess proton in solution. The addition of stronger acids like formic acid ($pK_a$ = 3.7) to these solvents increases the concentration of excess protons in the solution and also produces a greater reaction rate.

We assign the absorption band at 600 nm to the $S_1 \rightarrow S_n$ transition of the ROH* form of curcumin, whereas the narrower band at 500 nm is assigned to the transient absorption of the $ROH_2^{+}$* form. As seen in the figure, the ROH absorption decreases with time, whereas the $ROH_2^+$ absorption band increases. The opposite changes in the time dependence of the two absorption bands is direct evidence of the protonation reaction of curcumin that takes place in the excited state.

Figure 2c shows the transient spectra at the longer times of 8–69 ps. The transient spectra in this time window show no time-dependence of the signal intensity at a wavelength of ~560 nm. At longer wavelengths, a broad, asymmetric emission band with a maximum at ~630 nm is formed with time. At short wavelengths ($\lambda$ < 560 nm), the intensity of the $ROH_2^+$ absorption band increases with time. The transient absorption at 600 nm and the emission at 540 nm assigned to the ROH form are rather small and decrease with time.

Figure 2d shows the transient spectra at long times of 69–820 ps. Both the emission and absorption bands of the $ROH_2^+$ form decrease with time and, at very long times (820 ps), the transient pump–probe signal approaches zero at the same rate. The decay time is ~200 ps. This time constant was also observed previously[21] for the decay time of the time-resolved fluorescence signal of curcumin in acetic acid at 630 nm.

Figure 3 (panels a–d) shows the pump–probe transient spectra of curcumin in a solvent mixture of acetic and formic acids of $\chi_{acetic}$ = 0.8.

The addition of formic acid to the acetic acid solution increases the free-proton concentration and also provides an additional direct proton donor with larger proton-donating power and thus enhances the rate of the excited-state proton transfer to curcumin to form the $ROH_2^+$ form. A comparison of the time windows of Figure 3 (panels b–d) with that of curcumin in neat acetic acid shows that the rate of proton transfer to curcumin indeed increases in the presence of formic acid. The shape of the transient pump–probe spectra of curcumin in the solvent mixture is similar to that of curcumin in neat acetic acid, except that the reaction rates are larger.

When formic acid is added to an acetic acid solution, not only does the protonation reaction rate increase but also the relaxation rate of the state to the ground state increases. This formic acid effect is also observed in the steady-state emission spectrum of curcumin in acetic acid mixtures with formic acid. The steady-state fluorescence quenching is greater in the presence of formic acid. The study of time-resolved emission[17] showed a trend similar to that found in the current pump–probe study. With an increase in the mole fraction of formic acid, the protonation reaction rate increases and the


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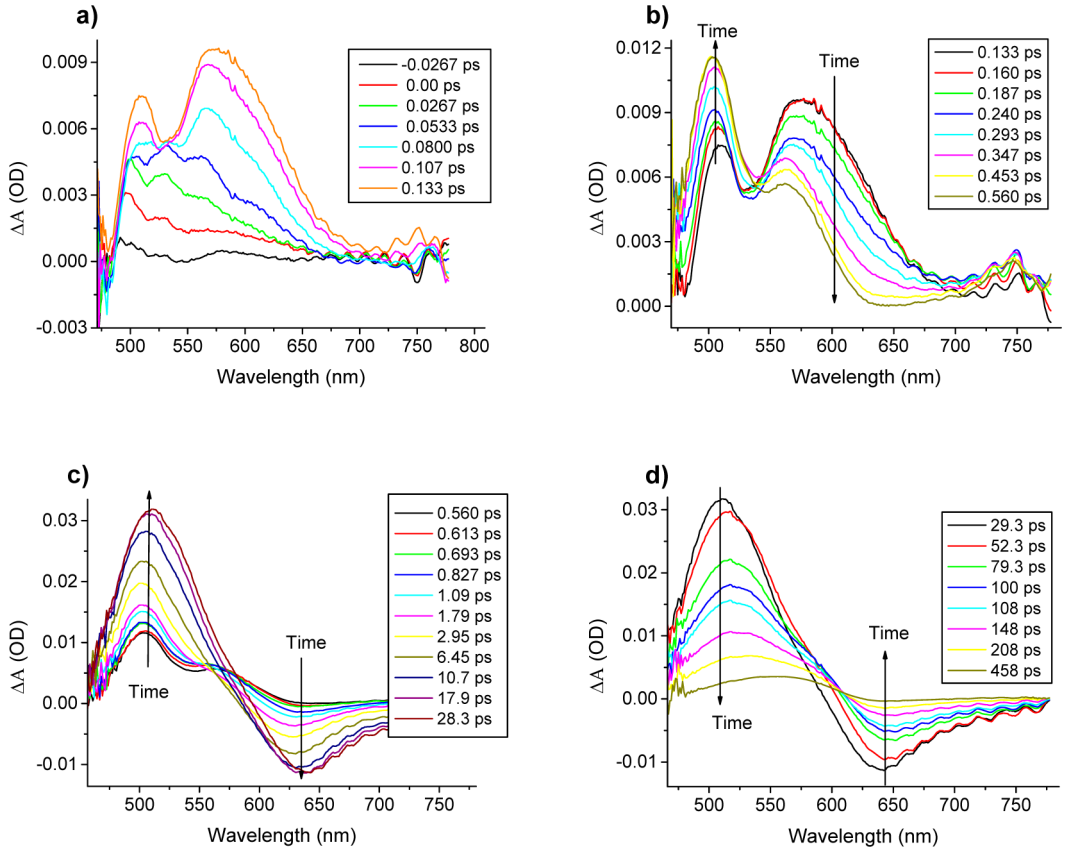


