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Effect of Deuterated Solvents on the Excited State Photophysical Properties of Curcumin

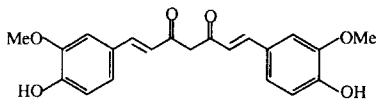
A. Barik[a], N. K. Goel[b], K. I. Priyadarsini[a] and Hari Mohan[a*]
[a]Radiation Chemistry & Chemical Dynamics Division, Bhabha Atomic Research Center, Trombay, Mumbai 400085, India.
[b]Radiation Technology Development Section, Bhabha Atomic Research Center, Trombay, Mumbai 400085, India.

Optical absorption and emission studies have been carried out to understand the effect of deuterium on the solvent dependent photophysical properties of curcumin in deuterated solvents such as $CDCl_3$, $(CD_3)_2SO$, $(CD_3)_2CO$, CD_3OD and CD_3CN. Optical absorption spectral studies showed that there is no significant shift in absorption maxima compared to the non-deuterated solvent. The fluorescence maxima shows significant shift with polarity of solvent but not much affected by the deuteration. The fluorescence quantum yield of curcumin increased marginally in almost all the deuterated solvents, indicating reduction in the non-radiative pathways. The fluorescence decay was biexponential in all the solvents and the average fluorescence lifetime was not much affected with deuteration, but showed decrease with increasing solvent polarity. Based on these studies, it is concluded that intermolecular hydrogen transfer is only partially responsible for the excited state deactivation of curcumin.

key words: Curcumin; Deuterated solvents; Photophysical properties

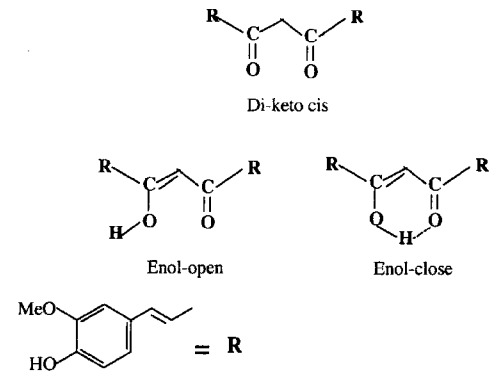
INTRODUCTION

Curcumin, bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (structure given in scheme 1), is a yellow pigment found in the rhizomes of *Curcuma longa*, popularly known as turmeric. The antibacterial activity of curcumin is greatly enhanced by light [1-3]. The photoproducts of curcumin are not toxic, therefore the toxicity is due to the excited state of curcumin [4]. The photophysics of curcumin is very much dependent on solvent polarity [5, 6], predominantly due to the existence of the keto-enol tautomerism, which involves intramolecular proton transfer. Depending on the polarity and protic nature of the solvents the keto-enol tautomeric equilibrium is influenced and therefore plays an important role in the excited state properties of curcumin. The enolic form can exist in different cis and trans isomeric forms (scheme 2) depending upon temperature, polarity or hydrogen bonding nature of solvents [7, 8]. If either intra- or inter-molecular proton transfer is mainly responsible for these solvent dependent photophysical properties, one should see a significant change in deuterated solvents.



Curcumin

Scheme 1.



Different tautomeric forms of curcumin

Scheme 2.

It has been reported that the fluorescence spectrum of curcumin is highly sensitive to the solvent polarity showing large dipole moment change in the excited state. The fluorescence quantum yield was very low in most of the solvents and is slightly higher in non-polar solvents. It is known that non-polar solvents prefer enolic from and polar solvents favor keto form of di-keto compounds [7, 8]. Normally, the extra stabilization to the molecule is achieved in polar protic solvents as compared to the polar aprotic

*To whom correspondence should be addressed.
E-mail :harim@apsara.barc.ernet.in
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solvents of same polarity, because of hydrogen bonding. With these objectives, the present studies have been carried out to understand the effect of deuteration on the photophysical properties of curcumin. Optical absorption and emission properties of curcumin were studied in different deuterated solvents to know if the shift in the absorption and fluorescence spectra is due to (1) the polarity effect, (2) intermolecular hydrogen bonding or (3) hydrogen transfer mechanism. In this paper absorption and fluorescence spectra of curcumin were recorded in deuterated analogues of solvents like chloroform ($CDCl_3$), dimethyl sulfoxide $(CD_3)_2SO$, acetonitrile (CD_3CN), acetone $(CD_3)_2CO$ and methanol (CD_3OD). Fluorescence quantum yield and lifetime measurements were also carried out in these deuterated solvents. The solvents were selected with different properties such as non-polar, polar aprotic and protic. The studies were performed mainly to assess the role of intermolecular hydrogen transfer on the excited state deactivation of curcumin.

