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Spectroscopic and photophysical studies of the anticancer drug: Camptothecin

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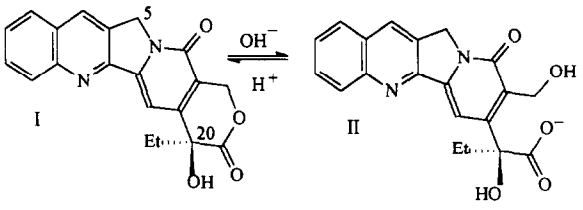
Abstract

Electronic absorption and fluorescence spectra as well as the photophysical properties of 20-(S)-camptothecin were studied in a series of organic solvents and in aqueous solution. The fluorescence quantum yield and lifetime were measured. In contrast to previously reported results, this study has demonstrated that there is a marked increase of fluorescence quantum yield and fluorescence lifetime with an increase in solvent polarity and hydrogen-bonding capacity. The solvent dependence of the fluorescence quantum yield and lifetime are explained. The solvent effects indicate that the S_1 state of CAM is $^1\pi,\pi^*$-type with some contribution from a close-lying higher energy $^1n,\pi^*$ state. The fluorescence lifetime of the drug was measured in sucrose solutions of varying viscosity and it was observed that the lifetime decreased with an increase in viscosity. The fluorescence quenching of both camptothecin lactone and camptothecin carboxylate in interactions with I^- ion in aqueous medium is reported. The fluorescence intensity of both forms are mainly quenched by the dynamic process. The quenching constant of the lactone form is twice as high as that of the carboxylate form.

Keywords: Camptothecin; Fluorescence spectra; Quantum Yield; Lifetime; Solvent effect; Quenching

1. Introduction

Camptothecin (CAM) I (Scheme 1), a plant alkaloid was first isolated and characterized by Wall and coworkers [1] in 1966 from a Chinese tree Camptotheca acuminata (family Nyssaceae). This pentacyclic alkaloid contains a quinoline ring system (ring A and B), a pyridone ring (ring D) and a terminal α-hydroxy lactone ring (ring E). It has a chiral center (C-20) within the lactone ring. The lactone ring is labile and will easily hydrolyze to form the sodium camptothecin (CAM-Na), II, in sodium hydroxide media [1].



Scheme 1.

Following its discovery and chemical identification, CAM was found to be active in tests against L1210 leukemia [2] and Walker 256 carcinosarcoma [1, 3, 4]. It was also found that CAM inhibits both DNA and RNA synthesis in mammalian cells. This attracted immediate interest in CAM as a potential cancer chemotherapeutic agent. Its high antitumor activity against a wide range of experimental

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tumors has been confirmed [5]. Because of poor aqueous solubility, CAM was clinically evaluated as its water soluble sodium salt (II) and was found to be one-tenth as active as the parent drug [6]. The patients under clinical phase I trial, mostly with cancer of gastrointestinal origin, encountered severe dose-dependent toxicities such as vomitting, diarrhoea, hemorrhagic enterocolitis, leukepenia, and thrombocytopenia [7]. As a result, despite apparent success in gastrointestinal cancer and neck tumor, the interest in CAM decreased. The results of preclinical and clinical developments of CAM and its analogs have been reviewed in the literature [8, 9].

One of the important effects of CAM is its rapid and reversible fragmentation of cellular DNA in cultured mammalian cells [10] in the presence of an enzyme, topoisomerase I (Top-I). The detailed mechanism of DNA single strand scission by CAM has been discussed in two recent reports [11, 12]. This discovery resulted in a renewed clinical interest in CAM. Assuming that the undesirable and unpredictable toxicities of CAM is partly due to its poor solubility in water, several water soluble CAM analogs have been synthesized [13] and clinically evaluated [14–22]. The drug activity of CAM, like many other anticancer drugs, involves its binding with DNA, proteins and membranes. Electronic absorption and fluorescence spectral behavior are often used to study the binding properties of drugs and hence, to throw light on the mechanism of its drug activity. Therefore, it is necessary to know the detail spectral properties of the drug in solution. Recently, in a series of publications Burke et al. have reported the binding properties of CAM and its amino- and hydroxy-analogs with liposomes and human serum albumins by exploiting the high fluorescence efficiency of these compounds [23–29]. We also have reported the excited-state tautomerization of CAM and the effect of pH on the camptothecin lactone – carboxylate equilibrium in the ground state [30]. Although much research has been done on the structure-activity relationship and the mechanism of DNA strand cleavage by CAM and its synthetic analogs, to our knowledge, except for a few scattered studies [23–29], no study on the detailed spectroscopic and photophysical properties of the parent drug has been reported. Therefore, we have undertaken a systematic study of the electronic absorption and fluorescence spectral properties of CAM in solution. In this manuscript, we discuss (i) the effects of organic solvents on the absorption and fluorescence spectra and photophysical properties of camptothecin, and (ii) the fluorescence quenching of camptothecin lactone and the carboxylate form by I^- ion in aqueous solution.

