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# Intramolecular charge transfer and solvation dynamics of Nile Red in the nanocavity of cyclodextrins

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Received 18 November 2003; in final form 27 February 2004

Published online: 20 March 2004

## Abstract

The relative retardation of non-radiative rate and solvation dynamics of Nile Red (NR) in the nanocavity of cyclodextrins (CDs) ( $\beta$ -cyclodextrin,  $\beta$ -CD and  $\gamma$ -cyclodextrin,  $\gamma$ -CD) have been investigated by steady state and time resolved fluorescence spectroscopy. The non-radiative rate for twisted intramolecular charge transfer process is (TICT) retarded almost 2.5 times in presence of  $\beta$ -CD whereas in presence of  $\gamma$ -CD a little retardation of non-radiative rate compared to pure water is observed. We have observed time-dependent Stokes' shift of NR in aqueous solution of  $\beta$ -CD but the same is not observed in case of  $\gamma$ -CD. The different feature of TICT and solvation of NR in two CDs ( $\beta$ -CD and  $\gamma$ -CD) arises due to the different types of complexation of NR with  $\beta$ -CD and  $\gamma$ -CD. © 2004 Elsevier B.V. All rights reserved.

## 1. Introduction

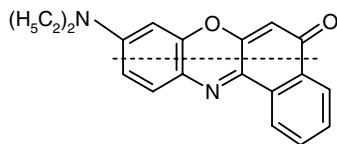
The excited state intramolecular charge transfer (ICT) of various organic molecules containing separate electron donor and acceptor moieties is currently studied in order to discern its importance in various photochemical and photobiological processes [1–3]. The ICT state has been proposed to become efficient by coupling with conformational change, as observed in para-substituted *N,N*-dimethylaniline derivatives. In these molecules upon photoexcitation internal rotation around the dialkyl amino group with the benzene ring leads to the formation of the 'twisted intramolecular charge transfer (TICT)' state [3]. The existence of TICT state of *N,N*-dimethylaniline derivatives in polar solvents is experimentally verified by dual emission. One of the emission bands corresponds to the emission from the locally excited state (LE) and another emission band is originally from the TICT state, which is largely Stokes' shifted with respect to normal emission [3]. Though there is some confusion regarding the TICT mechanism,

but various theoretical [4] and experimental evidence from the studies of *p*-(*N,N*-dimethylamino)benzonitrile (DMABN) and ethyl *p*-(*N,N*-diethylamino) benzoate (DEAEB) in supercritical fluids [5] and vapor phase [6] supports the TICT mechanism.

In recent years there has been a tremendous interest in studying the photophysical and photochemical properties of molecules incorporated in restricted geometries [7,8]. Nile red (NR, Scheme 1) is a hydrophobic, highly fluorescent, solvatochromic dye, which has been extensively used as a fluorescent probe for biological systems [9,10]. Due to the remarkable sensitivity of the absorption and fluorescence properties of NR to the microenvironment, NR has been exploited in studies of local polarity in zeolites [11–13], reverse micelles [14], Langmuir–Blodgett films [15] and micelles [16]. Moreover, it has been used to infer the heterogeneity of membranes [17], and the formation of dendrimer-surfactant supramolecular assemblies [18]. It has been also used extensively as a staining agent for biological tissues [19], and an active material for white light emitting diodes (LEDs) [20]. The most interesting feature of the NR molecule is its structure. The electron donating diethylamino group is attached to the electron withdrawing rigid aromatic system by a single bond. The rotation

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Scheme 1. Structure of Nile Red.

of diethylamino group along this single bond leads to the formation of TICT geometry. Unlike most other TICT molecules, NR molecule does not exhibit dual fluorescence in polar solvent and TICT is the main non-radiative decay process in the excited electronic state of NR [13,14]. Recently, it is established that NR molecules exhibit polarity dependent TICT characteristics [13–15,27]. It is revealed from the previous studies that the geometry of the restricted environments plays a crucial role to control the photophysical properties and dynamics of excited NR molecules [11–18,27].

The cyclodextrins (CDs) have been recognized as some of the most important host materials for organic molecules in aqueous media [21–23]. The CD-based systems have diverse applications particularly in the field of biomimetic chemistry, in the design of a particular enzyme [21] and various other fields [22,23]. The CD forms a hydrophobic and restricted cavity with hydrophilic external walls in aqueous solutions, providing two different microenvironments with an incorporated guest molecule. Three types of CDs are commonly available, each having different diameter [22]. The  $\beta$ -CD and  $\gamma$ -CD have relatively larger diameter of  $\sim 6.5$  and  $\sim 8.5$  Å, respectively compared to  $\alpha$ -CD, having a diameter of  $\sim 5$  Å. CDs have been used as host materials for investigating the solvation dynamics using various probes by Fleming et al. [24]. Recently, Srivatsavoy [25] studied inclusion complexes of NR in  $\beta$ -CD and  $\gamma$ -CD with the help of UV–Vis absorption, steady state and time-resolved fluorescence spectroscopy. The author concluded that multiple inclusion complexes are formed both in  $\beta$ -CD and  $\gamma$ -CDs. But the author did not discuss about the nature of host–guest inclusion complexes and the stoichiometry of host–guest complexes, which might give idea about the observed differences between  $\beta$ -CD and  $\gamma$ -CD results. Very recently, Wagner et al. [26] have used UV–Vis absorption, steady state fluorescence spectroscopy and electrospray mass ionization spectroscopy, to experimentally study the nature of these NR–CD complexes. They have also used molecular modeling for the study of geometries and stoichiometries of the association complexes. In this Letter, we report a systematic study of the effect of both  $\beta$ - and  $\gamma$ -CDs on the TICT, which is main non-radiative decay pathway of NR. We have also studied solvation dynamics of NR in aqueous solution of  $\beta$ -CD. Moreover, we have also measured time-resolved fluorescence anisotropy to understand the rotational behavior of NR in presence of  $\beta$ -CD and  $\gamma$ -CD.