EXPERIMENTAL

Curcumin (Sigma Chemicals) and spectrograde solvents from Spectrochem India Pvt. Limited were used as received. Coumarin 153 laser dye (Lambda phyzik) was used as a reference for quantum yield measurements. Deuterated solvents $CDCl_3$ (99.8 atom%), CD_3OD (99.95 atom%), $(CD_3)_2SO$ (99.9 atom%), $(CD_3)_2CO$ (99.5%) and CD_3CN (99.8%) were obtained from Aldrich Chemicals and used as received. $(CD_3)_2CO$ contained 0.03% TMS whereas all other deuterated solvents were free from any additive. Optical absorption measurements were carried out with a Jasco V-530 spectrophotometer. Emission spectra of curcumin in different solvents were recorded using Hitachi 4010 spectrofluorimeter. Proper care was taken to prevent any exchange while using deuterated solvents. Quartz cell of low volume capacity (1 ml) with path length of 1 cm was used for measuring absorption and emission spectra because of the limited availability of deuterated solvents.

Fluorescence quantum yield (Φ_f) of curcumin in different solvents was determined using coumarin-153 laser dye as a standard with a known Φ_f of 0.56 in acetonitrile [9]. The emission spectrum was integrated using the software available in the instrument and the quantum yield was calculated according to the equation given below:

$$\frac{\Phi_S}{\Phi_R} = \frac{A_S}{A_R} \times \frac{(Abs)_R}{(Abs)_S} \times \frac{n_S^2}{n_R^2} \quad (1)$$

F_S and F_R are the fluorescence quantum yield of sample and reference respectively. A_S and A_R are the area under the fluorescence spectra of the sample and reference respectively, $(Abs)_S$ and $(Abs)_R$ are the optical densities of the sample and reference solution respectively at the wavelength of excitation, n_S and n_R are refractive index of the solvents used for sample and reference respectively. Dielectric constants (e) and refractive indices (n) of the solvents were taken from literature [10].

Fluorescence anisotropy was measured in the same spectrofluorimeter (Hitachi 4010) using parallel and perpendicular polariser. The steady state anisotropy <r> is defined as:

$$\langle r \rangle = (I_{II} - I_A)/(I_{II} + 2I_A). \quad (2)$$

Where I_{II} and I_A are the polarized fluorescence intensities in parallel and perpendicular directions respectively to the excitation polarization. Fluorescence lifetimes were measured using a time-correlated single photon counting spectrometer, the details of which are available elsewhere [11]. Second harmonic pulses of Ti-sapphire laser (443 nm and 19 ps pulse width) were used for excitation. The decay curves were fitted using a nonlinear iterative least square fit method using equation (3).

$$G(t) = \sum_i B_i \exp(t/\tau_i) \quad (3)$$

G (t) is the fitted decay curve usually assumed to be a sum of exponentials, where B_i is the pre-exponential factor for the i^{th} component and τ_i is the corresponding fluorescence life time. For all the fluorescence measurements the absorbance of the solution was kept quite low (~0.2) at the excitation wavelength.

RESULTS AND DISCUSSIONS

Steady state absorption and fluorescence

Absorption and fluorescence spectra (Fig. 1) of curcumin were recorded in chloroform ($CDCl_3$), dimethyl sulfoxide ($(CD_3)_2SO$), acetone ($(CD_3)_2CO$), acetonitrile (CD_3CN) and

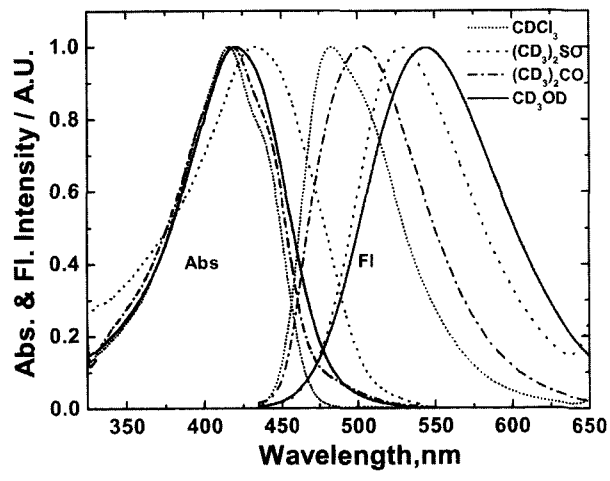

Figure 1. Normalized absorption and fluorescence spectra of curcumin in deuterated chloroform ($CDCl_3$), acetone ($(CD_3)_2CO$), dimethyl sulfoxide ($(CD_3)_2SO$) and methanol (CD_3OD).

