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Protocols

Significant bile salt induced perturbation of niosome membrane: A molecular level interaction study using 1-Naphthol fluorescence

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ABSTRACT

This study demonstrates that significant perturbation of tween20:cholesterol(1:1) niosome membrane takes place even at premicellar concentration of bile salts. Here, 1-naphthol (1-NpOH), a known and sensitive excited state proton transfer (ESPT) probe, was used to understand the nature of perturbation of the membrane in an unbuffered medium. The significant decrease in 1-NpOH fluorescence intensity in niosome-bile salt mixed system at both lower (10 °C) and higher (50 °C) temperatures indicates the bile salts [sodium cholate (NaC) and sodium deoxycholate (NaDC)] induce perturbation of niosome membranes. Variations in the fluorescence lifetime values of both the prototropic emissions (neutral and anionic species) along with the proton transfer rate of 1-NpOH confirm the bile salts perturb up to the hydrophobic core domain of the niosomal membranes. Bile salts induce size change of the niosomal membrane is confirmed through dynamic light scattering study.

1. Introduction

Bile salts are physiological surfactants synthesized in the human intestine [1]. It comprises a steroidal skeleton and an ionic head group. The steroidal skeleton is large, rigid and planar [2,3]. It has two different faces, i.e., one site is hydrophilic while the other is hydrophobic. Because of this amphiphilic nature, it assembles together to form different types of aggregates. Dimers are formed at lower concentration whereas higher order micellar aggregates are formed at higher concentration. Cholate and deoxycholate conjugate with the amino acid taurine or glycine to form their analogous cholates and deoxycholates in the human body [2,4]. The critical micellar concentration (CMC) value is reported as 3–19 mM for sodium cholate (NaC) whereas 2–10 mM for sodium deoxycholate (NaDC) [5]. The physiological importance of bile salts lies in their ability to solubilize and emulsify cholesterol, bilirubin, lecithin, and fat-soluble vitamins in mammalian intestines [6,7]. These solubilizing and emulsifying capacities enable them as subtract carrier for medicines, cosmetics and several other chemicals [8]. Bile salts are considered than other conventional surfactants because of the presence of both hydrophobic and hydrophilic surfaces which provides facial polarity to the molecules [9]. On the other hand, for a conventional surfactant, there is a polar head group and a nonpolar tail part [10].

Niosomes are colloidal particles made of nonionic surfactant and cholesterol. The structure of the niosomes is similar to liposomes having a concentric bilayer surrounding an aqueous compartment. They are more biocompatible, nontoxic and stable than liposomes. Niosomes act as solubilizer of both hydrophobic and hydrophilic substrates due to the presence of hydrophobic bilayer domain and aqueous compartment [11–14]. Different niosomal formulations have been modified using additives to improve the drug delivery efficiency and their chemical stability [15–18]. Nonionic surfactant and bile salt assemble together to form biolosomes in the presence and absence of cholesterol [15,16]. Yuksel and his co-workers prepared niosomes from nonionic surfactant (span 60), pluronics (PF127, L64 and P85) and charging agent [dicetyl phosphate (DCP), stearylamine (SA)] having different compositions. Niosomes prepared from span 60 and DCP/SA is stabilized by bile salt as compared to pluronics niosome. This conclusion is drawn from the turbidity change of the niosomal suspension [17]. The optimum percentage of sodium cholate and deoxycholate in the tween20:cholesterol:bile salt niosomes prepared in buffered solution (pH 6.8) were 2:1:2 and 2:1:3 for highest entrapment efficiency as reported by Wagh et. al [18]. With increase in bile salt concentration (i.e after these optimized concentrations) the entrapment efficiency was found to decrease due to the formation of micelle or mixed micelle [18]. Literature survey indicates that there is no systematic study available to understand the effect of bile salt on niosome membrane, although the interaction between different bile salts and phospholipid bilayers is well explored [19–26]. Bile salts form different types of aggregates with phospholipid vesicle depending upon their concentration, i.e., below

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CMC vesicle-bile salts monomer systems are formed whereas above CMC mixed vesicle-mixed micelle systems are formed. The mixed vesicles become solubilizes with further increase in bile salt concentration [19–21].

Both bile salts and niosomes are two biologically important systems so the interaction between them has significant importance. The present study was under taken to understand the effect of bile salts on tween20:cholesterol(1:1) niosome membrane using dynamic light scattering study and fluorescence properties of 1-NpOH. Among other fluorescent molecular probes we have chosen 1-NpOH due to its multi prototropic emission and distributive nature. ESPT process results in the origination of multiple emission i.e neutral emission (λ_{em} = ~ 360 nm) and anionic emission (λ_{em} = ~ 460 nm). Neutral emission is known to originate from hydrophobic environment whereas anionic emission originates from hydrophilic environment [20,21,27,28]. This distributive nature enables its use as an efficient probe to understand the microenvironmental changes of different organized media with variation in temperature and in presence of additives, as reported by Mishra and his co-workers [20,21,28]. Swain has studied the effect of bile salts (NaC and sodium taurodeoxycholate (NaTC)) on pluronic F127 using the fluorescence intensities and fluorescence lifetime values of 1-NpOH [29]. Bhattacharyya and his co-workers used 1-NpOH as a fluorescent molecular probe to understand the polymer-surfactant interaction [30]. Like other organized media (lipid bilayer membrane, pluronic F127, polymer-micelle mixted system), 1-NpOH is also sensitive towards the temperature and sodium dodecyl sulfate (SDS) induced microenvironmental changes of tween20:cholesterol(1:1) niosome membrane as reported in our previous studies [31,32]. The primary objective of this study is to understand the effect of biosurfactants (NaC and NaDC) on tween20:cholesterol(1:1) niosome membrane in unbuffered media using the fluorescence properties (fluorescence intensity and fluorescence lifetime values) of the well-known membrane-hydration sensing ESPT probe 1-NpOH [20,21,28], as bile salts are known to significantly increase membrane hydration levels. The choice of unbuffered media is to understand the effect of concentration-dependent natural aggregation of bile salts on niosome membranes, without any constraint imposed by buffered media on the aggregation behavior of bile salts (Scheme 1).

