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An investigation of Pluronic P123-Sodium cholate mixed system: Micellization, Gelation and Encapsulation behavior

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Abstract

The effect of Sodium Cholate (NaC) on the micellization and gelation characteristics of Pluronic P123 in aqueous media have been explored using tensiometry, rheology, dynamic light scattering (DLS), and densitometry. The aggregation characteristics were altered drastically with the addition of NaC as signified by increase in critical micelle concentration (CMC), critical micellization temperature (CMT) and critical gelation temperature (CGT). The results were explained on the basis of electrostatic and steric destabilization of P123 micelles by cholate. The apparent hydrodynamic diameter (D_H) of P123+NaC binary systems decreased upon addition of NaC upto 1.5wt%. Further, in addition to an increase in D_H , presence of two types of scattering species was also evidenced with increase in NaC concentration from 2.5-10wt%. The effect of NaC on the encapsulating capacity of P123 was also studied using naproxen and pyrene as two model hydrophobes. The work could give a sound understanding of the interaction and self-assembling behavior of Pluronics with the important physiological component i.e, bile salts which is important to consider in any pharmaceutical formulation involving Pluronics as drug delivery agent.

Keywords: Gelation; Micellization; CMC; CMT; CGT.

INTRODUCTION:

Non-ionic amphiphilic triblock copolymers of type ABA with poly(propylene oxide) PPO as the hydrophobic central block and poly(ethylene oxide) PEO as the hydrophilic terminal blocks are commercially known as Pluronics or Poloxamers. Due to their amphiphilic character, the copolymer monomers can aggregate to form micelles above critical micelle concentration (CMC) at a fixed temperature or above critical micelle temperature (CMT) at a fixed concentration.[1,2] With increase in temperature beyond CMT, the micelles formed undergo restructuring leading to an increase in their aggregation number, core size, micellar shape and reduction in their degree of hydration. At still higher temperatures, large micellar clusters are formed before being phase separated at the cloud point (CP) forming two coexisting isotropic phases[3-5] Moreover, with increase in concentration of copolymer well above its CMC, gels are formed and depending on the molecular architecture, molecular weight, concentration and temperature, such gels may assume cubic (face centered cubic (fcc), body centered cubic (bcc)), lamellar, and hexagonal crystalline structures.[6] For example, F127 forms a cubic gel at concentrations below 50 wt% and at temperatures above 5°C. The structures of P123 gels in the same concentration range are more complex, with cubic, hexagonal, and lamellar regions.[7] It has also been observed that aqueous solutions of P123 show time dependent sphere–to-rod micellar growth on approaching their cloud points, the rate of micellar growth being dependent on temperature, heat cycling[8] and nature of the electrolyte.[9]

Pluronics have extensively attracted attention of researchers in view of their unique solution behaviour, core-shell aggregate formation, phase behaviour, rheological properties[10-15], dispersion stabilization, lubrication, low toxicity and minimal immune response[16-18] rendering their immense applicative role in detergency, biomedical and pharmaceutical fields, tissue engineering, food protection, coating and painting, synthesis of different nanostructures etc.[19-22] The properties of pluronics (rheological, colloidal, dispersion etc.) are known to get affected by the presence of additives. Numerous studies have been carried out to investigate the influence of a variety of additives such as electrolytes, non-electrolytes, organic solvents, homopolymers, hydrotropes and surfactants on the aggregation and gelation characteristics of Pluronics.[23-32] Such studies are productive in optimizing the performance based properties of Pluronics for different applications due to their use in complex environments where the concomitant presence of many such additives is certain.

Understanding copolymer-surfactant interaction is essential not only from academic point of view but also from their high potential in industrial, biomedical and pharmaceutical applications.[33] There are reports available in the literature where the interaction between various types of triblock copolymers and conventional surfactants like Sodium dodecylsulfate (SDS), Hexadecyltrimethylammonium bromide (HTAB), Tetradecyltrimethylammonium bromide (TTAB), dimeric cationic surfactants, twin tailed and non-ionic surfactants have been investigated using various techniques. [1,31,32, 34-38] Several groups have explored the association mechanism and break up of Pluronic micelles upon addition of ionic surfactants by calorimetry, light scattering, cyclic voltammetry, time resolved fluorescence, and NMR techniques.[1,31,32,37,38] Studies on the ionic surfactant-block copolymer mixed systems[36,37] in aqueous media reveal that, depending on their concentrations and temperature, interactions between them lead to the formation of either mixed micelles or different type of mixed aggregates. Besides, the rheological properties of pluronics are significantly affected in presence of surfactants. Hetch et al.[37] studied the effect of adding SDS to 20-30 wt% aqueous gels of copolymer F127. As the SDS concentration was increased at a given copolymer concentration, the temperature range of the hard cubic gel became narrower and the gel eventually dispersed. Ganguly et al.[31] studied the effect of adding SDS to 20-30 wt% solutions of copolymer P123 and reported the enhancement of gel formation and dispersion of the gel phase at SDS/P123 mole ratio of 2 and 5.5 respectively. The gelation temperature of F88 is observed to increase with the addition of SDS due to solubilization of SDS into F88 micelles.[39]