## 2. Experimental

Nile red (Aldrich) was purified by thin layer chromatography using spectroscopic grade chloroform as an elutant as used earlier [13,26]. A single spot was observed in the TLC plate indicating that the compound is pure. NR is not stable in aqueous solution and decomposes with a half-life of  $\sim 20$  min [10,14]. Solutions for steady state and time-resolved measurements were prepared by the following methods. An excess of NR was taken in a 10 ml volumetric flask and triple distilled water was added up to the mark. The solution was sonicated for 10 min and then filtered through Whatman-43 filter paper. We have performed steady state (absorption and fluorescence) and time-resolved fluorescence measurements of aqueous solution of NR within 20 min. After that we have gradually added CD ( $\beta$ -CD or  $\gamma$ -CD) to this solution and performed steady state and time-resolved measurements. After addition of CD in aqueous solution, NR is stable for many hours. The  $\beta$ -CD and  $\gamma$ -CD are from Wako-chemicals, Japan and used without further purification. The excitation wavelength for all steady state and time resolved experiments were 550 nm and 408 nm respectively. The steady state absorption and emission spectra were measured using a Shimadzu (model no: UV1601) UV–Vis absorption spectrometer and Spex Fluorolog-3 (model no: FL3-11) fluorescence spectrophotometer. The fluorescence spectra were corrected for spectral sensitivity of the instrument. The experimental setup for picosecond time correlated single photon counting is described elsewhere [28]. The typical system response of the system is  $\sim 80$  ps. The decays were analyzed using IBH DAS-6 decay analysis software. The same software is also used for anisotropy analysis. The temperature was kept at  $298 \pm 1$  K for all measurements. We have calculated the fluorescence quantum yield ( $\phi_f$ ) of NR in different CDs considering the quantum yield of NR in water as 0.018 [14].

## 3. Results and discussions

### 3.1. Steady state absorption spectra

Representative absorption spectra of NR in water and on addition of  $\beta$ -CD are shown in Fig. 1a. The solubility of the NR is very low in water and consequently we have observed very little optical density of  $\sim 0.02$  at the absorption maxima ( $\sim 600$  nm) of NR in aqueous solution. On addition of  $\beta$ -CD, the optical density gradually increases with the blue shift in absorption spectra and in 13.2 mM of  $\beta$ -CD the absorption maximum is shifted to 590 nm. This behavior has been attributed to the enhanced dissolution of NR molecule through the hydrophobic interaction between NR and non-polar cavity of  $\beta$ -CD. Moreover, the

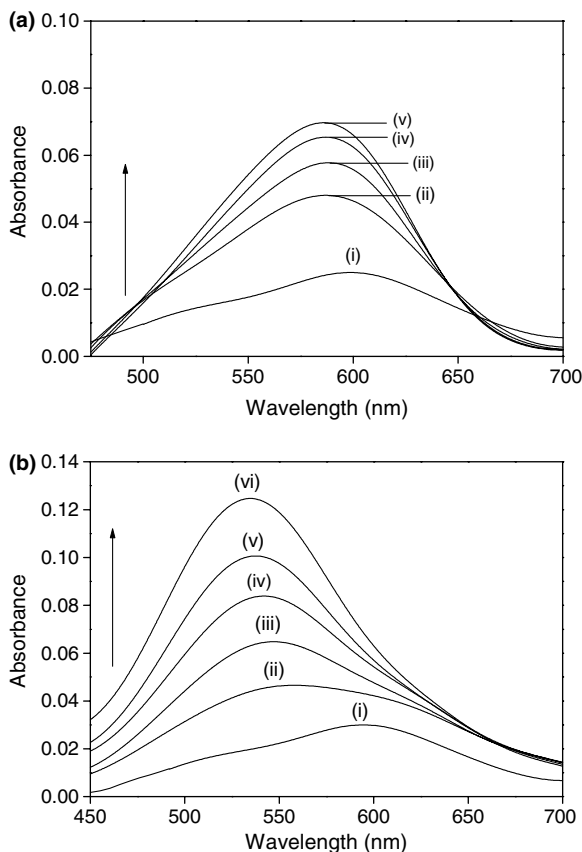


Fig. 1. (a) Absorption spectra of NR in water (i) and in presence of  $\beta$ -CD (ii–v) containing (ii) 8 mM, (iii) 10 mM, (iv) 12 mM, (v) 13.2 mM  $\beta$ -CD. (b) Absorption spectra of NR in water (i) and in presence of  $\gamma$ -CD (ii–vi) containing (ii) 3.85 mM, (iii) 5.77 mM, (iv) 7.7 mM, (v) 11.55 mM and (vi) 15.4 mM  $\gamma$ -CD.

existence of an isosbestic point at  $\sim 650$  nm is indicative of the formation well-defined 1:1 complex between  $\beta$ -CD and NR.