**Figure 3.** Pump−probe transient spectra of curcumin in a solvent mixture of acetic and formic acids of $\chi_{acetic} = 0.8$.

fluorescence decay time of the $ROH_2^+$ is strongly influenced by the formic acid. The decay time of the pump−probe signal of the transients shown in Figure 3d is 76 ps versus 200 ps in the case of curcumin in neat acetic acid (see Figure 2d).

Figure 4 (panels a−d) shows the pump−probe transients of curcumin in trifluoro-ethanol (TFE) at several times in the spectral range of 440−800 nm.

Figure 4a shows the transient signals at short times (up to 100 fs) where the supercontinuum pulse of the probe overlaps in time with the pump laser at 410 nm. The signal consists of a bleaching spectral component of the tail of the ground-state absorption band at ∼450 nm. The signal also consists of the ROH $S_1 \rightarrow S_n$ absorption at ∼600 nm as well as the ROH emission band at 530 nm and a small absorption assigned to the $S_1 \rightarrow S_n$ absorption of the newly formed $ROH_2^+$ form of curcumin with a band peak at 490 nm. Figure 4b shows the build-up of the $ROH_2^+$ transient absorption at the 490 nm spectral region, the decay of the ROH emission at 530 nm, and the ROH decay of the transient absorption at 600 nm.

Figure 4c shows the build-up of $ROH_2^+$ emission at 635 nm and further increase of the $ROH_2^+$ absorption at 490 nm. The ROH absorption and emission decrease further as the protonation reaction depletes the ROH* population.

Figure 4d shows the long-time pump−probe transients in the time window of 32−320 ps. Over this period, the $ROH_2^{+}$* population decays to the ground state, and the positive and negative signals approach zero intensity at long times.

Figure 5 (panels a−d) shows the pump−probe transient spectra of curcumin in a TFE/formic acid mixture of $\chi_{TFE} \approx 0.76$.

The shape and intensity of the transient spectra are similar to those shown in Figure 4 for curcumin in neat TFE. The main difference between the transient spectra of curcumin in neat TFE and in mixtures with $\chi_{formic} \approx 0.24$ is that the kinetic rate constants of the ROH* + AH $\overset{k'}{\rightarrow}$ $ROH_2^{+}$* + $A^-$ and the relaxation rate of the $ROH_2^{+}$* back to the ground state are larger in the TFE/formic acid mixture. Formic acid enhances the formation rate of $ROH_2^{+}$* and increases the nonradiative rate of $ROH_2^+$ back to the ground state. The same formic acid effect is observed in the transient spectra of curcumin in acetic acid. This effect was also observed in our previous study that was based on time-resolved emission measurements of curcumin in acetic acid and TFE and in mixtures of acetic acid with formic acid.[21]

An important point to notice is that the emission band maximum of $ROH_2^{+}$* in neat TFE is at ∼610 nm, while that of the TFE/formic acid mixture is at 645 nm. The emission peak in neat acetic acid is at 630 nm, while that of acetic/formic acid the emission peak of $ROH_2^{+}$* is at 640 nm.

As a control experiment in which intermolecular proton transfer reactions in curcumin are unlikely, we measured the pump−probe spectrum of curcumin in dichloromethane, as seen in Figure S1 of the Supporting Information. In dichloromethane, the pump−probe transient absorption spectrum between 0.12 ps (immediately after the pump excitation) and up to 0.8 ps consists of the ROH absorption band with a maximum at ∼580 nm and a bleaching of the ground state absorption with a tail at 475 nm > $\lambda \geq$ 450 nm. At long times from 0.8−14.4 ps, the $S_1 \rightarrow S_0$ ROH emission band at 540 nm could be observed in the spectrum, and the ground state bleaching, $\lambda$ < 470 nm, is also seen. A rather weak and narrow transient absorption at ∼480 nm is also observed, and an isosbestic point at 505 nm is also clearly seen. This weak


