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SOLUBILIZATION OF DIPHENYLAMINE IN MICELLAR MEDIA

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ABSTRACT

The solubilization process finds extensive applications in industrial, pharmaceutical and biological field. Studies were made on the solubilization of diphenylamine in different concentrations of anionic, cationic and nonionic micellar media. The solubilization phenomenon has also been confirmed by absorption spectral studies. Spectral parameters like quantum yield, Stokes' shift and molar extinction coefficient were calculated in micellar media at different concentration of compound. The changes in curve area and quantum yield has been indicated the change in solubilization pattern in presence of different class of surfactants. The maximum effect on solubilization of diphenylamine in its aqueous solution has been shown by non-ionic solvents.

KEYWORDS: diphenylamine, solubilization, micellar media, spectral studies, non-ionic surfactant.

INTRODUCTION

Term solubilization is broadly defined as "the preparation of a thermodynamically insoluble or very slightly soluble compound in a given solvent by the interaction of the additional amphiphilic component or components". The solubility of predominantly hydrophobic molecule in aqueous solution is enhanced by the addition of surfactants to solutions. The added surfactant molecule self-assemble to form micelles or vesicles which by providing a more compatible environment for the sparingly soluble molecules increase their solubilization. Surfactants have been widely used in numerous fields such as foodstuff, cleaning products, paints, cosmetics, oil recovery, waste water treatment, various separation process and pharmaceutical industry[1-4]. Surfactant are a class of substance which have a tendency to associate in solution forming particles of colloidal dimension called micelle,

which get adsorbed at interface[5]. Micellization has been treated either as a stepwise association phenomenon or as a phase transition prosses[6].

Fluorescence spectroscopy is one of the most widely used techniques in many field of science. The technique has become quite popular because of its acute sensitivity to changed structural dynamic properties of molecules. Due to its high sensitivity, it is the best method for monitoring interaction between surfactants and organic molecules and to determined critical micellar concentration. Solubilization studies at low concentration are possible by this method. High sensitivity results from the difference in wavelength between the exciting and fluorescence radiation[7].

Diphenylamine(DPA) is an colourless, crystalline organic compound with the formula (C6H5)2NH. The compound is a derivative of aniline, consisting of an amine group bonded to two phenyl groups. Diphenylamine is a redox indicator dye that has been studied for its antioxidant properties which reduce the scald of apples in storage[8]. Diphenylamine potentially exhibits fluorescence and is useful for the detection of nitrates and chlorates. The diphenylamine assay is a very useful tool in measuring apoptosis by determining the percentage of fragmentation of known amount of DNA into oligosomal fragments[9]. It is a common structure of non steroidal anti-inflammatory drugs to uncouple mitochondrial oxidative phosphorylation and to cause a decrease in hepatocellular ATP content and heptocyte injury[10]. Diphenylamine stimulates basal oxygen consumption and inhibits ADP-stimulated respiration. The increased interest in fluorescence appears to be due to advance in time resolution, method of data analysis and improved instrumentation[11]. In the absence of a detailed crystallographic database, approaches based on fluorescence spectroscopy have proved useful in elucidating the organization, topology and orientation of membrane proteins.[12]

Hilary F. Gooncwardene investigated an improved method for controlling the development of storage scald of apples, and more particularly an improved method of applying and producing extremely minute surface residues of diphenylamine derivatives on apples to prevent, control and /or retard the development of apple scald. It also relates to new formulations for utilizing the improved method for controlling the development of storage scald of apples.