2. Materials and methods

Tween20 (TW20) (Merck Chemical), Cholesterol (95 %, Alfa Aesar), NaDC (99 %, SRL), NaC (98 %, SRL) were purchased and used as received. 1-NpOH (GR grade) purchased from SRL, India, was purified by sublimation and used after checking its purity. All spectroscopic grade solvents were used for the experiments. For the preparation of experimental solution triple-distilled water was used which was prepared by using alkaline permanganate solution and sodium hydroxide.

2.1. Niosome preparation

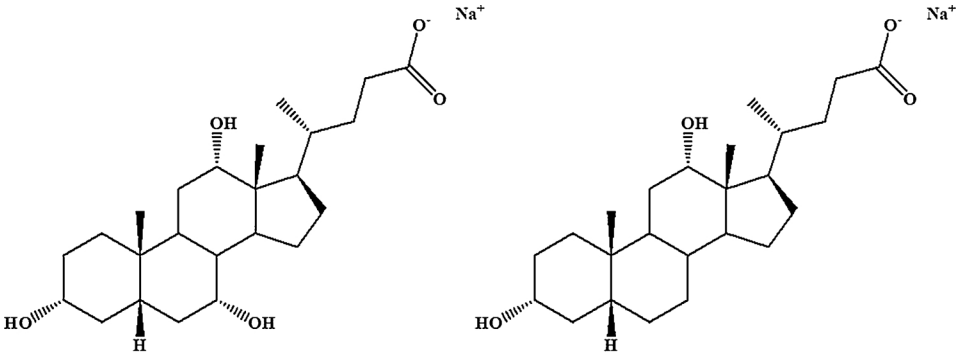
In this study, TW20:cholesterol(1:1) niosomes were prepared by solvent evaporation method [14,31,33–37]. TW20 and cholesterol (concentration 1.25 mM) were dissolved in chloroform:methanol mixture (2:1). The solvent was evaporated by using a rotary evaporator and the residual solvent present, if any was removed by keeping the round bottom flask in vacuum for 1 h. Niosome solution was prepared by adding appropriate volume of water, to the lipid film with vigorous vortexing above 60 °C. The dispersion was then sonicated for 10 min at 60 °C using a probe-sonicator. After sonication the solution was centrifuged to remove the larger vesicles. All the experiments were performed with freshly prepared solution of niosome and fluorescent probes.

2.2. Incorporation of bile salts

The stock solutions of bile salts were prepared in triple-distilled water. The experimental solutions were prepared by adding the required volume of bile salt stock to the niosome solution at 60 °C in order to achieve the final concentration of bile salts. The solutions were equilibrated for 2 h before carried out the experiment. All the experiments were carried out with freshly prepared solutions.

2.3. Methods

Malvern Zeta-sizer nano series instrument was used for Dynamic light scattering (DLS) analyses. The excitation wavelength was 632.8 nm and scattering angle 90°. Philips CM12 120 kV instrument was used for Transmission electron microscopy (TEM) imaging. The niosomal dispersion was drop cast on a carbon-coated copper grid and allowed to dry before capturing the TEM images. Fluoromax-4 fluorescence spectrophotometer was used for the measurement of fluorescence intensity. Temperatures (10 and 50 °C) were maintained by circulating water through a jacketed cuvette holder from a refrigerated bath (JULABO, Germany). The fluorescence lifetime measurement was carried out using Horiba Jobin Yvon TCSPC (time correlated single photon counting) lifetime instrument where 295 nm nano-LED was used as light source for the experiment. The pulse repetition rate was set to 1 MHz, and the pulse width was ~1.1 ns for 295 nm LED. The detector response time is less than 1 ns. Instrument response function was collected using Ludox AS40 colloidal silica which acts as a scatter. The decay data were analyzed using IBH software. The value of χ^2, between 0.99–1.45 and symmetrical distribution of residual was considered as a good fit. The average fluorescence lifetime (τ_{avg}) values were calculated by the following Eq. (1) [39,40]



Scheme 1. Chemical structure of **(A)** sodium cholate (NaC), **(B)** sodium deoxycholate (NaDC).

$$\tau_{avg} = \left(\sum_{i=1}^{n} \alpha_i \tau_i\right) / \left(\sum_{i=1}^{n} \alpha_i\right) \quad (1)$$

Where, τ_i represents the individual lifetime with corresponding amplitude α_i.