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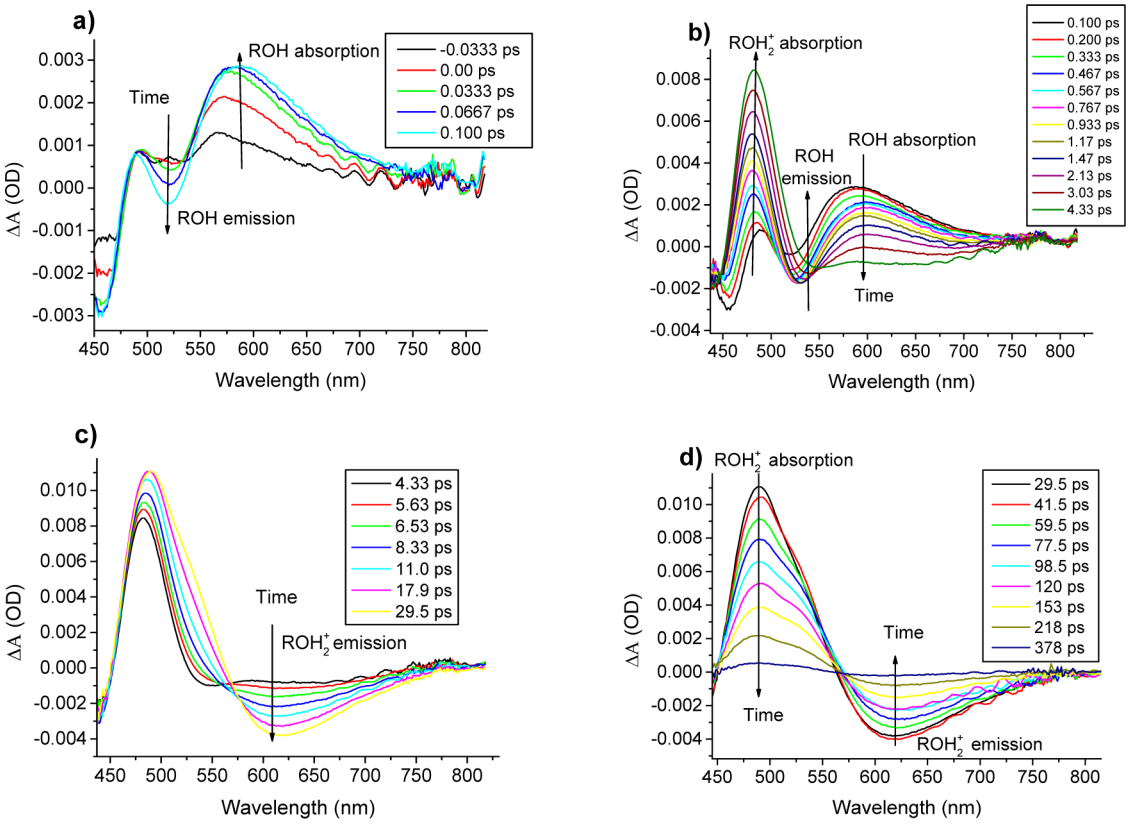


**Figure 4.** Pump−probe transients of curcumin in trifluoroethanol (TFE) at several times in the spectral range of 440−800 nm.

