Photophysical studies of indole and methylindoles in microheterogeneous medium (*)

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Summary. — The present paper reports the studies of the effect of inclusion of probe molecules indole, 1-methylindole (1MI) and 3-methylindole (3MI) within β-cyclodextrin (β-CD) cavity on excited state proton transfer (ESPT) or deprotonation reactions by steady state fluorescence and time-correlated single photon counting measurements. From Bensi-Hildebrand reciprocal plots it was inferred that the stoichiometry of the complex of β-CD with excited 3MI (or 1MI) should be of 1:1 type. The dynamic quenching rate constant k_q values for indole or 3MI in aqueous solution in the presence of quencher sodium hydroxide (NaOH) were found to be enhanced (nearly doubled) with addition of β-CD. No significant quenching in the fluorescence emission of 1MI or its β-CD included complex was found in the presence of NaOH. In the former case it is apparent that the incorporation of probes in CD cavity enhances the rate of deprotonation (abstraction of H-atom from N-position of indole or 3MI) owing to the interaction between excited β-included complex of indole or 3MI and OH^- of NaOH. From this observation the mode of inclusion of probe molecules in CD cavity or location of probe moiety in cavity could be determined. In case of 1MI no such ESPT reaction occurs with OH^- due to lack of N-position H-atom in this molecule. In the quenching phenomena observed in the fluorescence spectra of indole and 3MI or their β-CD included complex due to addition of NaOH, static quenching process, apart from deprotonation, was also found to have a significant role.

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1. – Introduction

Cyclodextrins are linked glucopyranose rings forming a torus structure. The interior of this torus forms a hydrophobic cavity that is capable of complexing hydrophobic molecules of suitable size to form host-guest enzymelike complexes [1]. A considerable interest has been directed towards these inclusion complexes as these systems have been employed as good models for protein-ligand interaction, enzymatic catalysis [2] and catalyzed chemical reaction [3]. Complexation of guest molecules into

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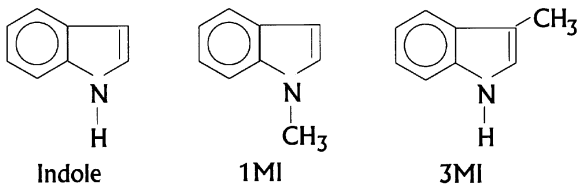

Fig. 1. – Molecular structures of indole, 1MI and 3MI.

cyclodextrin (CD) cavity results in a number of interesting photophysical and photochemical effects [4-6].

Photoinduced proton transfer reaction is a fundamental process in photochemistry and photobiology. In general acid-base properties of molecules in the excited state differ largely from those in the ground state. Recently there has been an increasing interest in using photoacids (*e.g.*, some aromatic amines or alcohols) as probes for the heterogeneous environment of aqueous solution [7]. A large number of investigations have been made on excited state proton transfer (ESPT) reactions of aromatic molecules in homogeneous solution [8, 9] but studies of the effect of heterogeneous medium (*e.g.*, micellar, cyclodextrin) on ESPT are still limited in number [10-12]. Reports regarding the inclusion effect of cyclodextrin (CD) on ESPT reaction show that in some cases [12] inclusion in CD enhances the rate of deprotonation reaction by a factor of 2 as compared with that of the rate in bulk solution but in other cases [13, 14] decrease in the deprotonation rate is observed. Authors have described these discrepancies by the difference in mode of inclusion of the molecules in the hydrophobic CD cavity. Nevertheless, it seems that more studies on several aromatic amines or phenolic systems would be needed to investigate the effect of inclusion or complexation of the molecule with CD on the ESPT process. In order to have more detailed information about the effect of inclusion on ESPT process, we have studied ESPT reaction of the probe molecules indole, 1-methylindole (1MI) and 3-methylindole (3MI) (fig. 1) complexed with β-CD by using steady state and time-resolved spectroscopic methods in the nanosecond time domain. The importance of indole lies in the fact that it is responsible for most of the absorption and intrinsic fluorescence of proteins and 3MI can be used as a model for tryptophan. The results obtained from the photophysical and photochemical studies of the cyclodextrin complexes of indoles and MIs might be useful in protein studies since CD can play the model role of complex protein systems.