3. Results and discussion

3.1. Structural characterization of niosomes: DLS and TEM studies

The characterization of the niosomes is done using DLS and TEM imaging techniques. Fig. S1 A represents the DLS histogram plot whereas Fig. S1 B represents the TEM images of niosomes. Niosomes are having nanometer size and spherical shape as observed from Fig. S1. The size of the niosomes obtained from both the techniques is different as both the measurements (DLS and TEM) are done under very different conditions. The sizes reflected in DLS, taken in aqueous suspensions, reflect the actual size distribution of niosomes. Sample preparation for TEM involves the step of drying. Thus although information on the overall morphology of the vesicles are obtainable, the data do not correlate well with niosome sizes. The size of the niosomes (~100 nm) correspond well with the size (100–200 nm) reported in literature [10,31,33–37].

4. Effect of bile salts on niosome membrane

4.1. Effect of Solution pH (aqueous solution of bile salt) on Niosome Membrane and ESPT Dynamics of 1-NpOH

The bile salt aggregation behavior is known to change under the constraint of a constant buffer condition (PBS) since the natural tendency of a bile salt (salt of a weak acid and a strong base) to hydrolyze and to form neutral acid and to change aggregation behavior gets prevented [38]. A study of the effect of natural bile salt aggregation on niosomal membranes can only be studied in unbuffered media. The pH of the bile salt aqueous solution varies from ~7.1–8.3 with increase in NaC concentration from 0 to 20 mM whereas it changes from ~7.1 to ~7.4 for NaDC in a concentration range 0–10 mM. With regards to the effect of pH change from 7.1–8.3 on niosome membrane, the corresponding niosome suspension was prepared in buffer solution having pHs 7.1 and 8.3 in the absence of bile salt for fluorescence measurements. The emission behavior of 1-NpOH in distilled water (pH 7.1) and phosphate buffer saline (pH 8.3) is represented in Fig. S2 A and the corresponding emission spectra in niosome suspension is represented in Fig. S2 B. It is observed that pH change does alter neither the ESPT behavior of 1-NpOH nor the structure of niosomes. Thus 1-NpOH as a fluorescent probe is well suited to help understanding the effect of the natural bile salt aggregation in unbuffered media on niosomal membranes.

4.1.1. DLS study

Additives (like sodium dodecyl sulfate, glucose) inducing size change of the niosomal membrane have been studied using DLS [32,41]. Here, Fig. 1 represents the DLS spectra of niosomes in the presence and absence of bile salts. The diameter of the niosome increases ~ 20 nm in the presence of both NaC (20 mM) and NaDC (10 mM). The increase in diameter indicates the bile salt induced size changes of the niosomal membrane. To understand the effect of bile salts (NaC and NaDC) on niosome membrane at a molecular level we have done both steady-state and time- resolved fluorescence studies using 1-NpOH fluorescence properties, which are discussed as follows.

4.1.2. Fluorescence intensity study

Fig. 2 represents the normalized fluorescence emission spectra of 1-NpOH in water, different bile salts micellar solution, niosome membrane and niosome-bile salt mixed system at 10 °C and 50 °C. In this study, all the experiments were performed at two temperatures (i.e 10 and 50 °C) only because there was no significant change observed in the spectral profile in a narrow temperature range (≤ 2 °C) for this formulation of niosomes [31]. This observation strongly implies the absence of thermotropic phase change behavior of TW20:cholesterol (1:1) niosome due to the presence of cholesterol which is known to smother the thermotropic phase transition temperature. The temperature, however had perceptible effect on the hydration level of the niosomal interface hence we have chosen two temperatures (10 and 50 °C) and focused on the effect of bile salts which are known to significantly disturbed membrane hydration [20,21]. In pure water, 1-NpOH gives only anionic emission (λ_{em} = 460 nm) whereas in different micellar solution (NaC, NaDC) it gives neutral emission (λ_{em} = 360 nm) along with its anionic emission. The intensity of the neutral emission in different micellar solution follows the same trend both at lower and higher temperatures (Fig. 2**A** and B). In case of niosome-bile salt mixed system, the neutral form intensity decreases in the presence of both NaC and NaDC (Fig. 2**C, D**). The anionic form intensity is known to originate from water accessible environment whereas neutral form intensity originates from water deficient environment [20,21,28,31]. In our recent study, we have reported that 1-NpOH neutral species (NpOH*) are distributed in the core and interfacial domain whereas anionic species (NpO^-*) are distributed in the interfacial domain and bulk water phase of niosome membranes [31]. So the change in NpOH* intensity can be related with the bile salt induced niosomal membrane change, which is further examined through temperature dependent fluorescence intensity and fluorescence lifetime studies, as discussed later.