2. Experimental details

2.1. Materials

The 20-(S)-Camptothecin was procured from Aldrich and was used without further purification. Analytical grade diethyl ether (Baxter), tetrahydrofuran (Chempure), chloroform (Mallinckrodt), and acetonitrile (Fischer Scientific Co.) were further purified and dried by standard methods [31]. Anhydrous spectrograde methanol (Aldrich), ethanol (Aldrich), ethylene glycol (Aldrich), 1,2-dimethoxyethane (Aldrich), dioxane (Aldrich) and cyclohexane (Aldrich) were used as received. Sodium hydroxide (CEM), sodium perchlorate (Fischer Scientific Co.), sodium acetate (Fischer Scientific Co.), sodium iodide (Fischer Scientific Co.), sodium thiosulfate (Fischer Scientific Co.), sodium bicarbonate and sodium carbonate (Fischer Scientific Co.) and sucrose (AMRESCO) were all of analytical grade and were used directly from the bottle. Megapore deionized water was used for making aqueous solutions.

2.2. Methods

Fluorescence quantum yield was determined by Parker's method [32]. Dilute solutions of CAM were prepared in an appropriate solvent and the absorbance was measured in reference to the corresponding solvent. The absorbance was maintained below 0.05. Quinine sulfate in 0.1N H_2SO_4 was used as the fluorescence standard ($\Phi = 0.545$) [33]. All solutions, including that of quinine sulfate, were excited at 350 nm. The sample over reference fluorescence spectra were recorded in the wavelength region of 370 to 600 nm. The following

equation was used to calculate quantum yields:

$$\Phi_u = \left(\frac{\Phi_s A_s}{F_s}\right) \times \frac{F_u}{A_u} \times \frac{n^2(\text{solv})}{n^2(\text{water})}, \quad (1)$$

where Φ, F and A are, respectively, the quantum yield, peak area, and absorbance at the excitation wavelength; 'n' is the refractive index; 's' and 'u' represent standard and unknown, respectively.

The quenching studies of CAM and CAM-Na were conducted, respectively in acetate(pH 5.1) and bicarbonate (pH 9.2) buffer. A 0.5 ml aliquot of the stock solution of CAM (or CAM-Na) was diluted to 10 ml in volumetric flasks by the same buffer solution. The I^- concentration varied from 0 to 0.2 M. All solutions contained 0.01 M sodium thiosulfate to prevent oxidation of the I^- ion. The ionic strength was kept constant at 0.4 by adding appropriate amounts of sodium perchlorate solution. The same solutions were used for lifetime measurements. A modified form of the Stern–Volmer equation [34] was used to analyze the static and dynamic processes:

$$F_0/F = 1 + (K_D + K_S)[Q] + K_D K_S [Q]^2, \quad (2)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively, Q, K_S and K_D are the static and dynamic quenching constants, respectively. The K_D value was obtained from a Stern–volmer plot of the lifetime data using equation:

$$\tau_0/\tau = 1 + K_D[Q], \quad (3)$$

where τ_0 and τ are the fluorescence lifetime in the absence and presence of quencher, respectively. The collisional quenching rate constant, k_q was calculated from the relation, $K_D = \tau_0 k_q$. The absorption and all fluorescence measurements were performed at 25°C unless noted otherwise.

2.3. Apparatus

The absorption spectra were recorded on a double beam Shimadzu UV-3101PC spectrophotometer equipped with a constant temperature circulator. Absorbance measurements were performed with reference to corresponding solvents using 1 cm^2 quartz cuvettes. The fluorescence spectra were measured with a Spex-Fluorolog model F2T21I spectro-fluorometer equipped with a cell compartment thermostated with VWR model 1160 constant temperature circulator.

