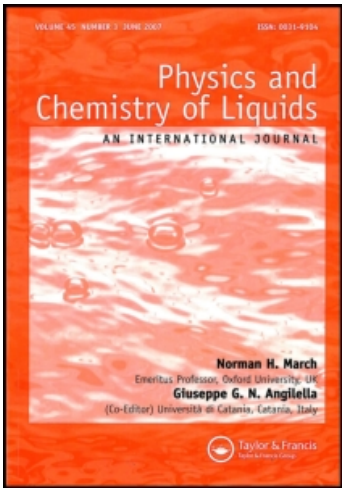


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J. Premakumari[a]; G. Allan Gnana Roy[a]; A. Antony Muthu Prabhu[b]; G. Venkatesh[b]; V. K. Subramanian[b]; N. Rajendiran[b]
[a] Department of Chemistry, Scott Christian College, Nagercoil 629002, Tamil Nadu, India [b] Department of Chemistry, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India

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# Spectral characteristics of sulphadiazine, sulphisomidine: effect of solvents, pH and β-cyclodextrin

J. Premakumari[a], G. Allan Gnana Roy[a], A. Antony Muthu Prabhu[b], G. Venkatesh[b], V.K. Subramanian[b] and N. Rajendiran[b]*

[a]*Department of Chemistry, Scott Christian College, Nagercoil 629002, Tamil Nadu, India;* [b]*Department of Chemistry, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India*

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The absorption and fluorescence spectra of sulphadiazine (SDA), sulphisomidine (SFM) and sulphanilamide (SAM) have been analysed in different solvents, pH and β-cyclodextin. The inclusion complexes of the above sulphonamides with β-CD were investigated by UV–Vis, fluorometry, DFT, FT–IR and $^1$H-NMR. The solvent study shows that the absorption and emission maxima of the SDA and SFM are more red-shifted than SAM molecule. In non-aqueous solvents, a single fluorescence band (340 nm) is observed, whereas in water and β-CD solutions, dual emission (at 340 and 430 nm) is noticed for SDA and SFM molecules. The dual emission is due to twisted intramolecular charge transfer band (TICT). Studies on β-CD solutions reveal that (1) sulphonamides form 1 : 1 inclusion complex with β-CD; and (2) the red-shift and the presence of TICT in the β-CD medium confirm that the heterocyclic ring is encapsulated in the hydrophobic part and aniline ring is present in the hydrophilic part of the β-CD cavity.

**Keywords:** sulphonamides; β-cyclodextrin; TICT; inclusion complex



## 1. Introduction

Sulphonamides constitute a class of drugs frequently used in pharmaceutical preparations, especially in veterinary practice. They are widely used as antibiotics and antimicrobial agents and are used for both therapeutic and prophylactic purposes in addition to their application as growth promoters. Sulphadiazine (SDA) is one of the sulphonamides used in the treatment of urinary tract infections, pneumocystic pneumonia, chronic bronchitis, meningococcal meningitis, acute otitis media and toxoplasmosis [1–3]. Further, these compounds have been found in surface and ground water, liquid manure and soil [4–8]. Interest in this field of drug studies is motivated not only by gaining some fundamental insight into the determination of drugs in pharmaceutical samples [9–11], but also by obtaining information about the structure and the chelating behaviour of the sulpha drugs [12–15].

*Corresponding author. Email: drrajendiran@rediffmail.com

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Photo-induced intramolecular charge transfer (ICT) is one of the most attractive topics of interest as a primary function for photoelectronic devices [16] as well as a basic mechanism for biological and chemical energy conversion [17]. The photo-induced charge separation is maximised when an electron donor group and an acceptor group are mutually perpendicular (in order to minimise electronic coupling between the two groups), which results in the formation of a twisted intramolecular charge transfer (TICT) state. Indeed, in some molecules such as 4-(*N*,*N*′dimethylamino) benzonitrile (DMABN) derivatives [18], aminodiphenyl sulphone (DPS) [19], aminodiphenyl amine (DPA) [20] and aminodiphenyl methane (DPM) [21] molecules, formation of TICT in the excited state followed by solvent relaxation plays an important role in determining the overall charge transfer process [18]. The TICT rate depends significantly on the ground state twist angle of the rotating groups [22]. However, some authors have suggested that the specific hydrogen bonding between the solvent and the solute is also important in order to maintain a large twist angle between the electron donor and the acceptor group [23]. Moreover, the charge separations in biological molecules are known to be coupled to proton motion, which may be a proton transfer process [24]. Thus, the hydrogen bonding effect of solvent on TICT (ICT) would be an interesting subject to explore with regard to the proton transfer coupled-charge transfer.



In order to substantiate the effect of TICT on sulphonamides in the inclusion complex, the absorption and emission spectra were measured in aqueous $\beta$-cyclodextrin ($\beta$-CD) solutions. Cyclodextrins (CD) are water-soluble cyclic oligosaccharides which form hydrophobic cavities with hydrophilic external walls [25]. The ability of CDs to accommodate guest molecules of the appropriate size in their cavities has been utilised by many investigators to control the photophysical and photochemical properties such as fluorescence enhancement [26] and intramolecular excimer/exciplex formation [27] of some organic compounds. Further, the restrictive environments of the CDs are known to affect the excited state geometry [28,29], which is closely related with the formation of the excited ICT state. Thus, there still remain several arguments on whether the ICT emission is always enhanced upon the formation of any CD complex and the excited state geometry is influenced by the CD complex formation. From this point of view, it would be interesting to see how the CD systems affect the TICT emission and the excited state geometry of a different type of the TICT molecule.

Recently, we have studied solvent, pH and $\beta$-CD dependences of different molecules [30–35] which show TICT emission in the excited state. This study has shown that the TICT takes place upon excitation of SDA, as demonstrated by large Stokes-shifted fluorescence emission in polar solvents. Especially in hydrogen bonding solvents, the TICT emission is further shifted into red. This has been attributed to the increased TICT emission as in the case of TICT molecules, such as DPS, DPA and DPM. In our continuous efforts to explore the H-bonding effects on the TICT process of sulphonamides in the excited state, we have studied the effects of $\beta$-CD on the ICT fluorescence properties in aqueous solution. The $\beta$-CD cavity provides an incorporated guest molecule with a non-polar and restrictive environment [36]. Thus, if sulphonamides embedded in the inner cavity of $\beta$-CD the excited state hydrogen bonding and geometry change will be greatly affected. From this point of view, in this article, the TICT emission of SDA ($N^1$-(2-pyrimidinyl) sulphanilamide), sulphisomidine (SFM, $N^1$-(2,6-dimethyl 4-pyrimidinyl)

sulphanilamide) and sulphanilamide (SAM, 4-aminobenzene sulphonamide) molecules in different solvents and the formation of inclusion complexes are reported.

## 2. Experimental

### 2.1. *Instruments*

Absorption spectral measurements were carried out using a Hitachi Model U-2010 UV-visible spectrophotometer and fluorescence measurements were made using a Shimadzu RF 5301 spectrofluorimeter. The pH values in the range 2.0–12.0 were measured on Elico pH meter model LI-120. Fourier Transform Infrared (FT–IR) spectra were obtained with Avatar-330 FT–IR spectroscopy using KBr pellets. The range of spectra was from 500 to 4000 $cm^{-1}$. The $^1$H-NMR spectra were recorded by Bruker 400 MHz spectrometer.

### 2.2. *Reagents and materials*

SDA, SFM, SAM and $\beta$-CD and all the solvents were of spectrograde and were obtained from Sigma–Aldrich chemical company. The purity of the compounds was checked by similar fluorescence spectra by the excitation at different wavelengths. Triply distilled water was used for the preparation of aqueous solutions. Solutions in the pH range 2.0–12.0 were prepared by adding the appropriate amount of NaOH and $H_3PO_4$. A modified Hammett's acidity scale ($H_0$) for the solutions below pH $\sim$ 2 (using a $H_2SO_4$–$H_2O$ mixture) and Yagil's basicity scale ($H_-$) for solutions above pH $\sim$ 12 (using a NaOH–$H_2O$ mixture) were employed. The solutions were prepared just before taking measurements. The concentration of the sulphonamide solutions were of the order of $2 \times 10^{-2}$ M. The concentration of $\beta$-CD solution was varied from $1.135 \times 10^{-3}$ to $1.135 \times 10^{-2}$ M. The stock solution was prepared in methanol and the methanol content of the solution was about 2%.



### 2.3. *Preparation of solid inclusion complexes*

Accurately weighed $\beta$-CD (1.2 g) was taken in a 50 ml conical flask and dissolved in 30 ml of distilled water. Then the sulphonamides (SDA, SFM and SAM) were taken in a 50 ml beaker and 20 ml of distilled water was added and stirred on an electromagnetic stirrer until it was dissolved. The $\beta$-CD solution was poured into the sulphonamide solution slowly. This mixed solution was continuously stirred for 48 h at 50°C. The reaction mixture was refrigerated for 48 h. White crystals were precipitated, which were filtered with $G_4$ sand filter funnel and washed with distilled water. After drying in an oven at 60°C for 12 h, a white powder product was obtained, this was the inclusion complex of sulphonamides with $\beta$-CD.