On addition of  $\gamma$ -CD to the aqueous solution of NR the absorption spectra become blue shifted compared to the absorption spectra of NR in water (Fig. 1b). The absorption maximum of NR in 15.4 mM  $\gamma$ -CD is at  $\sim 540$  nm, i.e., the absorption spectra has been shifted  $\sim 60$  nm to the blue end compared to the absorption spectrum of NR in water. There is a considerable increase of the optical density with the increase in  $\gamma$ -CD concentration. The blue shift in absorption spectra and increase in optical density arises due to the formation of capped complexes, which is discussed in last paragraph of Section 3.2. The absence of isosbestic point rules out the possibility of formation of 1:1 inclusion complex.

### 3.2. Steady state emission spectra

The emission spectra of NR in water and on successive addition of  $\beta$ -CD are shown in Fig. 2a. NR is very weakly fluorescent in water but on addition of  $\beta$ -CD the fluorescence quantum yield increases significantly (Table 1a).

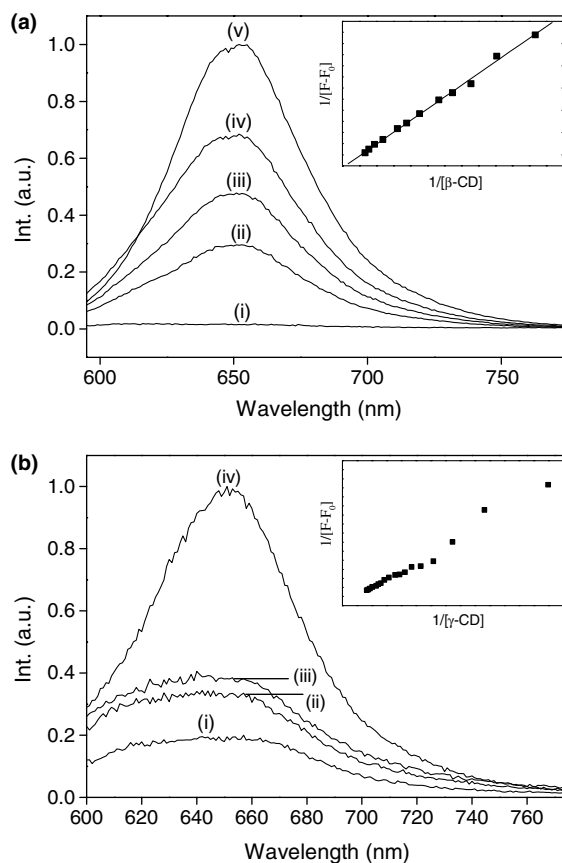


Fig. 2. (a) Steady-state emission spectra of NR in water (i) and in presence of  $\beta$ -CD (ii–iv) containing 4.4 mM (ii), 8.8 mM (iii), 13.2 mM (iv)  $\beta$ -CD. (v) Represents emission spectrum of NR in presence of 13.2 mM  $\beta$ -CD and 7M urea. (b) Steady state emission spectra of NR in water (i) and in presence of 7.7 mM (ii) and 15.4 mM (iii)  $\gamma$ -CD. (iv) Represents emission spectrum of NR containing 15.4 mM  $\gamma$ -CD and 7 M urea. Insets are double reciprocal plot of  $F - F_0$  against concentration of CD.

In pure water the emission peak of NR is  $\sim 660$  nm. On successive addition of  $\beta$ -CD, the spectrum gradually exhibits hypsochromic shift and finally at 13.2 mM  $\beta$ -CD the peak maximum is at  $\sim 650$  nm. The blue shift in emission spectra and increase in fluorescence quantum yield indicates the formation of inclusion complex between NR and  $\beta$ -CD. The value of the binding constant  $K_b$  for the inclusion complex is calculated using the following equation [29]:

$$\frac{1}{F - F_0} = \frac{1}{K_b(F_1 - F_0)[\text{CD}]} + \frac{1}{F_1 - F_0}, \quad (1)$$

where  $[\text{CD}]$  represents the analytical concentration of CD,  $K_b$  is the binding constant,  $F_0$  represents the fluorescence intensity of free NR,  $F_1$  is the fluorescence intensity of the inclusion complex and  $F$  is the observed fluorescence intensity at its maximum. Inset of Fig. 2a shows a straight line from the plot of the  $1/F - F_0$  versus reciprocal of the  $\beta$ -CD concentration for the present system, indicating 1:1 stoichiometry in the