Fluorescence lifetimes were obtained by using a PTI Inc LS-100 luminescence spectrometer. A N_2 and He gas mixture was used in the discharge tube for excitation. A dilute solution of colloidal starch was used as a scatterer to determine the exciting lamp flash profile. The 337 nm emission of N_2 was used for sample excitation. The decay curves were obtained by use of time correlated single photon counting (TC-SPC). To obtain fluorescence decay curves $(2–5) \times 10^4$ counts were collected in the peak channel. Each data set was collected in 256 channels. The data were analyzed by using a multiexponential decay analysis program. The goodness of fit between experimental and computed decay curves was evaluated by the reduced χ^2(0.9–1.2) and Durbin–Watson (> 1.7) parameters and the randomness of the plot of weighed residuals and the autocorrelation functions. The measurements were repeated more than once to obtain the best data set. The fluorescence lifetimes in sucrose solutions were measured at 20° C. All other measurements were performed at 25°C.

3. Results and discussion

3.1. Absorption and fluorescence spectra

The absorption and fluorescence spectra (Fig. 1) of CAM in nonpolar solvents are characterized by structured bands. However, the structure is lost in polar solvents. The absorption and emission maxima and the molar absorptivity of CAM in the solvents studied are compiled in Table 1 along with the solvent polarity parameters ($E_T(30)$) [35]. The molecule has a strong absorption at ~ 370 nm as indicated by the high molar absorptivity (ε) at this wavelength. The molar absorptivity is in good agreement with a previously reported value (19 900 l mol^{-1} cm^{-1}) [1]. The molecule has a strong fluorescence with λ_{max} ~ 425 nm in all solvents examined. The absorption spectra exhibit a blue shift in hydrogen-bonding solvents, including chloroform. However, the fluorescence spectral

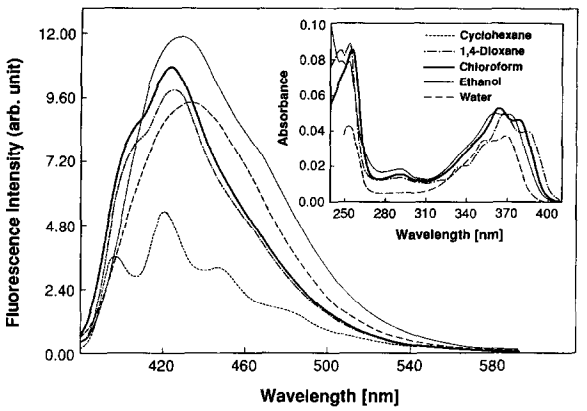

Fig. 1. Absorption (inset) and fluorescence Spectra of CAM in organic solvents and water.

shift is slightly towards the longer wavelength with an increase in solvent polarity.

The above spectral characteristics of CAM can be explained on the basis of its structure. Due to the extended conjugation of the quinoline ring, the four rings (rings A–D) are planar in the molecule. This shifts the spectrum to red with respect to that of quinoline [36]. The rigid structure of the molecule also explains the high molar absorptivity and fluorescence quantum yield (Φ_f). The strong absorption and emission spectra suggest that the lowest excited singlet state of the molecule is of π, π^* character. This is further indicated by the red shift in the fluorescence spectra with an increase in solvent polarity. However, contribution from the $n \rightarrow \pi^*$ transition is also indicated by the blue shift of the long-wave absorption band in hydrogen-bonding solvents. This blue shift is probably due to hydogen-bonding interactions with the solvent molecules through the lone-pair electrons of the quinolinic as well as pyrrolic ring nitrogen of the molecule. The observed shift in the absorption spectrum is small although a CNDO/2 calculation of CAM by Flurry and Howland [37] has indicated that the dipole moment of the molecule is 7.1D in the S_0 state. The data in Table 1 indicate that the molar absorptivity of the lowest energy transition of CAM remains almost unchanged upon increase in solvent polarity, which suggests that the radiative rate constant should be independent of solvent polarity. Absence of any significant change in the position of emission maxima with the solvent polarity suggests that the dipole moment of the molecule does not change significantly upon electronic excitation to the S_1 state.

