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Spectroscopic behaviour of the dye safranine T in aqueous polyethylene glycol medium

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Abstract

The interaction of the dye safranine T (ST) in the ground and excited states with polyethylene glycols (PEGs) of five different molar masses was studied. The binding parameters of the combinations were estimated in terms of Langmuir and Scatchard equations. The spectroscopic study has enabled the solvent parameters of the aqueous PEG media to be estimated by comparison with such parameters for pure solvents.

Keywords: Safranine T; Polyethylene glycol; Fluorescence; Absorbance; Binding isotherm; Solvent parameters

1. Introduction

The spectral behaviour of dyes is significantly dependent on the nature of the environment. Interaction of a dye with a solvent at the molecular level is reflected in its visible and fluorescence spectra. Interesting features of such phenomena may occur in surfactant and micellar solutions, which are of general and particular interest in view of the special role of surfaces in guiding and modifying physicochemical processes [1–10].

We have studied the spectroscopic behaviour of the dye safranine T (ST) in micellar and other environments [10,11]. The surfactants used fall in the Tween series (esters of long-chain fatty acids having a fixed polyethylene oxide (PEO) group as the polar head). It has been established that the Tween molecules undergo charge transfer interaction with the dye ST. The complexing strengths of the non-ionic micelles follow the order Tween 80 > Tween 60 > Tween 40 > Tween 20. In the Tween series, the non-polar tails of Tween 80, 60, 40 and 20 are oleic, stearic, palmitic and lauric acids respectively and the head groups are the same, i.e. a sorbitan moiety attached to $-(CH_2-CH_2-O)_{20}-$. The critical micelle concentrations of the Tweens are different [12,13], and so are their aggregation numbers (both are of the order Tween 80 > Tween 60 > Tween 40 > Tween 20). It has therefore been surmised that, to a large extent, the charge transfer interaction is controlled by the aggregation number of the micelles. The ST–micelle association constant K_M has been found to increase with the aggregation number as Tween 80 > Tween 60 > Tween 40 > Tween 20 [8,9]. Since the head groups of all the Tweens are the same, an increased aggregation number means an increased local concentration (population) of the PEO head groups. Thus there is a possibility that the PEO group has a direct bearing on the charge transfer process between ST and Tween. A preliminary study using aqueous solutions of polyethylene glycols (PEGs) of varying degrees of polymerization has shown that the absorption and emission spectra of ST can be affected in such media. It was therefore felt that a separate investigation of the spectral behaviour of ST in aqueous PEG solutions of graded molecular weights was needed to understand the overall influence of the Tween micelles on the photophysical behaviour of the dye.

In this report, results of a detailed study on the ground and excited state spectra of the dye ST in aqueous solutions of PEG with average molecular weights of 200, 300, 400, 600 and 1000 are presented. Results of the spectral investigation of the interaction of ST with ethylene glycol (EG), propylene glycol (PG) and amylose are also presented. A rational analysis of the spectral data has been attempted.

2. Experimental details

ST (E. Merck) was recrystallized twice from an ethanol–water medium. PEG 200, 300, 400, 600 and 1000 were BDH products. The numbers correspond to

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their average molar masses [14]. Doubly distilled conductivity water was used for solution preparation.

Absorption spectra were recorded on a Shimadzu UV-visible spectrophotometer with matched pairs of silica cuvettes (path length, 1 cm). Fluorescence spectra were measured using a Fluorolog F111A spectrofluorometer (Spex. Inc., NJ, USA) with a slit width of 2.5 nm. The excitation and emission wavelengths were 520 and 587 nm respectively. All spectral measurements were duplicated in a constant-temperature water bath (accurate within ±0.5 °C) and the mean values were processed for data analysis.

3. Results

3.1. Absorption behaviour of ST–PEG system

ST exhibits a maximum absorption at 522 nm with an extinction coefficient (ϵ) of 28 457 $dm^3\ mol^{-1}\ cm^{-1}$ at 298 K. In the presence of PEG (approximately 0.1 mol dm^{-3}), an enhancement in absorbance with a bathochromic shift takes place. Fig. 1 shows the spectral features of ST in aqueous PEG 200 medium. An isosbestic point at 400 nm is observed. The binding isotherm for ST–PEG interaction was constructed by taking the maximum absorbance of a fixed amount of dye (2.5×10^{-5} mol dm^{-3}) with increasing [PEG]. The amount of bound dye at each PEG concentration was estimated by comparing the absorbance with its maximum value at higher [PEG]. The concentration of the free dye D_f then followed from its total concentration. The estimates of the amounts of free dye D_f and bound dye $\bar{\nu}$ per mole of $-CH_2-CH_2-O-$ in PEG at equilibrium were then used in the reciprocal relation of Langmuir (Eq. (1)) and the Scatchard equation [15–17] (Eq. (2)) to derive information about the binding constant K and the average number of binding sites $\bar{n}$ per binding centre (here $-CH_2-CH_2-O-$).