methanol (CD_3OD). These absorption and fluorescence spectra were compared with the respective non-deuterated solvents. In general the spectra were broad with a shoulder and the fluorescence spectra were also broad and featureless, indicating the presence of more than one isomeric form in the ground and excited state. The absorption (λ_a) and fluorescence (λ_{fl}) maxima of curcumin, which are calculated from the full width at half maxima (FWHM) in different solvents, are shown in Table 1. The comparison of absorption maxima in deuterated and non-deuterated solvents showed very little effect on deuteration (Table 1), and solvent polarity function (Δf) (Fig 2a). The emission maxima however increased with increase in solvent polarity (Fig 2b) and good linearity could be observed for both deuterated and non-deuterated solvents in a similar manner. In all these solvents DMSO showed a different behavior, while the absorption maximum is unaffected by the solvent polarity, it is red shifted by about 15 nm in DMSO. Even in Fig 2, the results for DMSO showed significant deviation from linearity. This shows that there are specific interactions between DMSO and curcumin, probably through the diketo and phenolic groups [12]. When we compare the width of the fluorescence spectra in all these solvents, it can be seen that the width is less in deuterated solvents than that in non-deuterated solvents. This suggests that interchanging of different isomeric forms in the excited state is not so favorable in deuterated solvents. The comparison of emission maxima in deuterated solvent with the non-deuterated analogue showed that fluorescence maxima is blue shifted in deuterated solvents, except in methanol (Table 1). This is attributed to the reduction of zero point energy on deuteration [13]. Based on these results in different deuterated and non-deuterated solvents, it can be concluded that the polarity of the solvent plays an important role as compared to inter-molecular hydrogen bonding or hydrogen transfer. If inter-molecular hydrogen transfer was responsible for the change in fluorescence maxima, one would expect a much larger shift on deuteration.

As curcumin exists in different tautomeric and isomeric forms, excitation spectra are often important to get an idea about the emitting species in the excited state. Excitation spectra of curcumin were determined in different deuterated solvents (Fig 3). The width of the excitation spectra became more compared to the absorption spectra and appearance of shoulder in red wavelength has become more prominent. Excitation spectra were also measured with changing the emission maxima by 20 nm both in the blue and red side, but no appreciable change was observed.

These steady state spectral measurements of curcumin suggest that S_1 state is highly polar than the ground state. The large solvatochromic red shift observed in a polar protic solvent like methanol may be due to the effect of polarity and/ or hydrogen bonding ability of the solvents on the excited

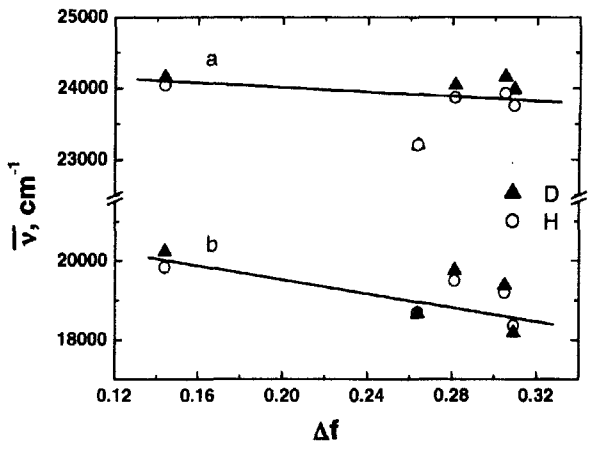

Figure 2. Plot of absorption - (a) and fluorescence (b) maxima (in cm^{-1}) of curcumin as a function of solvent polarity function (Δf).

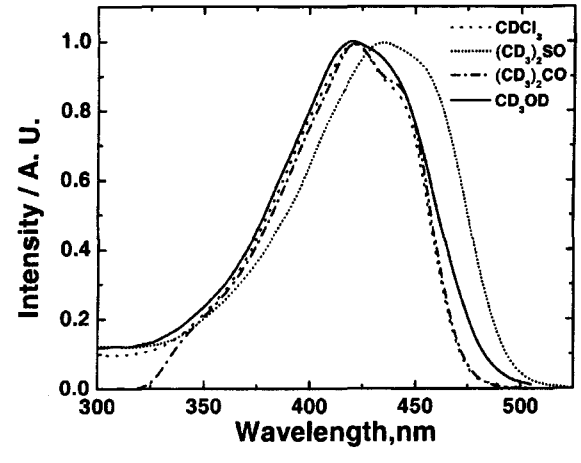

Figure 3. Normalized excitation spectra of curcumin in deuterated chloroform, acetone, dimethyl sulfoxide and methanol.