MATERIAL AND METHODS

The stock solution of diphenylamine (Sigma Aldrich, 99%) was prepared in double distilled water. The concentration of the compound was kept at $1x10^{-5}$ M throughout the experiment. The surfactants used to made the studies are (a) non-ionic surfactants (i) Tx-100 polyoxyethylenetertoctyl phenyl ether (ii) Tween-40 polyoxy ethylene sorbitan monopalmitate (iii) Tween-20; polyoxyethylenesorbitanmonolaurate (b) Cationic surfactants (i) CTAB: cetyltrimethylammonoum bromide (ii) CPB: cetylpyridinium bromide (iii) CPC: cetylpyridinium chloride and (c) Anionic surfactants (i) DBSS: Dodecylbenzyl sodium sulphonate (ii) DSSS: Dioctyl sodium sulphosuccinate (iii) SLS: Sodium lauryl sulphate. All the surfactants were either of Sigma(USA) or BDH (UK) products. The fluorescence spectrum was taken with Perkin Elmer Fluorescence Spectrophotometer model number 204A with a synchronized model number 056 strip chart recorder and absorption spectra were taken on Hewlett Packard (HP) 8452A diode array spectrophotometer. The absolute fluorescence quantum yield of the compound relative to anthracene solution is taken as standard. The area of the fluorescence spectrum were recorded over the whole range of emission under identical conditions were used to calculate quantum yield of sample in different micellar media.

RESULTS AND DISCUSSION

For aqueous solution of diphenylamine the maximum excitation wavelength was found at 290nm and the wavelength of maximum emission were obtained at 335nm(Fig.1).

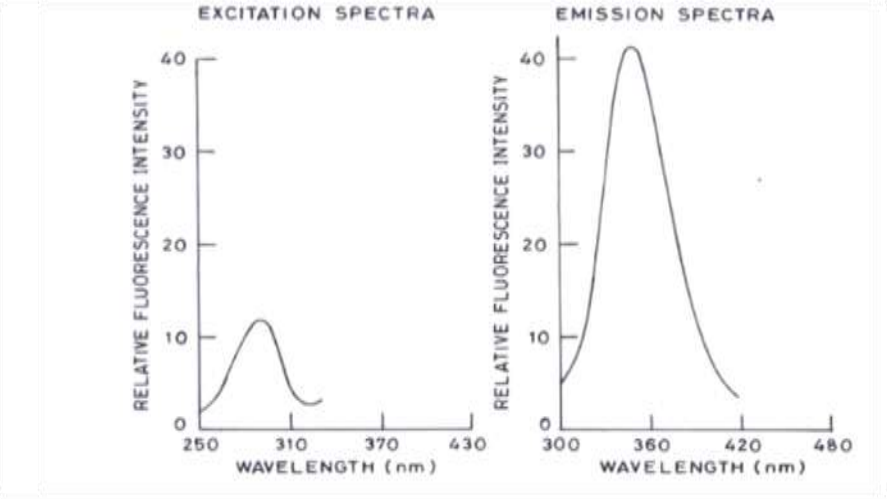

Figure 1: Fluorescence excitation and emission spectra.

The non-ionic surfactants used for the study were Tweeen-20, Tween-40 and Tx-100. The range of concentration for all non-ionic surfactants was varied from 0.003%-0.1%. All three types of non-ionic surfactants showed an enhancement in fluorescence intensity. With Tween-20 and TX-100, on increasing surfactant concentration a continuous increase in fluorescence intensity with 5-25nm blue shift in emission wavelength was observed. Out of three non-ionic surfactants maximum increase in fluorescence intensity was shown by TX-100. At its higher concentration fluorescence intensity reached out of scale. The change in emission intensity with various concentration of TX-100 is shown in Fig.2.

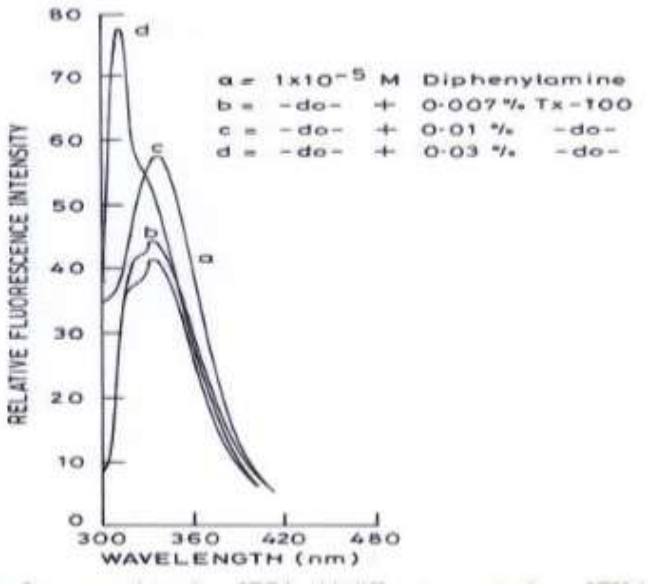

Figure 2: Relative fluorescence intensity of DPA with different concentrations of TX-100.