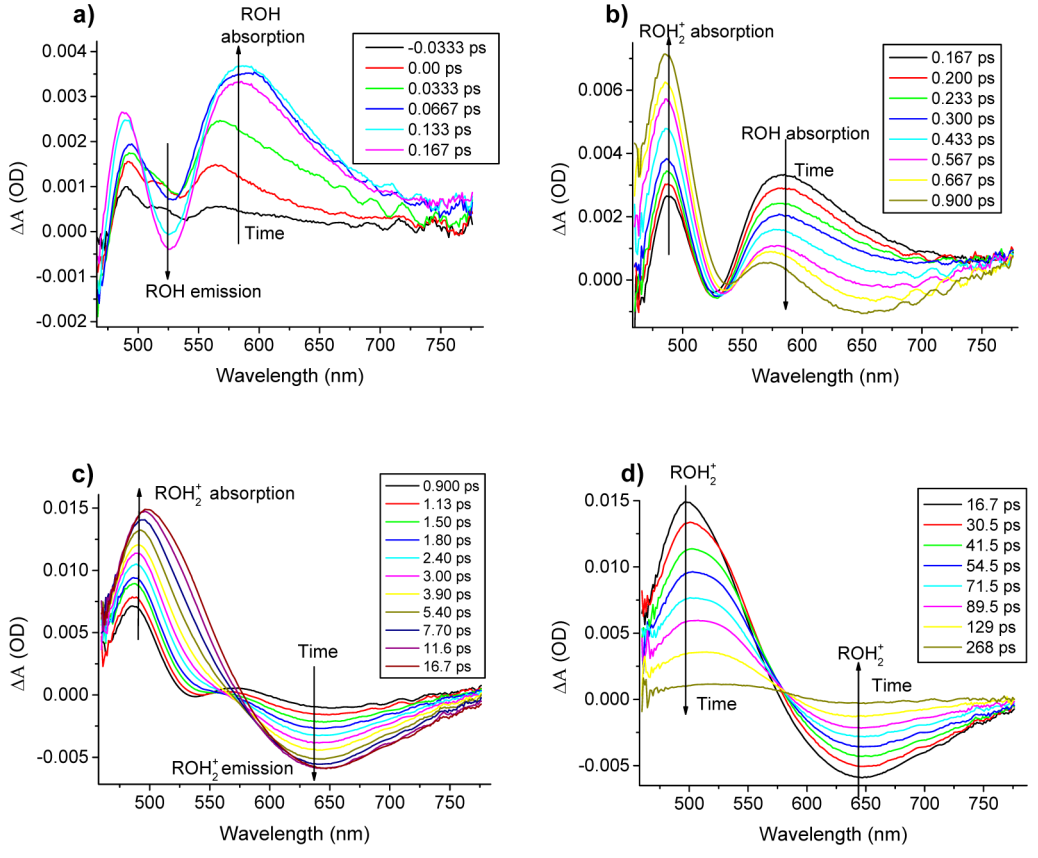


**Figure 5.** Pump−probe transient spectra of curcumin in a TFE/formic acid mixture of $\chi_{TFE} \approx 0.76$.


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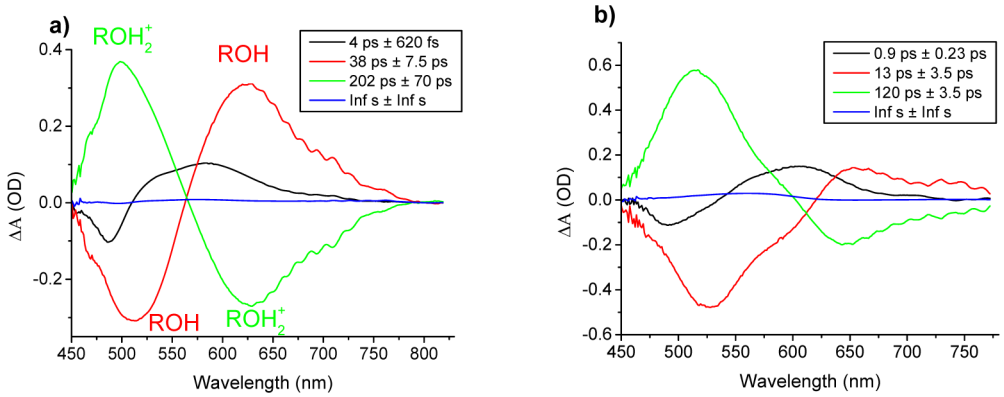


**Figure 6.** Transient spectra of curcumin ROH form in (a) acetic acid and (b) a mixture of acetic and formic acids with $\chi_{formic}$ = 0.2.

absorption is clearly different in its intensity, width, and shape than the broad and intense transient absorption at 500 nm of curcumin in both acetic acid and TFE as well as in mixtures of these two solvents with formic acid. The broad band in the acidic protic solvents we assign to the $ROH_2^{+*}$ excited state absorption, whereas the weak and narrow absorption at 480 nm of curcumin in dichloromethane we assign to a hot ground state absorption $S_0(\nu)\rightarrow S_1(\nu')$, where $\nu$ and $\nu'$ are the vibrational state quantum numbers.

**Main Findings.** (1) Femtosecond transient pump−probe spectra over the spectral range of 450−800 nm, consist of four spectral bands. (2) An emission band with a band peak at ∼530 nm and an absorption band with a peak at ∼600 nm are assigned to curcumin in its neutral ROH form. (3) Both the absorption and emission bands of ROH arise from optical transitions of the curcumin in the $S_1$ state. (4) With time, the transient spectra change and two new bands appear, whereas the intensity of the two ROH bands decreases. (5) We assign the new bands to the excited protonated form of curcumin $ROH_2^{+*}$ with an absorption peak at ∼500 nm and emission peak at 620 nm. (6) The rate of the protonation reaction depends on the properties of the solvent; in acetic acid ($pK_a$ = 4.7), the protonation time-constant is 33 ps, while in trifluoroethanol (TFE), it is only 14 ps. (7) The protonation rate of curcumin in acetic acid increases in the presence of formic acid ($\chi_{formic} \approx 0.24$), which is a stronger acid with $pK_a \sim 3.7$. (8) The excited-state lifetime of the $ROH_2^{+*}$ form of curcumin decreases by about a factor of 2 in solvent mixtures of acetic acid and TFE with formic acid ($\chi_{formic} \approx 0.24$).

## DISCUSSION

**Global-Fitting Analysis.** We used the global-fitting procedure to deconvolute the complex transient pump−probe spectra shown in Figures 2−5 and obtain the relevant absorption and emission spectra of the chemical species associated with the photoprotolytic processes of the protonation reaction of curcumin in hydrogen-bond-donating solvents. In short, we used singular-value decomposition,[27] imposing three time components. The kinetic traces of these components (Figures S2 and S3 of the Supporting Information) were used for globally fitting the transient spectra to a multiexponential decay. Figure 6 (panels a and b) show the pre-exponential spectra derived from the analysis of the transient spectra shown in Figure 2 for curcumin in neat acetic acid and in Figure 6b for curcumin in a mixture of acetic acid and formic acid with $\chi_{formic}$ = 0.2.

Basically, we find three time components in the transient pump−probe spectra of curcumin. The first is an ultrafast time component of about 1.2 ± 0.2 ps.

The short time-component may be associated with either of two processes. The first plausible process is an excited-state intramolecular hydrogen transfer (ESIHT) between the oxygen atoms of the diketone moiety. The stable ground-state conformation is the enol form.[14] The excited-state stable conformation is not yet known. Several studies suggest that if a hydrogen transfer reaction occurs in the excited-state, then the most plausible process is an enol−keto isomerization. Excited-state intramolecular hydrogen transfer is observed in many compounds in which a hydrogen donor and acceptor groups are next to each other. Usually in aromatic hydroxy compounds, the OH group is a proton donor and an aromatic nitrogen atom situated next to the hydroxyl hydrogen atom is the proton acceptor. The hydroxyl hydrogen and the nitrogen atoms are already hydrogen-bonded in the ground state (prior to the photoexcitation process). The rate of the excited-state intramolecular hydrogen-transfer (ESIHT) reaction is ultrafast $\tau_{PT}$ < 100 fs. Several studies have shown that it occurs in about 50 ± 10 fs.[28,29] The fast time-component of curcumin is about 1.2 ps, more than twenty times longer than the given rate for the ESIHT process. The hydrogen-bonding process has been extensively studied with the use of IR pump−probe spectroscopy as well as 2D IR time-resolved spectroscopy.[30−33] In accordance with most of the transient IR studies, the timescale of hydrogen-bond making and breaking is about 1.4 ± 0.2 ps. Thus we assign the short time component of the global analysis to hydrogen bonding that occurs in the excited state, prior to the actual proton transfer to the curcumin.