The techniques of UV–Vis, fluorimetry, $^1$H-NMR, FT–IR, scanning electron microscope, thermodynamic parameters and semi-empirical quantum calculations (DFT) methods are used to examine the effects of $\beta$-CD complexation with SDA, SFM and SAM. The stoichiometry and formation constants were determined from $\beta$-CD measurements. The formation constants of the complexes were estimated in order to predict and understand the factors affecting complexation between $\beta$-CD and sulphonamides in an aqueous solution. The solid state studies on the inclusion

complex of $\beta$-CD playing the role of guest molecules were performed to obtain direct evidence for the formation of the inclusion complexes.

## 3. Results and discussion

### 3.1. *Effects of solvents*

Table 1 depicts the absorption maxima, $\log \varepsilon$, fluorescence maxima and Stokes shifts of SDA, SFM and SAM in the solvents having different polarities and tendencies to form hydrogen bond (Figure 1). Because of the very low solubility of sulphonamides in cyclohexane, the absorption maxima were obtained using 1% dioxane solution of cyclohexane. The absorption maxima should be very near to one if obtained from pure cyclohexane since the polarity of dioxane is close to cyclohexane. Further, the trend observed in the absorption maxima of the sulphonamides in cyclohexane is similar to other solvents. Data in Table 1 indicate that the absorption spectra are red-shifted from cyclohexane to methanol. When compared to methanol, a blue-shift is noticed in water. The absorption maxima of SDA, SFM are close to sulphamethoxazole [37] (SMO, methanol, $\lambda_{absorption} \sim 270$ nm, $\lambda_{fluorescence} \sim 340$ nm, acetonitrile, $\lambda_{absorption} \sim 270$ nm and $\lambda_{fluorescence} \sim 337$ nm), sulphisoxazole (SFO, methanol, $\lambda_{absorption} \sim 269$ nm, $\lambda_{fluorescence} \sim 340$ nm, acetonitrile, $\lambda_{absorption} \sim 270$ nm and $\lambda_{fluorescence} \sim 338$ nm), and sulphathiazole (STO, methanol, $\lambda_{absorption} \sim 269$ nm, $\lambda_{fluorescence} \sim 340$ nm, acetonitrile, $\lambda_{absorption} \sim 270$ nm and $\lambda_{fluorescence} \sim 338$ nm). The absorption spectral characteristics of SDA and SFM are slightly red-shifted compared to those of SAM. This shows that the replacement of hydrogen atom from $–SO_2–NH_2$ group by pyrimidine ring has little effect on the electronic energy levels of SAM. The above results suggest that the position of the substituent (oxazole, thiazole or pyrimidine ring) in the $RSO_2–NH–$ group does not effectively change the absorption behaviour of these molecules. Compared to aniline [21] (cyclohexane, $\lambda_{absorbtion} \sim 283$, 235 nm, $\lambda_{fluorescence} \sim 320$ nm, methanol, $\lambda_{absorption} \sim 278$, 230 nm and $\lambda_{fluorescence} \sim 335$ nm) the absorption spectra are blue-shifted in all solvents, but the fluorescence spectra are red-shifted. Further, in polar solvents, only a small absorption and emission spectral shift was observed; this is because of the tautomeric structures present in sulphonamides [38] (Figure 1). For this reason, sulphonamides are insoluble in non-polar cyclohexane solvent.

The electronic spectra of aryl amines have been studied extensively [19–21, 39–41] and the red-shift produced in the absorption spectra of the parent hydrocarbon is due to the resonance interaction of the lone pair of the amino nitrogen with the $\pi$-cloud of the parent hydrocarbon. This interaction increases if an electron withdrawing group is attached at the *para* position. It is well known that in sulphonamides the electron withdrawing group is the tertiary nitrogen atom or sulphonyl group [38]. Thus, the nature of the electron withdrawing group in the *para* position decides the percentage charge transfer character of the $\pi$–$\pi^*$ transition as well as values of the band maximum. In sulphonamides, the $SO_2$ group (present in the *para* position) will act as the electron withdrawing group, and therefore the electron donor amino group is conjugated with the electron withdrawing group (Figure 1) and can also inhibit more interaction between (1) solvents with the sulphonamide molecules and (2) aniline ring to pyrimidine ring. Further, it is well known that the amino proton becomes a strong acid and the tertiary nitrogen and

Table 1. Absorption and fluorescence spectral data (nm) observed for SFM, SDA and SAM in different solvents.

| | SFM | | | | SDA | | | | SAM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Solvents | $\lambda_{absorption}$ | log $\varepsilon$ | $\lambda_{fluorescence}$ | Stokes shift | $\lambda_{absorption}$ | log $\varepsilon$ | $\lambda_{fluorescence}$ | Stokes shift | $\lambda_{absorption}$ | log $\varepsilon$ | $\lambda_{fluorescence}$ | Stokes shift |
| Cyclohexane | 262.8 | sat | 302 | 1384 | 262.6 | Sat | 301 | 1384 | 260.0 | 3.40 | 320 | 7211 |
| | | | 327 | | | | 326 | | | | | |
| 1,4-Dioxane | 267.4 | 4.42 | 317 | 5623 | 267.6 | 4.52 | 315 | 5623 | 260.0 | 3.45 | 336 | 8700 |
| THF | 268.4 | 4.40 | 317 | 5103 | 268.4 | 4.43 | 315 | 5103 | 261.2 | 3.38 | 336 | 8523 |
| Ethyl acetate | 268.4 | 4.51 | 305 | 4503 | 267.4 | 4.61 | 304 | 4503 | 261.4 | 3.38 | 336 | 8494 |
| | | | 330 | 7002 | | | 329 | 7002 | | | | |
| Dichloromethane | 268.6 | 4.38 | 330 | 6503 | 268.6 | 4.42 | 303 | 6503 | 261.6 | 3.53 | 336 | 8464 |
| | | | | | | | 328 | | | | | |
| Acetonitrile | 268.8 | 4.45 | 305 | 4419 | 268.0 | 4.48 | 304 | 4419 | 261.0 | 3.38 | 336 | 8552 |
| | | | 340 | 6918 | | | 330 | 6918 | | | | |
| *t*-Butyl alcohol | 268.8 | 4.33 | 305 | 4613 | 268.4 | 4.23 | 304 | 4613 | 261.6 | 3.38 | 340 | 8815 |
| | | | 340 | 7776 | | | 336 | 7776 | | | | |
| 2-Butanol | 271.4 | 4.39 | 305 | 4060 | 270.6 | 4.47 | 304 | 4060 | 261.4 | 3.35 | 340 | 8844 |
| | | | 340 | 6467 | | | 338 | 6467 | | | | |
| 2-Propanol | 271.4 | 4.39 | 315 | 6689 | 271.0 | 4.31 | 315 | 6689 | 261.2 | 3.53 | 340 | 8874 |
| | | | 343 | 5553 | | | 343 | 5553 | | | | |
| Glycol | 269.2 | 4.44 | 315 | 6845 | 270.0 | 4.47 | 316 | 6835 | 261.4 | 3.52 | 340 | 8844 |
| | | | 345 | 9298 | | | 345 | 9298 | | | | |
| Methanol | 269.0 | 4.59 | 318s | 6696 | 269.6 | 4.69 | 318s | 6696 | 261.0 | 2.52 | 340 | 8640 |
| | | | 345 | 5645 | | | 345 | 5645 | | | | |
| Water (pH = 6.5) | 263.0 | 4.39 | 305 | 5252 | 263.6 | 4.37 | 306 | 5257 | 258.0 | 2.21 | 342 | 9160 |
| | 214.2 | 4.07 | 342 | 8697 | | | 342 | 8697 | | | | |
| | | | 432 | 14788 | | | 432 | 14788 | | | | |
| Dipole moment | 7.19 | | 9.11 | | 5.32 | | 7.21 | | 6.95 | | 8.57 | |
| Onsagar cavity radius (Å) | 6.43 | | | | 6.26 | | | | 4.14 | | | |
| Correlation coefficient $E_T(30)$ *vs.* $\Delta\bar{\nu}_{ss}$ | | | | 0.8864 | | | | 0.8852 | | | 0.8726 | |
| BK *vs.* $\Delta\bar{\nu}_{ss}$ | | | | 0.6416 | | | | 0.6358 | | | 0.6276 | |

Note: s, shoulder.