Bile acid/salts are known to possess hydrophilic concave α-face due to presence of multiple hydroxyl groups and the hydrophobic convex β-face (hydrocarbon like). This facial amphiphilicity drives their self-aggregation to form micelles and other supramolecular networks.[40-42] Bile Salt micelles have been utilized as solubilizing media for various hydrophobic moieties with their solubilizing efficiency modulated by mixed micellization with other conventional surfactants. [43] However, little emphasis has been given to the mixed micellization of bile salts with Pluronics. Very recently, Roy et al[44] investigated the role of NaTc (Sodium Taurocholate) and NaDc (Sodium deoxycholate) on the self-assembled architecture of P123 micelles. The study revealed that the bile salts reside at the core-corona interface of the P123 micelle, thereby increasing the polarity of the bile salt-P123 mixed micelle. Also, the study evidenced the formation of two types of complexes at higher mole fractions of bile salt-P123 (>1:3). In addition, Gomez et al[2,45] studied the interaction between Pluronics (F127/F68) and bile salt NaTDc(Sodium Taurodeoxycholate) at the oil water interface with the emphasis on the role of Pluronics in delaying the lipid digestion. Furthermore, the effect of bile salts on the aggregation characteristics of Pluronics at both low and high concentration regimes i.e, micellization and gelation is still unexplored. It has been reported[46-48] that the aggregation characteristics of OE based nonionic surfactants are significantly affected by the bile salts In view of this, bile salt induced modification of Critical Micellization Temperature (CMT), Critical Gelation Temperature (CGT) and loading capability of Pluronic deserves attention as most of the pharmaceutical and pharmacological applications rely on the temperature dependence of self-assembly and its properties. A sound understanding of Pluronic-Bile Salt interactions and self-assembling behaviour could be of utmost importance to understand micellar stability of Pluronics in presence of the physiological components, and therefore promises to contribute to the efficient design of stable pluronic based delivery systems with the desired drug carrying capacity. Therefore main objective of the present study was to provide an insight on the effect of sodium cholate (NaC) on micellization, gelation and encapsulation behavior of model triblock biocompatible copolymer Pluronic P123, being widely used in pharmaceutical and cosmetic formulations, in aqueous media using tensiometry, rheology, dynamic light scattering, densitometry and spectrophotometric measurements.

EXPERIMENTAL:

Materials: Triblock copolymer having average composition of $(PEO)_{20}(PPO)_{70}(PEO)_{20}$ (denoted as Pluronic P123) and nominal molar mass of 5600 $gmol^{-1}$ was an Aldrich product. Sodium cholate (NaC), Pyrene and Naproxen were also Aldrich products and were used without further purification. Triple distilled water was used in the preparation of all solutions. The structure of the copolymer and the bile salt used in the study are presented in **Scheme 1**.

Solution Preparation for Rheological, Dynamic Light Scattering (DLS) And Density Measurements: Stock solutions of Pluronic P123 and NaC were prepared and left to equilibrate overnight in a refrigerator at the temperature of ca. 6^{o}C. The following day P123 was mixed with

NaC forming mixed systems containing constant copolymer concentration of 25wt% and varying concentration of NaC (0.1, 0.5, 1, 1.5, 2.5, 5 and 10 wt% of NaC). The binary solutions were again left to equilibrate for at least one week prior to the rheological and DLS measurements.

While as for CMT determination by densitometric measurements, total concentration of binary mixtures was fixed at 1mM and mole fraction of P123: NaC varied as 1:9, 3:7, 5:5, 7:3, 9:1.

Surface Tension Measurements: Surface tension measurements of Pure P123, NaC and their associated binary mixtures of varying mole fractions 1:9, 2:8, 3:7, 5:5, 7:3, 8:2, 9:1 (P123: NaC) were carried out by the Platinum ring detachment method with the Kruss K9 tensiometer equipped with a thermostable vessel holder. The total amphiphile concentration was varied by adding their concentrated stock surfactant solution (pure and binary systems) of known concentration in small installments using a Hamilton Syringe to 20 mL water contained in the sample vessel placed in a vessel holder. Readings were taken after thorough mixing and temperature equilibration at 25°C (±0.1°C) by circulating water from a Brook field TC-102 thermostat through the vessel holder. The CMC values of all the surfactant solutions in pure and mixed states were determined from the plot of surface tension (γ) vs logarithm of total amphiphile concentration (Fig. 1).