The anionic surfactants used for the study were DBSS, SLS and DSSS. The concentration of all anionic surfactants varies from 0.003% to 0.1%. On initial addition, all anionic surfactants showed a gradual increase in the peak height. At higher concentration it showed a small decrease in fluorescence intensity. The change in spectra with DBSS is shown in Fig 3.

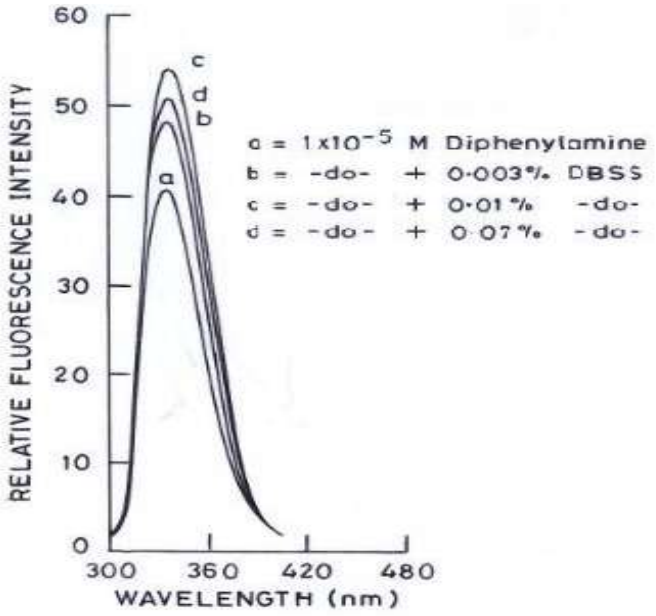

Figure 3: Relative fluorescence intensity with different concentrations of DBSS.

The cationic surfactants used for study were CTAB, CPC and CPB. The concentration of all three cationic surfactants varied from 0.003% to 0.1%. A very small decrease in fluorescence intensity was observed in presence of all three cationic surfactants. The fluorescence intensity in absence of surfactants and maximum fluorescence intensity obtained in presence of surfactants are shown in Table 1.

Table 1: Minimum and maximum fluorescence intensity of diphenylamine in absence and presence of surfactants

S. No.	Name of surfactants	Fluorescence intensity in absence of surfactants	Concentration of surfactants at which maximum fluorescence intensity obtained	Maximum fluorescence intensity obtained
1	Tween-20	40	0.1	95
2	Tween-40	42	0.1	98
3	TX-100	42	0.03	78
4	DBSS	42	0.01	54
5	SLS	42	0.03	67
6	DSSS	42	0.03	55
7	CTAB	41	0.003	41
8	CPC	42	0.003	41
9	CPB	42	0.003	41

The absorption wavelength for the solution of $1x10^{-4}$M diphenylamine was found at 285nm.

The concentration of all non-ionic, anionic and cationic surfactants employed was varied from 0.003% to 0.1%. A continuous increase in the absorbance with 5nm blue shift was observed on adding Tween-20 and Tween-40 to diphenylamine solution. Maximum increase in absorbance with 5-10nm blue shift was observed with TX-100. All anionic surfactants caused a gradual increase in absorbance at first while at higher concentration it showed a decrease in absorbance. A very slight decrease in absorbance was recorded on addition of various concentrations of CTAB, CPC and CPB.

The Stokes' shift of Diphenylamine at its various concentrations at room temperature was calculated. The variation in Stokes' shift indicates that energy changes occur in the excited state of the molecule on varying the concentration of the compound at room temperature. Calculated values are given in Table-2 and the recorded spectra are shown in fig.4.