4.1.3. Temperature dependent emission behavior of 1-NpOH in Niosome Membranes with variation in NaC and NaDC concentrations

The emission spectra of 1-NpOH in niosome membranes with increasing concentrations of NaC and NaDC are represented in Figs. S3 and S4. The neutral form intensity decreases with increase in both NaC and NaDC concentrations at 10 °C and 50 °C (Fig. 3). The corresponding control study i.e. emission spectra of 1-NpOH in pure NaC and NaDC solutions with increasing bile salt concentrations at 10 and 50 °C are represented in Figs. S5 and S6. *It is interesting to see that the NpOH* fluorescence intensity increases with increase in NaC concentration as well as NaDC concentration which is opposite to the trend observed for NpOH* fluorescence in niosome suspension.* This anomalous behavior indicates that the bile salts induce perturbation to the niosome membrane. Partitioning of the NaDC bile salt (hydrophobicity index 0.72) into the niosome membrane is more than NaC (hydrophobicity index 0.13) which is also reflected in sharper decline of NpOH* intensity (Fig. 3).

4.1.4. Discussion

Fluorescence intensity of NpOH* in micellar solutions (NaC and NaDC solutions) is very weak as compared to its intensity in the niosome membranes. The low intensity of NpOH* emission in micellar solution and the reverse trend in the emission behavior of NpOH* both in the micellar solution and niosome membrane with variation in NaC and NaDC concentrations imply that there is no contribution of unpartition 1-NpOH present in micellar bulk phase of niosomes towards the variation in the NpOH* intensity with increase in NaC and NaDC concentrations [20,21]. NpOH* emission is known to originate from hydrophobic environment, so the significant decrease in the NpOH* intensity with variation in both NaC and NaDC concentrations indicates the increase in medium hydrophilicity of the niosome membrane. This finding is further confirmed through fluorescence lifetime study.

4.1.5. Fluorescence lifetime study

4.1.5.1. Fluorescence lifetime study of 1-NpOH in Niosome Membranes with variation in NaC concentration. Fluorescence lifetime values of both the prototropic emissions of 1-NpOH are sensitive towards the bile salts induced change in the lipid bilayer membrane and pluronic hydrogel

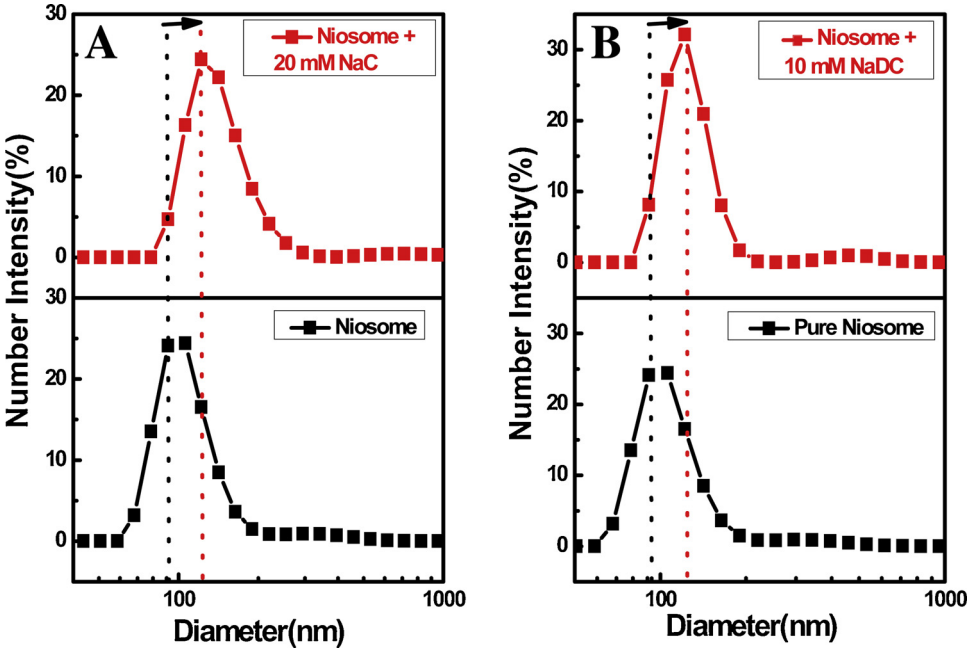


Fig. 1. DLS plot of niosomes in the absence and presence of NaC **(A)**, and NaDC **(B)** at room temperature, niosomal diameter increases in presence of both NaC and NaDC, [NaC] = 20 mM, [NaDC] = 10 mM.

[20,21,29]. Fluorescence lifetime values of 1-NpOH decrease with increase in hydration [20,21,28,29]. Fluorescence lifetime decays and corresponding lifetime values of NpO^{-}* in niosome membranes with increase in NaC concentration at 10 °C and 50 °C are represented in Fig. S7 and Table 1. It follows bi-exponential decay in the absence and presence of NaC. There is a significant decrease in the longer lifetime value whereas the variation is not much significant for shorter component. The longer lifetime component is known to originate from interfacial domain whereas the shorter component originates from bulk water domain of niosomal membrane [31]. A control study (fluorescence lifetime study of NpO^{-}* in NaC solution with increasing concentration of NaC) has been made to understand the slight decrease in the shorter lifetime component of NpO^{-}* in niosome membrane with variation in NaC concentrations. Fluorescence lifetime decay and

