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Fluorescence Approach to Micellar Solubilization of A Heterocyclic Alkaloid: Quinine Hydrochloride

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ABSTRACT

Quinine hydrochloride (QH) is a pharmaceutically important heterocyclic alkaloid derived from the bark of cinchona tree. Micellar solubilization of QH in nonionic and ionic surfactant heteromicroenvironment monitored by fluorescence and absorption spectral techniques has been reported by the authors in this communication. The relatively week fluorescence of QH was significantly enhanced in nonionic micellar media formed by Tween-20 surfactant. The influence of the surfactant, concentration and working experimental conditions on the fluorescence spectra of QH is thoroughly evaluated and discussed. The solubilizing action of the surfactants has been confirmed by the theoretically calculated spectral parameters like, empirical fluorescence coefficient, quantum yield, molar extinction coefficient and Stokes' shift. The authors provide a unique format for the analytical and medicinal application of QH based on micellization of the surfactants which makes them promising drug carriers, employing fluorescence, absorption and light scattering measurements.

Keywords : Micelles, Quinine hydrochloride, Fluorescence, Solubilization.

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INTRODUCTION

Surfactants play a vital role in various drug delivery. To formulate compounds sparingly soluble in water, pharmaceutically acceptable cosolvents or surfactants are typically employed to increase solubility. Polymeric micelles made by surfactants have a whole set of unique characteristics, which make them a very promising drug carriers for a wide range of drugs. The low solubility in biological fluids displayed by about 50% of the drugs still remains the main limitation in oral, parentral and transdermal administration. To overcome these drawbacks, inclusion of hydrophobic drugs into polymeric micelles which composed of surfactants are one of the most effective alternatives [1]. The formation of colloidal sized clusters in solutions, known as micelles have particular significance in pharmacy because of their ability to increase the solubility of a sparingly soluble substances in water [2], thus increasing their bioavailability. The ambivalence of amphiphiles towards an aqueous environment is responsible for the phenomenon of self - association of individual surfactant molecules resulting in a variety of micellar aggregate structures [3]. The concentration of a monomeric surfactant molecule at which micelles appear is called the critical micelle concentration (cmc). The occurrence of cmc results from a delicate balance of intermolecular forces. The main attractive force results from the hydrophobic interaction among the nonpolar surfactant tails, whereas the main opposing repulsive force results from steric and electrostatic interactions in case of ionic surfactants between the surfactant polar head groups [4]. Micelles are known to have an anisotropic water distribution within their structure, i.e., the water concentration decreases from the surface towards the core of the micelle, with a completely hydrophobic core. These aggregates exhibit an interfacial region separating the polar bulk aqueous phase from the hydrocarbon like interior. As a consequence, micellar solutions consist of a special medium in which hydrophobic, amphiphilic or ionic compounds may be solubilized and the reagents may be concentrated or separated in aqueous solution [5]. The spatial position of a solubilized drug in micelle will depend on its polarity, nonpolar molecules will be solubilized in the micellar core and compounds with intermediate polarity will be distributed along the surfactant molecules in certain intermediate positions.

The utilization of micelles as drug carriers presents some advantages when compared to other alternatives such as soluble polymers and liposomes. Micellar systems can solubilize poorly soluble drugs and thus increase their bioavailability, stay in the blood long enough to provide gradual accumulation in the required area, their size permit them to accumulation areas with leaky vasculature. Moreover, specific ligands can be attached to their outer surface in order to optimize the controlled release and specificity of pharmacological effect. Micelles can be obtained in an easy and reproducible manner in large scale [6]. Therefore, the utilization of aqueous solutions for drug solubilization can be advantageous for drug delivery purposes, with two possibility of increasing water solubility of poorly soluble drugs, improving bioavailability, reducing toxicity and other side effects, enhancing permeability across the physiological barriers, and substantial change in drug distribution [6].

In recent years [7-9], extensive investigations have been made on micellar effect of the surfactants on polynuclear heterocyclic compounds. QH is widely used as antimalarial drug. It is also a mild antipyretic and analgesic effects and has been used in common cold preparations for that purpose. It is also useful in some muscular disorders, especially nocturnal leg cramps and mytonia congenita, because of its direct effect on nuclear membrane in sodium channels.