Table 2: Stokes' shift data of diphenylamine at room Temperature.

S.No.	Concentration of compound (M)	F.I.	λ_{ex} (nm)	F.I.	λ_{em} (nm)	P.M. Gain	Sensitivity	Stokes' Shift (cm^{-1}) 10^3
1.	1×10^{-5}	11	290	335	40	2	0.1	4.0475
2.	3×10^{-5}	14	290	335	48	2	0.1	4.0475
3.	5×10^{-5}	16	300	335	49	2	0.1	4.4825
4.	7×10^{-5}	17	300	335	52	2	0.1	4.4825
5.	9×10^{-5}	20	305	340	53	2	0.1	4.3751
6.	3×10^{-4}	18	305	340	62	2	0.1	4.3751
7.	9×10^{-4}	30	310	340	78	2	0.1	4.8462
8.	1×10^{-3}	45	310	340	95	2	0.1	4.8462

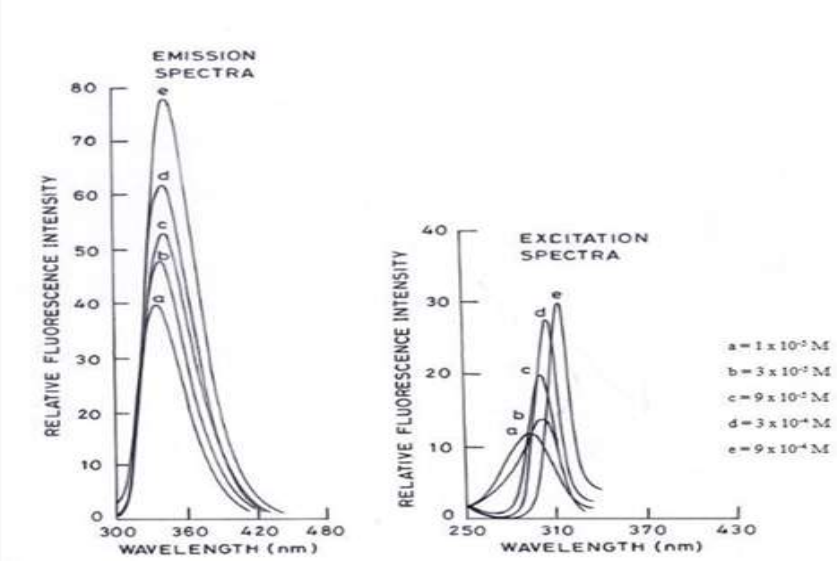

Figure 4: Fluorescence spectra showing Stokes' shift.

Molar extinction coefficient was calculated for diphenylamine with different non-ionic, anionic and cationic surfactants i.e. in micellar media. For non-ionic concentration the molar extinction coefficient value increases on increasing surfactant concentration. For anionic surfactant molar extinction coefficient value increased initially and it was decreased at higher concentration of surfactants. With cationic surfactants the values of molar extinction coefficient show a very slight decrease (not remarkable). The fluorescence quantum yield has been calculated for the aqueous solution of diphenylamine relative to anthracence in ethanol as standard.

The results indicated that non-ionic surfactants had a stronger enhancement effect on fluorescence and absorption behavior of DPA. The maximum enhancement was obtained for Tx-100, which has been supported by absorbance, log ε, k_f and Φ_f values. The quantum yield values, molar extinction coefficient values for Tx-100 added solution is shown in Table-3.

Table-3: Absorption maxima, molar extinction coefficient, fluorescence maxima and quantum yield of p-Nitroacetophenone at different concentration of Tx-100.

S.no.	Concentration of TX-100 used(%)	Absorption maxima(nm)	Molar extinction coefficient(dm^3 /mol cm)	Fluorescence maxima	Quantum yield
1.	0.000	285	4.6674	335	0.7935
2.	0.003	285	4.6794	335	0.8983
3.	0.007	285	4.7489	335	0.9342
4.	0.03	285	4.8419	310	0.9754

Fluorescence coefficient value of DPA with different surfactants was synchronized with the fluorescence intensity of the compound with different surfactants. The empirical fluorescence coefficient values obtained with non-ionic surfactants are shown in Table-4.