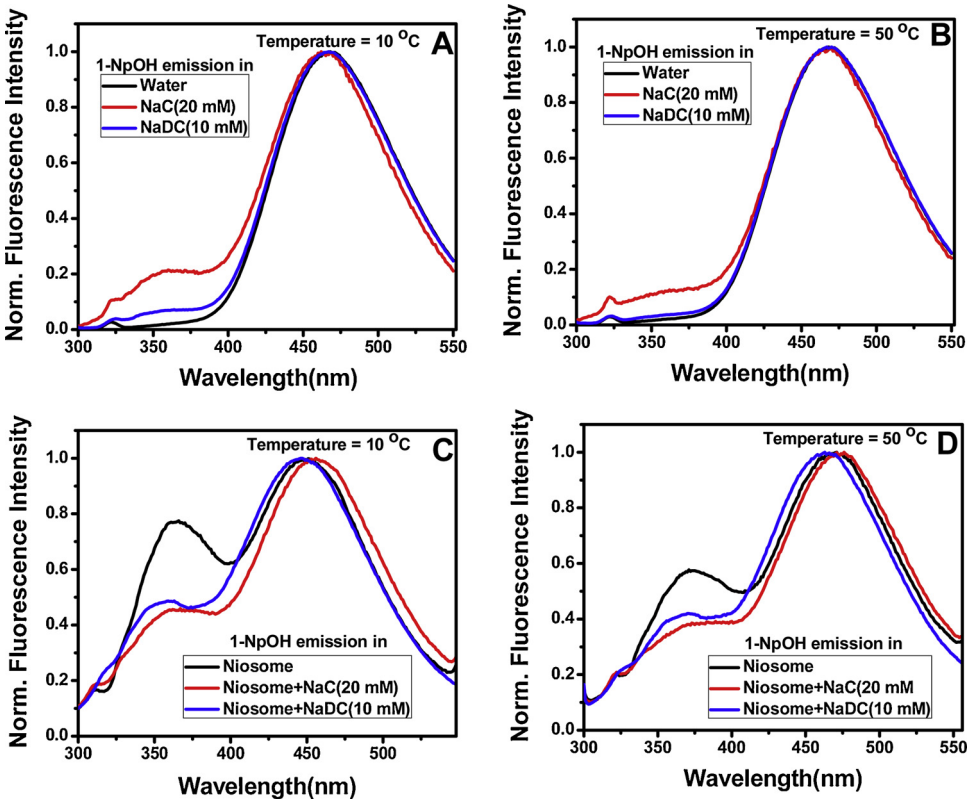


Fig. 2. Normalized emission spectra of 1-NpOH in water and micelle media at **(A)** 10 °C, **(B)** 50 °C and in niosomes and niosome-bile salt mixed system at **(C)** 10 °C, **(D)** 50 °C, [1-NpOH] < 4 μM, [NaC] = 20 mM, [NaDC] = 10 mM, λ_{ex} = 290 nm, slit width = 5/5 nm.

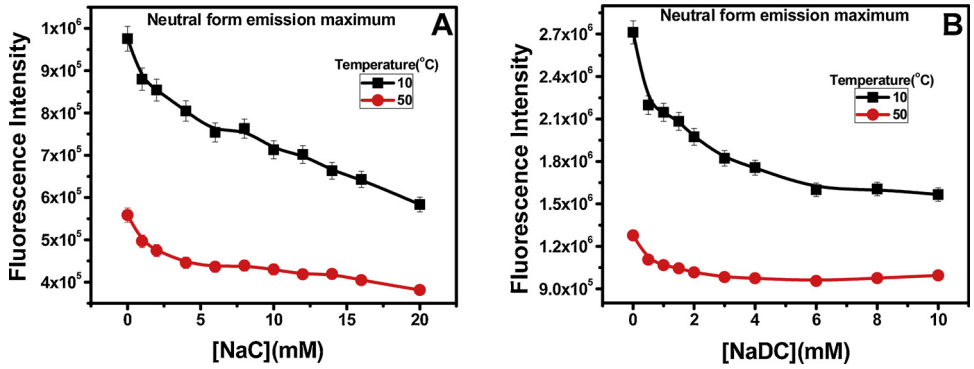


Fig. 3. Point plot of the emission maximum of NpOH* (360 nm) with increasing NaC **(A)** and NaDC **(B)** concentrations at 10 °C and 50 °C, λ_{ex} = 290 nm, [1-NpOH] < 4 μM, slit width = 5/5 nm, error = ± 3 %.

Table 1
Fluorescence lifetime values of NpO^-* in niosome membranes with increase in NaC concentration at 10 °C and 50 °C, λ_{ex} = 295 nm, λ_{em} = 460 nm, [1-NpOH] < 4 μM, error = ± 5 %.