2. – Experimental

Indole and 3MI, obtained from Aldrich, were purified by vacuum sublimation. 1MI (Aldrich) was purified by distillation under reduced pressure. Analytical grade β-CD was purchased from Aldrich and was used as such. Millipore water was used as solvent. All solutions were freshly prepared just before experiment and degassing of the solutions was found to be unnecessary. The electronic absorption and emission spectra were recorded with the help of Shimadzu UV-VIS 2101PC spectrophotometer and Hitachi F-4500 fluorescence spectrophotometer, respectively. The fluorescence lifetimes were measured by using a time-correlated single photon counting (TCSPC) technique. For this, a coherent synchronously pumped, cavity dumped rhodamine 6G

dye laser (702-1) pumped by a coherent CW mode-locked Nd:YAG laser (Antares 76-S) was used. The fundamental laser light at 600 nm was frequency doubled to produce exciting light at 300 nm. The emissions were detected at magic angle (54.7°) polarisation using a Hamamatsu MCP photomultiplier tube (2809 U). The resolution of the set-up is 0.02 ns. The deconvolution of the fluorescence decays was done by using global lifetime analysis software.

3. – Results and discussion

3·1. *Spectral characteristic.* – Indole, 1MI and 3MI possess low solubility in water. The association constant value between indole and β-CD ($\sim 184\ dm^3\ mol^{-1}$) at 25 °C has been reported [5]. In case of 1MI and 3MI, the electronic absorption spectra of their aqueous solutions showed an increase in absorbance accompanied by a broadening of the spectra by gradual addition of β-CD. This is expected as the guest molecule is dissolved in hydrophobic core of β-CD due to detergent action of CD. In contrast to the absorption spectra of indole [5] and 1MI, no isosbestic point is observed in the case of 3MI (figs. 2(a)(b)). Following the observations made by earlier authors [15, 16] it could be proposed that the possible cause of the absence of isosbestic point in the case of 3MI

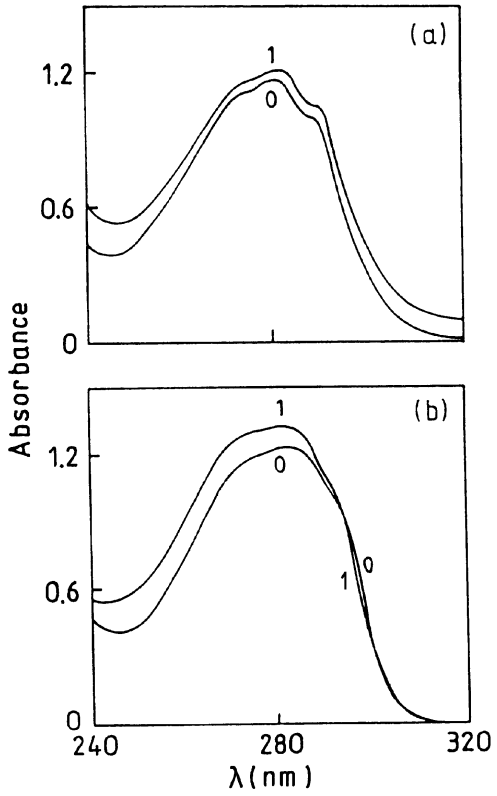

Fig. 2. – Electronic absorption spectra of (a) 3MI ($C = 2.0 \times 10^{-4}\ mol\ dm^{-3}$) and (b) 1MI ($C = 8.0 \times 10^{-5}\ mol\ dm^{-3}$) in water without (curve 0) and with (curve 1) β-CD ($C = 5.0 \times 10^{-3}\ mol\ dm^{-3}$) at 298 K ($l = 1$ cm).