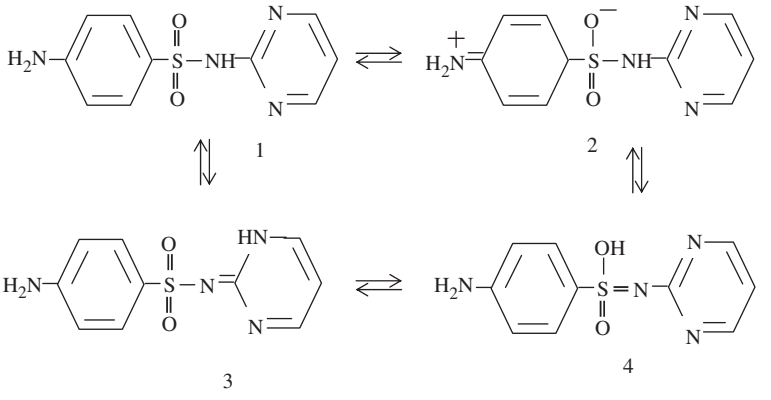


Figure 1. Various resonance structures of SDA.

$SO_2$ groups become a strong base in $S_1$ state. This is reflected in sulphonamides; hence, no larger red-shift is observed in protic solvents.

Figure 2 shows the fluorescence spectra of SDA, SFM and SAM in different solvents. In contrast to the weak solvent dependent absorption spectra, the emission properties of SDA and SFM molecules are strongly solvent dependent indicating the possibility of a change in the character of the electronic state. The emission maxima of both molecules are expected to be red-shifted relative to SAM. In all non-aqueous solvents, sulphonamide molecules give two emission maxima, whereas in water they give three emission maxima. Among the three maxima, one occurs in the shorter wavelength region (SW) at around ~305 nm, the second in the middle wavelength region (MW ~ 340 nm) and the third in the longer wavelength region (LW ~ 430 nm). In cyclohexane, the intensity of SW band is greater than MW and LW, whereas in non-aqueous polar solvents, the MW intensity is greater than SW and LW. Further, the fluorescence intensity is weaker in water than other non-aqueous polar solvents. As the solvent polarity is increased, the emission maximum of MW and SW band shifts to the red range, which is the greatest one for the MW band. It should also be pointed out that the fluorescence intensity ($I_F$) of the above band maxima does not undergo any marginal change with increase of the excitation (265, 275, 285 and 295 nm). Furthermore, in all solvents, the feature and position of the MW band is very similar to those of SMO, SFO, STO and SAM. The excitation spectrum corresponding to the SW, MW and LW resembles the absorption spectrum of SDA and SFM. As mentioned earlier, the fluorescence spectra are highly solvatochromic. The polarity dependent shift of the emission spectra of SDA and SFM in water containing various concentrations of dioxane is given in Figure 3. As the percentage of dioxane increases, the fluorescence intensity of LW band decreases and it is accompanied by a large increase in the MW fluorescence intensity.

The appearance of LW fluorescence in glycerol and $\beta$-CD solutions implies that the spectral behaviour of the sulphonamides is not due to solute–solvent specific interaction (complex formation). The excitation spectra do not differ from each other, indicating that they should originate from two different excited states or two different forms of the excited molecule. The possibility of excimer formation can also

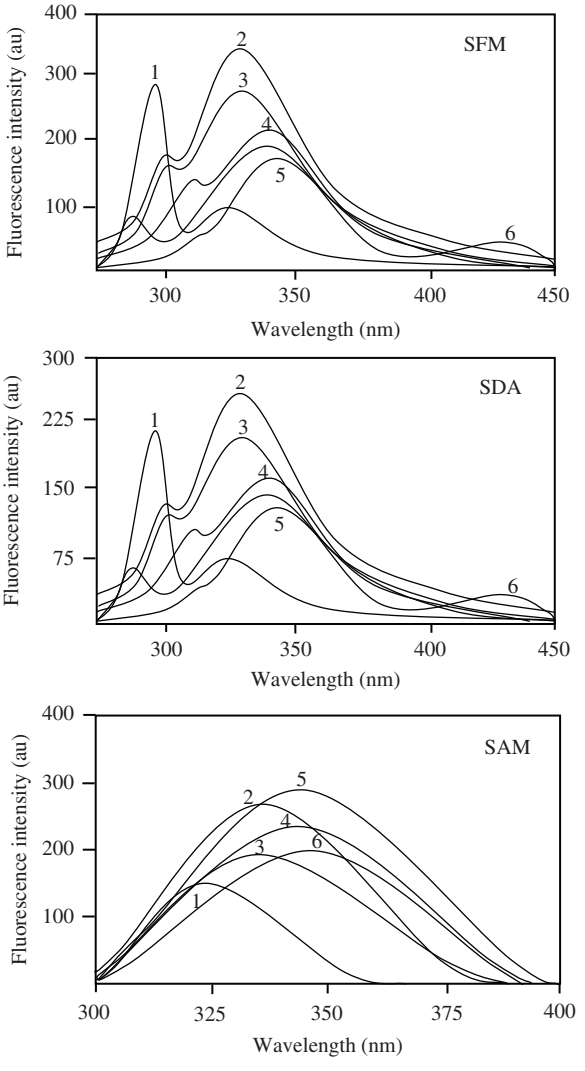



Figure 2. Fluorescence spectra of SFM, SDA and SAM in selected solvents at 303 K: (1) cyclohexane, (2) ethyl acetate, (3) acetonitrile, (4) 2-propanol, (5) methanol and (6) water.

be rejected on the basis of the following reasons: (1) the ratio of the intensities in the band maxima does not change with an increase in the concentration of the sulphonamides in the range from $2 \times 10^{-6}$ to $2 \times 10^{-4}$ M; (2) the LW band of the molecule is also observed in glycol at room temperature. The similarity between the fluorescence excitation spectra of the corresponding SW, MW and LW emission bands suggests the presence of TICT in SDA and SFM. In water, the LW emission intensity (432 nm) is higher than that of SW, which suggested that the TICT is present in these molecules.

In non-aqueous solvents, the emission from the LW of SDA and SFM seems to be very weak or absent as compared to the MW band and therefore, the LW band is assumed to be in TICT state. This is because the LW was red-shifted in water,

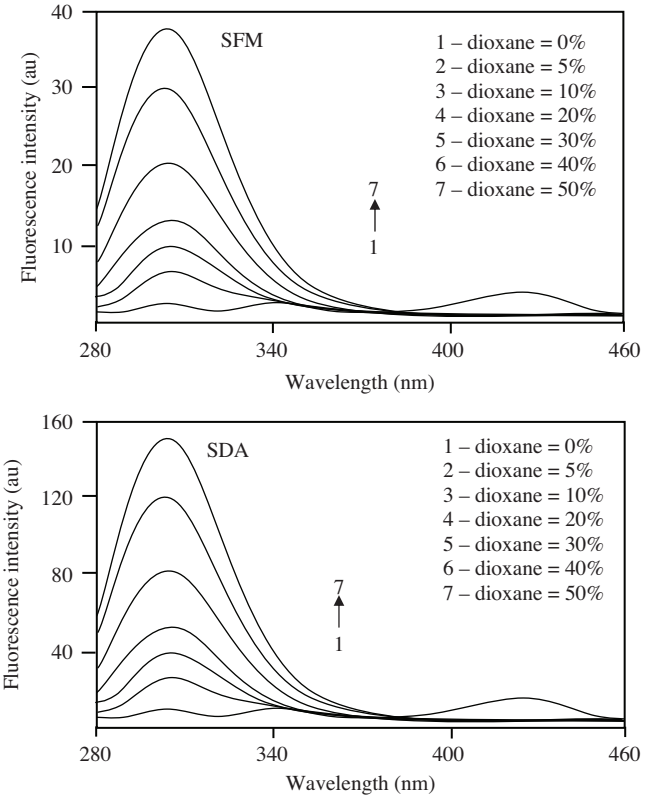



Figure 3. Fluorescence emission spectra of SFM and SDA in water–dioxane mixed solvents. Excitation wavelength, 275 nm.



suggesting that the emitting state is TICT type. This is indicated by the large increase in dipole moment upon excitation. The increase in dipole moment is caused by a change in the electronic configuration of the amino group from tetrahedral ($sp^3$) in $S_0$ state to the trigonal ($sp^2$) in the excited state. In order to check the TICT, the fluorescence spectrum of SDA at 303 K was recorded in solutions consisting of different compositions of glycerol–$H_2O$ mixtures (Figure 4). As expected, the fluorescence intensity of the MW band increased with an increase in glycerol content. This is according to the fact that as viscosity increases, the free rotation of the aniline group decreases.