Table 1
Absorption and fluorescence maxima [λ_{max}(nm)] and molar absorptivity (ε) of CAM in various solvents

Solvent	$E_T(30)$[a]	λ_{max}(abs)	ε (L mol^{-1} cm^{-1})	λ_{max}(flu)
Cyclohexane	30.9	385	—	474(s)
		368	—	444
		278	—	420
		257	—	396
Diethyl ether	34.5	384	—	422
		368	—	405(s)
		285	—	
		255	—	
1,4-Dioxane	36.4	385	16962	425
		368	21266	409(s)
		292	7679	
		254	33629	
Tetrahydrofuran	37.4	385	—	424
		369	—	408(s)
		280	—	
		256	—	
1,2-Dimethoxy-ethane	38.2	383	17257	424
		367	21941	408(s)
		290	8270	
		254	39620	
Chloroform	39.1	378	(s)	422
		364	21856	406(s)
		290	6667	
		256	35189	
Acetonitrile	45.6	377	(s)	422
		364	21266	
		289	7173	
		253	35189	
Ethanol	51.9	369	—	428
		290	—	
		253	—	
Methanol	55.4	369	21489	428
		289	6979	
		253	33659	
Ethylene glycol	56.3	372	21350	431
		290	6835	
		255	32457	
Water (pH 5.2)	63.1	370	—	428
		252	—	

[a]The values are taken from Ref. [35]; s shoulder.

3.2. Fluorescence quantum yields and lifetimes

The fluorescence quantum yields and lifetimes of CAM were measured in a number of solvents. The data are collected in Table 2. It can be seen that the fluorescence quantum yield increases with an increase in solvent polarity and hydrogen-bonding capacity, except in chloroform in which the quantum yield as well as lifetime is low. The fluorescence lifetimes were obtained by the TC-SPC decay method. A representative decay profile obtained in acetonitrile solvent is depicted in Fig. 2. All decay curves were best fit to double exponential decay. However, the amplitude of the second component was very small (< 0.001) and the corresponding lifetime was unusually high. This is probably due to background noise. Therefore, the decays were assumed to be single exponential as expected. The fluorescence lifetimes of CAM in water is less than the values reported (4.7 and 4.2 ns) [23, 25] by Burke and coworkers. However, the lifetime in methanol is higher than the value (3.5 ns) [25] reported by the same authors. The fluorescence lifetime is lower in ethylene glycol as compared to other protic solvents. Among the aprotic solvents, the fluorescence lifetime of CAM is smaller in chloroform. The data in Table 2 also indicate that the fluorescence lifetime of the drug increases parallelly with the quantum yield from cyclohexane to water. However, Burke and coworkers assumed that the fluorescence lifetime of CAM is independent of the nature of the solvent in their analysis of fluorescence anisotropy data [28] on the basis of which they calculated the relative population of CAM and CAM-Na bound to liposomes and proteins. The radiative (k_r) and nonradiative (k_{nr}) rate constants calculated by using the relation, $k_r = \Phi_f/\tau_f$ and $k_{nr} = (1 - \Phi_f)/\tau_f$, respectively, are also listed in Table 2. The radiative rate constant of CAM, within the experimental error, remains unchanged upon increasing the solvent polarity. However, the nonradiative rate constant decreases with an increase in solvent polarity and hydrogen-bonding capacity.

It has been reported that in nitrogen heterocycles, e.g. quinoline, isoquinoline and acridine there is a $^1n,\pi^*$ state very close to the $S_1(\pi, \pi^*)$ state [38, 39]. In fact, in hydrocarbon solvents the lowest excited singlet state in these molecules is of n, π^*-type. Most of these nitrogen heterocycles are weakly or nonfluorescent in aprotic solvents. However, in protic solvents, e.g. alcohol and water, they become strongly fluorescent due to a reversal of $^1n, \pi^*$ and $^1\pi, \pi^*$ states [37, 40]. Thus, $\pi \rightarrow \pi^*$ is the lowest energy transition in these molecules in protic solvents. Lim [41] and Hochstrasser [42] have explained this by the vibronic interaction between the $^1n,\pi^*$ and $^1\pi,\pi^*$ states. Since CAM is a quinoline alkaloid, its fluorescence properties can be similarly explained. Although $^1\pi,\pi^*$ is the lowest excited singlet state in CAM in the solvents studied in this work, a small contribution from a close-lying $^1n,\pi^*$ state is evident from the absorption and fluorescence data. In aprotic solvents, the energy gap between these two states is probably small. As a result the vibronic interaction between the states is high. This reduces the symmetry of the $^1\pi,\pi^*$ state and thus increases the Franck–Condon factor. Consequently, the rate of radiationless decay increases. This explains the low fluorescence quantum yield of CAM in cyclohexane. However, in protic solvents, the hydrogen-bonding interaction of the solvent molecules with the lone pair of quinoline and/or pyrrolic nitrogen(s) destabilizes the $^1n,\pi^*$ state but stabilizes the $^1\pi,\pi^*$ state, thus reducing the energy gap between the states and thereby decreases the vibronic interaction. The role of solute–solvent hydrogen-bonding interaction on the