We assign the second spectrum derived from the global-fitting analysis to the absorption and emission spectra of the ROH* form of curcumin. The red lines in Figure 6 (panels a and b) show the transient spectra of the ROH form of curcumin in acetic acid and in a mixture of acetic and formic acids with $\chi_{formic}$ = 0.2, respectively.

The decay time of the spectra of the ROH form in acetic acid and in the acetic/formic mixture is 38 and 13 ps, respectively. Formic acid enhances the decay rate of the ROH form of curcumin. We therefore conclude that the ROH* form reacts with either the acetic or formic acids and with the excess protons in the acetic/formic mixture.

$$ROH^* + AH \rightarrow ROH_2^{+*} + A^-$$

The reaction rate depends on the strength of the acid ($pK_a$). The stronger the acid the greater the proton-transfer rate


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constant. The protonation occurs in the excited state in the rather short time of a few tens of picoseconds in neat acetic acid and in about 13 ps when formic acid is added to the solution. The steady-state emission spectrum of curcumin in neat acetic acid shows that at long wavelengths ($\lambda$ > 600 nm), there is a small contribution of an emission band that is not seen in methanol or ethanol solutions. When formic acid is added to the acetic acid solution, there is an increase in the relative intensity of the long-wavelength band at 620 nm with respect to the major emission band with a band peak at ∼540 nm (see Figure 1). The greater decay rate of the global-analysis spectrum assigned to the ROH in the acetic/formic mixture is simply explained by the greater protonation rate of the ROH to form the $ROH_2^+$, since formic acid is a much stronger acid ($pK_a$ = 3.7) than acetic acid ($pK_a$ = 4.7). The rate of the proton-transfer reaction depends on the properties of both the proton donor and the proton acceptor. The larger the proton-donor tendency (small $pK_a$ value) of the solvent, the greater the rate of proton transfer to curcumin, the proton acceptor.

Table 1 provides the rate constants derived from the global analysis of the transient pump−probe signals of curcumin in both acetic acid and TFE as well as their mixtures with formic acid.

**Table 1. Global Analysis Rate Constants of Curcumin**

| decay time (ps) | TFE | TFE/formic acid | acetic acid | acetic acid/formic acid |
|---|---|---|---|---|
| $\tau_1$ | 1.4 ± 0.2 | 1.3 ± 0.14 | 4.0 ± 0.620 | 0.9 ± 0.23 |
| $\tau_2$ | 14 ± 2.0 | 9.2 ± 2.0 | 38 ± 7.5 | 13 ± 3.5 |
| $\tau_3$ | 112 ± 40 | 69 ± 50 | 202 ± 70 | 120 ± 3.5 |

Figure S4 (panels a and b) in the Supporting Information shows the global-analysis spectra of curcumin in trifluoroethanol (TFE) and in a TFE/formic acid mixture of $\chi_{formic}$ = 0.24.

The ROH spectrum consists of an emission band that appears as a negative signal with a minimum at 530 nm and absorption at 620 nm for TFE and at 650 nm for the TFE/formic acid mixture. The curcumin ROH* decays slightly faster in TFE than in acetic acid ($\tau \cong$ 14 ps). When formic acid is added to the solution, the decay rate of the ROH* spectra increases slightly ($\tau \cong$ 9 ps).

We assign the third global-analysis spectrum of curcumin to the protonated $ROH_2^+$ form. The $ROH_2^{+}$* spectra of curcumin are shown in both Figure 6 and Figure S4 of the Supporting Information. The curcumin $ROH_2^+$ absorption peak is at 510 nm for acetic acid and 500 nm for TFE solution. In the solvent mixture, the absorption band maximum is shifted to the red by about 10 nm. The emission band of the $ROH_2^{+}$* is at 630 nm in acetic acid and 615 nm in TFE. In the acetic acid/formic acid mixture, the $ROH_2^{+}$* emission band shifts to the red by about 20 nm and by 30 nm in the TFE/formic acid mixture. Figure S5 of the Supporting Information shows the $ROH_2^+$ transient spectrum of curcumin in TFE and TFE/formic acid mixtures as derived from the global analysis. The decay rate of the $ROH_2^{+}$* global-analysis spectrum depends on the solvent. The decay is assigned to the relaxation of the excited state back to the ground state. The $ROH_2^+$ lifetime is much shorter than the pure radiative rate of curcumin,[34] which is estimated to be ∼5 ns on the basis of the molar absorption coefficient, ∼40000 $M^{-1}$ $cm^{-1}$ at the band maximum (about 400 nm). We explain this acid effect on the nonradiative rate, which is partially dependent on the back-protonation process:

$$A^- + ROH_2^{+}* \rightarrow ROH(g) + AH$$

where AH and $A^-$ are the protonated and deprotonated solvent forms, and ROH(g) is the ground-state neutral form of curcumin.