The Langmuir equation is given by

$$\frac{1}{\bar{\nu}}=\frac{1}{\bar{n}}+\left(\frac{1}{\bar{n}K}\right)\left(\frac{1}{D_f}\right) \tag{1}$$

The Scatchard equation is given by

$$\bar{\nu}/D_f=\bar{n}K-K\bar{\nu} \tag{2}$$

The validity of Eqs. (1) and (2) in the present situation is supported by the presentations in Figs. 2 and 3 respectively. The results are given in Table 1. The binding phenomenon occurs with different degrees of affinity following the order PEG 1000 > PEG 600 > PEG 400 > PEG 300 > PEG 200, the order of the molar masses of the polymer. The polar oxygen centres of ($-CH_2-CH_2-O-$) in PEG are considered to be responsible for holding the ST molecules by way of charge transfer interaction. This is elaborated in a subsequent section.

3.2. Fluorescence behaviour of the dye–PEG system

The enhancement of the fluorescence intensity of dyes and other compounds in micellar media has been

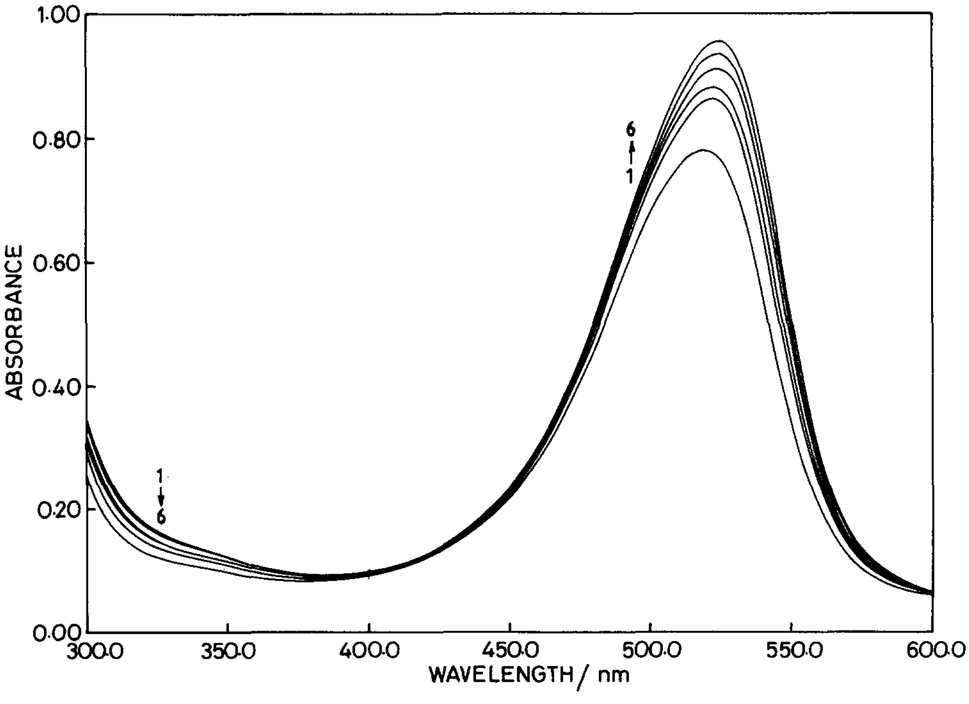

Fig. 1. Visible absorption spectra of ST and PEG 200 at 298 K. [ST] = 25 μmol dm^{-3}; [PEG 200] (mol dm^{-3}): (1) 0; (2) 0.1; (3) 0.2; (4) 0.3; (5) 0.4; (6) 0.5.