Table 2
Photophysical properties of CAM in various solvents

Solvent	Φ_f[a]	τ_f(ns)[b]	$k_r \times 10^8\ s^{-1}$	$k_{nr} \times 10^8\ s^{-1}$
Cyclohexane	047	2.78	1.69	1.91
Diethyl ether	0.50	2.94	1.70	1.70
1,4-Dioxane	0.52	2.97	1.75	1.62
Tetrahydrofuran	0.52	2.96	1.76	1.62
1,2-Dimethyoxyethane	0.53	3.05	1.74	1.54
Chloroform	0.45	2.51	1.79	2.19
Acetonitrile	0.54	3.15	1.71	1.46
Ethanol	0.60	3.52	1.70	1.14
Methanol	0.61	3.69	1.65	1.06
Ethylene glycol	0.62	3.54	1.75	1.07
Water (pH 5.2)	0.64	3.99	1.60	0.90

[a]Uncertainty in the values is in the range of $\pm$ 0.005.
[b]Uncertainty in the values is $\pm$ 0.02.

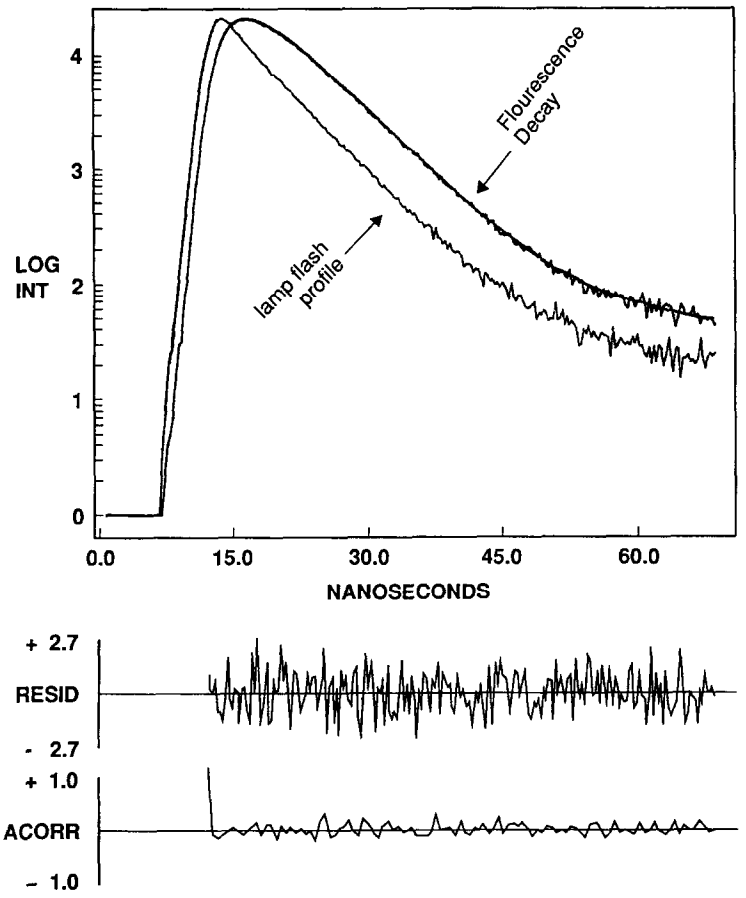

Fig. 2. Fluorescence decay of CAM in acetonitrile solvent, $\lambda_{ex} = 337$ nm, $\lambda_{em} = 430$ nm.

fluorescence quantum yield is demonstrated in Fig. 3. It can be seen that the fluorescence intensity of CAM in cyclohexane increases on addition of a small quantity of methanol. The low value of Φ_f in chloroform can be attributed to fluorescence quenching by the solvent molecules. This is supported by the low τ_f value in chloroform as compared to other solvents.