In this study, the Stokes shifts (Table 1) of the sulphonamide molecules determined in different solvents of different polarities have been correlated with the $E_T(30)$ (energy transfer) [42] and BK (Bilot and Kawaski) [43] parameters (Figure 4). Aprotic solvents indicate that the unspecific interactions are the key factors in shifting the fluorescence maxima to the red range for these molecules. The slope of the plot suggests a large change in the dipole moment of the molecule upon electronic excitation and hence a strong CT character of the emitting state. The dipole moment of the molecule in the excited state can be obtained from the slope of the Lippert–Mataga plot. Assuming that '*a*' is calculated from the optimised distance between two end atoms of the molecules, this is calculated from the DFT level optimised

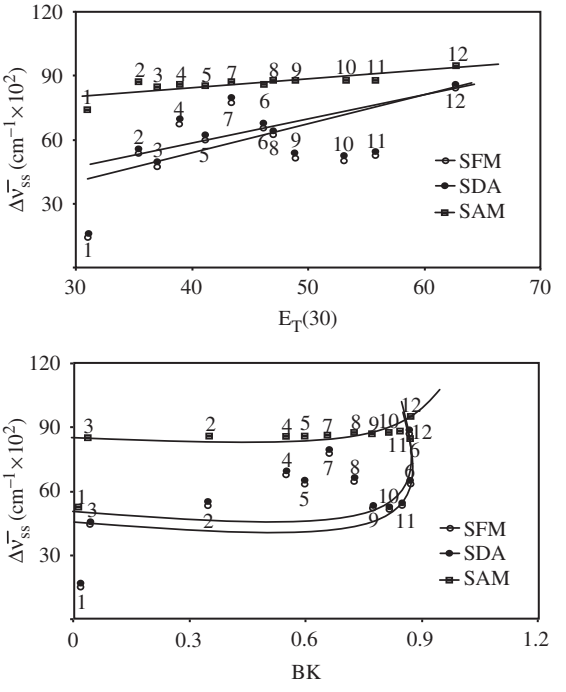



Figure 4. Plot of Stokes shifts ($cm^{-1}$) of SFM, SDA and SAM *vs.* $E_T(30)$ and BK solvent parameters: (1) cyclohexane, (2) diethyl ether, (3) 1,4-dioxane, (4) ethylacetate, (5) dichloromethane, (6) acetonitirle, (7) t-butyl alcohol, (8) 2-butanol, (9) 2-propanol, (10) ethanol, (11) methanol and (12) water.



structure of the molecules by using Cache (7.5) program. We get ($\Delta\mu = \mu_e - \mu_g$) the excited dipole moment value from the following equation:

$$\Delta\bar{\nu}_{ss} = \frac{2(\mu_e - \mu_g)^2}{hca^3} f(D, n) + c, \tag{1}$$

where $\Delta\bar{\nu}_{ss}$ is the Stokes shift ($\Delta\bar{\nu}_{ss} = \bar{\nu}_{abs}\,\max - \bar{\nu}_{flu}\,\max$), $\mu_g$ and $\mu_e$ are, respectively, the ground and excited state dipole moments of the solute molecule, ($\mu_e - \mu_g$) the difference between the excited and ground dipole moments, $a$ the Onsager cavity radius and $f(D, n)$ the Onsager polarity function defined by the equation:

$$f(D, n) = \frac{D-1}{2D+1} - \frac{n^2-1}{2n^2+1}, \tag{2}$$

where $n$ is the refractive index and $D$ the dielectric constant of the solvent, respectively. The ground state dipole moment ($\mu_g$) of the molecule is obtained from DFT calculations, as shown in Table 1.

The $E_T(30)$ parameter is the most effective and practical polarity scale, which takes both dispersive and specific (hydrogen bonding) interactions into account. The Stokes shifts of SDA and SFM molecules were plotted against the parameter $E_T(30)$ (Figure 4). As expected, a good correlation of the Stokes shift with the $E_T(30)$ scale was obtained. This indicates the fact that the solute–solvent interactions are

responsible for the solvatochromic shifts of these molecules. As evident from the slope of the plots, these interactions are increased in the case of SAM and may be attributed to the increase of dipole moment in the excited state. Rigid structures with limited internal degrees of freedom change the structure of the solvent cage by changing their dipole moment in the excited state. This process induces a large Stokes shift in SAM in polar solvents.

### 3.2. *Effect of hydrogen ion concentration*

The absorption and fluorescence spectra of the SDA, SFM and SAM molecules are studied in various acid and base concentrations in the $H_0$/pH/H_ ranging from −10 to 16.0. The spectral properties of the different prototropic species are presented in Table 2. None of the molecules show any change in absorption and emission characteristics in the region of pH ∼ 12–8, indicating that in this pH range only neutral species (N) are present. When the pH is lower than 8, a regular red-shift is noticed in the absorption and fluorescence spectra and the maxima of this species do not resemble SAM, indicating the formation of monocation (MC) in SDA and SFM systems. On increasing the hydrogen ion concentration from pH ∼ 2, again a large red-shifted maximum is observed in the absorption and fluorescence spectra revealing that dication (DC) is formed. On further increase in the acid concentrations from $H_o$-2 to H-10, no significant shift was noticed in the absorption in maxima. Compared to the neutral or MC species, the absorbance of the DC species is very weak. While increasing the pH up to 11, a slight red-shifted absorption maximum is noticed, suggesting the formation of monoanion. However, in the $S_1$ state, above pH ∼ 12, the fluorescence intensity decreases without the appearance of any new band. In general, the monoanion formed by deprotonation from amino group with some exceptions [44, 45] are non-fluorescent [46–49]. We attribute the decrease in fluorescence intensity to the formation of monoanion. In SAM, with an increase of acid and base concentrations, the fluorescence intensity of the 340 nm maximum decreases indicating that the MC and monoanion are formed and they are non-fluorescent.

In the absorption spectra, the presence of two isosbestic points (at 250 and 298 nm) clearly indicates that different species are involved in the equilibrium. The presence of two isosbestic points (at 250 and 298 nm) in the absorption spectra suggests the presence of equilibrium between the MC and the neutral species or between the different MCs and the neutral. The MC can be formed by protonation either at the ring nitrogen or in the $–NH_2$ group. It is well established that the protonation at $NH_2$ group will lead to the blue-shift while the protonation at = N- atoms will lead to the red-shift in the absorption and fluorescence spectra of the neutral species. Dogra and coworkers [46–49] have clearly established that the first protonation takes place in the pyrimidine nitrogen atom. A regular red-shift formed in the DC reveals that intramolecular proton transfer (IPT) is present between both = N- atoms and amino group. Interestingly, even though three protonation sites are present in sulphonamides, trication does not form even at high acid concentrations. This is because (1) both = N- atoms are strong electron withdrawing in nature; and (2) the presence of strong IPT and tautomeric structure in SDA and SFM molecules (Figure 1) prevent the formation of trication. Further, the DC absorption and emission maxima of SFM

Table 2. Various prototropic maxima (absorption and fluorescence) of SFM, SDA and SAM in aqueous and $\beta$-CD mediums.

| | SFM | | | | SDA | | | | SAM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous medium | | $\beta$-CD medium | | Aqueous medium | | $\beta$-CD medium | | Aqueous medium | | $\beta$-CD medium | |
| Species | $\lambda_{absorption}$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | $\lambda_{fluorescence}$ | $\lambda_{absorbtion}$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | $\lambda_{fluorescence}$ |
| Dication | 308 | 442 | 307 | 440 | 308 | 442 | 308 | 440 | | | | |
| | 272wsh | 380 | 266wsh | 380 | 272wsh | 382 | 271 | 378 | | | | |
| | 220 | 295 | 221 | 294 | 220 | 295 | 221 | 294 | | | | |
| Monocation | 265 | 440 | | 440 | 265 | 440 | | 440 | 272wsh | 326 | 270 | 326 |
| | | 359 | | 360 | | 360 | | 337 | 263wsh | Q | 269 | Q |
| | | 294 | | 295 | | 294 | | 295 | 218 | | 219 | |
| Neutral | 254 | 430 | 269 | 430 | 254 | 430 | 269 | 430 | 258 | 340 | 258 | 340 |
| | 240 | 340 | | 338 | 240 | 342 | | 338 | | | | |
| | | 288 | | 294 | | 288 | | 294 | | | | |
| Monoanion | 259 | Q | | | 259 | Q | | | | Q | | |

Notes: wsh, weak shoulder, Q, quenching.

and SDA molecules do not resemble SAM, indicating that IPT is present in both molecules (Table 2). This is probably due to the protonation taking place in the amino group with the absorption and emission maxima shifting to the blue range [35,36,38,39]. On the basis of the above results (Tables 2 and 3), we conclude that MC could be formed at = N- atoms and strong IPT interactions are present in DC species. A similar behaviour (i.e. red-shift in absorption spectrum) was observed during the first protonation of 2-(4′-aminophenyl) benzoxazoles [53].

The ground and excited state acidity constants ($pK_a$ and $pK_a^*$) values for the above equilibrium were determined, respectively, using absorbance data and fluorimetric titration methods (Table 3). The $pK_a$ value obtained for the DC–MC and MC–N prototropic equilibrium using the Henderson equation, is found to be 6.2 and 0.94, respectively (Table 3). The $pK_a$ value for the prototropic equilibrium so obtained (DC–MC, MC–N) is similar to SMO, STO and SFO systems [37].