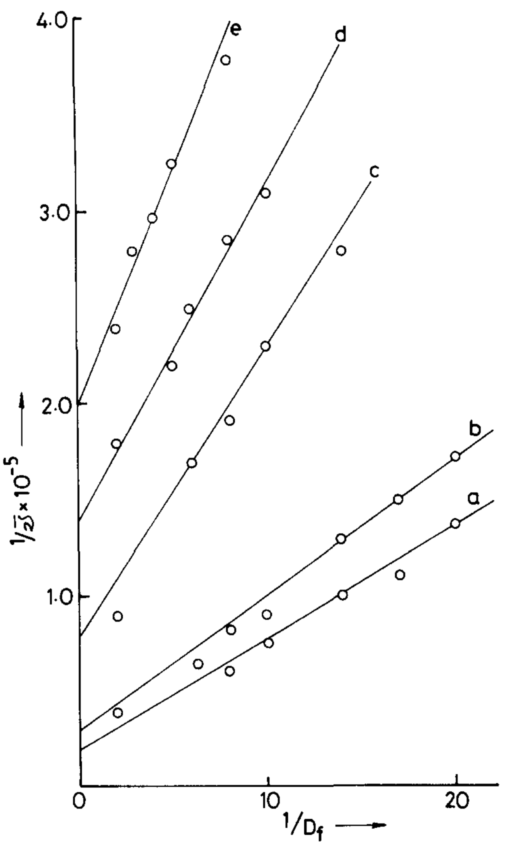

Fig. 2. Plot of $1/\bar{\nu}$ vs. $1/D_f$ according to Eq. (1): (a) PEG 200; (b) PEG 300; (c) PEG 400; (d) PEG 600; (e) PEG 1000.

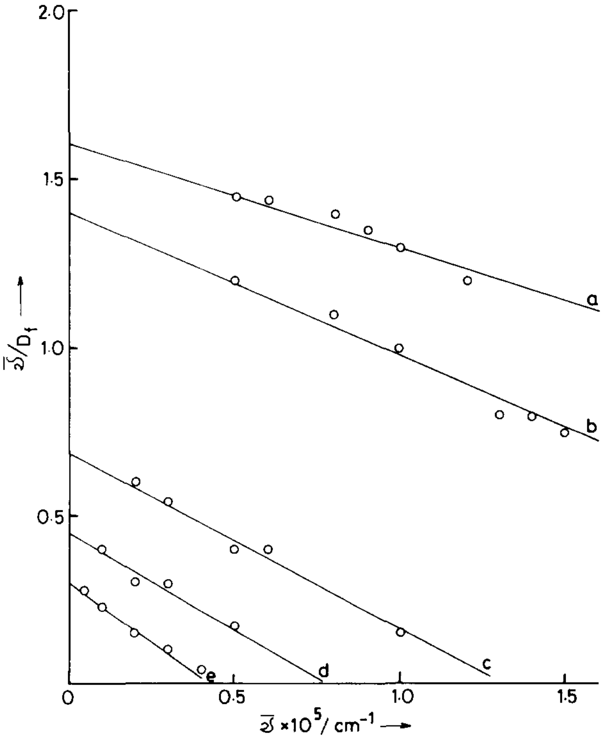

Fig. 3. Plot of $\bar{\nu}/D_f$ vs. $\bar{\nu}$ according to Eq. (2): (a) PEG 200; (b) PEG 300; (c) PEG 400; (d) PEG 600; (e) PEG 1000.

Table 1
The K and n values for ST–PEG interaction obtained from absorption and emission measurements at 298 K

PEG	Absorption measurements		Emission measurements	
	$K_{abs} \times 10^{-5}$ (dm^3 mol^{-1})	n_{abs} ($\times 10^5$)	$K_{em} \times 10^{-5}$ (dm^3 mol^{-1})	n_{em} ($\times 10^5$)
200	3.4 (3.1)	5.0 (5.1)	2.0 (2.0)	2.5 (2.1)
300	4.2 (4.2)	3.3 (3.3)	2.3 (2.4)	1.6 (1.7)
400	5.3 (5.3)	1.3 (1.3)	2.7 (2.8)	1.0 (1.3)
600	7.4 (6.0)	0.71 (0.75)	3.1 (4.0)	0.5 (0.8)
1000	8.3 (7.2)	0.5 (0.42)	3.8 (4.4)	0.4 (0.7)

First value, from Eq. (1). Second value (in parentheses), from Eq. (2).