The increase of fluorescence lifetime from cyclohexane to water can also be explained along the same lines as for fluorescence quantum yield discussed in the preceding paragraph. Since the molar absorptivity of the lowest energy transition does not change significantly with the solvent polarity, according to the Strickler–Berg equation [43], the radiative rate constant of CAM should remain unchanged. Therefore, any change in the measured lifetime must be due to a change in the nonradiative decay rates. Consequently, the fluorescence quantum yield becomes directly proportional to the measured lifetime as indicated by the data in Table 2. It should be noted that the radiative rate constant (k_r) remains almost constant and the nonradiative decay rate decreases with an increase in solvent polarity and hydrogen-bonding capacity in going from cyclohexane to water. To further confirm the role of nonradiative decay processes, we have measured the fluorescence lifetime of CAM in acetate buffer (pH 5.1) as a function of temperature. As can be seen from the plot of τ_f versus T in Fig. 4, the τ_f value decreases with a rise in temperature. The decrease in fluorescence lifetime with an increase in

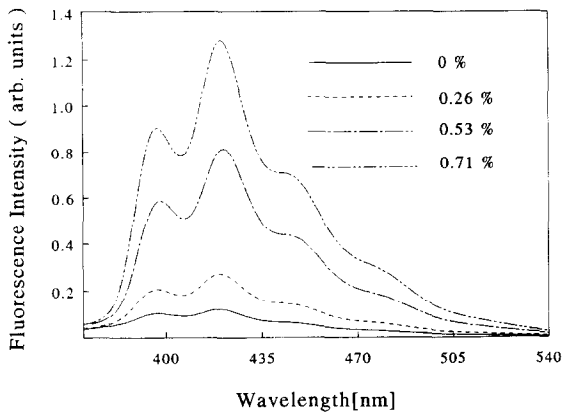

Fig. 3. Fluorescence spectra of CAM in cyclohexane containing various percentage of methanol, $\lambda_{ex} = 350$ nm.

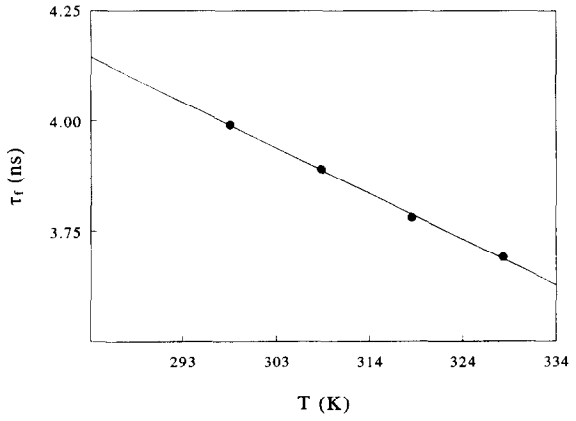

Fig. 4. Plot of fluorescence lifetime(τ_f) versus Temperature (T) in water.

temperature also supports the existence of vibronic interaction between the close-lying $^1\pi,\pi^*$ and $^1n,\pi^*$ states of CAM. The increase in temperature enhances the vibronic interaction between the states thus increases the nonradiative decay rate. The low τ_f value in chloroform is a result of fluorescence quenching by solvent molecules through collisional interactions.

The inconsistency of the measured τ_f value in water with the results reported in literature is probably due to higher pH and ionic strength of the buffer solution employed by the above mentioned authors. They measured the fluorescence lifetime of CAM in phosphate-buffered saline (PBS) solution (pH 7.4) containing 8 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl and 3 M KCl. However, the half life of the lactone ring hydrolysis reaction of the drug in the above experimental condition as reported by the authors as well as other researchers is $\sim$ 16.8 min. Our measurements of the rates of the hydrolysis reaction by direct spectrophotometric method also resulted in similar half life value [44]. In our recent publication [30], we have shown that at pH > 8.0 CAM is completely hydrolyzed to the carboxylate form. Since the carboxylate form will be more favored in solutions of high ionic strength, the rate of lactone ring hydrolysis will be further enhanced in the PBS buffer as reported. Moreover, the rise of temperature to 37°C would also increase the reaction rate. Consequently, a high concentration of the carboxylate form will be present in the PBS buffer employed by the authors. Therefore, the measured lifetime will correspond to the carboxylate form rather than the lactone form. When the fluorescence lifetime of CAM-Na was measured in bicarbonate buffer (ionic strength = 0.2) at pH 9.2, a τ_f value of 4.48 ns was obtained from the single exponential fluorescence decay [30]. This value is very close to the reported τ_f value for CAM. The lower τ_f value in methanol obtained in this study as compared to that reported by Burke et al. could be due to the lower temperature (25°C) at which the measurement was performed. This has been discussed in the previous paragraph.

