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Spectrofluorimetric Study of 3-Hydroxy-4-Nitrobenzoic Acid in Micellar Media.

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ABSTRACT

Micellar solubilization is an effective method for dissolving hydrophobic substrates in aqueous solutions. Due to their amphiphilic nature, micelles are recognized to have an important role in a variety of processes that are studied in both fundamental and technological sciences. The present study investigated the solubilization of 3-Hydroxy-4-Nitrobenzoic Acid in micellar media of various surfactants in order to access its medicinal/ pharmaceutical potential. Fluorescence spectroscopy and absorption techniques were utilized to monitor 3-Hydroxy-4-Nitrobenzoic Acid (3-H-4-NBA) micellar solubilization. Non-ionic surfactants exhibited a higher enhancing impact on fluorescence and absorption behavior of 3-H-4-NBA. According to the findings, Tween-20 showed the greatest enhancements, which was confirmed by theoretically calculated spectral parameters, like quantum yield, empirical fluorescence coefficient, molar extinction coefficient and Stoke's shift. An attempt has been made to provide a unique format for analytical and medicinal/ pharmaceutical application of 3-H-4-NBA based on micellization and solubilization process.

Keywords: Micelles, Solubilization, 3-Hydroxy-4-nitrobenzoic Acid (3-H-4-NBA), Fluorescence.

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INTRODUCTION

Surfactants have a wide range of applications, including detergency, as an emulsifying agent, drugs, farming, improved petroleum recovery, and so on, due to their strong interfacial properties [1-4]. Polymer surfactant systems have recently received a lot of attention [5]. Surfactants potential to form colloidal-sized clusters in solutions, known as micelles, is one of their most significant properties in pharmacology since it allows them to improve the solubility of sparingly soluble drugs in water (6). From this point of view on comparing with the other options such as soluble polymers and liposomes, the use of micelles as drug carriers has several benefits. According to spectroscopic studies, different solubilized compounds are detected in different regions of micelles [7-8]. The fluorescence spectroscopy technique is useful for studying the interactions between micelles and molecules. Micelles can develop spontaneously under specific conditions and are more thermodynamically stable when it comes to dissociation and aggregation [9]. 3-Hydroxy-4-nitrobenzoic acid inhibits Ras proteins, making it a promising option for cancer treatment [10]. 3-H-4-NBA is a fluorescence aromatic compound and used as pharmaceutical intermediate. Therefore, the present fluorescence study of micellar solubilization of 3-H-4-NBA and their role in pharmacy is of paramount importance, particularly within context of their proficiency of solubilizing the hydrophobic drugs in biomimetic systems like micelles. The solubilization phenomenon has also been confirmed by absorption spectral studies. Spectral parameters, like quantum yield, molar extinction coefficient, empirical fluorescence coefficient (k_f), and Stoke's shift have been theoretically calculated which are found to be in good agreement with the experimental observations. This proves the validity of the investigation made. The present study revealed the solubilization capabilities of conventional surfactant solutions towards (3-H-4-NBA) solute, hence the process of micellization followed by solubilization of the solute would catalyse their pharmaceutical activities which may serve better results for fast drug delivery system.

MATERIALS AND METHODS

3-H-4-NBA was a sigma sample in terms of purity. The following surfactants were employed: (A) Nonionic surfactants (i) TX-100: Polyoxyethylene Tert–octyl Phenol (ii) Tween-20: Polyoxyethylene Sorbitain Monolaurate (iii) Tween-80: Polyoxyethylene Sorbitain Monooleate (B) Anionic surfactants (i) DBSS: Dodecylbenzene Sodium Sulphonate (ii) DSSS: Dioctyl Sodium Sulphosuccinate (iii) SLS: Sodium Lauryl Sulphate (C) Cationic surfactants (i) CPC: Cetylpyridinium Chloride (ii) CTAB: Cetyltrimethyl Ammonium Bromide (iii) MTAB: Myristyltrimethyl Ammonium Bromide. BDH (UK) or Sigma (USA) products were used for all of the surfactants.