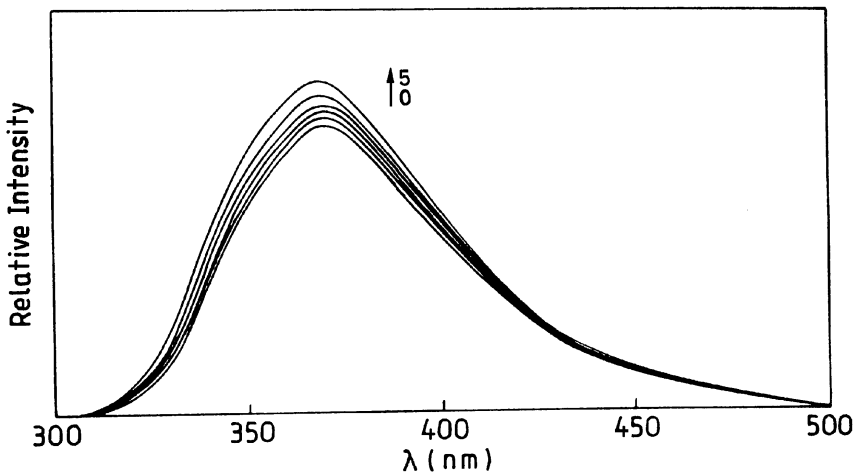

Fig. 3. – Effect of β-CD on the fluorescence emission spectra of 3MI ($C = 2.0 \times 10^{-4}$ mol dm^{-3}) in water at 298 K ($\lambda_{exc} = 290$ nm). Concentration of β-CD (mol dm^{-3}) in (0) 0; (1) 3.5×10^{-4}; (2) 7.1×10^{-4}; (3) 1.2×10^{-3}; (4) 1.9×10^{-3}; (5) 3.5×10^{-3}.

might be due to the formation of more than one kind of inclusion complex, *e.g.*, one with the 3MI molecule completely accommodated in the CD cavity and the other in which a part of the molecule is projected out of the cavity.

Figure 3 shows the effect of β-CD on the fluorescence emission spectrum of 3MI, studied in the present investigation, in water solution. As the concentration of CD is increased the fluorescence intensity of 3MI increases with a red shift of around 2 nm. These observations indicate the environmental change around the guest molecule.

3·2. *Association constant.* – It has been noticed that fluorescence measurements are more sensitive compared to absorption measurements because of the larger changes in emission intensity induced by CD. Hence we used the intensity values obtained from fluorescence measurements for calculating the association constant, K. We carried out a double reciprocal analysis for the binding constant of the complex in order to study the different stoichiometry between CD and a guest molecule (MI). In case of a simple 1:1 complex, the equilibrium can be written as

$$[S] + [CD] \overset{K}{\rightleftharpoons} [SCD]. \tag{1}$$

For this equlibrium, one obtains the following expression of Bensi-Hildebrand double reciprocal plot [17, 18]:

$$\frac{1}{I - I_0} = \frac{1}{K(I_1 - I_0)} \frac{1}{[CD]} + \frac{1}{I_1 - I_0}, \tag{2}$$

where I_0, I_1 denote fluorescence intensities of the probe (MI) in bulk water and in the complex, respectively; I is the fluorescence intensity at a given CD concentration and K is the association constant.

Figure 4 shows the double reciprocal plot for complexation of a MI with β-CD. The

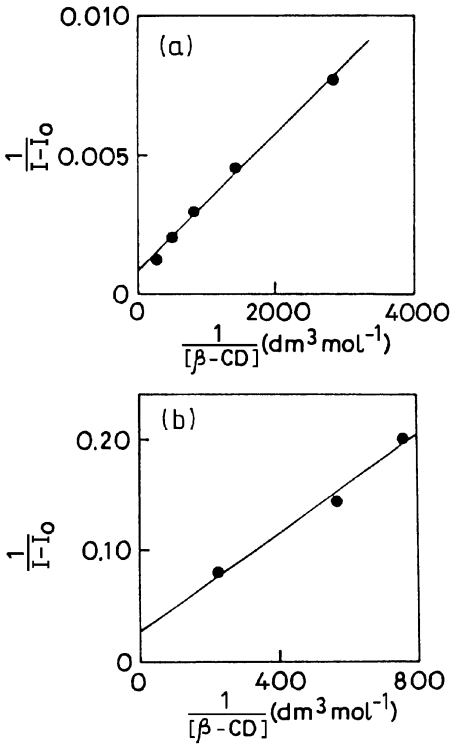

Fig. 4. – Double reciprocal plots (using eq. (2), see text) for the probe molecules (a) 3MI and (b) 1MI complexed with β-CD at 298 K.