Table 1  
Emission characteristics of Nile Red (NR) in  $\beta$ -CD and  $\gamma$ -CD

System	$\phi_f$	$\langle\tau_f\rangle$ (ns)	$K_r \times 10^{-9}$ (s $^{-1}$ )	$K_{nr} \times 10^{-9}$ (s $^{-1}$ )
(a) $\beta$ -CD				
NR in water	0.018	0.422	0.047	2.322
NR in water + 4.40 mM $\beta$ -CD	0.051	0.601	0.085	1.579
NR in water + 8.80 mM $\beta$ -CD	0.082	0.797	0.102	1.152
NR in water + 13.2 mM $\beta$ -CD	0.116	0.845	0.137	1.046
NR in water + 13.2 mM $\beta$ -CD + 7 M urea	0.158	1.050	0.150	0.80
(b) $\gamma$ -CD				
NR in water	0.018	0.422	0.047	2.322
NR in water + 6.16 mM $\gamma$ -CD	0.025	0.454	0.055	2.147
NR in water + 15.4 mM $\gamma$ -CD	0.031	0.503	0.062	1.926
NR in water + 15.4 mM $\gamma$ -CD + 7 M urea	0.062	0.633	0.098	1.481

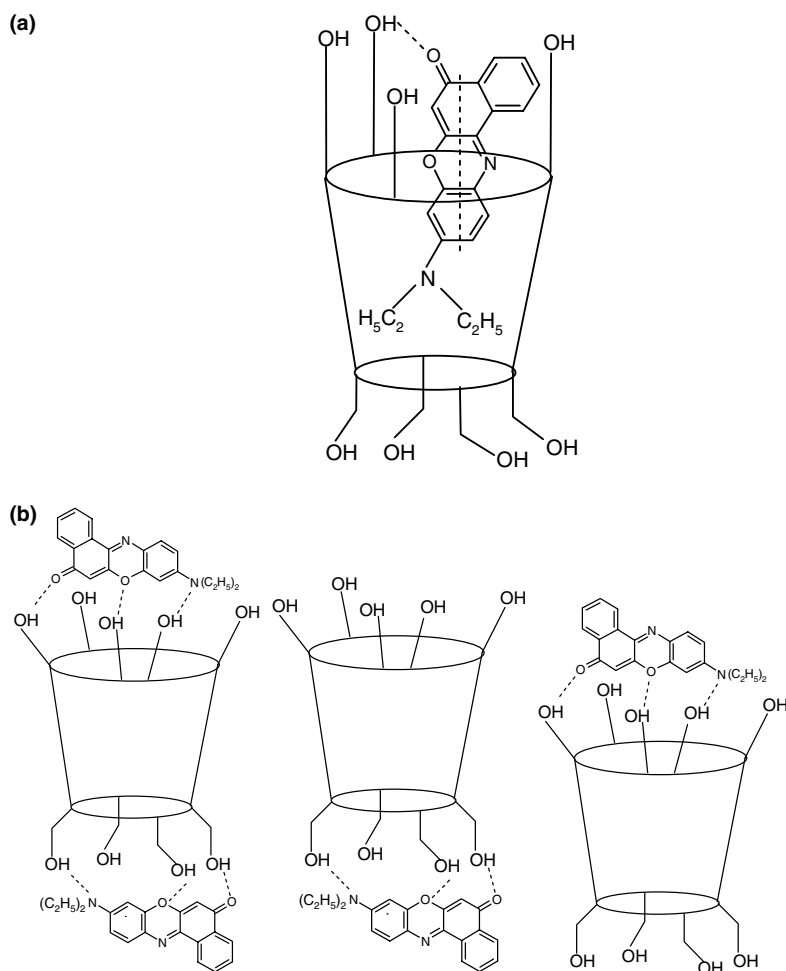
NR: $\beta$ -CD inclusion complex. From the slope and intercept, the formation constant was determined to be  $10 \text{ M}^{-1}$  at 298 K.

On addition of  $\gamma$ -CD in the aqueous solution of NR, a little change in the intensity and quantum yield (Table 1b) is observed. A representative fluorescence spectrum of NR in aqueous solution on addition of  $\gamma$ -CD is shown in Fig. 2b. The double reciprocal plot of  $F - F_0$  against concentration of  $\gamma$ -CD (inset of Fig. 2b) cannot be fitted with a straight line and on the other hand the plot exhibits an upward concave curvature suggesting that 1:1 host:guest complex formation is not possible but higher order complex may form.