All the experiments were made at room temperature (23-25^{o}C). A stock solution of 3-H-4-NBA in methanol solvent was taken. For both the fluorescence and absorption studies the concentration was maintained at $5x10^{-4}$ M and $4x10^{-4}$ M respectively. During the entire process, the concentration of 3-H-4-NBA was kept constant. The fluorescence spectrum was recorded on a synchronized Perkin-Elmer model 056 strip chart recorder, and the absorption spectra were recorded on a Systronics UV-VIS Spectrophotometer. The purity of surfactants was determined by using the drop weight technique to calculate their critical micelle concentration (CMC) value using surface tension measurement. The total fluorescence quantum efficiency values of 3-H-4-NBA were determined in comparison to anthracene solution, which was employed as a reference. Under the identical conditions, the entire range of emission was used to record the fluorescence spectrum for the reference and sample.

RESULTS AND DISCUSSION

A methanolic solution of 3-H-4-NBA showed the excitation maxima at 315 nm, whereas the emission spectrum showed a peak at 445 nm. Nonionic surfactants had the greatest influence on compound solubilization of the three types. All nonionic surfactants increased the fluorescence intensity of 3-H-4-NBA, with Tween-20 having the highest rate of enhancement in fluorescence intensity of all nonionic surfactants (Fig.1). On addition of anionic surfactants, DBSS and SLS, the fluorescence intensity initially remained constant and then decreased at higher concentration of surfactants with a hypsochromic shift of 25 nm and 5 nm respectively. While DSSS showed no marked change in fluorescence intensity. Each cationic surfactants reduced fluorescence intensity while leaving the peak location unchanged. Table-1 shows the fluorescence intensity maxima and minima for 3-H-4-NBA, with or without the interaction of all the above mentioned surfactants.

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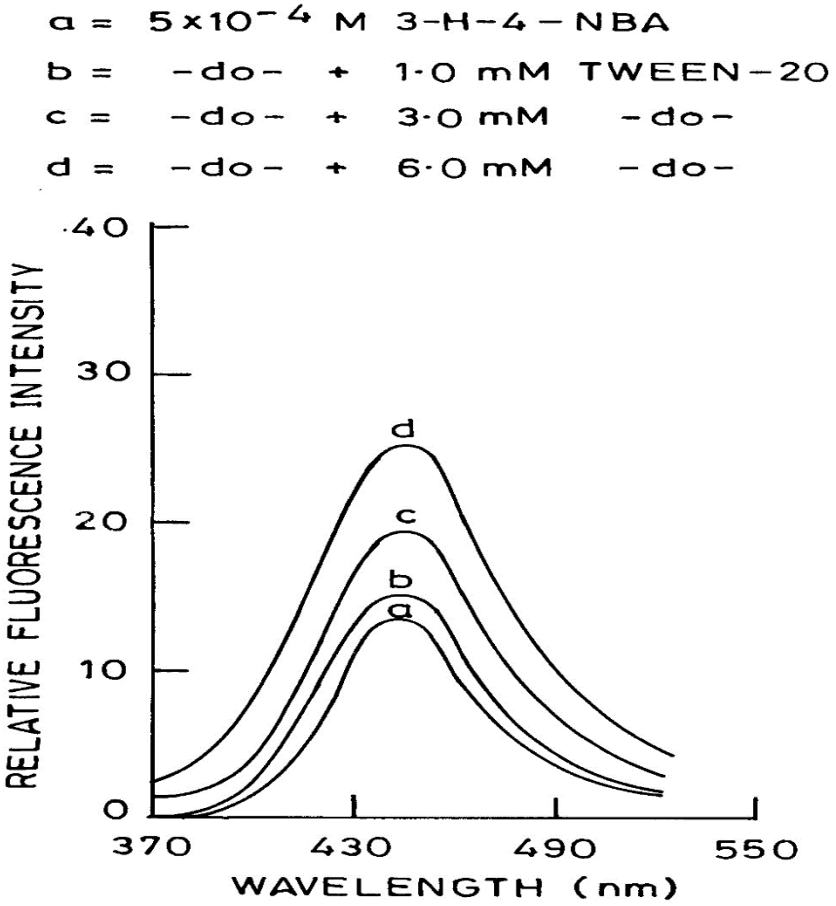