plot is described by a straight line as it should follow eq. (2). From the slope and intercept of linear plots of the $1/(I - I_0)$ *vs.* 1/[CD] (fig. 4), we computed the values of the association constant, K which is found to be 314 dm^3 mol^{-1} for 3MI and 119 dm^3 mol^{-1} for 1MI at 25 °C. The linearity indicates that the stoichiometry of the complex of β-CD with 3MI (or 1MI) should be 1:1.

3·3. *Excited-state quenching process.* – The emission intensity of steady-state fluorescence, produced by the singlet excitation (290 nm) of probe molecules (indole or 3MI) was found to decrease regularly with increasing concentration of sodium hydroxide (NaOH) in water solution at room temperature. This quenching of fluorescence intensity was not observed in the case of 1MI with increasing concentration of NaOH.

Excited state deprotonation reaction with NaOH has been recognized to quench indole fluorescence and the kinetics of proton transfer from photoexcited indole to NaOH in water have been reported [9]. In the present investigation, from the observed changes in the fluorescence emission spectra of 3MI and 1MI, it can be proposed that the fluorescence quenching in the case of 3MI might be due to deprotonation or excited state proton transfer reaction because fluorescence of 1MI does not quench with addition of NaOH. In the case of the latter molecule as H-atom in the N-position of indole chromophore is replaced by —CH_3 group, deprotonation is not possible.

Absorption and excitation spectra of the probes (indole, 3MI) were found to remain unchanged with increase of concentration of the base NaOH. This seemingly indicates that no association complex is formed between the reactants in the ground state. Thus, a possible reaction mechanism for excited state proton transfer of indole (and 3MI) can be schematically described as

$$\text{AH} + \text{OH}^- \underset{k_r}{\overset{h\nu}{\rightleftharpoons}} \text{AH}^* + \text{OH}^- \xrightarrow{k_1} \text{A}^{-*} + \text{H}_2\text{O} \xrightarrow{k_{nr}} \text{A}^- + \text{H}_2\text{O}\,, \tag{3}$$

where k_r, k_{nr} and k_1 are radiative, non-radiative and deprotonation rate constants of the above mentioned reaction shown in scheme (3).

As anionic species of indole derivatives are non-emissive [9] there is the lack of spectral arguments in favor of such a proposed mechanism. Nevertheless, following the observations made by earlier workers [9] with several similar systems, it can be argued that the mechanism shown in (3) might be operative in the case of 3MI-NaOH systems.

The simple Stern-Volmer (SV) plot for the fluorescence quenching of probe molecules by OH^- ion is shown in fig. 5(a). The simple SV equation (4*a*) is shown below. By plotting F_0/F *vs.* [Q] an upward curvature was noticed. This seemingly indicates that the static quenching reaction between indole (and 3MI) and OH^- ion might be present. Thus the quenching data were analyzed by transient effect model with the help of eq. (4*b*) which includes static quenching parameter V_f [19, 20].

$$\frac{F_0}{F} = 1 + K_{SV}[\text{Q}], \tag{4a}$$

$$\frac{F_0}{Fe^{V_f[\text{Q}]}} = 1 + K_{SV}[\text{Q}]. \tag{4b}$$

V_f is the static type and K_{SV} is the dynamic quenching constant. F_0, F are corrected fluorescence emission intensities of the fluorophore in the absence and presence of quencher, respectively and [Q] is the concentration of the quencher. K_{SV} is equal to the product of bimolecular quenching rate constant (k_q) and τ_0, fluorescence lifetime of the fluorophore in the absence of the quencher. By treating the data according to eq. (4*b*) and by plotting $F_0/(F\exp[V_f[\text{Q}]])$ *vs.* [Q] for varying V_f (using a linear least-squares curve-fitting procedure) [5], until a linear plot is attained, the static (V_f) and dynamic (K_{SV}) quenching constants were estimated. The values of these constants are shown in table II.