Both the  $\beta$ - and  $\gamma$ -CD cavities are less polar than pure water and the polarity of the cavity is in between acetonitrile and 1,4-dioxane [8]. It was shown from various studies that the increase in the non-radiative rate constant for NR increases with solvent polarity [13–15,27]. It is a diagnostic of a TICT in the molecule [30]. In general TICT state is non-emissive in NR and TICT is the main non-radiative deactivation pathway in case of NR [13–15,27]. On addition of  $\beta$ -CD in an aqueous solution of NR, a significant increase of emission intensity is observed. This is because of the formation of 1:1 complex formation between NR and  $\beta$ -CD. Since the rotational freedom of diethylamino group is prevented (Scheme 2) and the polarity is decreased inside the CD cavity, the non-radiative rate is retarded in the  $\beta$ -CD cavity. Thus, increase in the fluorescence quantum yield of NR in presence of  $\beta$ -CD is observed. The cavity diameter of  $\gamma$ -CD is much higher  $\sim 8.5 \text{ \AA}$  compared to  $\beta$ -CD ( $\sim 6.5 \text{ \AA}$ ). So, the possibility of inclusion of NR in the  $\gamma$ -CD is much higher with respect to the  $\beta$ -CD from the consideration of size. But the increase in fluorescence quantum yield on addition of  $\gamma$ -CD in aqueous solution of NR is much less compared to that of  $\beta$ -CD. This result is contrary to the absorption result. Recently, Cser et al. [31] showed that viscosity barely influences the fluorescence behaviour of NR. Taking a series of alcohols and other non-hydrogen bonding solvents they showed that hydrogen bonding of NR with alcohols could occur both

in the ground state and excited states. The fluorescence quantum yield and lifetime are found to be diminishing significantly with increasing hydrogen bond donating capacity of the medium. Hence, hydrogen bonding between NR and  $\gamma$ -CD may play a role to control the TICT process of NR in presence of  $\gamma$ -CD. This conjecture is further supported from the fact that the intramolecular hydrogen bonding is very strong between adjacent  $-\text{OH}$  groups in  $\beta$ -CD [22]. Therefore, the  $\beta$ -CD is rather rigid structure and a complete secondary belt is formed by these hydrogen bonds [22].  $\gamma$ -CD is a non-coplanar molecule and this type of hydrogen belt is not possible here [22]. So, the possibility of intermolecular hydrogen bonding with other external guest is more favorable in  $\gamma$ -CD. To verify the effect of hydrogen bond of NR: $\beta$ -CD and NR: $\gamma$ -CD complexes, we have investigated the effect of addition of urea on these systems. Urea is commonly known as a strong water structure breaker, i.e., hydrogen bond breaker [21,32]. On addition of urea (up to 7 M) to NR in 15.4 mM  $\gamma$ -CD solution, the fluorescence intensity is gradually increased. The fluorescence quantum yield and lifetime is also increased in presence of 7 M urea to NR in 15.4 mM  $\gamma$ -CD solution (Table 1). On addition of 7 M urea in 15.4 mM  $\gamma$ -CD solution containing NR, there is two fold increase in fluorescence quantum yield ( $\phi_f$ ) from 0.031 to 0.062 and increase in lifetime from 0.503 to 0.633 ns (Table 1 and Fig. 2). The increase in quantum yield ( $\phi_f$ ) on addition of urea suggests that urea breaks the hydrogen bond between NR and  $-\text{OH}$  groups of CDs. Consequently, the NR molecules have easier access to the less polar cavity of the  $\gamma$ -CD and leads to the increase in fluorescence quantum yield. On addition of 7 M urea in of NR: $\beta$ -CD, there is a little increase in the fluorescence quantum yield ( $\phi_f$ ) from 0.116 to 0.158. It suggests that most of the NR molecules are included in the  $\beta$ -CD cavity before urea addition. The slight increase in quantum yield ( $\phi_f$ ) indicates the presence of some weak hydrogen bonding between NR and  $-\text{OH}$  group of  $\beta$ -CD.

The presence of isosbestic point in absorption spectra and the linear fit of double reciprocal plot of



Scheme 2. (a) The pattern of inclusion complex between NR and  $\beta$ -CD. (b) The pattern of capped complexes between NR and  $\gamma$ -CD.

NR in  $\beta$ -CD, confirms the formation of 1:1 inclusion complex formation between NR and  $\beta$ -CD. Wagner et al. [26] also reported that the formation of 1:1 complex formation between NR and  $\beta$ -CD. For  $\gamma$ -CD, 1:1 complex formation is ruled out as the double reciprocal plot is non-linear. The absorption spectra indicate that NR molecule feels less polar environment in presence of  $\gamma$ -CD. The molecular modeling studies of Wagner et al. [26] have showed that the full inclusion of NR molecule inside the  $\gamma$ -CD cavity is not stable and will quickly eject the guest molecule (NR). Thus higher order complexes are involved. If 2:1 (host:guest) complex forms, then significant fluorescence enhancement should occur. The small fluorescence enhancement observed on addition of  $\gamma$ -CD, rules out the possibility of 2:1 (host:guest) complex formation. The formation of 1:2 (host:guest) inclusion complex should be reflected by the decrease in fluorescence quantum yield ( $\phi_f$ ) compared to NR in aqueous solution due to self quenching inside the  $\gamma$ -CD cavity. But we have observed a slight increase in