Figure 1

Table 1: Fluorescence intensity of 3-H-4-NBAin absence and presence of surfactants

λ_{ex} = 315 nm; λ_{em} = 445 nm; P.M. Gain= 3; Sensitivity Range= 3

S. No.	Name of Surfactant	Relative fluorescence intensity in absence of surfactant	Concentration of surfactant used (mM)	Relative fluorescence intensity at maximum concentration of surfactant	λ_{em} (nm)
1.	TX-100	13	7.0	16	445
2.	Tween-20	13	7.0	26	445
3.	Tween-80	13	7.0	22	445
4.	DBSS	13	7.0	9	420
5.	DSSS	13	7.0	11	440
6.	SLS	13	7.0	33	410
7.	CPC	13	1.5	0	445
8.	CTAB	13	3.0	2	445
9.	MTAB	13	3.0	2	445

The absorption spectra exhibited a peak at 275nm. A constant increase in absorbance was observed when any of the nonionic surfactants were added, with no shift in peak location. On addition of

anionic surfactants, absorbance initially decreased and then increased at their higher concentrations. DBSS and SLS showed hypsochromic shift of 10 nm in λ_{max}. For cationic surfactant, CPC absorbance spectra enhanced with 10 nm hypsochromic shift in λ_{max}, while CTAB and MTAB initially increased and then decreased the absorbance with a hypsochromic shift of 5 nm. Molar extinction coefficient (logε) calculations showed an enhancement in logε values with the increase in nonionic surfactants concentration. For anionic surfactants logε values initially decreased, then increased at their higher concentrations. For cationic surfactants, CTAB and MTAB, initially logε values increased but at their higher concentration it decreased, while CPC increased the logε values continuously. For nonionic surfactants added 3-H-4-NBA solution, quantum yield (ϕ_f) values increased on increasing the concentration. For all ionic surfactants a decrease in ϕ_f values was observed except for DSSS. The empirical fluorescence coefficient (k_f) values showed parallelism with fluorescence intensity as well as with quantum yield (ϕ_f) values. Table- 2 shows the spectral parameters (quantum yield values, empirical fluorescence coefficient values and molar extinction coefficient values) for Tween-20 added solution.

Table 2: Absorption maxima (λ_{max}), fluorescence maxima (λ_{em}), molar extinction coefficient (log ε) and quantum yield (ϕ_f) of 3-H-4-NBA at different concentration of Tween-20 (mM)

S. No.	Concentration of Tween-20 used (mM)	λ_{max} (nm)	log ε ($dm^3mol^{-1}cm^{-1}$)	λ_{em} (nm)	ϕ_f
1.	0.00	275	3.4599	445	0.0465
2.	1.0	275	3.4707	445	0.0530
3.	3.0	275	3.4882	445	0.0690
4.	6.0	275	3.4969	445	0.0926

The Stokes' shift for 3-H-4-NBA at room temperature decreased on its dilution. Table- 3 shows the calculated Stokes' shift data of 3-H-4-NBA. Non-ionic surfactants exhibited a larger enhancing effect on 3-H-4-NBA fluorescence and absorption behavior, according to the findings. Tween-20 showed the greatest enhancement, which was verified by absorbance, logε, and ϕ_f values.