Temperature = 10 °C, λ_{ex} = 295 nm, λ_{em} = 470 nm				
[NaC](mM)	τ_1,α_1	τ_2,α_2	τ_{avg}	χ^2
0	$8.0_1(18)$	$16.9_7(82)$	15.3_5	1.33
1	$7.9_5(17)$	$16.0_5(83)$	14.6_7	1.22
2	$7.8_0(17)$	$15.9_2(83)$	14.5_3	1.28
4	$7.6_4(20)$	$15.2_5(80)$	13.7_2	1.22
6	$7.5_8(22)$	$15.0_4(78)$	13.3_9	1.08
8	$7.4_6(23)$	$14.5_5(77)$	12.9_1	1.18
10	$7.3_1(25)$	$14.4_8(75)$	12.6_8	1.13
12	$7.0_1(29)$	$13.9_0(74)$	12.1_0	1.10
14	$7.0_1(29)$	$13.8_4(71)$	11.8_5	1.07
16	$6.9_0(29)$	$13.2_1(71)$	11.3_7	1.03
20	$6.7_0(30)$	$12.6_7(70)$	10.8_7	1.20
Temperature = 50 °C, λ_{ex} = 295 nm, λ_{em} = 470 nm				
[NaC](mM)	τ_1,α_1	τ_2,α_2	τ_{avg}	χ^2
0	$7.1_3(47)$	$11.6_2(53)$	9.5_0	1.09
1	$7.3_0(47)$	$11.0_5(53)$	9.2_8	1.06
2	$7.2_8(48)$	$10.8_1(52)$	9.1_1	1.35
4	$7.1_2(48)$	$10.4_8(52)$	8.8_6	1.13
6	$7.1_6(48)$	$10.0_2(52)$	8.6_4	1.03
8	$7.2_9(55)$	$10.0_3(45)$	8.5_2	1.10
10	$7.2_0(56)$	$9.8_5(44)$	8.3_6	1.31
12	$7.1_6(58)$	$9.6_7(42)$	8.2_1	1.28
14	$6.8_3(60)$	$9.9_5(40)$	8.0_7	1.11
16	$6.7_4(60)$	$9.9_4(40)$	8.0_2	1.22
20	$6.6_5(62)$	$9.9_4(38)$	7.9_0	1.32

corresponding lifetime value of NpO^-* in NaC solution with increasing NaC concentrations are represented in Fig. S8 and Table S1. NpO^-* follows mono-exponential fitting having a single lifetime value ~ 8 ns in water and also in presence of NaC. There is no change in the lifetime value with increasing concentration on NaC from 0 mM to 20 mM, which indicates that there is no contribution of unpartition 1-NpOH towards the slight variation in the shorter lifetime component of NpO^-* in niosomes. It is expected that NaC-TW20 mixed micelle may be responsible for this variation, as there is some free TW20 also present in the niosomal suspension. The decrease in the longer lifetime component may be attributed to the NaC induced interfacial hydration of the niosome membrane as the presence of water in membrane influences the prototropic lifetimes of 1-NpOH. There is an increase in the relative amplitude of shorter component along with a decrease in the relative amplitude of longer component both at 10 and 50 °C. Like NpO^-*, NpOH* also follows bi-exponential fitting in niosome membrane in the absence and presence of NaC (Fig. S9, Table S2). The shorter lifetime value is known to originate from the interfacial domain whereas the longer lifetime value originates from the core domain [31]. There is a decrease in both shorter and longer lifetime values with increase in NaC concentration. The relative amplitude of shorter lifetime component decreases along with an increase in the relative amplitude of longer lifetime component. The decrease in the lifetime value indicates that NaC induced perturbation of niosome membrane [20,21]. The formation of mixed micelle between NaC and niosome membrane may result in the variation in fluorescence lifetime values of 1-NpOH neutral species with increase in NaC concentrations.

The variation pattern of the relative amplitudes of both NpO-* and NpOH* indicates that there is redistribution of 1-NpOH molecules between different domains of niosomal membrane. The decrease in the fluorescence intensity and average lifetime value of NpOH* with increase in NaC concentration at 10 °C and 50 °C are represented in Fig. 4.

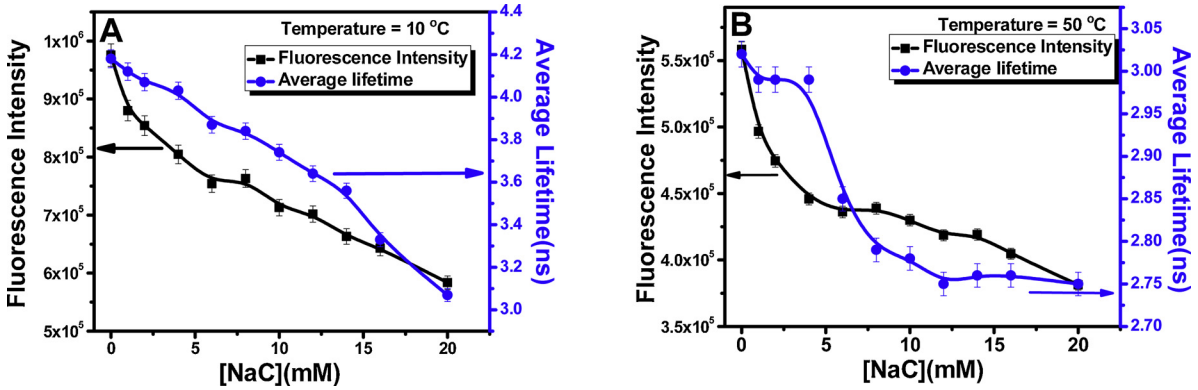


Fig. 4. Variation in steady state fluorescence intensity and average lifetime value of NpOH* in niosome membrane with variation in NaC concentration at **(A)** 10 °C, **(B)** 50 °C, λ_{ex} = 295 nm, λ_{em} = 360 nm, [1-NpOH] < 4 μM, error = ± 5 %.