Table 1 shows that the decay time of the $ROH_2^{+}$* is ∼200 ps in acetic acid and 120 ps in the acetic/formic mixture. Thus, formic acid enhances the nonradiative rate. A similar acid effect on the nonradiative rate is observed for the decay time of $ROH_2^+$ in TFE and in TFE/formic acid mixtures. The decay times are 112 and 70 ps, respectively.

Figure S6 of the Supporting Information shows the global analysis results in a three exponential fit of the pump−probe transient spectrum of curcumin in dichloromethane, for which intermolecular photoprotolytic processes are not expected. As seen in the figure, the global fit shows an absorption band which we assign to the ROH excited state absorption. At short times, the absorption band peak is at ∼550 nm and at long times its position is at 600 nm. The $ROH_2^{+}$* absorption and emission are missing in the global analysis. The control experiment of curcumin in a protic solvent supports the main finding of this article in which protonation of curcumin takes place in the excited state in hydrogen-bond-donating solvents.

**Pump−Probe Spectral Analysis.** We fit the shape and height of the two absorption and two emission spectral bands [$F_i(\lambda)$] seen in the pump−probe transient spectra of curcumin for several measured times over the time range of 0.1−28 ps, by a log-normal spectral-line-shape function (Suzuki[35] and Maroncelli[19]):

$$I(\nu) = h\begin{cases} \exp[-\ln(2)\{\ln(1+\alpha)/\gamma\}^2] & \alpha > -1 \\ 0 & \alpha \le -1 \end{cases} \quad (1)$$

$$\alpha \equiv 2\gamma(\nu - \nu_p)/\Delta \quad (2)$$

The log-normal function has four adjustable parameters: $h$, $\nu_p$, $\Delta\nu_p$, and $\gamma$. $\nu_p$ is the band-peak position, $h$ is the band intensity, $\Delta\nu_p$ the bandwidth, and $\gamma$ is the asymmetry of the band. When $\gamma$ = 0, the line shape is Gaussian. For the absorption bands [$S_1(0) \rightarrow S_n(\nu = n)$], the asymmetry parameter of the bands is positive, whereas for emission bands [$S_1(0) \rightarrow S_0(\nu = n)$] of both ROH and $ROH_2^+$ forms of curcumin, it is negative.

Figure 1 (panels a and b) shows the steady-state emission of curcumin in acetic acid and in TFE as well as in mixtures of these solvents with formic acid. We also display in the figures, the fit of the log-normal band shape to the experimental results. Table S2 of the Supporting Information provides the fitting parameters of the steady-state emission spectra shown in Figure 1. The steady-state spectrum of curcumin in formic acid mixtures of the two solvents consists of two bands, that of the ROH form and of the protonated form $ROH_2^+$. We used the computed steady-state emission spectra of both the ROH and $ROH_2^+$ forms in the fit of the transient pump−probe spectra of curcumin shown in Figures 2−5. The transient spectrum of curcumin consists of two absorption bands and two emission bands. The protonation reaction of curcumin in the excited state decreases both the ROH absorption and emission bands in the transient spectrum, whereas the $ROH_2^+$ absorption and emission increase with time. The absorption band at ∼500 nm is assigned to the $S_1 \rightarrow S_n$ transition of $ROH_2^+$, and the absorption band at ∼600 nm to the $S_1 \rightarrow S_n$ transition of ROH. As shown for example in Figure 2 (panels b and c), in acetic acid the intensity of the ROH band decreases with a decay time of about 30 ps, while that of the $ROH_2^+$ increases with the same time constant. The two emission bands in the transient spectrum are assigned to the ROH form at ∼530 nm and to the $ROH_2^+$


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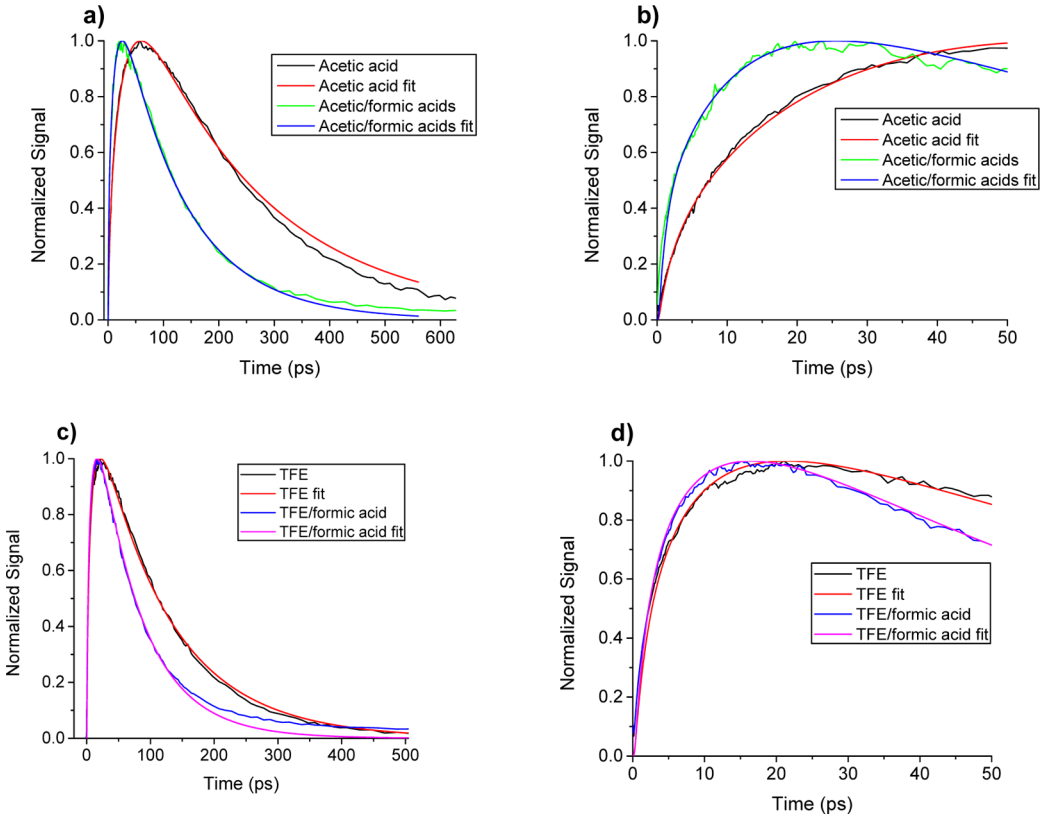