### 3.3. *Effects of β-CD with sulphonamides*

Table 4 depicts the absorption and emission spectra of SFM, SDA and SAM recorded in different $\beta$-CD concentrations (pH $\sim$ 7) and are shown in Figures 5 and 6. Upon the addition of $\beta$-CD, the absorption maxima of the SDA and SFM molecules are red-shifted with gradual increase in the molar coefficient, whereas in SAM the absorbance increased at the same wavelength. The increase in the absorbance is due to the encapsulation of both molecules into the $\beta$-CD cavity and it is attributed to the detergent action of $\beta$-CD [25–32]. Further, when the absorbtion spectra were recorded after 24 h, no significant change was observed in the absorbance, indicating that these sulphonamides do not decompose in the $\beta$-CD solution. The above behaviour may be attributed to the enhanced dissolution of the sulphonamide molecules through the hydrophobic interaction between the guest and non-polar cavity of the $\beta$-CD. These results suggest that sulphonamides are entrapped into the $\beta$-CD cavity to form the inclusion complex. Moreover, the red-shift observed in pH $\sim$ 7-$\beta$-CD shows that the pyrimidine nitrogen atom interacts with $\beta$-CD hydroxyl groups, because it is well known that CDs are good H donors [50–54].

No clear isosbestic point was observed in the absorption spectra, but the changes observed in the absorbance were very small. In general, the existence of an isosbestic point in the absorption spectra is an indication of the formation of well-defined 1 : 1 complex [25–36,38,39]. The possibilities proposed for this deviation are: (1) more than one guest molecule could have been accommodated in the $\beta$-CD cavity; (2) due to the space restriction of $\beta$-CD cavity more than one type of complex, each having 1 : 1 stoichiometry, could have been formed; and (3) sulphonamide solutions containing methanol (1%) which are added to $\beta$-CD could have made the interaction between these components. Since in these experiments the concentration of methanol is practically constant with respect to the $\beta$-CD concentration, it might have affected the isosbestic point [30–35].

Figure 6 shows the fluorescence spectra of the above sulphonamides in aqueous solution as a function of $\beta$-CD concentration. Since no clear isosbestic point was observed in the absorption spectrum, the excitation wavelength is selected in such a manner that the absorbance changes are very small. In pure aqueous medium, the

Table 3. Ground ($S_0$) and excited states ($S_1$) acidity constant ($pK_a$ and $pK_a^*$) values of different prototropic equilibriums of SFM, SDA and SAM.

| | SFM | | | | SDA | | | | SAM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous medium | | β-CD medium | | Aqueous medium | | β-CD medium | | Aqueous medium | | β-CD medium | |
| Equilibrium | $pK_a$ absorption | $pK_a^*$ FT | $pK_a$ | $pK_a^*$ FT | $pK_a$ absorption | $pK_a^*$ FT | $pK_a$ absorption | $pK_a^*$ FT | $pK_a$ absorption | $pK_a^*$ FT | $pK_a$ absorption | $pK_a^*$ FT |
| Dication monocation | 0.94 | 1.0 | 0.84 | 1.1 | 0.94 | 1.0 | 0.84 | 1.1 | | | | |
| Monocation neutral | 6.2 | 5.8 | 6.0 | 5.6 | 6.3 | 5.8 | 6.1 | 5.6 | 0.83 | 0.82 | 0.80 | 0.79 |
| Neutral monoanion | 14.2 | 12.5 | | | 13.6 | 14.1 | | | 13.6 | 14.1 | | |

Table 4. Absorption and fluorescence maxima (nm) of SFM, SDA and SAM at different $\beta$-CD concentrations.

| | SFM | | | SDA | | | SAM | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration of $\beta$-CD M | $\lambda_{absorption}$ | $\log \varepsilon$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | $\log \varepsilon$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | $\log \varepsilon$ | $\lambda_{fluorescence}$ |
| Water (without $\beta$-CD) | 263.0 | 4.42 | 300 | 264.0 | 4.40 | 300 | 258 | 3.43 | 340 |
| | 214.6 | 4.04 | 340 | 214.6 | 4.01 | 339 | | | |
| | | | 425 | | | 424 | | | |
| 0.001 | 265.0 | 4.39 | 300 | 265.0 | 4.36 | 300 | 258 | 3.51 | 340 |
| | 210.0 | 4.45 | 340 | 210.0 | 4.48 | 339 | | | |
| | | | 427 | | | 427 | | | |
| 0.002 | 267.6 | 4.40 | 301 | 267.6 | 4.37 | 301 | 258 | 3.52 | 340 |
| | 210.0 | 4.50 | 340 | 210.0 | 4.51 | 340 | | | |
| | | | 440 | | | 438 | | | |
| 0.003 | 268.2 | 4.44 | 302 | 268.2 | 4.38 | 301 | 258 | 3.58 | 340 |
| | 211.8 | 4.49 | 340 | 211.8 | 4.45 | 338 | | | |
| | | | 440 | | | 438 | | | |
| 0.004 | 268.6 | 4.47 | 302 | 268.6 | 4.40 | 300 | 258 | 3.60 | 340 |
| | 213.2 | 4.55 | 340 | 213.2 | 4.51 | 336 | | | |
| | | | 440 | | | 438 | | | |

(*Continued*)

Table 4. Continued.

| Concentration of $\beta$-CD M | SFM | | | SDA | | | SAM | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\lambda_{absorption}$ | log $\varepsilon$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | log $\varepsilon$ | $\lambda_{fluorescence}$ | $\lambda_{absorption}$ | log $\varepsilon$ | $\lambda_{fluorescence}$ |
| 0.005 | 269.0 | 4.49 | 302 | 269.0 | 4.41 | 301 | 258 | 3.62 | 340 |
| | 209.0 | 4.57 | 340 | 209.0 | 4.46 | 336 | | | |
| | | | 440 | | | 437 | | | |
| 0.006 | 269.2 | 4.52 | 301 | 269.2 | 4.42 | 301 | 258 | 3.64 | 340 |
| | 211.8 | 4.59 | 340 | 211.8 | 4.51 | 338 | | | |
| | | | 436 | | | 436 | | | |
| 0.007 | 269.8 | 4.54 | 301 | 269.8 | 4.44 | 301 | 258 | 3.62 | 340 |
| | 213.2 | 4.60 | 340 | 213.2 | 4.44 | 338 | | | |
| | | | 440 | | | 440 | | | |
| 0.008 | 270.0 | 4.55 | 302 | 270.0 | 4.46 | 302 | 258 | 3.68 | 340 |
| | 212.2 | 4.61 | 340 | 212.2 | 4.49 | 338 | | | |
| | | | 440 | | | 440 | | | |
| 0.009 | 270.0 | 4.56 | 302 | 270.0 | 4.47 | 302 | 258 | 3.68 | 340 |
| | 209.8 | 4.63 | 340 | 209.8 | 4.54 | 338 | | | |
| | | | 440 | | | 440 | | | |
| 0.010 | 270.0 | 4.56 | 302 | 270.0 | 4.47 | 302 | 258 | 3.74 | 340 |
| | 213.2 | 4.64 | 340 | 213.2 | 4.52 | 338 | | | |
| | | | 440 | | | 440 | | | |
| Excitation wavelength | 270 | | | 270 | | | 260 | | |
| Binding constant ($M^{-1}$) | 165 | | 580 | 158 | | 560 | 207 | | 417 |
| $\Delta G$ (kJ M) | −12.84 | | −16.02 | −12.74 | | −15.92 | −13.42 | | −15.19 |

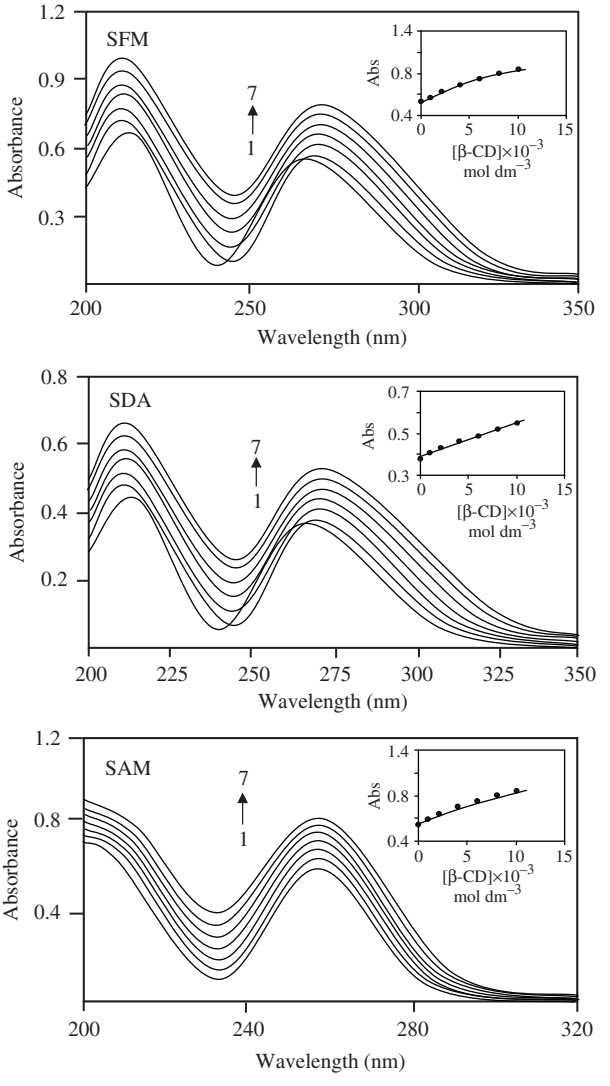



Figure 5. Absorption spectra of SFM, SDA and SAM for different $\beta$-CD concentrations (M): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008 and (7) 0.01. Insert: figure absorbance *vs.* $\beta$-CD concentration.

locally excited (LE) intensity is greater than TICT intensity. However, on addition of $\beta$-CD, both the LE and TICT intensities were equally increased. With an increase in the $\beta$-CD concentration, a regular red-shift was observed in the TICT band (425–440 nm), whereas no significant change was observed in the LE band (340 nm). As observed in absorption in $\beta$-CD medium, the sulphonamide MC and DC emission spectral maxima were similar to aqueous solutions (Table 2), suggesting that the structural geometry of SDA: $\beta$-CD and SFM: $\beta$-CD inclusion complexes are the same in terms of orientation of the guest molecules. In contrast, for SAM system without any dual emission, the emission intensity at 340 nm was gradually decreased at the same wavelength.