3˙4. *Effect of inclusion on excited state quenching process.* – In the β-CD included complex of probes quenching experiments were performed with OH^- (of NaOH) in order to get information about the factors affecting the prototropic processes. SV plots for the quenching of indole and 3MI in water with β-CD, shown in fig. 5(b), also showed an upward curvature indicating the presence of static quenching. The quenching data for solutions containing both free and bound molecules were analyzed by using the following form of SV equation [5]:

$$\frac{F_0}{F} = \left[\frac{f_f}{1 + K_f[\text{Q}]\,e^{V_f[\text{Q}]}} + \frac{f_b}{1 + K_b[\text{Q}]\,e^{V_b[\text{Q}]}}\right]^{-1}, \tag{5}$$

where f_f and f_b are fractional contributions of free and bound (with β-CD) indole (or

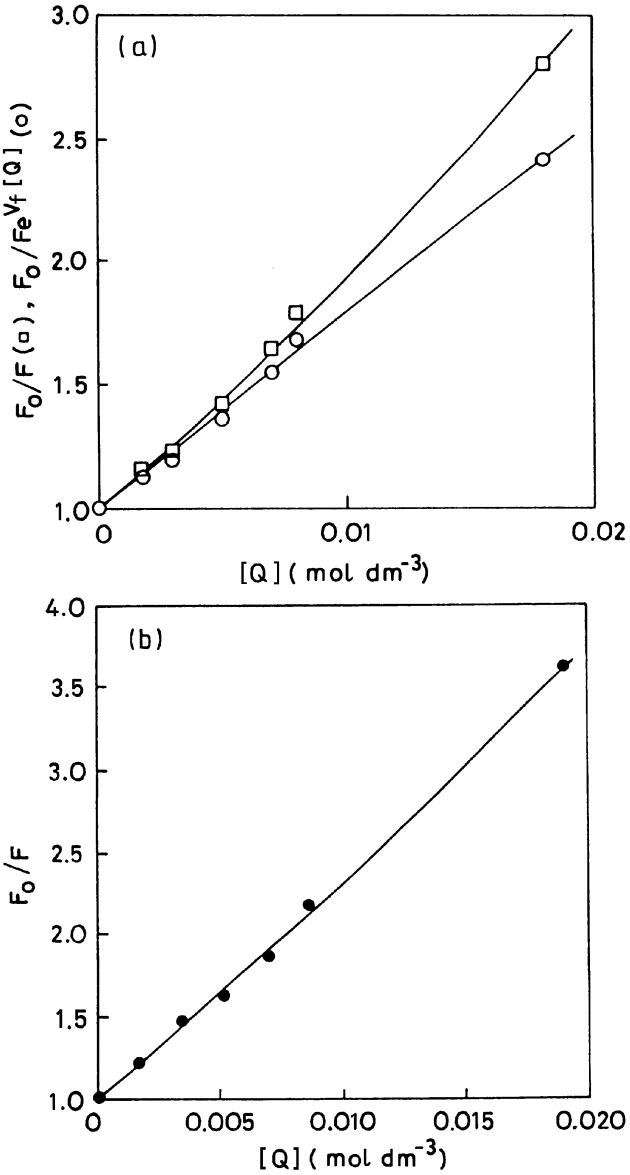

Fig. 5 – (a) Stern-Volmer plot for OH^- ion quenching of 3MI in water without β-CD (using eq. (4b), see text) at 298 K. (b) The same as (a) but in the presence of β-CD (using eq. (5), see text) at 298 K.