**Figure 7.** Time-resolved pump−probe signals of curcumin at the peak of the $ROH_2^+$ absorption band at ∼500 nm in (a) acetic acid and acetic/formic acid mixtures of $\chi_{formic} \approx 0.2$, (b) short times of panel (a), (c) TFE and TFE/formic acid mixture of $\chi_{formic} \approx 0.24$, and (d) short times of panel (c).

form at ∼620 nm. The transient absorption and emission bands overlap and thus positive absorption and negative emission interfere with each other. As a result, the transient spectra do not show, for example, the ROH emission band at 540 nm as a negative signal but only as a minimum between the two strong, positive absorption-band signals, that of the ROH at 600 nm and of the $ROH_2^+$ at 500 nm. To fit the transient pump−probe spectrum, we used the fit of the steady-state emission spectrum shown in Figure 1. The fitting parameters are given in Table S1 of the Supporting Information. We also fit the strong absorption bands of ROH and $ROH_2^+$. Figure S7 (panels a and b) in the Supporting Information show pump−probe transient spectra of curcumin in acetic acid and in TFE, respectively, measured at several times along with their log-normal fit on the basis of the procedure described above. The fits are rather good at all wavelengths. Table S2 of the Supporting Information provides the log-normal line-shape-fitting parameters of the ROH* and $ROH_2^{+}$* transient absorption bands as well as the ROH and $ROH_2^+$ emission bands.

With time, the relative amplitude of the $ROH_2^+$ absorption and emission bands increases while that of the ROH band decreases. This fit clearly shows that the protonation of the excited ROH form of curcumin occurs with a time constant that depends on the solvent properties.

**Time-Resolved Pump−probe Signals of $ROH_2^+$.** Figure 7 (panels a−d) shows the time-resolved pump−probe signals of curcumin at the peak of the $ROH_2^{+}$* transient-absorption band at ∼500 nm.

Figure 7 (panels a and b) show the signals of curcumin in acetic acid and in acetic/formic acid mixtures of $\chi_{formic} \approx 0.2$. At 500 nm, the overlap with the other emission and absorption bands of the transient spectra is minimal and, therefore, the time-resolved signal provides the excited-state solute/solvent dynamics and the protonation kinetics of the $ROH_2^{+}$* form of curcumin. The signal shows a multiexponential rise in acetic acid and also in acetic/formic acid mixtures. The signal rise is followed by a long decay attributed mainly to a nonradiative process promoted by deprotonation of $ROH_2^{+}$* to form the ground-state ROH(g).

The short-time-rise components of the signal in the mixture are ∼1.4 and 3.5 ps. These time components are associated with the hydrogen-bonding dynamic prior to the actual curcumin protonation reaction. For the acetic/formic acid mixture, we assign the long-time-rise component of about 13 ps to the protonation reaction of curcumin by the formic acid. The slower rise component is 33 ps in neat acetic acid and is assigned to the rate of protonation of curcumin by the weaker acetic acid. The decay time of $ROH_2^+$ of curcumin is slow in acetic acid and much faster in the acetic/formic acid mixture.

Figure 7 (panels c and d) show similar values of the transient pump−probe signal versus time measured at ∼500 nm, the $ROH_2^+$ form absorption peak. The two signals shown in the two panels belong to two samples, one of curcumin in TFE and one of a TFE/formic acid mixture with $\chi_{formic} \approx 0.24$. Surprisingly, the rise of both signals is nearly the same, whereas the decay time in the TFE/formic acid mixture is half that in neat TFE. The rise of the signal in neat acetic acid is much longer than in neat TFE. We explain these results in terms of faster protonation kinetics in TFE than in acetic acid. The rise time of the $ROH_2^+$ signal in neat acetic acid is ∼33 ps, whereas in TFE it is ∼14 ps. The nonradiative rate in TFE/formic acid mixture is larger and the decay times of the $ROH_2^+$ signal are