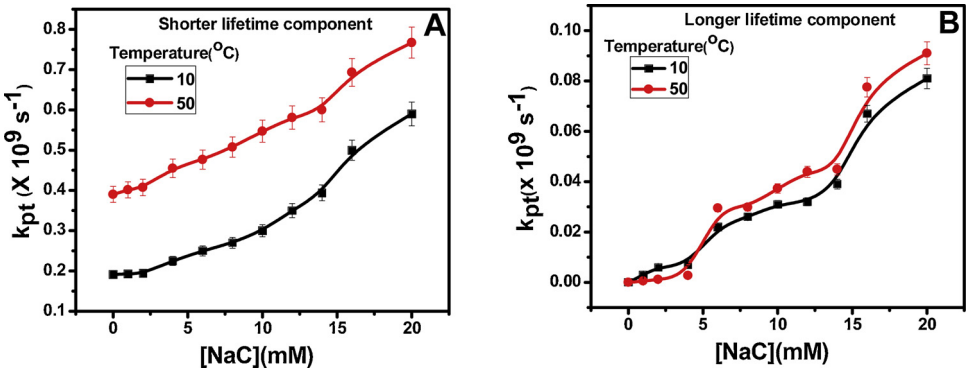


Fig. 5. Variation in ESPT rate constant (k_{pt}) of NpOH* in niosome membrane with increase in NaC concentration for shorter lifetime component **(A)** and longer lifetime component **(B),** [1-NpOH] < 4 μM, error = ± 5 %.

It indicates the NaC induced perturbation both at interfacial and core domains of the niosome membranes. The availability of water molecules around NpOH* present at different domains of the niosome membrane is estimated by calculating the proton transfer efficiency using Eq. 2 [21,42]. The proton transfer efficiency is calculated using both shorter and longer lifetime components of NpOH*. It increases with increase in NaC concentration (Fig. 5). This indicates the availability of water molecules around 1-NpOH in niosomes in presence of NaC.

$$kpt = \frac{1}{\tau} - \frac{1}{\tau o} \quad (2)$$

here k_{pt} is the ESPT rate constant and τ_o represents the lifetime value of 1-NpOH in the absence of ESPT process. The longer lifetime component of NpOH* originates from membrane core region is considered as τ_o for the calculation of k_{pt} with variation of temperature.

4.1.5.2. Non-extensive distribution analysis of lifetime data. The fluorescence lifetime distribution data and the corresponding distribution graphs of

NpO^-* and NpOH* in niosome membrane with variation in NaC concentrations at 10 °C and 50 °C are represented in Tables S3, S4 and Figs. S10–S13. The full width half maximum (FWHM) values of both shorter and longer lifetime components decrease with increase in NaC concentrations both at 10 °C and 50 °C which suggest the decrease in medium heterogeneity both at interface and bulk water domain (Tables S3 and S4). NaC induced perturbation of the niosome membrane is further supported by the distribution data.

4.1.5.3. Fluorescence lifetime study of 1-NpOH in Niosome Membrane with variation in NaDC concentration. The fluorescence lifetime decays and corresponding lifetime values of NpO^-* with increase in NaDC concentration at 10 °C and 50 °C are represented in Fig. S14 and Table S5. It follows bi-exponential decay having two lifetime values in niosome membranes with and without NaDC. A control study has been made (fluorescence lifetime study of 1-NpOH in NaDC micellar solution) to understand the slight decrease in the shorter lifetime component in niosome membrane with variation in NaDC concentrations. Fluorescence lifetime decay and the corresponding lifetime value of NpO^-* in NaDC solution with increasing concentration of NaDC are represented in Fig. S15 and Table S6. It follows mono-exponential fitting having a lifetime value of ~ 8.6 ns at 10 °C and ~ 7.5 ns at 50 °C. It can be concluded that there is no role of unpartition 1-NpOH in the variation pattern of the smaller lifetime component of NpO-* in niosome membrane. NaDC-TW20 mixed micelle may be responsible for this slight variation in lifetime value. Like NpO-*, NpOH* also follows bi-exponential fitting in niosome membrane with and without NaDC. The lifetime decay and the corresponding lifetime values are represented in Fig. S16 and Table S7. Both the shorter and longer lifetime values decrease with increase in NaDC concentrations both at 10 °C and 50 °C. As the lifetime values of NpOH* is significantly sensitive towards the presence of water molecules this variation pattern indicates the NaDC induced perturbation followed by hydration both at the interface and core domains of the niosome membrane. The shorter component is known to originate from interface whereas longer component originates from hydrophobic core domain. Another interesting observation is that the relative amplitude of shorter component decreases along with an increase in the relative amplitude of longer component both at 10 °C and 50 °C (Table S7). Redistribution of 1-NpOH molecules between core and interfacial domain of the niosomal membrane results in varied relative amplitude values. Both the fluorescence intensity and average lifetime value of NpOH*