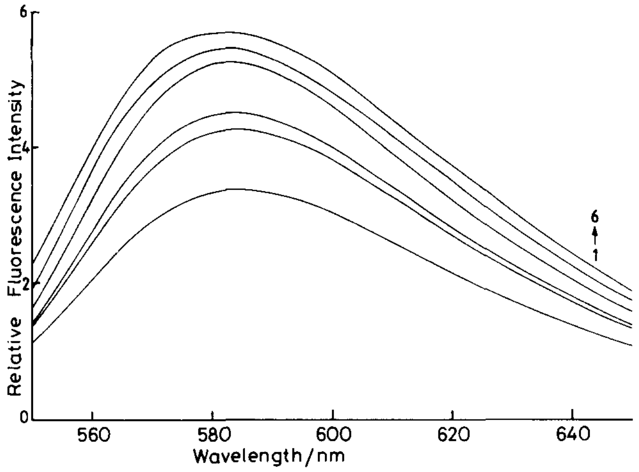

Fig. 4. Fluorescence spectra of ST in aqueous PEG 200 at 298 K. [ST] = 25 μmol dm^{-3}; curves 1–6 represent [PEG 200] = 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mol dm^{-3}.

reported previously [10,18]. The fluorescence of many compounds is enhanced by being adsorbed on the micellar surface leading to altered polarity and viscosity [12]. Aqueous solutions of PEG enhance the fluorescence intensity of ST, which is appreciable at [PEG] = 0.1 mol dm^{-3}. This is depicted in Fig. 4. The curve is Langmuir in type (Fig. 5). The D_f and $\bar{\nu}$ values were estimated using the F vs. [PEG] isotherm as performed for the absorbance measurements (presented above). The data were then analysed in the light of Eqs. (1) and (2) (presented in Fig. 6) and the results (K and n) are presented in Table 1. They compare reasonably well with those from absorption. Both the ground and excited state spectroscopic attempts give the same estimation of the ST–PEG interaction.

3.3. Estimation of the polarity of the aqueous PEG environment

The absorption and emission spectra of ST in acetonitrile, acetone, ethanol, ethylene glycol, dioxan, formamide and tetrahydrofuran have been reported [11].

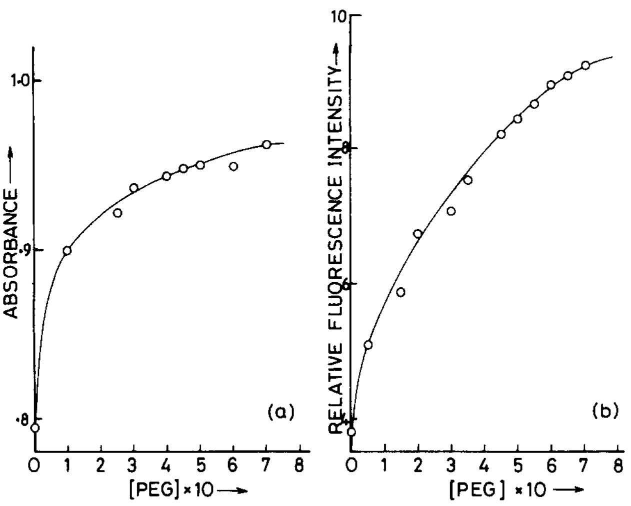

Fig. 5. Plot of absorbance at 520 nm (a) and fluorescence intensity at 583 nm (b) of ST (25 μmol dm^{-3}) in PEG 300 solutions at 298 K.

The spectrum of ST in PEG medium shows shifts in the 0–0 transition in the ground and excited states of the molecule due to solvent interaction, and the intensities are also changed. The visible spectral transitions occur in the range 528–537 nm. The spectral shift observed in pure solvents and aqueous PEG can be used as a guide to the interaction of the dye with the PEG molecules.

The solvent parameters, dielectric constant and refractive index can be correlated with the Stokes' shift [19–22] (the shift between the ground and excited state transitions of ST). Within the zero-order approximation, solute–solvent interaction is considered to be primarily of dipole–dipole nature including dispersion interactions.