fluorescence quantum yield ( $\phi_f$ ) compared to NR in aqueous solution. Again the effect of urea addition suggests the formation of strong hydrogen bonds between NR and  $-\text{OH}$  groups of  $\gamma$ -CD. Thus considering above fact it may possible to imagine complexes with no actual inclusion but in which the NR molecule make flat hydrogen bonded complex against  $\gamma$ -CD cavity opening, thus serving as a cap and such capped complexes would be expected to be much less robust than fully inserted complexes. Furthermore, the capped complexes would still result in a large change in the NR environment as compared to free dye in aqueous solution. Thus we observed a blue shift in absorption spectra in case of NR in presence of  $\gamma$ -CD. Wagner et al. [26] concluded from the steady state spectra as well as molecular modeling study that the capped 1:1 and 1:2 association complexes are formed between  $\gamma$ -CD and NR. Our observed result of NR in presence of  $\gamma$ -CD also fully consistent with the formation of capped 1:1 or 1:2 association complexes between host and guest (Scheme 2).

### 3.3. Time-resolved studies

#### 3.3.1. Non-radiative rate constant ( $k_{nr}$ ) for TICT processes

As we mentioned earlier, the TICT is the main non-radiative decay pathway of NR [13–15,27]. Using the following familiar relationships, we can calculate the radiative ( $k_r$ ) and non-radiative rate constant ( $k_{nr}$ ) using quantum yield ( $\phi_f$ ) and lifetime ( $\tau_f$ ) value

$$k_r = \frac{\phi_f}{\tau_f}, \quad (2)$$

$$\frac{1}{\tau_f} = k_r + k_{nr}. \quad (3)$$

The different photophysical parameter and radiative ( $k_r$ ) and non-radiative rate constant ( $k_{nr}$ ) are summarized in Table 1. The anomalous decrease of non-radiative rate of NR in presence of  $\beta$ -CD compared to that of  $\gamma$ -CD may be due to the different locations of the probe in these two CDs. In case of NR: $\beta$ -CD inclusion complex, the rotational freedom of diethylamino group is hindered and NR molecule experience a less polar environment inside the cavity of  $\beta$ -CD. Hence, the formation of TICT state of NR is some extent prevented in presence of  $\beta$ -CD. Consequently, non-radiative rate of NR is retarded in presence of  $\beta$ -CD. In case of  $\gamma$ -CD, NR molecule make flat hydrogen bonded complex against  $\gamma$ -CD cavity opening, thus serving as a cap and in such capped complexes the free rotation of diethylamino group is not restricted compared to that of NR: $\beta$ -CD inclusion complex. Thus, non-radiative rate of NR in presence of  $\gamma$ -CD is not severely affected. Table 1 indicates that the  $k_{nr}$  value of NR is affected in a similar way in both  $\beta$ - and  $\gamma$ -CD in presence of urea. So, we cannot draw any definite conclusion from this observation. But the significant change of quantum yield in NR: $\gamma$ -CD system in presence of urea, definitely indicates the role of hydrogen bonding in this system.

#### 3.3.2. Dynamics of solvent relaxation in CD

The time-resolved fluorescence decays of NR in  $\beta$ -CD and  $\gamma$ -CD are fitted with a bi-exponential function. In case of  $\gamma$ -CD, the fluorescence decays at the blue end and red end are very similar. We have observed wavelength dependent fluorescence decays in case of NR in  $\beta$ -CD. The decay at the red end becomes slower compared to blue end. The situation is rather different with respect to the emission lifetime of NR in chloroform or methanol. Krishna et al. [27] observed that the amplitudes of the short and long component of lifetime of NR in viscous solvents like octanol and glycerol are wavelength dependent. Moreover, in case of octanol and glycerol the amplitude of the short component lifetime becomes negative at longer wavelength. In our system (NR in 13.2 mM  $\beta$ -CD) the amplitudes for the short

and long lifetime become wavelength dependent but the amplitude for the short component is not negative even at longer wavelength.

In the case of NR, the change in dipole moment upon excitation to the first excited state was estimated to be 7.4 D higher than that of the ground state from the solvatochromic Stokes shift with the solvent polarity using the Lippert–Mataga equation [13]. The large change in dipole moment results in reorganization of the solvent dipoles around the NR molecule in the excited state. We have constructed time-resolved emission spectra (TRES) from the fluorescence decays at different wavelength by applying spectral reconstructive method of Maroncelli et al. [33]. The TRES of NR in 13.2 mM of  $\beta$ -CD are shown in Fig. 3a. The maximum shift observed in the TRES is  $327\text{ cm}^{-1}$ . The presence of isosbestic point at  $14,800\text{ cm}^{-1}$  in TRES again confirms the formation of 1:1 inclusion complex between NR and  $\beta$ -CD. We have measured solvation dynamics of NR in presence of  $\beta$ -CD with the help of solvent correlation function,  $C(t)$ . The decay of the solvent correlation function ( $C(t)$ ) for NR in 13.2 mM  $\beta$ -CD is shown in Fig. 3b and the decay parameters of solvent correlation