Raymond F. Chen [10] studied some anomalous features of fluorescence of quinine the fluorophore of QH, i.e, in acid solution its excitation spectrum fails to coincide with its absorption spectrum at long wavelengths and few other unusual optical features should be considered when quinine is used as either a fluorescence test substance or a quantum yield standard. Quinine may be a useful probe to detect inhibition of a specific liver activity with in an individual [11]. W.A. Laurie et al. [12] studied photoreactions of quinine in aqueous citric acid solution. D. Pant et al. [13] explained that quinine is less applicable to time resolved studies, because its fluorescence decay is not monophasic. Xue-Mei Wang and Hong-Yuan Chen [14] studied FTIR and NMR studies of the complexation of aqueous QH with β-cyclodextrine and support the presence of hydrogen bonds which stabilize the complexes. T. Yata et al. [15] measured the transient membrane potential in response to QH in a membrane filter impregnated with phospholipids and 1-octanol. H.J. Jeuring et al. [16] determined quinine in soft drinks by fluorescence, UV spectrophotometry and reversed-phase ion-pair chromatography. Accounting for its importance in medicinal research, we chose to study QH for its micellar solubilization by fluorescence, absorption and light scattering techniques.

EXPERIMENTAL

Materials :

Analytically pure QH was a Merck sample. The following surfactants were employed : (A) Nonionic (i) TX-100 : Polyoxyethylene tert-octyl phenyl ether (ii) Tween-80 : Polyoxyethylene sorbitain monooleate (iii) Tween-20 : Polyoxyethylene sorbitain monolaurate (B) Anionic (i) SLS : Sodium lauryl sulphate (ii) DBSS : Dodecylbenzyl sodium sulphonate (iii) DSSS : Dioctyl sodium sulphosuccinate (C) Cationic (i) CPC : Cetylpyridinium chloride (ii) CTAB : Cetyltrimethyl ammonium bromide (iii) MTAB : Myristyltrimethyl ammonium bromide. All the surfactants were either of Sigma (USA) or BDH (UK) products.

Methods :

The stock solution was prepared in double distilled water. All the experiments were performed around 23-25°C and final concentration of compound was kept at 1 x 10^{-6} M for fluorescence studies. For absorption studies the concentration of QH was kept at 1 x 10^{-4} M throughout the experiments.

All the fluorimetric experiments were carried out with Perkin Elmer Fluorescence Spectrophotometer (Model No. 204 A) with a synchronized strip chart recorder (Model no. 056). A Xenon lamp was used as a light source. For recording the fluorescence excitation and emission spectra, its slit width was kept at 10 nm and a cell of 1 cm path length was used.

The absorption measurements were made with Hewlet Packard (HP) 8452, and diode array spectrophotometer respectively. The light scattering studies were made with a Brice - Phoenix universal light scattering photometer, model No. 2000, in conjunction with a multiflex galvanometer. Measurements are made at an angle of 90° to the incident beam and the scattering intensity was measured in terms of galvanometer deflection.

The purity of the surfactants was checked by determining their CMC values the help of surface tension measurements, employing drop-weight method. The values obtained coincided

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with the reporded values. The absolute fluorescence quantum yield (ϕ_f) of QH was calculated relative to anthracene solution as standard. Fluorescence emission of anthracene is in the same range as that of QH. Approximate corrections were made to compensate for different absorption of the compound and the standard. Each time the total intensity of fluorescence emission was measured for the standard and the sample from the area of the fluorescence spectrum recorded over the whole range of emission under identical conditions. Molar extinction coefficient data have been reported as its logarithm ($\log \varepsilon$). The Stokes' shift [17] data have been calculated with change in its concentration.