The spectral shifts $\Delta\bar{\nu}=(\bar{\nu}_a-\bar{\nu}_f)$, where $\bar{\nu}_a$ and $\bar{\nu}_f$ refer to the frequencies of absorption and fluorescence respectively, for the solvents and aquo-PEG media are presented in Table 2. Several workers [19–22] have correlated the solvent polarity parameters, involving the dielectric constant, with the Stokes' spectral shift. For ST, the shifts in the solvent system have been correlated with the Kosower Z value [23] and the transition energy for intramolecular charge transfer (E_T^{30}) [23,24] in Fig. 7. The correlations of $\Delta\bar{\nu}$ with both Z and E_T^{30} are fairly linear. However, $\Delta\bar{\nu}$ of the solvents does not show a direct correlation with the dielectric constant D.

Assuming that similar correlations of $\Delta\bar{\nu}$ with both Z and E_T^{30} also hold in aquo- PEG, the Z and E_T^{30} values for the PEG medium have been estimated using the measured $\Delta\bar{\nu}$ values shown in Fig. 7. The values are presented in Table 2. Since $\Delta\bar{\nu}$ does not show a direct correlation with the dielectric constant D, an indirect method has been adopted for its evaluation in PEG solutions. This is presented in the next section.

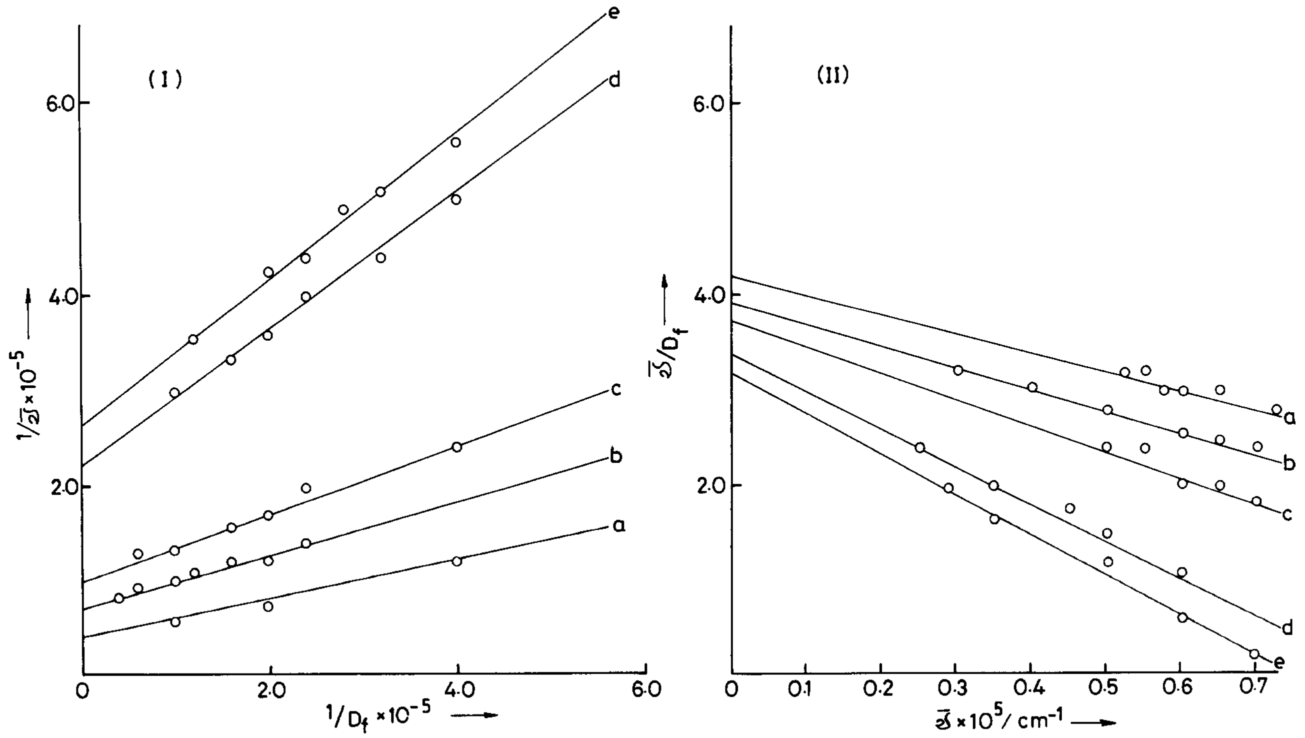

Fig. 6. Plot of $1/\bar{\nu}$ vs. $1/D_f$ according to Eq. (1) (I) and $\bar{\nu}/D_f$ vs. $\bar{\nu}$ according to Eq. (2) (II) from fluorescence spectra: (a) PEG 200; (b) PEG 300; (c) PEG 400; (d) PEG 600; (e) PEG 1000.