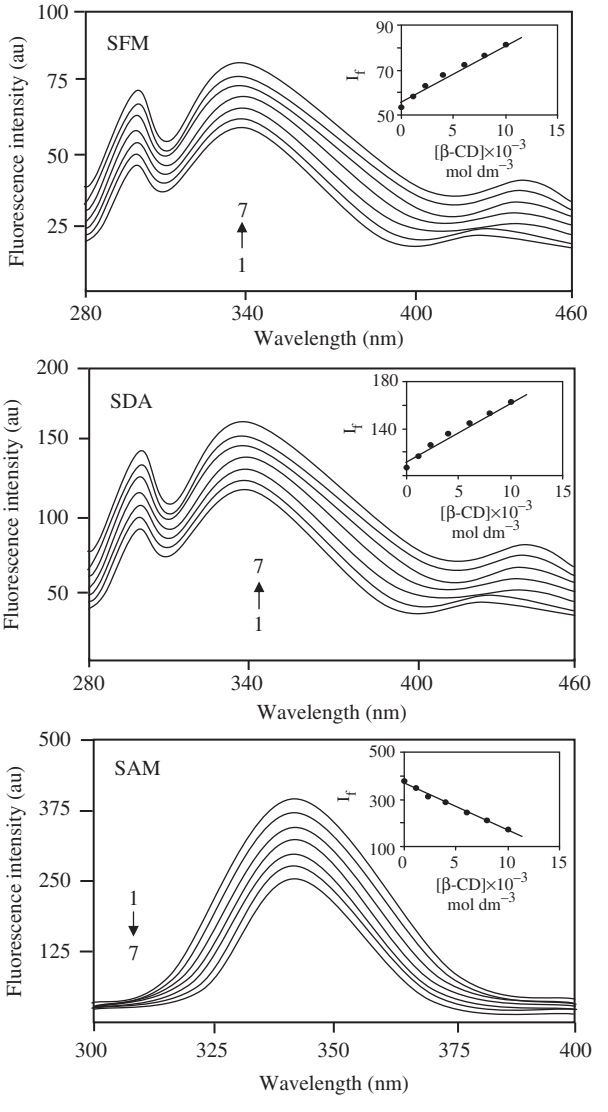



Figure 6. Fluorescence spectra of SFM, SDA and SAM for different $\beta$-CD concentrations (M): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008 and (7) 0.01. Insert: figure fluorescence intensity *vs.* $\beta$-CD concentration.

In $\beta$-CD solutions, the enhancement of both LE and TICT bands of sulphonamides may be explained as follows. The enhancement of the LE band in $\beta$-CD may be due to lowering of solvent polarity at higher $\beta$-CD concentration [51–55]. Inside the $\beta$-CD cavity, sulphonamides are in a much less polar environment and the main non-radiative path of the LE band through ICT or TICT is restricted, which also causes an enhancement of the LE and TICT band. Furthermore, the geometrical restriction of the $\beta$-CD cavity would restrict the free rotation of the pyrimidine ring in the $\beta$-CD cavity and thus it favours the formation of TICT state. From our earlier studies, we found that in SMO, SFO and STO with aqueous $\beta$-CD

solution [37], hydrophobicity is the driving force for encapsulation of the molecule inside the cavity and naturally the hydrophobic part goes inside the deep core of the non-polar cavity and the polar group will be projected in the hydrophilic part of the β-CD cavity [51–56]. From the above findings, it is clear that in β-CD solutions, the surrounding polarity of –$SO_2$NH– group does not change very much, as there is no hypsochromic shift of the most polar (TICT) state [56]. This may be possible only if the orientation of sulphonamide is such that the aromatic ring goes inside the β-CD cavity and the amino group is present in the lower part of the β-CD cavity. Moreover, if the amino group is present in the interior part of the β-CD cavity, a hypsochromic shift or TICT emission should not be present in the β-CD medium.

The above results indicate that SDA and SFM molecules are partially entrapped in non-polar β-CD cavity, whereas SAM molecule is completely included in the β-CD cavity. Since the size of SAM is smaller than β-CD cavity size, this molecule is completely included in the cavity; therefore, the emission intensity is decreased. However, the sizes of SDA and SFM molecules are larger than the β-CD cavity and they are partially included in it. Noteworthyly, the LW emission intensity gradually increased along with red-shift by increasing the β-CD concentrations. It can be suggested that the LW emission band is related to the formation of β-CD inclusion complex. Such a spectral shift may correspond to an energy stabilisation of the emitting state and is characteristic for the fluorescence of TICT state. The LW emission from the above sulphonamides are originated from the TICT state with twisting occurring at the amide S–N bond between the aniline ring (electron donor) and the $SO_2$ group or pyrimidine ring (electron acceptor). Modiano *et al.* [57] reported that whenever two aromatic rings are separated by the groups like $SO_2$, $CH_2$, CO, NH, etc., they form a TICT state. Thus, it can be speculated that the enhancement of the band at 440 nm in the above sulphonamide emission should have originated from the TICT state. The TICT emission observed in β-CD suggests that the inclusion process plays the major role in the TICT emission. The excitation spectra at 440 nm emission are similar to the 340 nm emission spectra, which suggest that TICT is present in these molecules.



The binding constant for the formation of inclusion complex was determined by analysing the changes in the absorbance and fluorescence intensities with the β-CD concentrations. The binding constant ($K$) values determined by using Benesi–Hildebrand relation [58] indicate that 1 : 1 complexes are formed between β-CD and sulphonamides. The 1 : 1 complex formation between sulphonamides and β-CD has been analysed using the following equation:

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon} + \frac{1}{K[\mathrm{SAM}]_0 \Delta\varepsilon[\beta - \mathrm{CD}]_0} \tag{3}$$

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{K(I - I_0)[\beta - \mathrm{CD}]^2}, \tag{4}$$

where $\Delta A$ is the difference between the absorbance of sulphonamides in the presence and absence of β-CD, $\Delta\varepsilon$ the difference between the molar absorption coefficients of sulphonamides and the inclusion complex, and $[\mathrm{SAM}]_0$ and $[\beta\text{-CD}]_0$ the initial concentrations of sulphonamides and β-CD, respectively. Figure 7 and 8 shows the plots of $1/\Delta A$ and $1/[I–I_0]$ as a function of $1/[\beta\text{-CD}]$ and $1/[\beta\text{-CD}]^2$ for these

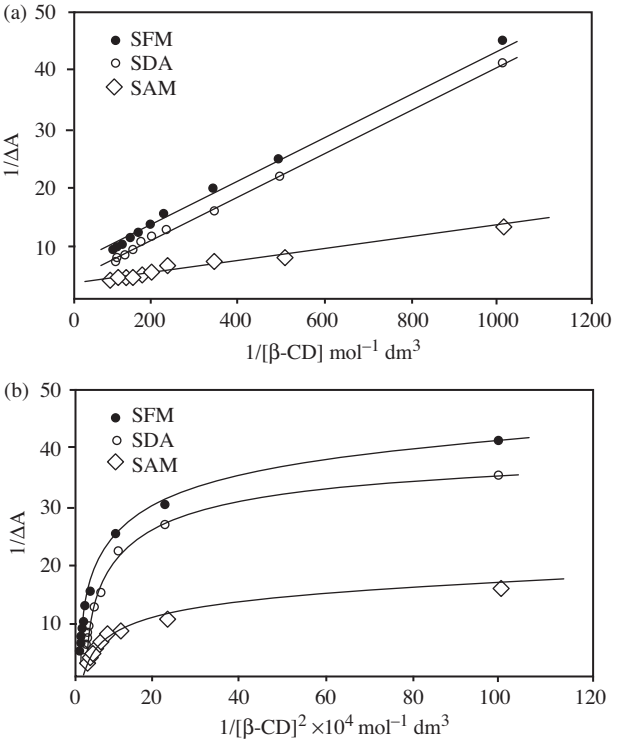



Figure 7. Absorption spectra of Benesi–Hildebrand plot for the complexation of SFM, SDA and SAM with $\beta$-CD. Plot of 1/ΔA *vs*. 1/[$\beta$-CD].



molecules. The plots of $1/\Delta A$ *versus* $1/[\beta\text{-CD}]^2$ and $1/[I–I_0]$ *versus* $1/[\beta\text{-CD}]^2$ give upward or downward curves and $1/\Delta A$ *versus* $1/[\beta\text{-CD}]$ and $1/I–I_0$ *versus* $1/[\beta\text{-CD}]$ gives linear plots. This analysis reflects the formation of 1 : 1 inclusion complex formed in all sulphonamides. Good linear correlations were obtained, confirming the formation of 1 : 1 inclusion complexes. From the intercept and slope values of these plots, '*K*' values were calculated. The binding constant values are so small compared to other guest molecule/$\beta$-CD complexes such as dialkyl aminobenzonitrile and its derivatives [51–55]. This is probably because (1) sulphonamide molecules are partially included into the cavity; (2) pyrimidine group might have been included in the cavity; and (3) these molecules are not tightly encapsulated in the $\beta$-CD cavity.