Table 1. Photophysical properties of curcumin in different solvents

Solvent	Δf^H	λ_a^H	λ_a^D	λ_{fl}^H	λ_{fl}^D	Φ_{fl}^H	Φ_{fl}^D	Φ_{fl}^D/Φ_{fl}^H
Chloroform	0.1473	416 ± 3	414 ± 3	504 ± 20	494 ± 20	0.154	0.136	0.88
DMSO	0.2637	431 ± 3	431 ± 3	535 ± 20	536 ± 20	0.045	0.050	1.11
Acetone	0.2812	418 ± 3	416 ± 3	513 ± 20	506 ± 20	0.159	0.182	1.14
Acetonitrile	0.3050	415 ± 3	414 ± 3	521 ± 20	516 ± 20	0.141	0.164	1.16
Methanol	0.3093	421 ± 3	417 ± 3	545 ± 20	550 ± 20	0.032	0.047	1.47

Due to broad nature of the spectra the absorption and fluorescence maxima are calculated from the full width at half maxima.

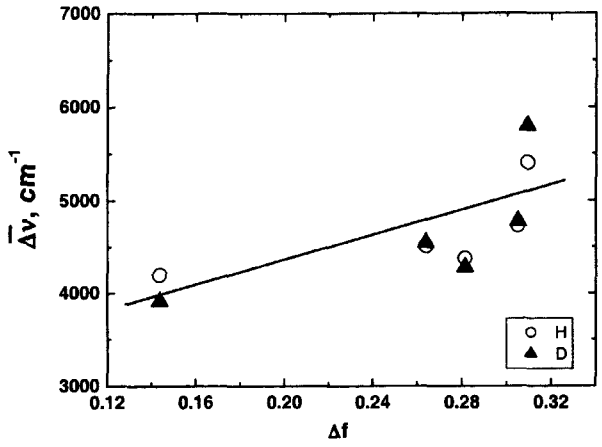

Figure 4. Plot of Stokes' shift (in cm^{-1}) of curcumin as a function of solvent polarity function (Δf).

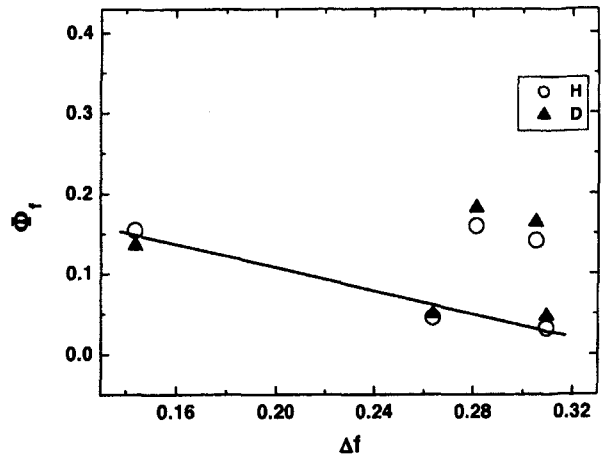

Figure 5. Plot of quantum yield of curcumin as a function of solvent polarity function (Δf).

state of curcumin. To understand if this Stokes' shift is due to polarity effect or due to the combination of both polarity and hydrogen bonding effect, the Stokes' shift (in cm^{-1}) has been plotted (Fig. 4) as a function of solvent polarity parameter (Δf) according to Lippert and Mataga relationship [14, 15]. The Stokes' shift is observed to increase with solvent polarity function with very little effect of deuteration.

Fluorescence quantum yield and life time

The fluorescence quantum yield of curcumin was determined in the deuterated solvents and compared with the non-deuterated analogues and the results listed in Table 1. The fluorescence quantum yield values increased in the deuterated solvent as compared to the non-deuterated analogue. The ratio of the fluorescence quantum yield in deuterated and non-deuterated solvent ($\phi_{fl}^{D}/\phi_{fl}^{H}$) is given in Table 1. Except for chloroform, the ratio was more than 1. The maximum change (1.4) was observed in polar protic solvent methanol. The increase in the fluorescence quantum yield observed in deuterated solvents suggests that non radiative processes are reduced on deuteration. Also it is likely that the weakness of the hydrogen bonding between the deuterated solvents with curcumin is also responsible for the increase in the fluorescence quantum yield of curcumin in the deuterated solvents. Intermolecular hydrogen transfer is one of the non-radiative processes contributing to the decrease in fluorescence. However other non-radiative processes like intramolecular hydrogen transfer and cis-trans isomerisation may also contribute significantly to the fluorescence loss. The fluorescence quantum yield was found to decrease with increase in the solvent polarity function (Fig. 5) with very small effect of deuteration. These results suggest that intermolecular hydrogen transfer is only partially responsible for the non-radiative decay process.