Table 3: Stokes' shift data of 3-H-4-NBA at room temperature

S. No.	Concentration of Compound	λ_{ex} (nm)	F.I.	λ_{em} (nm)	F.I.	P.M. Gain	Sensitivity Range	Stokes' Shift (cm^{-1})
1.	$1x10^{-2}$ M	315	1	445	1	4	3	9274
2.	$7x10^{-3}$ M	315	3	445	1	4	3	9274
3.	$5x10^{-3}$ M	315	4	445	2	4	3	9274
4.	$3x10^{-3}$ M	315	1	445	1	3	3	9274
5.	$1x10^{-3}$ M	315	10	445	8	3	3	9274
6.	$7x10^{-4}$ M	315	13	445	11	3	3	9274
7.	$5x10^{-4}$ M	315	16	445	13	3	3	9274
8.	$3x10^{-4}$ M	315	18	440	15	3	3	9018
9.	$1x10^{-4}$ M	315	9	430	8	3	3	8490

Many properties of organic compounds alter significantly in micellar medium. The solubilizing effect of surfactant micelles can thus explain the following data. This process is most likely to be visible around a surfactant's critical micelle concentration (CMC). The greatest boost was shown in Tween-20, which can be ascribed to an increase in quantum efficiency. Because most of the other deactivation

mechanisms that compete with fluorescence have a smaller influence in non-ionic nonpolar media, the quantum yield of fluorescence is larger [11]. According to the findings, incorporating the hydrophobic solubilizate 3-H-4-NBA molecule into the nonpolar environment of Tween-20 micelle interior may be desirable. The hydrophobic character of Tweens increases with the Tween number. The order of hydrophobicity of Tween-20 is due to the nonpolar component laurate [12]. On addition of anionic surfactants, an initial constant value of emission intensity is assumed to be due to anionic nature of the compound and presence of electron withdrawing nitro- group, which decreases electron density on the benzene ring, thus there should not be any interaction with the anionic surfactants. While, the later decrease may be due to hydrophobe dilution by the increasing surfactant concentration [13]. The quenching in fluorescence intensity of 3-H-4-NBA occurred in cationic micellar media may be attributed to the electrostatic preferential interaction between the π-electrons of the solubilizate molecule and cationic head group of the surfactant which may lead to changes in the configuration of the 3-H-4NBA molecule, leading to a loss of co- planarity and a reduction in fluorescence intensity. These interactions occur in excited state of the fluorophore. Due to the presence of nucleophilic pyridine ring in the structure of CPC molecule which makes it act as quencher via a hydrogen bond between the proton donor and acceptor. This will cause the excited state's π- electrons to delocalize, resulting in fluorescence depletion [14]. According to Shizuka et al., the increase in fluorescence peak value and ϕ_f values in ionic micellar media of different surfactants strongly implies that most of the non-radiative process occur at a lower intensity in micellar systems than in water, which might be due to a decrease in the rate of intersystem crossing [15]. Absorption is less sensitive to its environment than fluorescence, hence introducing surfactants has a lower impact on absorption spectra than fluorescence spectra. The actual gap in solute's solvation energy in the ground state versus the excitation state causes hypsochromic shift in λ_{max} in micellar medium. This is explained by considering the spectra in terms of "energy levels". When the so called ground state is more polar than the excited state, a hydrophobic environment stabilizes the excited state more than the ground state. So overall there is increase in the energy gap between excited and ground state. More energy means high frequency (E= hυ) and thus lower wavelength (λ= c/υ) thus resulting in hypsochromic (blue) shift. This is the theoretical background behind this (hypsochromic shift) phenomenon. General it is often seen that more hydrophobic environment means hypsochromic (blue) shift.

In very dilute solutions of 3-H-4-NBA, the decreasing trend in Stokes' shift values may be explained by water-water hydrogen bonding interactions, which lowers the overall hydrogen donation to the solute [16].

CONCLUSION

After analyzing and comparing the data for 3-H-4-NBA, it was discovered that all of the theoretically derived spectral parameters agree well with the experimental results. This proves the validity of the investigations made. As a result, the current fundamental work can be applied to a better understanding of the assimilation of several critically important medications in the human body by phospholipids, which operate as micelles in physiological fluids. Micellization followed by solubilization of the 3-H-4-NBA substrate would accelerate drug delivery process with the possibility of increasing water solubility of poorly soluble drugs, improving bioavailabity, reducing toxicity and other effects, enhancing permeability across the physiological barrier and substantial change in drug distribution. Micellar solubilization is widely used in biochemical and medicinal research.

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