3MI), respectively to the total fluorescence intensity in the absence of quencher. In the case of β-CD-3MI system, fractional contribution of bound (f_b) 3MI and free (f_f) 3MI were computed, following the procedure of Ross *et al.* [5], and these values were found to be 0.57 and 0.43, respectively. K_b and V_b are collisional and static quenching constants for bound molecules. The ratio of the fluorescence lifetimes (obtained by

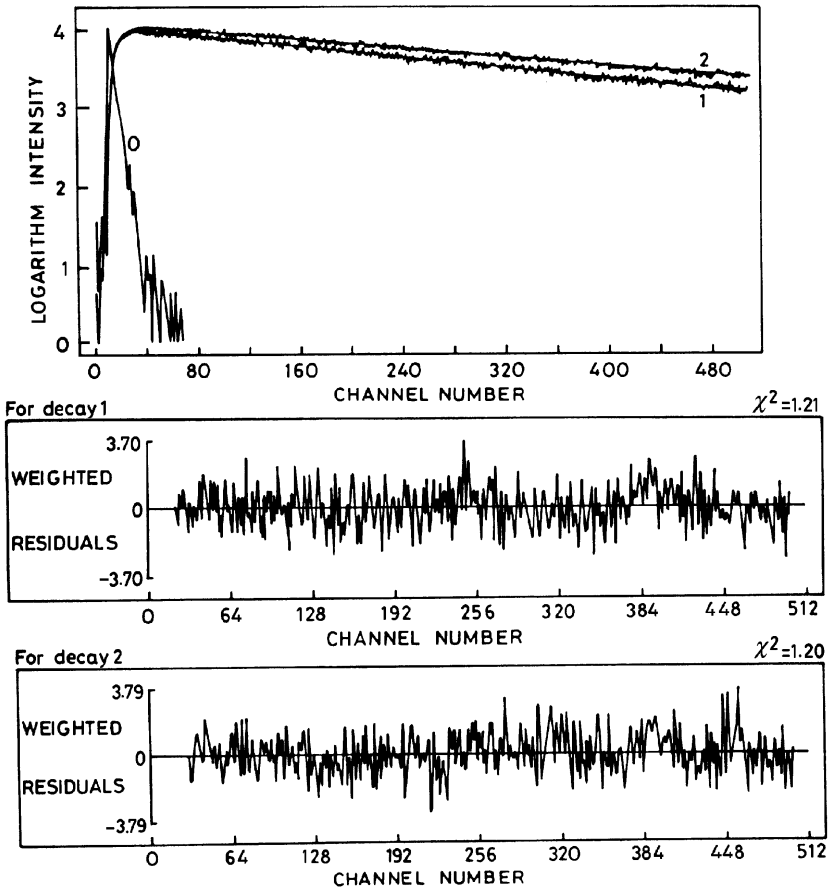

Fig. 6. – Fluorescence decay curves of 3MI in aqueous solution at 298 K ($\lambda_{exc} = 300$ nm, $\lambda_{em} = 370$ nm) in the absence of β-CD (curve 1) and in the presence of β-CD (curve 2) associated with the lamp profile (curve 0). The curves show the single component fits and residuals ($DW \sim 1.45$).

TCSPC technique, fig. 6) of bound and free 3MI (1.1) is in close agreement with the ratio of quantum yields of bound and free 3MI. The values of K_b and V_b were obtained by fitting the quenching data to eq. (5) by using a non-linear least-squares curve-fitting procedure. Experimental results are summarized in tables I, II.

From the values of dynamic quenching rate constant $k_q (= K_f/\tau_0,\ K_f \approx K_{SV}$ of expression (4*b*)) (table I), it is clear that diffusion plays a significant role in the deprotonation process. However, from fluorescence quenching experiment of indole with acrylamide the value of static quenching constant (V_f) was reported as 2.5 $dm^3\,mol^{-1}$ [5], whereas in the present case of OH^- quenching the value of V_f is found to be 8.1 $dm^3\,mol^{-1}$. This difference in the value of V_f can be explained in terms of much higher mobility of OH^- ion due to Grotthus type of migration [21, 22]. Hence, V_f which describes the distribution of quencher molecules around probes can be explained with higher OH^- ion mobility. k_q for 3MI in water was found to be less than that of indole. This observation is in accord to our expectation as it is known [23] that 3MI is much