The free energy change of the inclusion complexes was calculated from the following equation

$$\Delta G = -\mathrm{RT}\ \ln K. \tag{5}$$

As it can be seen from Table 4, $\Delta G$ is negative, which suggests that the inclusion process proceeded simultaneously at 303 K. The negative values in the experimental temperature indicate that the inclusion process is an exothermic and enthalpy-controlled process. The hydrophobic interaction between the internal wall of $\beta$-CD and guest molecules is an important factor for the stability of inclusion complexes. In sulphonamides, it may safely be considered that the change in the magnitude of the

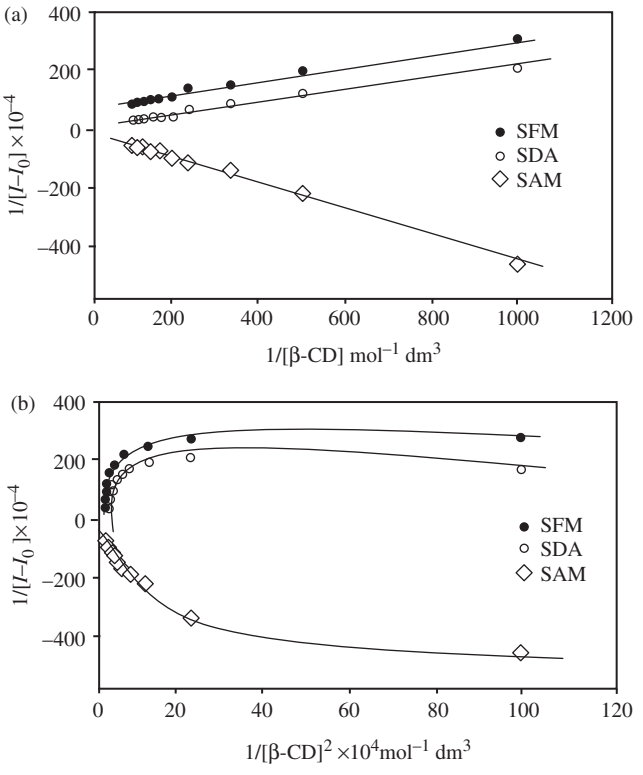



Figure 8. Fluorescence spectra of Benesi–Hildebrand plot for the complexation of SFM, SDA and SAM with $\beta$-CD. Plot of $1/[I–I_0]$ *vs.* $1/[\beta\text{-CD}]$.



hydrophobic interaction is related to that of the contact area of the guest molecule for the internal wall of $\beta$-CD. The negative enthalpy change arose from the van der Waals and the steric barrier caused by molecular geometrical shape and the limit of $\beta$-CD cavity to the freedom of shift and rotation of guest molecule.

### 3.4. *Prototropic reactions in β-CD medium*

To know the effect of $\beta$-CD on the prototropic equilibrium between neutral, MC and DC, the pH-dependent changes in the absorption and emission spectra of the sulphonamides in aqueous solution containing $\beta$-CD were recorded, and they are shown in Table 2. The absorption and emission maxima of these sulphonamides were studied in $6 \times 10^{-3}$ M $\beta$-CD solutions in the pH range 0.1–11. In $\beta$-CD solutions, on comparing with aqueous medium, the absorption and emission maxima of these sulphonamide neutral maxima are red-shifted (Table 2). The ground and excited state $pK_a$, $pK_a^*$ values of these molecules in $\beta$-CD medium are slightly lower than that of aqueous medium (Table 3). In $\beta$-CD solutions, a red-shift was observed in the neutral maxima than aqueous solutions, suggesting that the heterocyclic ring part of the sulphonamide molecules is encapsulated in the $\beta$-CD cavity.

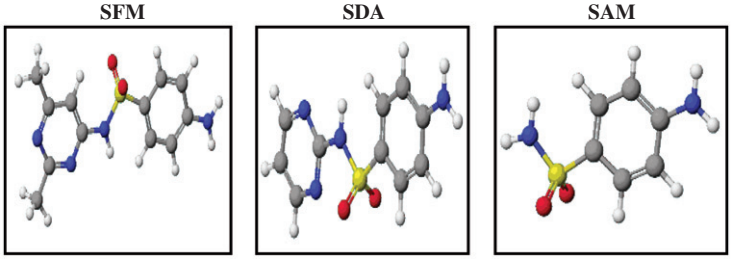
### 3.5. *Possible inclusion complex*

From the above discussions, the possible inclusion mechanism is proposed as follows: naturally, two different types of inclusion complex formation between SDA and SFM molecules with $\beta$-CD are possible: (1) aniline ring is captured in the $\beta$-CD cavity; and (2) pyrimidine ring is captured in the $\beta$-CD cavity. First, let us consider the Type I arrangement. If the aniline part is encapsulated with the $\beta$-CD cavity, like SAM, in higher $\beta$-CD concentrations the fluorescence intensities should be decreased at the same wavelength. If this type of inclusion complex is formed, SDA and SFM molecules should not show any dual emission in the $\beta$-CD medium. Furthermore, if the amino group is entrapped within the $\beta$-CD cavity the DC maxima [30–35] (i.e. protonation of amino group) should be blue-shifted in $\beta$-CD than in the aqueous medium. But the results in Table 2 show that in SDA and SFM, no significant difference is observed in the spectral shifts of both $\beta$-CD and aqueous media; i.e. DC maxima follow the same trend both in aqueous and $\beta$-CD media.

For Type II encapsulation, if the pyrimidine ring includes the $\beta$-CD cavity, the MC maxima of the sulphonamide molecule (protonation of tertiary nitrogen atom) should red-shift in $\beta$-CD than in aqueous medium. The results in Tables 2 and 4 show a red-shift in $\beta$-CD (pH $\sim$ 7) solution, indicating that the heterocyclic ring interacts with $\beta$-CD hydroxyl groups, confirming the fact that CDs are good hydrogen donors [37,57,58]. The tendencies of these shifts in $\lambda_{absorption}$ and $\lambda_{fluorescence}$ for these molecules can be attributed to their inclusion into the $\beta$-CD cavity and are explained in Figure 9. Further, the results observed in sulphonamides are similar to SMO/SFO/STO molecules [37]. Furthermore, it is well known that, due to the formation of the inclusion complex, the $pK_a$ ($pK_a$*) values are known to change depending upon relative affinity of the guest and host [51–53].

In Type II complex, the $\beta$-CD cavity will impose a restriction on the free rotation of the $CH_3$ group or pyrimidine group in its excited state. In this type of inclusion, TICT emission should increase in the $\beta$-CD medium. The question may arise why TICT emission is not observed in SAM? This is because the size of SAM is smaller than $\beta$-CD cavity; therefore, the complex formation does not affect the free rotation of $–SO_2–NH_2$ group. Another question may arise, namely why SDA and SFM do not exhibit the TICT emission in non-aqueous polar and non-polar solvents. It may be because of a weak dipole–dipole interaction between the $R–SO_2–NH–$ group with solvent and fast back charge transfer. These features support the idea that the TICT state in $\beta$-CD is stabilised through complex formation between SDA and SFM with $\beta$-CD. This confirms that the environments around the anilino group in $\beta$-CD medium are the same as in the bulk aqueous medium. These features indicate that Type II complex is favoured more than Type I.