Table 2
Solvent parameters and spectral characteristics of ST in aqueous PEG medium

PEG	$\bar{\nu}_{max}^{ab}$ (cm^{-1})	$\bar{\nu}_{max}^{fl}$ (cm^{-1})	$\Delta\bar{\nu}$ (cm^{-1})	Z	E_T	D
200	18939	17212	1727	84	54	67.5
300	18850	17271	1579	78	50	53
400	18744	17301	1443	74	47	41.5
600	18674	17361	1313	69	44	29
1000	18622	17361	1261	67	42.5	24.5

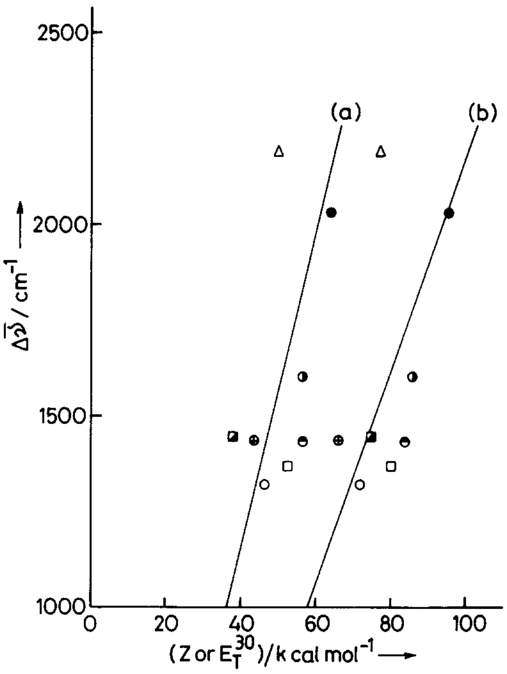

Fig. 7. Plot of $\Delta\bar{\nu}$ vs. E_T^{30} (a) and Kosower Z value (b) for various solvents.

4. Discussion

From the ground and excited state spectral properties, it is evident that dye–PEG complex formation takes place and the association constants are of a similar magnitude. The dye ST may form a 1 : 1 dye–micelle complex. ST complexation with Tween micelles with different degrees of affinity has been reported [10]. With an increase in the length of the hydrocarbon chain (tail end of the surfactant), the affinity increases. In the Tween series, the number of head groups is identical. The increased affinity for ST binding is related to the increased micellar aggregation number, which is directly proportional to the length of the non-polar tail. The increased aggregation number as a result of the increased length of the non-polar tail provides an increased number of head groups (containing $-CH_2-CH_2-O-$) per micelle. It is the polar head groups that are primarily responsible for the spectral phenomena. This is supported by the ST–PEG interaction reported here. PEGS with increased molar mass have an increased effect. Ethylene glycol and amylose have no effect, whereas propylene glycol has a minor effect. This reveals that the $-CH_2-CH_2-O-$ unit is responsible for PEG interaction with ST; as it increases in number, it produces more effect. The values of $\bar{n}$ and K are of the order of 10^{-5} and 10^5 respectively. $-CH_2-CH_2-O-$ centres are required to bind one molecule of ST to produce the spectral effect. The polymer is considered to assume a special configuration in solution to introduce a concerted charge transfer effect on ST. Amylose is known to form a helix, each turn containing seven glucose residues, to undergo dipolar interaction with trapped iodine molecules to produce a blue colour in the amylose–I_2 complex. The order of K of the ST–PEG complex is very large, which is a consequence of the combination of one ST molecule with 10^5 $-CH_2-CH_2-O-$ assembled centres of PEG to form one unit of the complex on the basis of the general equilibrium, $ST + n(-CH_2-CH_2-O-) \rightleftharpoons ST-(CH_2-CH_2-O)_n$. According to a recent report [8], ST–micelle interaction also yields association constants of equal order of magnitude.