TABLE I. – *Values of dynamic quenching constant (k_q) for the probes in water and in the presence of β-CD at 298 K. Fluorescence lifetimes (τ_0) of the probe molecules in the absence of quencher are also shown.*

Probe	Quencher	β-CD (m mol dm^{-3})	τ_0 ($\pm$0.02) (ns)	K_{SV} (dm^3 mol^{-1})	k_q (dm^3 mol^{-1} s^{-1})
Indole	NAOH	0	3.97($\pm$0.05) (*)	56	14.1×10^9
		18	5.93($\pm$0.05) (*)	131	22.1×10^9
3MI	NAOH	0	8.1 —	79	9.7×10^9
		3.5	8.8 —	164	18.7×10^9
1MI (**)	NAOH	0	8.5	—	no quenching
		5.0	—	—	no quenching

(*) Reference [5].
(**) No quenching.

TABLE II. – *Values of static quenching constant for the probes indole and* 3MI *in water (V_f) and in the presence of β-CD (V_b) at* 298 K.

Probe	Quencher	β-CD (m mol dm^{-3})	Static quenching constant (dm^3 mol^{-1})	
			V_f	V_b
Indole	NaHO	0	7.0	—
		18	—	8.3
3MI	NaHO	0	8.1	—
		3.5	—	9.8

stronger base in the excited state than is the indole. Our experimental data show that the dynamic quenching constants (k_q) for both the inclusion complexes increase by approximately double than the values observed for their respective free probes. These results are not in agreement with the observations made by Shizuka and co-workers [13, 14] for 2-naphthol. They reported a decrease in the proton dissociation rate on addition of β-CD. This phenomenon was described as less availability of space which hinders hydroxyl ion of NaOH from coming in contact with the embedded molecule where deprotonation centers were completely buried in the non-polar cavity and thereby resulting in a decrease in the prototropic reaction. In the present investigation the plausible mechanism for excited state intermolecular proton transfer reaction between 3MI and NaOH in the presence of β-CD might be very similar to that reported on carbazole system [12] where the molecules have been suggested to be at the interface and increase in deprotonation was described by cooperative [24] flip-flop [25] proton transfer process.

Hence, it is clear from above observations that incorporation of probes in CD cavity enhances the deprotonation rate. From our spectral data, we can also presume about the location of probe moiety in the cavity. A small blue shift ($\approx$ 2 nm) in the

fluorescence spectra of indole, 1MI or 3MI was observed when CD is added to its aqueous solution. If the amino group (> NH) is buried within the non-polar CD cavity, then the solvation of the amino group by water would be eliminated and a large blue shift in the fluorescence spectra would be expected. But from the observed red shift in the electronic absorption spectra and very little change (a small blue shift) in the fluorescence spectra of indole and MI, presently studied, it can be said that the amino group is near the secondary hydroxyl rim of the CD cavity and the apolar part (phenyl ring) of the molecule is buried in the hydrophobic cavity.

It can be surmised that the large quenching rate constant values (table I) observed for the probe molecules (indole and 3MI) in aqueous solution in the presence of β-CD might be due to the fact that the elaborate interconnection of hydrogen bonds near secondary rim of CD cavity (for which the nature of microenvironment has the properties of ethanol-water binary mixture type) influences largely the prototropic processes of these probes and their amino protons would be abstracted by OH^- ions in the bulk.

4. – Conclusion

In ESPT process the deprotonation rate of indole and 3MI molecules increases on inclusion in cyclodextrin cavity. This phenomenon can be explained by the combined process of Saenger's flip-flop hydrogen bonding phenomenon and the cooperative proton transfer process. Cooperative proton transfer process explains the dissociation of amino proton by OH^- ion through the network of hydrogen bonds in flip-flop motion, *i.e.* hydrogen bonding interaction of probe molecules with the secondary hydroxyl rim is of prime importance for getting detached of the > NH proton by OH^- ion.

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