To examine the effect of hydrogen-bonding capacity of the solvents upon the fluorescence lifetime of CAM, we have measured the quantity in acetonitrile solvent containing varying concentration of water. It can be seen from Fig. 5 (inset) that the $\Delta\tau_f$ value initially increases with increasing concentration of water and then plateaus at higher concentrations. A similar trend can also be noted in the ΔF value. Since the addition of 1–2% of water is not likely to change the polarity of acetonitrile, the above change can be attributed to hydrogen-bonding interaction between the solute and solvent molecules.

The low lifetime of CAM in ethylene glycol compared to methanol may be attributed to higher bulk viscosity of the solvent. The effect of viscosity of

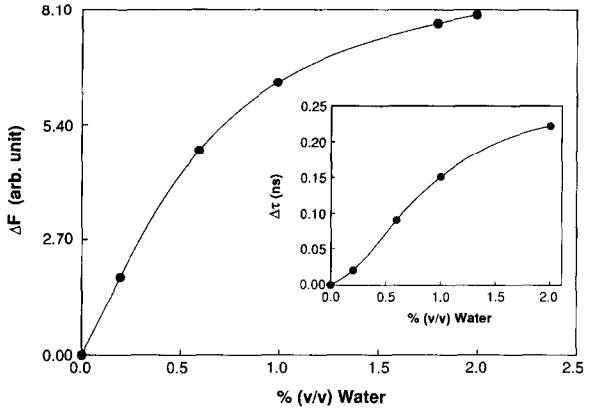

Fig. 5. Plot of fluorescence intensity change(ΔF) and change of lifetime($\Delta\tau_f$) (inset) as a function of %(v/v) water in acetonitrile.

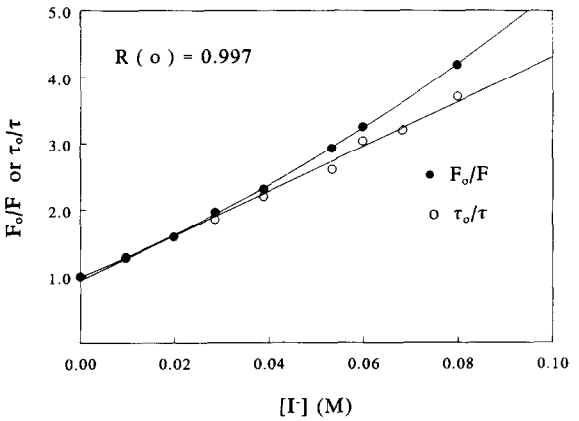

Fig. 7. Stern–Volmer plots for the fluorescence quenching of CAM (pH 5.1) by I^- ion.

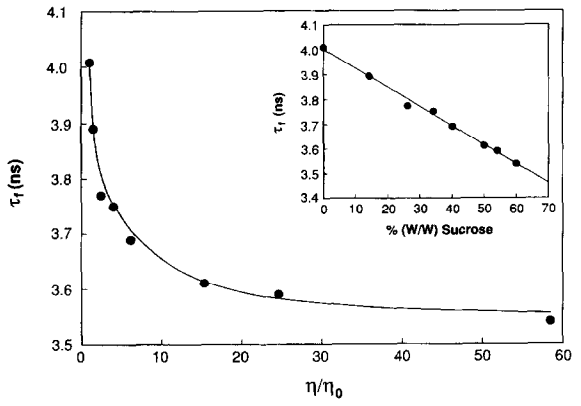

Fig. 6. Dependence of fluorescence lifetime (τ_f) on w% sucrose in water(inset) and relative viscosity (η/η_0).

solvent was examined by the measurement of fluorescence lifetime of CAM in aqueous solutions containing varying percentages of sucrose. The data are plotted in Fig. 6 as a function of weight percentage of sucrose (inset) and relative viscosity (η/η_0) [45]. The figure shows a linear decrease of τ_f with increasing percentage of sucrose which means a nonlinear decrease of lifetime with the increase of relative viscosity of the medium.

3.3. Fluorescence quenching

Burke and Mishra [23] have studied the fluorescence quenching of free as well as protein and liposome-bound CAM in phosphate buffer of pH 7.4. However, our recent studies [30] as well as earlier reports including that of the above mentioned authors have indicated that in aqueous solution at pH 7.4, there is a significant concentration(87%) [27, 47] of CAM-Na even at room temperature. According to these authors the half life for the reaction at 37°C is 16.8 min [25]. This suggests that there might be heterogeneity in ground state and thus if not in steady-state measurements, it will introduce an error in the lifetime measurements because of the longer time required for the latter. Consequently, the reported quenching constant thus obtained represents the quenching of both CAM and CAM-Na. Therefore, in the present work we have studied the fluorescence quenchig of CAM and CAM-Na by I^- ion to estimate individual quenching constants in aqueous solution.