RESULTS AND DISCUSSION

The aqueous solution of QH showed maximum excitation peak at 330 nm and emission peak at 390 nm. All the nonionic surfactants, on addition to QH solution caused a continuous enhancement in its fluorescence emission intensity with increasing concentration. Among them Tween-20 exerted the maximum effect. The changes in the fluorescence spectra of QH on addition of Tween-20 are given in Fig. 1. For anionic surfactants added QH solution fluorescence intensity initially decreased and then increased at their higher concentration with a blue shift of 15-20 nm in emission peak position. Maximum effect was exerted by DBSS. The changes in the fluorescence intensity of QH on adding DBSS are given in Fig. 2. For cationic surfactants added solution fluorescence intensity initially increased and then decreased at their higher concentration without any shift in emission peak position. Among them maximum effect was exerted by CPC. The fluorescence intensity in presence and absence of surfactants are given in Table 1. The absorption spectra gave a peak at 330 nm. All the nonionic surfactants showed enhancement in absorbance without any shift. For anionic surfactants added solution absorbance initially decreased and then increased on increasing concentration with a red shift in λ_{max}. For cationic surfactants added solution absorbance initially increased and then decreased at higher concentration without any shift. The changes in the absorption spectra with Tween-20 are shown in Fig. 3.

The light scattering studies of QH were made at an angle of 90° to the incident light. During addition of each surfactant there occurred a sharp decrease in the galvanometer deflection with increase in surfactant concentration. The nonionic surfactants show maximum deflection. The concentration required to reach the minimum value of scattering flux was different for each surfactant. Fig. 4 represents the results of galvanometer deflection as a function of the surfactants Tween-20, DBSS and CPC.

The calculated fluorescence quantum yield (ϕ_f) of surfactant added QH showed almost parallelism with changes in fluorescence intensity of the compound. In nonionic surfactant – micellar media the quantum yield values increased with the concentration. Highest (ϕ_f) values were obtained for Tween-20 micellar media, which are given in Table 2. With anionic surfactants the quantum yield values obtained initially decreased and then increased at their higher concentration, while with cationics (ϕ_f) values gave the reverse trend. Molar extinction coefficient ($\log \varepsilon$) values for all the nonionic and anionic surfactants showed an increasing

trend, while with cationic surfactants $\log \varepsilon$ values increased on initial additions but gradually decreased on raising the surfactant concentration. Empirical Fluorescence coefficient (k_f) values of QH with different surfactants were found to be parallel with the fluorescence intensity of the compound. The maximum fluorescence coefficient values were obtained with Tween-20. For higher concentration of QH, the Stokes' shift values obtained were high but on dilution, a decrease in Stokes' shift values was obtained. All the theoretically calculated spectral parameters are illustrated in Table 2 and 3 respectively.

The results indicate that the surfactant added system has a stronger emission intensity enhancement than the system in which there was no surfactant present. The maximum fluorescence enhancement was obtained by adding Tween -20. On addition of (0.01% to 0.4%) DBSS a gradual blue shift of 15-20 nm occurred in the emission intensity. This may be because of the difference in the solvation energy of the solute in the ground state and the excited state in the micellar medium [18].

The absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π^* excited state. This is because the absorption peaks for the transition fall in an experimentally convenient region of the UV-VIS spectrum. These transitions need an unsaturated group in the molecule to provide the electrons. The presence of lone pair of electrons on the nitrogen atom (hetero-atom) and the three double bonds in the heterocyclic ring induce the n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. On adding the surfactant, the lone pair lowered the energy of the n orbital. So the energy difference between the ground and excited states was slightly enhanced resulting in a small blue shift with water polarity due to n $\rightarrow \pi^*$ transitions, thus this shift is because of the difference in the solvation energy of the solute in the ground state and excited state in different microheterogenous micellar media.

The results obtained can be explained on the basis of solubilization by the microheterogenous environment of micelles present in the surfactant solution at or marginally above CMC. The maximum enhancement in the fluorescence emission intensity of QH was obtained with Tween-20 micellar media, which has also been supported by absorbance, light scattering flux (σ_f) values and $\log \varepsilon$ values. This may be attributed to the increase in quantum efficiency of fluorescence. Furthermore, the quantum yield of fluorescence is higher in nonpolar medium because of the lesser effect of other deactivation processes which compete with fluorescence [19]. Also that the rate of non-radiative processes are less in nonionic micellar medium in comparison to those in aqueous medium. Another cause may be due to the adsorption of the fluorophore at the micellar surface which decreases the rate of collision of the fluorophore by water molecules. It is assumed that the ionic micelles are too hydrophilic to