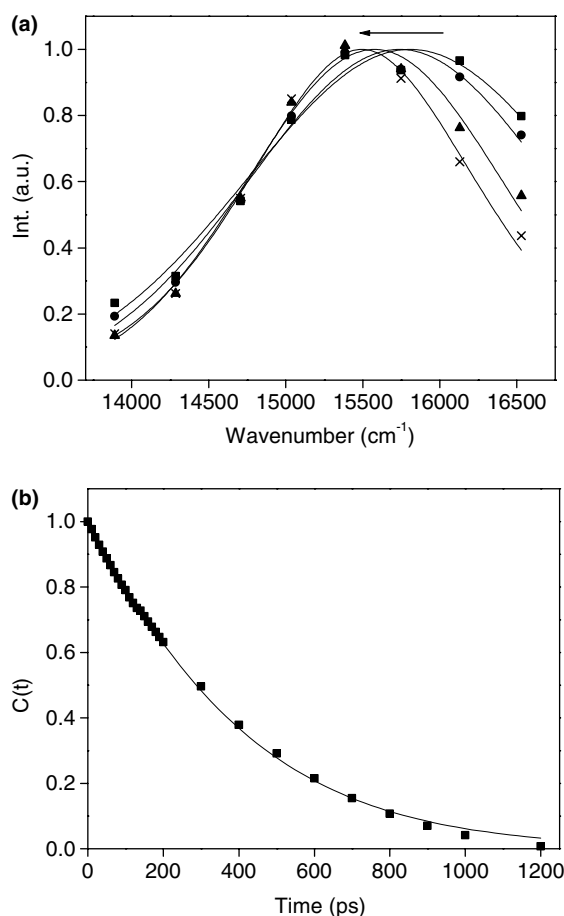


Fig. 3. (a) Time resolved emission spectra of NR in presence of 13.2 mM  $\beta$ -CD at 0 (■), 100 (●), 500 (▼) and 1000 (×) ps. (b) Decay of solvent correlation function ( $C(t)$ ) of NR in 13.2 mM  $\beta$ -CD.

function  $C(t)$  are summarized in Table 2. The solvation time of NR is 0.400 ns with single exponential in nature. Fleming and co-workers [24] observed the solvation dynamics of pure water using Coumarin 480 as a probe and the solvation time is  $\sim 310$  fs. To the best of our knowledge, this is the first report of solvation dynamics measurement of NR in  $\beta$ -CD cavity. The average solvation time of C-480 in  $\gamma$ -CD cavity is 475 ps [24], which is very close to that of our reported value in  $\beta$ -CD cavity. So, the solvation dynamics of the water molecules inside  $\beta$ -CD cavity are severely retarded compared to pure water.

### 3.3.3. Rotational relaxation

The time resolved fluorescence anisotropy ( $r(t)$ ) is used to determine the rotational dynamics of the chromophores. The  $r(t)$  was calculated using the following equation:

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)}, \quad (4)$$

where  $G$  is the correction factor for detector sensitivity to the polarization direction of the emission.  $I_{\parallel}(t)$  and  $I_{\perp}(t)$  are fluorescence decays parallel and perpendicular to the polarization direction of the excitation light, respectively. The results of anisotropy measurements are summarized in Table 3 and the representative anisotropy decays are shown in Fig. 4.

The rotational relaxation of NR in pure water is well characterised by a single-exponential decay rate and having a time constant of  $\sim 50$  ps. In presence of 13.2 mM  $\beta$ -CD, the rotational relaxation of NR is bi-exponential having time constant of 92 ps (80%) and 887 ps (20%). The presence of long relaxation time (887 ps) in  $\beta$ -CD suggests that some NR molecules are ‘caged’ in the cavity. The addition of urea (7 M) to NR in 13.2 mM  $\beta$ -CD solution the relaxation behavior of the probe is not severely effected (Fig. 4). It suggests that the hydrogen bonding between NR and  $\beta$ -CD is not so effective.

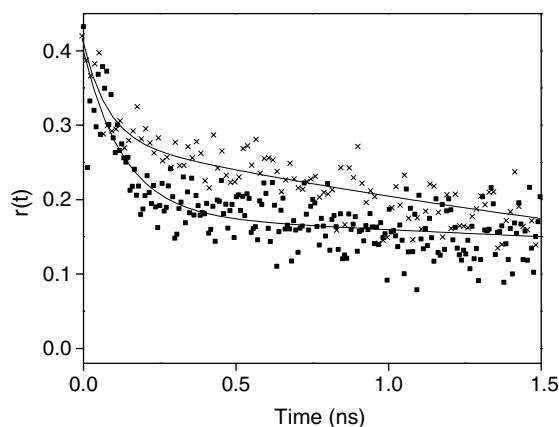


Fig. 4. Time-resolved fluorescence anisotropy ( $r(t)$ ) of NR in 13.2 mM  $\beta$ -CD (■) and in 13.2 mM  $\beta$ -CD containing 7 M urea (×).