In our earlier paper, we have demonstrated that camptothecin is present mainly in the lactone form (I) at pH < 5 and as the carboxylate form (II) at pH > 8 [30]. Therefore, we have chosen pH 5.1 and 9.2 to study the fluorescence quenching of CAM and CAM-Na respectively. A modified Stern-Volmer plot of the ratio (F_0/F) of the fluorescence intensities of CAM in the absence and presence of the quencher against the quencher concentration, [I^-], is shown in Fig. 7. An upward curvature in the plot for CAM suggests the presence of both static and dynamic quenching processes. To resolve these quenching processes, fluorescence lifetime measurements were also performed at different

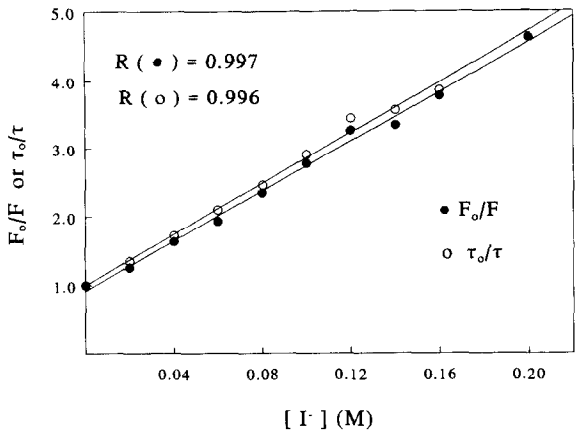

Fig. 8. Stern–Volmer plots for the fluorescence quenching of CAM-Na (pH 9.2) by I^- ion.

Table 3
Fluorescence quenching constants of CAM and CAM-Na

Molecule	K_D (M^{-1})	K_S (M^{-1})	τ_0 (ns)[a]	k_q ($M^{-1}s^{-1}$)
CAM (pH 5.1)	33.7	4.0	3.82	8.8×10^9
CAM-Na (pH 9.2)	18.6 18.2[b]	0.0	4.31	4.3×10^9

[a]Fluorescence lifetime in absence of quencher.
[b]Obtained from steady-state fluorescence measurement.

concentrations of I^- ion. The Stern–Volmer plot of τ_0/τ versus [I^-] demonstrates a decrease of fluorescence lifetime with an increase of I^- concentration (Fig. 7). However, unlike CAM, the Stern–Volmer plots (Fig. 8) of CAM-Na are linear, suggesting the presence of only dynamic quenching process. The results of fluorescence quenching of CAM and CAM-Na are summarized in Table 3. It is noted that the dynamic quenching constant (K_D) of CAM is higher than that of CAM-Na. This is likely because of unfavorable collisions between two negatively charged species. The quenching rate constants of the molecules are very close to the diffusion rate. The K_D value of CAM is less than the value (39 M^{-1}) [23] reported by Burke and coworkers. This is probably because of the higher temperature (37°C) employed by these authors in their measurements since the rate of collision is expected to increase with a rise in temperature.

4. Conclusions

Solvent effects on the photophysical properties of camptothecin are investigated. In contrast to previously reported results, our study has shown that both the fluorescence quantum yield and the lifetime of CAM are dependent on the nature of the solvent. Both quantities increase with an increase in polarity of the solvent. It has been demonstrated that enhancement of fluorescence intensity and lifetime of camptothecin lactone in organic solvents is due to both dipole–dipole and hydrogen-bonding interactions. The fluorescence intensity of CAM is quenched by chloroform. The increase in viscosity of solvent decreases fluorescence lifetime. The solvent effects suggest that the S_1 state of CAM is of π,π^*-type and there is vibronic interaction between the $^1\pi,\pi^*$ and a close-lying higher energy $^1n,\pi^*$ state. The fluorescence quenching of the lactone and the carboxylate form by I^- ion is mainly due to a dynamic process as suggested by the Stern–Volmer plots. The dynamic quenching rate constant of camptothecin lactone is twice as high as that of the carboxylate form.

Acknowledgements

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