solubilize the QH molecules to larger extent. The higher polarity of ionic micelles may be asserted to the loose, fluctuating and disordered structures of the micelles [20]. In ionic micelles, the QH molecules must leave its aggregate and exclude water molecules inside the ionic micelle. These processes cause slow solubilization. An initial decrease in fluorescence intensity of QH occurred on addition of anionic surfactant and then it enhanced on raising the surfactant concentration. The interactions here are mainly electrostatic in nature between the N^+ group in the exocyclic substituent of QH and the anionic head groups of the surfactant. At low ionic concentration, below CMC, solubilizate QH-anionic aggregate formation took place which accounts for initial decrease in fluorescence. But above CMC only the monomeric form of the compound exists which penetrated into the micellar interior and got solubilized, hence fluorescence enhanced [21]. The red shift observed in the absorption peak in anionic micellar media is because the interaction of water polarization with the lone pair decreased and the energy difference between π and π^* reduced and hence induced the $\pi \rightarrow \pi^*$ transitions which resulted into the red shift. Absorption is less sensitive to its environment, as compared to fluorescence, thus absorption spectra are less affected on addition of surfactants. However, the results obtained here, support those with fluorescence studies. However, the initial increase and then decrease in the fluorescence intensity on addition of cationic surfactants to QH solution may be attributed to the electrostatic preferential interaction between the polar substituent Cl^- of the QH molecule which convert the molecule into anionic radical/species and the cationic head group of the surfactant micelle which may result in the change of geometry of the QH molecule where in, it losses the coplanarity and hence the emission intensity started decreasing at higher concentration of the cationic surfactants. The increased light scattering flux values imply that the micelles have been possibly adsorbed on to the dispersed microcrystals of QH. The molecules of QH have been subsequently solubilized by incorporation into the interior nonpolar core of the micelles. Sufficiently large values of molar extinction coefficient ($\log \varepsilon$) may be due to $\pi \rightarrow \pi^*$ transitions, as on increasing the concentration of nonionic surfactant, $n \rightarrow \pi^*$ transitions decreased. The ($\log \varepsilon$) values of the QH molecules in different micellar media follow the same trend as their emission intensity. Hence it proves the well known fact that the fluorescence intensity of a fluorophore is directly related to its molar extinction coefficient [22].

The values of empirical fluorescence coefficient k_f obtained may be attributed to the increased sensitivity of the fluorimetric analysis of the solubilization of organic molecules by surfactants which offer a protective microenvironment, leading to enhanced fluorescence of the solubilizate by shielding the excited state from non-radiative decay that normally occurs in bulk aqueous solution. The decreasing trend in Stokes' shift in very dilute solution of QH is rationalizable in view of water-water hydrogen bonding interactions which lowers the overall hydrogen donations to the solute QH.

CONCLUSION

The present analysis and interpretation suggest that experimental results observed and the theoretically calculated spectral data are found to be in good aggrement. This proves the validity of the investigations made. Hence the process of micellization followed by solubilization

of QH substrate would catalyse its drug delivery activities. The authors have attempted to mimic the micellar solubilization of QH, an antimalarial drug in the laboratory. In this work, the influence of the surfactant head group on the extent of QH solubilization was investigated by fluorescence technique and supported by absorption and light scattering flux.The ionic surfactants present the medium profile for QH, as a result of electrostatic interactions between the drug and the surfactant head groups. The increased solubilization of QH in Tween-20 micellar solution was a consequence of interaction of Tween-20 head group i.e., its monomeric form and also of the molar fraction of surfactant in the micellar form that is higher for nonionic surfactants due to the low CMC.