The results are compared in Table 3 in terms of the aggregation numbers of the non-ionic Tween micelles (n_{agg}) and the average number of ethylene oxide (EO) groups of PEG ($\bar{n}_{EO}$). The contributions of the EO groups and other environmental effects (particularly in the micellar solutions) are apparent. The results given in Table 3 suggest an average of 100 molecules of Tween in a micelle. Twenty EO groups in a molecule offer 2000 groups in a micelle that can interact with a single ST molecule. Such a state involves a large number of EO groups undergoing charge transfer interaction with ST, but it is approximately 50 orders of magnitude lower than the composition derived from

Table 3
Comparison of ST interaction with Tween micelles and PEG at 298 K

System	$\bar{n}_{agg}$	$\bar{n}_{EO}$	$K \times 10^{-5}$ (dm^3 mol^{-1})
TX 100	136	–	3.5
Tween 20	86	–	2.0
Tween 40	90	–	2.6
Tween 60	110	–	4.0
Tween 80	122	–	5.2
PEG 200	–	4.5	2.7
PEG 300	–	6.8	3.25
PEG 400	–	9.1	4.0
PEG 600	–	13.6	5.25
PEG 1000	–	22.7	6.05

$\bar{n}_{agg}$ values taken from Ref. [1].

the ST–PEG interaction reported here. The difference may be ascribed to the unequal activities of the free EO groups in PEG and the EO groups bound to the polymethylene non-polar chains via a sorbitan moiety in the Tweens. Other factors due to the micellar environment may also contribute. Quantification of the difference would be a worthy exercise.

The derived K and n values for ST–PEG interaction follow the relations

$$K = 0.28n + 2.5 \tag{3}$$

from the absorption results and

$$K = 0.16n + 1.4 \tag{4}$$

from the emission results.

The spectral shifts of ST in the solvent systems have been correlated with the Kosower Z value and the transition energy for intermolecular charge transfer ($E_T{}^{30}$). It has already been stated that $\Delta\bar{\nu}$ values of the solvents do not show a direct correlation with the dielectric constant D. An indirect method has therefore been adopted for its evaluation in the aquo-PEG medium. It has been observed that D bears a linear correlation with both Z and $E_T{}^{30}$; such correlations are illustrated in Fig. 8. Both the Z and $E_T{}^{30}$ values for the ST–PEG system have been used to estimate the corresponding local D values from Fig. 8. The average values of the two sets of data are presented in Table 2.

The Z, $E_T{}^{30}$ and D values decrease with increasing molar mass of PEG with a consequent decrease in $\Delta\bar{\nu}$ (Table 2). The local polarity decreases with an increase in the population of EO residues in the molecule of PEG to yield a higher association constant. The linear correlation of $\Delta\bar{\nu}$ with the molar mass of PEG follows the best fit relation

$$\Delta\bar{\nu} = 1.87 \times 10^3 - 0.9M_{\mathrm{PEG}} \tag{5}$$

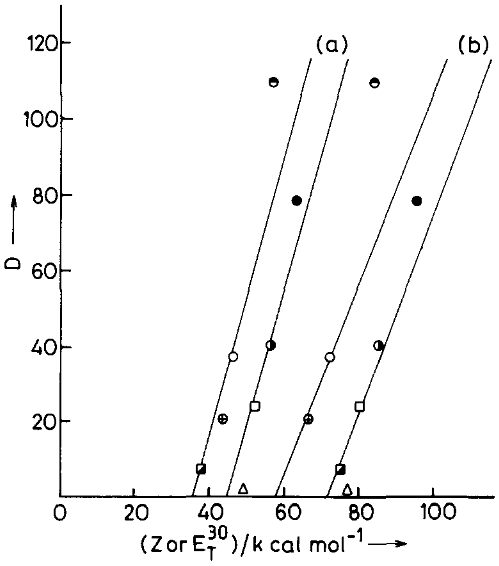

Fig. 8. Plot of dielectric constant D vs. $E_T{}^{30}$ (a) and Kosower Z value (b) for various solvents: ○, acetonitrile; ⊕, acetone; □, ethanol; ◐, ethylene glycol; △, dioxan; ●, water; ◆, formamide; ◪, tetrahydrofuran.

5. Conclusions

This study has led to the following conclusions.

(1) PEG containing EO residues in the molecule can undergo charge transfer interaction with ST in aqueous medium, the efficiency of which depends on the number of EO residues.
(2) One molecule of ST requires 10^5 EO residues to augment the charge transfer interaction with a large association constant.
(3) The Stokes' spectral shift $\Delta\bar{\nu} = (\bar{\nu}_a - \bar{\nu}_f)$ has a direct correlation with Z, $E_T{}^{30}$ and D. The local polarity in the medium systematically decreases with an increase in the number of EO residues in PEG.

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