This is further supported by semi-empirical quantum mechanical calculations: the ground state geometry of these sulphonamides are optimised by using the DFT method (Cache 7.5 program). The internal diameter of $\beta$-CD is approximately 6.5 Å and its height is 7.8 Å. The vertical distance between the sulphonamides is higher than the $\beta$-CD cavity size and the horizontal distance is smaller than the $\beta$-CD cavity size (Figure 9). Considering the shape and dimensions of $\beta$-CD and SDA/SFM, the only way sulphonamides can enter the $\beta$-CD cavity is lengthwise. In this situation, it is impossible for the sulphonamides to be encapsulated completely in one




**SFM**: ΔH = 6.55907 kJ

| | | | |
|---|---|---|---|
| $H_1 - H_3 = 7.03$ | $H_3 - H_4 = 4.38$ | $H_6 - S = 6.99$ | $H_2 - H_3 = 6.45$ |
| $H_3 - H_5 = 5.38$ | $H_5 - S = 7.00$ | $H_1 - H_4 = 11.19$ | $H_3 - H_6 = 5.37$ |
| $H_7 - S = 6.51$ | $H_1 - H_5 = 12.33$ | $H_3 - H_7 = 6.56$ | $H_8 - S = 5.49$ |
| $H_1 - H_6 = 12.34$ | $H_3 - H_8 = 6.03$ | $H_4 - H_7 = 5.87$ | $H_1 - H_7 = 12.87$ |
| $H_3 - H_9 = 6.03$ | $H_4 - H_8 = 6.43$ | $H_1 - H_8 = 11.81$ | $H_1 - H_9 = 11.78$ |
| $H_5 - H_8 = 5.88$ | $H_6 - H_9 = 5.89$ | $H_2 - H_6 = 11.62$ | $H_2 - H_7 = 12.76$ |
| $H_2 - H_8 = 11.85$ | | | |

**SDA** : ΔH = 66.94864 kJ

| | | | |
|---|---|---|---|
| $H_1 - H_6 = 12.200$ | $N_1 - N_2 = 6.616$ | $H_2 - H_6 = 12.510$ | $H_1 - O_2 = 7.056$ |
| $H_1 - H_7 = 10.936$ | $H_2 - O_2 = 6.833$ | $H_2 - H_5 = 11.211$ | $H_1 - O_1 = 7.055$ |
| $H_1 - H_8 = 6.538$ | $H_2 - O_1 = 6.833$ | $H_1 - N_2 = 6.999$ | $H_2 - N_2 = 7.308$ |

**SAM** : ΔH = –192.20380 kJ

| | | | |
|---|---|---|---|
| $H_1 - H_2 = 1.706$ | $H_1 - O_1 = 7.083$ | $H_5 - H_6 = 1.637$ | $H_1 - O_2 = 7.083$ |
| $H_1 - H_5 = 7.990$ | $H_2 - O_1 = 6.867$ | $H_2 - H_5 = 8.290$ | $H_2 - O_2 = 6.867$ |
| $H_1 - H_6 = 6.604$ | $N_1 - N_2 = 6.618$ | $H_2 - H_6 = 7.130$ | $N_1 - S = 5.895$ |

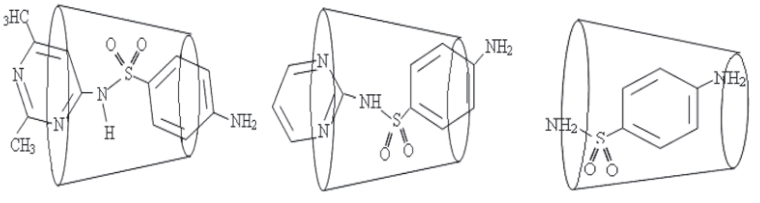



Figure 9. Cache structures, bond lengths and the proposed inclusion complex of SFM, SDA and SAM.

β-CD molecule. Taking into account the dimensions of the sulphonamides and β-CD, the complex can be located as shown in Figure 9.

## 4. Solid inclusion complex studies

### 4.1. *FT–IR spectra of sulphonamides*

#### 4.1.1. *Sulphadiazine/sulphisomidine*

The FT–IR spectra of SDA and the solid inclusion complex are also studied. The amino NH stretching frequency (sharp peak) at 3356 $cm^{-1}$ and the amido stretch at 3424 $cm^{-1}$ (sharp peak) became broad in the complex. The CH stretching at 3259 $cm^{-1}$ is shifted in the inclusion complex to 3107 $cm^{-1}$ and the CH bending vibration at 843–683 $cm^{-1}$ is not shifted in the complex. The C=C stretching

vibrations at 3076, 3040 $cm^{-1}$ and C=C bending vibrations at 1647 and 1594 $cm^{-1}$ are moved in the inclusion complex to 1653 and 1594 $cm^{-1}$. The $SO_2$ aromatic ring stretching at 1654 $cm^{-1}$ is moved in the inclusion complex to 1653 $cm^{-1}$. The $SO_2$ stretching at 1325 $cm^{-1}$ is shifted in the inclusion complex to 1326 $cm^{-1}$. The C–$NH_2$ stretching at 1262 $cm^{-1}$ appears at the same wavelength. The pyrimidine absorption peak at 796–639 $cm^{-1}$ appears at the same peak. The above results indicate that SDA and SFM molecules are encapsulated in the $\beta$-CD cavity.

#### 4.1.2. *Sulphanilamide*

The sharp amino stretching at 3477 $cm^{-1}$ and the amide NH stretching at 3373 $cm^{-1}$ are moved in the inclusion complex to 3473 $cm^{-1}$. $NH_2$ deformation at 1629 $cm^{-1}$ is moved in the inclusion complex to 1639 $cm^{-1}$. The C–N stretching at 1312 $cm^{-1}$ is moved in the inclusion complex to 1335 $cm^{-1}$. The CH stretch at 3065 $cm^{-1}$ is lost in the complex and the CH bending at 899–622 $cm^{-1}$ is moved in the inclusion complex to 858–706 $cm^{-1}$. The C=C stretching at 3098 $cm^{-1}$ disappears in the inclusion complex. The aromatic ring stretching at 1593 $cm^{-1}$ disappears in the inclusion complex. The S=O stretch at 1094 $cm^{-1}$ is moved in the inclusion complex to 1080 $cm^{-1}$ and the $SO_2NH$ symmetry stretch at 1148 $cm^{-1}$ and anti-symmetry stretch at 1312 $cm^{-1}$ are moved in the inclusion complex to 1156 and 1335 $cm^{-1}$, respectively. The overtone frequencies around 2600–2000 $cm^{-1}$ are lost in the inclusion complex. The above finding indicates that the SAM molecule is included in the $\beta$-CD cavity and forms an inclusion complex.



### 4.2. *$^1$H-NMR spectral studies*

Proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy has proved to be a powerful tool in the study of inclusion complexes [59, 60]. $^1$H-NMR spectroscopy provides an effective means of assessing the dynamic interaction site of $\beta$-CD with that of the guest molecules [59, 60]. The resonance assignments of the protons of $\beta$-CD are well established [59, 60] and consist of six types of protons. The chemical shift of $\beta$-CD protons reported are very close to those reported in this study. The H-3 and H-5 protons are located in the interior of the $\beta$-CD cavity and it is therefore likely that the interaction of the host with the $\beta$-CD inside the cavity will affect the chemical shifts of the H-3 and H-5 protons. A minor shift is observed for the resonance of H-1, H-2 and H-4 located on the exterior of $\beta$-CD [59, 60].

The addition of sulphonamides into the $\beta$-CD results in a downfield chemical shift for the sulphonamide protons in DMSO. The chemical shift values are given below: *SAM (inclusion complex)* values in ppm: $NH_2 \sim 5.76$ (5.78), $SO_2NH_2 \sim 6.86$ (6.92), $^2H/^6H \sim 7.45$ (7.50), $^3H/^5H \sim 6.59$ (6.64); *SFM (inclusion complex)* values in ppm: $NH_2 \sim 5.98$ (6.01), $SO_2NH \sim 10.95$ (11.02), $^2H/^6H \sim 7.577$ (7.581), $^3H/^5H \sim 6.599$ (6.602), $^3CH_3 \sim 2.363$ (2.367), $^5CH_3 \sim 2.263$ (2.268) and $^6H' \sim 6.751$ (6.756); *SDA (inclusion complex)* values in ppm: $NH_2 \sim 5.94$ (5.97), $SO_2NH \sim 10.97$ (11.00), $^2H/^6H \sim 7.575$ (7.578), $^3H/^5H \sim 6.601$ (6.605), $^{3'}H \sim 6.795$ (6.799), $^{4'}H \sim 6.642$ (6.646) and $^{5'}H \sim 6.714$ (6.719). As can be seen from the above values, the chemical shift data for the inclusion complex are different from those of the free compound. A small downfield shift on sulphonamides are observed, suggesting that the above molecules are encapsulated in the $\beta$-CD cavity.

## 5. Conclusions

From the above studies, we conclude the following: the absorption and emission maxima of the SDA and SFM are more red-shifted than the SAM molecule; in non-aqueous solvents, a single fluorescence band (340 nm) was observed, whereas in water and $\beta$-CD solutions, TICT (430 nm) is noticed in SDA and SFM molecules. $\beta$-CD studies indicate that: (1) sulphonamides form 1 : 1 inclusion complex with $\beta$-CD; (2) the red shift and the presence of TICT in the $\beta$-CD medium confirms that the pyrimidine ring is encapsulated in the $\beta$-CD cavity and the aniline ring is present outside the $\beta$-CD cavity.

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