Therefore, nonionic surfactants could e considered the best alternative for solubilization of QH, as well as other basic drugs. This class of surfactant provides a reasonable molar solubilization capacity combined with low CMC values, resulting in increased solubility. Moreover, the low toxicity of nonionic surfactants makes them particularly interesting for solubilization and drug delivery purposes. This leads to increase the bioavailability of the drug, stay in the body (blood) long enough to provide gradual accumulation in the required area and their size permit them to accumulate in areas with leaky vasculature. Thus aqueous micellar solution have large utilization for drug solubilization can be advantageous for drug delivery purposes, with the possibility of increasing water solubility of poorly soluble drugs, improving bioavailability, reducing toxicity and other side effects, enhancing permeability across the physiological barriers, and substantial change in drug distribution.

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Table – 1: Fluorescence intensity of Quinine hydrochloride in absence and presence of surfactant

λ_{ex} = 330 nm, λ_{em} = 390 nm, P.M. Gain = 3, Sensitivity Range = 1

Name of surfactant	Fluorescence intensity in absence of surfactant	Concentration of surfactant used (%)	Maximum fluorescence intensity (nm)
TX-100	20	0.3	36
Tween-80	20	0.3	38
Tween-20	20	0.3	45
CPC	20	0.03	39
CTAB	20	0.03	30
MTAB	20	0.005	27
DBSS	20	0.4	46
DSSS	20	0.4	18
SLS	20	0.4	18

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Table - 2: Absorption maxima (λ_a), fluorescence maxima (λ_{em}), molar extinction coefficient ($\log \varepsilon$) and quantum yield (ϕ_f) of Quinine hydrochloride at different concentration of Tween-20

S.No.	Tween-20 in %	λ_a (nm)	$\log \varepsilon$ (dm^3 mol^{-1} cm^{-1})	λ_{em} (nm)	ϕ_f Quinine hydrochloride
1.	0.000	330	5.6674	390	0.1404
2..	0.005	330	5.6812	390	0.1748
3.	0.1	330	5.7101	390	0.2545
4.	0.3	330	5.7323	390	0.4663

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Table – 3: Stokes' shift data of Quinine hydrochloride at room Temperature

S.No.	Concentration of the compound (M)	F.I.	λ_{ex} (nm)	F.I.	λ_{em} (nm)	P.M. Gain	Sensitivity Range (μ)	Stokes' shift (cm^{-1})
1.	1×10^{-6}	10	330	20	390	3	1	4662
2.	3×10^{-6}	25	330	47	390	3	1	4662
3.	5×10^{-6}	40	330	75	392	3	1	4792
4.	3×10^{-5}	5	330	9	392	1	0.3	5070
5.	7×10^{-5}	10	330	20	393	1	0.3	5135
6.	1×10^{-4}	14	330	27	393	1	0.3	5229