The fluorescence decay of curcumin at room temperature, in all the solvents (both deuterated and non-deuterated) is biexponential in nature. The two values of the excited state life times may probably correspond to different keto and enol form of curcumin. Due to biexonential nature, no systematic changes were observed in fluorescence lifetime. In most of the cases, one of the lifetime components is increased, while the other component decreased. Even their relative contribution changed. It was therefore not possible to find systematic isotope effect using one of the components. Therefore the average fluorescence lifetimes (t_{av}) were used for comparison and the ratio of average fluorescence lifetime in deuterated and non-deuterated solvent ($\tau_{av}^{D}/\tau_{av}^{H}$) are listed in Table 2.

Steady state anisotropy

Steady state anisotropy of curcumin was determined both in deuterated and non-deuterated solvents (Table 3). For small fluorophore in solution, the rotational motion depends on the viscosity of the solvent. The viscosity of the solvent does not change on deutaration. In the present case the measured steady state anisotropy of curcumin (Table 3) was found to be very small, because of low viscosity of the solvents. Small variation found between chloroform and acetonitrile may be due to the difference in the viscosity value. The slightly higher value of steady state anisotropy of curcumin in methanol and DMSO may be due to the hydrogen bonding between the solute and solvent and higher viscosity of the solvent respectively. Also due to low fluorescence intensity in these solvents, a large error as high as 10 to 20% is expected in the anisotropy values, which are listed in table 3. Because of very short fluorescence lifetime of curcumin, no rotational dynamics studies could be carried out.

CONCLUSIONS

The photophysical properties of curcumin were studied in deuterated solvents and compared with the non deuterated one. The small blue shift in the absorption spectra was attributed to the difference of zero point energy in the

Table 2. Fluorescence lifetime of curcumin in different solvents

Solvent	τ_{fl}^{H}(ps)	τ_{av}^{H}(ps)	τ_{fl}^{D} (ps)	τ_{av}^{D}(ps)	$\tau_{av}^{D}/\tau_{av}^{H}$
Chloroform	267 (50.9%)	372	304 (74.2%)	332	0.89
	481(49.1%)		414 (25.8%)		
DMSO	183 (86.2%)	238	177 (84.0%)	237	0.99
	577 (13.8%)		549 (16.0%)		
Acetone	141 (19.8%)	388	255 (24.9%)	567	1.46
	449 (80.2%)		671 (75.1%)		
Acetonitrile	342 (22.4%)	552	216 (17.1%)	613	1.11
	612 (77.6%)		695 (82.9%)		
Methanol	108 (65.3%)	316	189 (48.6%)	386	1.22
	706 (34.7%)		572 (51.4%)		

Table 3. Steady state anisotropy of curcumin in different solvents

Solvent	η(mN s m^{-2})	$\langle r \rangle^{H}$	$\langle r \rangle^{D}$
Chloroform	0.596	0.097	0.109
DMSO*	1.996	0.274*	0.278*
Acetonitrile	0.375	0.059	0.059
Methanol*	0.544	0.192*	0.153*

*Fluorescence intensity is very low in these solvents.

deuterated and non-deuterated solvents. Fluorescence spectra of curcumin in deuterated solvents behave differently compared to absorption spectra. This may be due to the different tautomer or isomer contribution in the excited state of curcumin. Excitation spectra of curcumin do not give much information about the excited state of curcumin. Fluorescence quantum yield and lifetime were increased in deuterated solvents because of decrease in non-radiative processes in the excited state of curcumin. Steady state anisotropy of curcumin remains unaffected in deuterated and non-deuterated solvents. With in the Born-Oppenhemier approximation isotopic replacement does not affect the potential energy surface of the molecule nor does it perturb the electronic energy levels [16]. The only change is in the molecular vibrations, rotations and translations. The reduced electronic coupling with the vibrational levels in deuterated solvents may be responsible for the small increase in fluorescence in deuterated solvents. Thus our studies conclude that intermolecular hydrogen transfer is only partially responsible for the excited state deactivation of curcumin.

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