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**Table 2. Fitting Parameters of the Time-Resolved Pump-Probe Signals of Curcumin[a]**

| solvent | $a_1$ | $\tau_1$ (ps) | $a_2$ | $\tau_2$ (ps) | $a_3$ | $\tau_3$ (ps) | $\tau_F$ (ps) |
|---|---|---|---|---|---|---|---|
| acetic acid | 0.11 | 1.5 | 0.11 | 5.3 | 0.78 | 33 | 220 |
| acetic/formic acids | 0.25 | 1.5 | 0.1 | 5.3 | 0.65 | 15 | 110 |
| TFE | 0.25 | 1.5 | 0.35 | 5.3 | 0.45 | 15 | 108 |
| TFE/formic acids | 0.23 | 1.5 | 0.32 | 5.3 | 0.45 | 15 | 66 |

[a] $y(t) = [\sum_{i=1}^{3} a_i (1 - \exp(t/\tau_i)] \times \exp(-t/\tau_F)$.

110 and 66 ps in neat TFE and TFE/formic acid mixture, respectively. Table 2 provides the multiexponential fitting parameters of the $ROH_2^+$ form of curcumin shown in Figure 7. These parameters are in good agreement with the global fit of the three kinetic parameters in both Figure 6 and Figure S4 of the Supporting Information.

## SUMMARY AND CONCLUSIONS

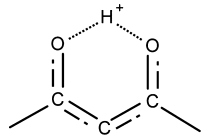
We have used femtosecond pump−probe spectroscopy to study the excited-state-protonation reaction of curcumin in the presence of weak organic acids. We used acetic acid ($pK_a = 4.7$) and trifluoroethanol ($pK_a = 12.4$) as acidic solvents. The transient pump−probe spectrum in the spectral range of 450−800 nm consists of four spectral bands. At short times, up to about 1 ps after excitation, we observe mainly one emission band with a maximum at ∼530 nm and a strong absorption band at 600 nm. We assign the two bands to the absorption and emission of the neutral, ROH, form of curcumin. The absorption band is attributed to an $S_1 \rightarrow S_n$ transition, and the emission is to the ground state and overlaps the blue part of the steady-state emission spectrum of curcumin in these solvents. With time, both band intensities decrease and two new bands are seen in the transient pump−probe spectra. We assign these new bands to the excited-state protonated $ROH_2^+$ form of curcumin. The absorption-band peak is at 500 nm, while the emission-band peak is at 620 nm. When formic acid ($pK_a \approx 3.7$) is added to acetic acid in a molar ratio of $\chi_{formic} \approx 0.2$ or to TFE in a molar ratio $\chi_{formic} \approx 0.24$, the rate of curcumin protonation $ROH^* + AH \rightarrow ROH_2^{+*} + A^-$ increases. In the presence of formic acid, the steady-state emission spectrum clearly shows a protonated emission band with a maximum at 620 nm. Pump−probe time-resolved spectral analysis by a global-fitting procedure provides the rate of the excited-state protonation reaction of the curcumin in acetic acid, TFE, and in mixtures of these two solvents containing formic acid $\chi_{formic} \approx 0.2$.

The time-constants for protonation are 14 ps and 33 ps for TFE and acetic acid, respectively. The rate of protonation in acetic acid solutions increases in the presence of formic acid, and the time constant is ∼13 ps. In TFE/formic acid mixtures, the protonation rate is almost unaffected and is similar to the rate in neat TFE. The transient pump−probe spectrum decreases and reaches zero intensity at longer times. The decay rate of the pump−probe spectrum depends on the solvent and is also strongly dependent on the presence of formic acid in solution. The pure radiative rate can be calculated from the ground-state absorption spectrum of curcumin with the use of the Strickler−Berg[34] formula. The pure radiative rate is about 5 ns, and the decay times of the transient spectra are 210 ps in acetic acid and 110 ps in TFE. The decay times are considerably shorter in the corresponding formic acid mixtures. The lifetimes are ∼100 ps for acetic/formic acid and 68 ps for TFE/formic acid solutions. We explain the considerable dependence of the excited-state lifetime of curcumin on formic acid by proposing that a large part of the overall nonradiative rate is due to the curcumin deprotonation reaction:

$$ROH_2^{+*} + A^- \rightarrow ROH(g) + AH$$

where ROH(g) denotes the neutral form of curcumin in the ground state and $A^-$ and AH the deprotonated and protonated solvent, respectively. Thus, the effect of acid on the steady-state emission spectrum of curcumin is also a high degree of quenching, which is indeed observed. At this point in our continuing research on curcumin spectroscopy, we are unable to place the protonation site of curcumin. Although previous studies show that the curcumin ground state is the keto−enol form,[36] we try and analyze the possible protonation sites. There are six oxygen atoms in curcumin. The two methoxy oxygen atoms are not involved in proton chemistry; two oxygen atoms form a diketo or keto−enol structure, and two are phenolic oxygen atoms. The phenolic oxygen atom is known to be a photoacid and not a photobase. Thus, it is plausible that the diketo oxygen atoms have the ability to act as a proton sponge and that the added proton forms a stable six-membered ring as shown in Scheme 2.

**Scheme 2. Diketone Form**




## ASSOCIATED CONTENT

### Supporting Information

(A) Fitting parameter tables and (B) pump−probe transient spectra, singular-value decomposition kinetic traces, and global analysis of curcumin. 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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