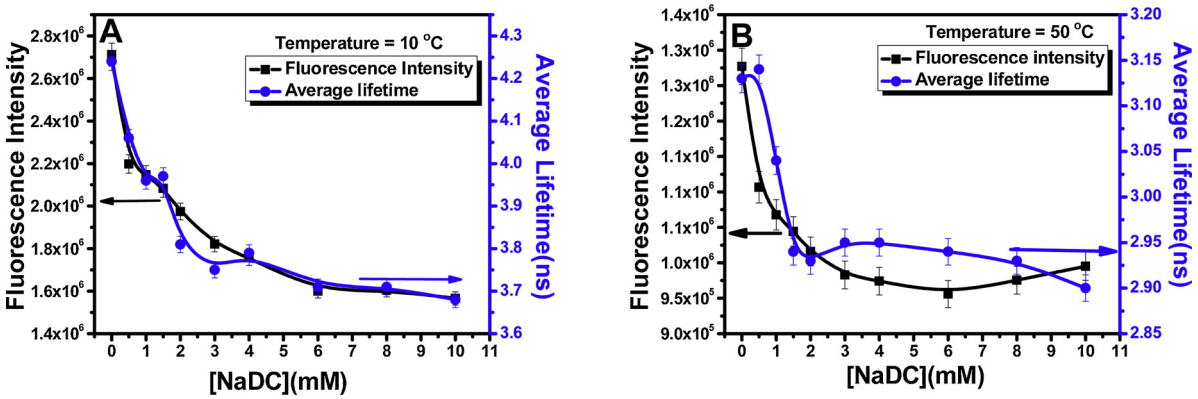


Fig. 6. Plot of steady state fluorescence intensity and average lifetime value of NpOH* in niosome membranes with increase in NaDC concentration at **(A)** 10 °C, **(B)** 50 °C, λ_{ex} = 295 nm, λ_{em} = 360 nm, [1-NpOH] < 4 μM, error = ± 5 %.

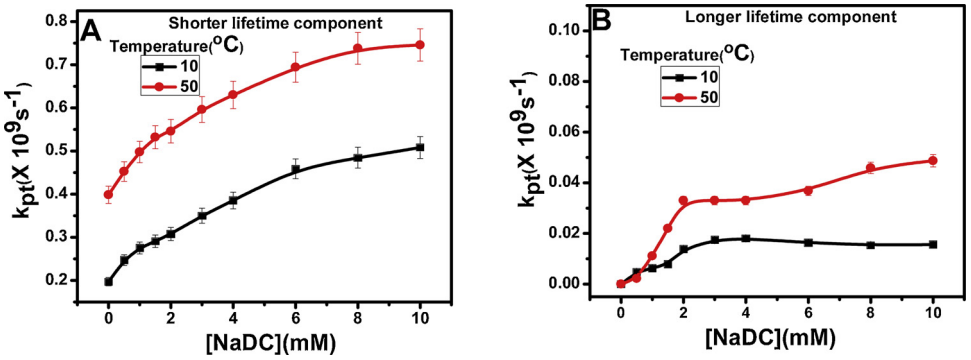


Fig. 7. Variation in the ESPT rate constant (k_{pt}) of NpOH* in niosome membrane with increase in NaDC concentration for shorter lifetime component **(A)** and longer lifetime component **(B),** [1-NpOH] < 4 μM, error = ± 5 %.

decrease with increase in NaDC concentrations as represented in Fig. 6. The sharper decrease in the both fluorescence intensity and average lifetime value of NpOH* indicates the more partitioning of NaDC into the niosome membrane as compare to NaC. The proton transfer efficiency is calculated using both the shorter and longer lifetime values of NpOH* emission and observed that k_{pt} rate increases with increase in NaDC concentrations both at 10 and 50 °C (Fig. 7). Like NaC, NaDC also disturbed the niosomal membrane both at interface and core domains with higher efficiency.

4.1.5.4. Non-extensive distribution analysis of lifetime data. The distribution data and the corresponding distribution plot of NpOH* and NpO-* in niosome membrane with variation in NaDC concentration at 10 °C and 50 °C are presented in Tables S8, and S10, and Figs. S17–S20. Like NaC, NaDC also disturbs the niosome membrane both at the interfacial domain and core domain. Here also the decrease in FWHM values of both shorter and longer lifetime components suggest that heterogeneity of the niosome membrane decreases with increase in NaDC concentrations.

5. Conclusions

This study reports the interaction of physiologically important unconjugated bile salts (NaC and NaDC) with niosome membranes in an unbuffered medium using the fluorescence properties (fluorescence intensity and fluorescence lifetime value) of 1-NpOH and DLS study. The decrease in fluorescence intensity of 1-NpOH neutral species in niosome membranes with increasing bile salts concentration (from premicellar to post micellar range) at 10 and 50 °C indicates the bile salt induce significant perturbation to the niosomal membrane both at premicellar and postmicellar concentration range. Variation in the fluorescence lifetime values along with the non-extensive distribution analysis confirm that bile salts induced perturbation both at the interface and core domain of the niosome membranes. The perturbation induced by NaDC bile salt on niosome membrane is found to be more than in the case of NaC. This finding may be useful in many biological studies, as bile salts and niosomes are pharmaceutically important.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.colsurfb.2019.110594.

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