In case of NR in 15.4 mM  $\gamma$ -CD, the anisotropy decay is fitted by a single exponential function and having rotational relaxation time constant of 90 ps. The slight increase in relaxation time may be due to the possibility of hydrogen bond of NR molecule with the –OH groups of the rim of  $\gamma$ -CD. On addition of 7 M urea to the NR in  $\gamma$ -CD, the anisotropy decay become biexponential having rotational relaxation times of  $\sim 50$  ps (67%) and 1.3 ns (33%). The presence of slow component in presence of urea indicates that urea break hydrogen bonds between NR and –OH groups of CD and leads to the accessibility of NR molecule to the  $\gamma$ -CD cavity. These results confirm the formation of capped complex between NR and  $\gamma$ -CD.

A diffusion in a cone model was used to describe the motion of chromophore inside the cavity [34]. In the diffusion in a cone model the unit vector  $\mu$  with orientation  $\Omega = (\theta, \phi)$  diffuses freely in the angular region  $0^\circ \leq \theta \leq \theta_{\max}$  and  $0^\circ \leq \phi \leq 2\pi$  with a diffusion co-efficient  $D_w$ . In this case the amplitude of short component, which characterizes the amount of the anisotropy loss from the internal motion, can be used to estimate the

Table 2

Decay characteristics of Nile Red (NR) in  $\beta$ -CD

Systems	$\Delta\nu^a$ ( $\text{cm}^{-1}$ )	$a_1$	$\tau_1$ (ps)	$a_2$	$\tau_2$ (ps)	$\langle\tau_s\rangle^b$ (ps)
NR in water + 13.2 mM $\beta$ -CD	327	1.00	400	0	0	400

<sup>a</sup>  $\Delta\nu = \nu_0 - \nu_\infty$ .

<sup>b</sup>  $\langle\tau_s\rangle = a_1\tau_1 + a_2\tau_2$ .

Table 3

Rotational relaxation times of Nile Red (NR) in  $\beta$ -CD and  $\gamma$ -CD

Systems	$r_0$	$a_1$	$\tau_1$ (ps)	$a_2$	$\tau_2$ (ns)
NR in water	0.40	0.40	50	–	–
NR in water + 13.2 mM $\beta$ -CD	0.40	0.32	92	0.08	0.887
NR in water + 13.2 mM $\beta$ -CD + 7 M urea	0.36	0.18	71	0.18	1.060
NR in water + 15.4 mM $\gamma$ -CD	0.40	0.40	90	–	–
NR in water + 15.4 mM $\gamma$ -CD + 7 M urea	0.37	0.25	46	0.12	1.300



angle  $\theta_{\max}$  of the cone. Taking the transition dipole of the fluorophore to be directed along the long symmetry axis of the guest and assuming that the long symmetry axis of the guest (Scheme 1) is perpendicular to the  $\beta$ -CD's open end (Scheme 2), it is possible to write [34],

$$\frac{r(\infty)}{r(0)} = \frac{A_{\text{long}}}{A_{\text{short}} + A_{\text{long}}} = \frac{1}{4} \cos^2 \theta_{\max} (1 + \cos \theta_{\max})^2. \quad (5)$$

Now from the rotational relaxation data (Table 3) for NR in  $\beta$ -CD shows  $A_{\text{long}}/(A_{\text{long}} + A_{\text{short}}) = 0.2$ . Using the above equation, one find  $\theta_{\max} = 55^\circ$  for NR in  $\beta$ -CD. Using the same equation,  $\theta_{\max}$  for NR in  $\gamma$ -CD is either  $0^\circ$  or  $180^\circ$ . It again supports the formation of capped complexes of NR in presence of  $\gamma$ -CD.

#### 4. Conclusions

The steady state and time-resolved studies indicate the formation of 1:1 inclusion complex between NR and  $\beta$ -CD. However, our observed results suggest the formation of 'capped' complex between NR and  $\gamma$ -CD. We have observed a retardation of non-radiative rate for TICT of NR both in the presence of  $\beta$  and  $\gamma$ -CD compared to pure water. But the retardation of non-radiative rate is much higher in case of  $\beta$ -CD compared to  $\gamma$ -CD with respect to water. We have observed a time-dependent Stokes' shift of NR in  $\beta$ -CD system but the same is not observed in  $\gamma$ -CD. The observed solvation time of NR is 400 ps for NR: $\beta$ -CD system. The increase in rotational relaxation time of NR in presence of  $\beta$ -CD compared to water indicates that the probe molecules are incorporated in the cavity of the CD. The little retardation in the non-radiative rate of NR in presence of  $\gamma$ -CD system may be due to the formation of hydrogen bonds between the  $-\text{OH}$  groups of  $\gamma$ -CD with NR molecules. This is also verified from the relatively large increase in rotational relaxation time and quantum yield of NR in  $\gamma$ -CD system on addition of urea compared to the same in  $\beta$ -CD and this suggest the formation of 1:1 or 1:2 capped complexes between NR and  $\gamma$ -CD.

#### Acknowledgements

N.S. is thankful to Department of Science and Technology (DST) and Council of Scientific and Industrial Research (CSIR), India for generous research grants. P.H., D.C. and A.C. are thankful to CSIR for research fellowships.

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