Table-4: Fluorescence coefficient(k_f) value of diphenyleamine in various nonionic surfactants at their different concentration.

S.No.	% of Tween-20 (w/v)	k_f x10^4 (per mole)	% of Tween-40 (w/v)	k_f x10^4 (per mole)	% of TX-100 (w/v)	k_f x10^4 (per mole)
1.	0.0	400	0.0	420	0.0	
2.	0.03	690	0.005	450	0.005	420
3.	0.05	750	0.07	610	0.03	580
4.	0.07	890	0.05	760		780
			0.07	860		

With increasing concentration of surfactants, the maximum enhancement in fluorescence intensity was obtained with non-ionic surfactants, while anionic surfactants showed initial increase and then small decrease in fluorescence intensity. Cationic surfactants did not showed remarkable effects on solubilization of DPA. Thus above observations can be explained by the solubilising action of surfactant micelles. Hydrophilic compounds can be adsorbed on the surface of the micelle[13], compounds with intermediate solubility should be located in intermediate positions within the micelle such as between the hydrophilic head groups of micelles[14] and in the palisade layer between the hydrophilic groups and the first few carbon atoms of the hydrophobic group, that is the outer core[15] and completely insoluble hydrophobic compounds may be located in the inner core of the micelle[16-18].

Non-ionic surfactants usually are better solubilising agents than ionic surfactants for hydrophobic compounds, because of their lower CMC values. For polar compounds it is more complicate to establish a general relationship between the degree of solubilization and the chemical structure of surfactant, since solubilization can be in both the inner and outer region of the micelle. The extent of solubilization into a particular micelle depends upon the locus of solubilization and therefore the shape of the micelle. As the micelle become more asymmetrical and volume of the inner core increases relative to that of the outer portion. Therefore, one can expect that the solubilization of compounds in core will increase with increase in asymmetry, whereas the solubilization of drugs in the outer region will decrease[18].

Tx-100 solubilizes the solute molecule very efficiently because of oblate ellipsoidal model of Tx-100. Kano et al[19] have found that interior of Tx-100 is more hydrophobic than those of ionic micelles. The highest solubilising effect of Tx-100 may also be due to presence of

ether linkage in it, while other non-ionic surfactants employed were esters. The increased solubility of DPA in anionic surfactants is due to resonance which made the molecule cationic in nature which is solubilized with anionic surfactant and cause increased in fluorescence intensity as well as quantum yield.

The increase in log ε and Φf values in anionic surfactants indicates that the rate of nonradiative process is less in micellar system in comparison to those in water. This may be due to decrease in intersystem crossing rate as pointed out by Shizuka et al[20]. Another reason is micellar surface, which decrease the rate of collision deactivation of the fluorophore by water molecule. A blue shift is due to the difference in solvation energy of the solute in the ground state[21]. The large Stokes' shift values for p-NAP are due to H-bond formation between the solute and solvent in the ground state. These bonds break following excitation, but reforms following proton transfer. The present analysis indicates that during solubilization of solute into the surfactant system, the incorporation of the solute influence the balance of favourable and unfavourable forces guiding micellization and the structural changes occurring due to aggregation, dissociation and hydrogen bonding.

CONCLUSION

Experimental data showed that all the non-ionic surfactants have increasing effect on solubilization of diphenylamine, but the maximum enhancement in solubilization was observed with TX-100. With anionic surfactants solubilization increased at lower concentration while it decreased at higher its concentration. Cationic surfactants did not show any remarkable effect on solublization. After interpreting and comparing the results obtained for p-NAP, it is found that the theoretically calculated spectral parameter like molar extinction coefficient, Stokes' shift, quantum yield values and empirical fluorescence coefficient are in good agreement with experimental results. This proves the validity of the assumption made.

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