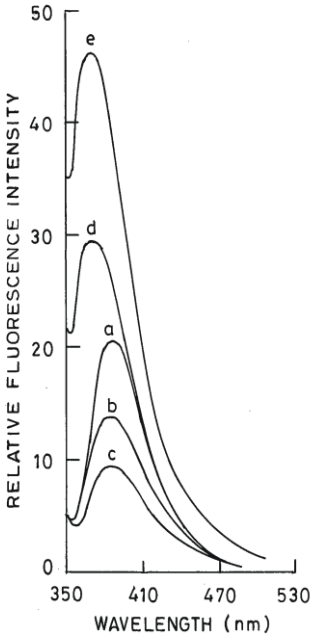

Fig. 1

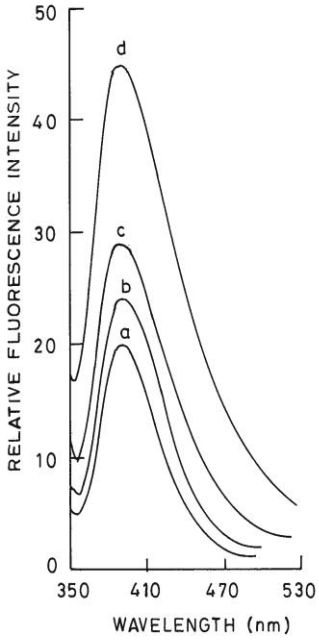

Fig. 2

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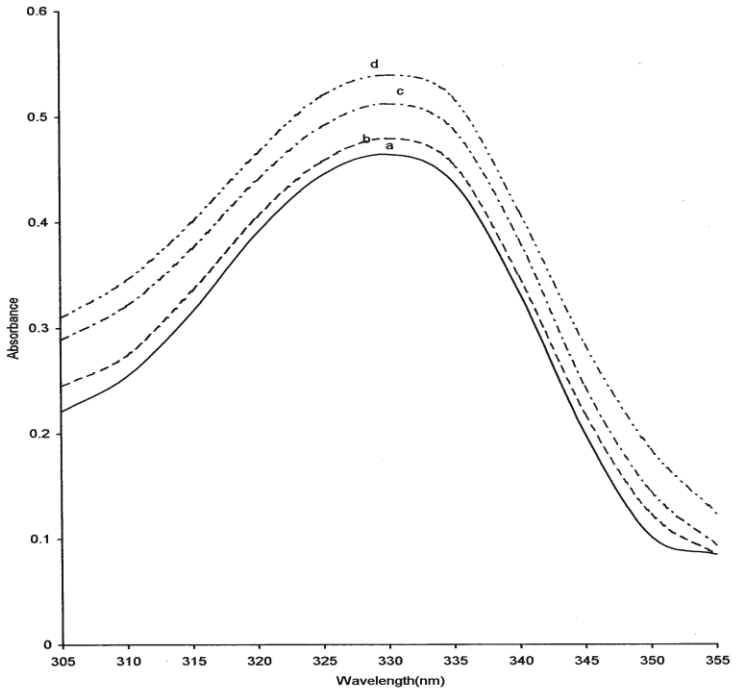


Fig. 3

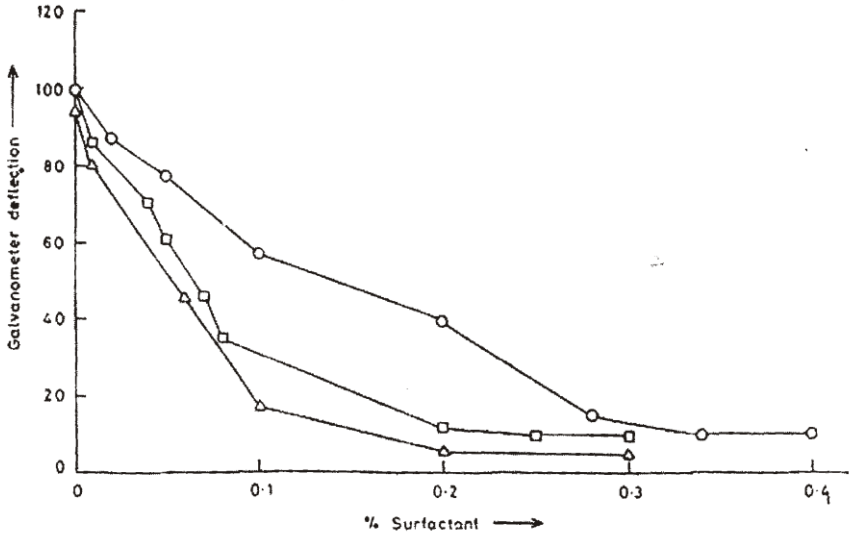


Fig. 4

Legend to Figures

Fig. 1 Influence of addition of Tween-20 on fluorescence intensity of 1×10^{-6} M QH solution

(a) No surfactant
(b) 0.005% Tween-20
(c) 0.1% Tween-20
(d) 0.3% Tween-20

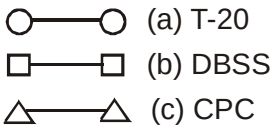
Fig. 2 Influence of addition of DBSS on fluorescence intensity of 1 x 10^{-6} M QH solution

(a) No surfactant
(b) 0.003% DBSS
(c) 0.007% DBSS
(d) 0.15% DBSS
(e) 0.4% DBSS

Fig. 3 Influence of addition of Tween-20 on absorbance of 1 x 10^{-4} M QH solution

(a) No Surfactant
(b) 0.005% Tween-20
(c) 0.1% Tween-20
(d) 0.3% Tween-20

Fig. 4 Influence of surfactants on light scattering of 1 x 10^{-6} M QH solution



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