Densitometry: The density measurements of triple distilled water, pure P123 and its binary mixtures with NaC containing their varying mole fractions but fixed total concentration of 1mM were performed on Anton Paar DMA 4500 densitometer at varying temperatures ranging from 6°C to 32°C. The instrument employs an oscillating tube technique, where the relationship between the period of oscillation and the density is utilized. The fundamental frequency of the U-tube is a function of the systems mass making the oscillating frequency function of the sample density. The relation holds as long as the sample has a relatively low viscosity. From the measured density, the apparent partial specific volume of the binary mixtures were calculated using the following relation[49]

$$v(c) = \frac{1}{\rho_{solvent}}[1 - \frac{(\rho_{solution} - \rho_{solvent})}{C_{solution}}] \quad 1$$

Where the $C_{solution}$ and ρ are expressed in grams per milliliter.

Rheometry: The rheological properties of the Pure P123 solution and its binary mixtures with NaC were investigated by Anton Paar MCR-102 Rheometer equipped with a peltier system of temperature control with an accuracy of ± 0.01^{o}C. Cone plate geometry (1^{o}-50mm, d=0.101mm) was used for the measurements. Test modes employed were: the shear rate sweep, frequency sweep and the temperature ramp. In steady shear experiments, shear rate was varied from 0.01 to 7000 sec^{-1}. Frequency sweep measurements were performed in angular frequency range of 0.1 to 500 rad sec^{-1}. All the experiments were carried out at 25^{o}C. However, the temperature was varied from 5-80^{o}C in the temperature ramp experiments at the fixed frequency of 1Hz. At such a low frequency, it is easy to meet the requirements of linear viscoelasticity. Temperature was varied at a heating rate of 2^{o}C per minute. A small amount of low viscosity silicone oil was applied on the peripheral surface between the upper and lower plates to prevent evaporation of water.

Dynamic Light Scattering: Size measurements were performed using Malvern Zetasizer Nano ZS90 dynamic light scattering instrument with 4 mW, 633 nm He-Ne laser. The angle of detector was set at 90^{o}. Prior to dynamic light scattering measurements, solutions were filtered using 0.22µm filter.

Solubilization experiment:

The solubility of two solubilizates viz Naproxen and Pyrene was determined in separate experiments in pure P123 and P123+NaC binary mixtures at their varying mole fractions and fixed total concentration of 1mM. Excess amount of solubilizate was added to vials containing 1mL of surfactant solutions (pure and binary) to ensure maximum solubility. The vials were then sealed with screw caps and agitated for a period of 24 hours on a magnetic stirrer at a temperature of 25±0.5^{o}C. The solutions were subjected to centrifugation at 13400 rpm in order to remove any undissolved solubilizate. The concentration of the solubilized probes in different micellar solutions were then determined Spectrophotometrically using Shimadzu Spectrophotometer (UV-1650) from their respective absorbance values following appropriate dilution of an aliquot of the supernatant with methanol. The solubility of Naproxen and Pyrene was determined at their characteristic wavelength of 332nm and 334nm respectively using the extinction coefficient of 2831.7 M^{-1}cm^{-1} for Naproxen[50] and 49mM cm^{-1} for Pyrene.[51]

Results and Discussion:

Mixed Micelle Formation between P123 and NaC: Representative plots of γ versus log [Total Amphiphile] for pure amphiphiles (P123 and NaC) and their mixtures at varying mole fractions are presented in Fig.1. The CMC values are summarized in Table 1. The CMC value of NaC obtained is 9.9 mM which compares well with the literature value.[52] The surface tension plot of pure P123 shows two breaks, where the high concentration break signifies the formation of multimolecular micelle and corresponds to the CMC.[53] This is because the PEO-PPO-PEO copolymers are polydisperse both in mass and composition and hence causes the micellization process to occur gradually rather than at specific concentration. The CMC of P123 obtained is 0.00097 mM. However, the CMC values obtained for binary systems increases by increasing the mole fraction of NaC (Table 1) with appreciable increase at higher mole fractions of NaC. This could be probably because of the increasing electrostatic and steric destabilization of the P123 micelles by NaC. Also, the experimental CMC values of binary systems were found to be less than the ideal CMC values calculated using Clint equation[54] which indicates negative deviation from ideal behaviour for mixed micelle formation. The estimate of negative deviation and hence non ideality of binary surfactant systems has been quantified using Rubingh's model[55]. The interaction parameter β , which accounts for deviation from ideality, is an indicator of degree of interaction between two surfactants in mixed micelle. β values along with the micellar mole fraction, X_i^M and activity coefficient f_i, of the ith surfactant within mixed micelles calculated through Rubingh's equation[55] are also presented in Table 1. A negative value of β indicates attractive interactions between the two surfactants in mixed micelles. In the binary mixtures of P123-NaC, negative β values of higher magnitude were observed indicating strong synergism between the two in the mixed micelle formation. This could be because of the reduction in the electrostatic self-repulsion of ionics and steric self-repulsion of non-ionics on mixing by dilution effect.[56,57] Also, the strong synergism could be because of the participation of hydroxyl groups of NaC in hydrogen bonding with the POE groups present in the micellar corona. A large value of X_1 indicates that the mixed micelles are rich in P123 component which is analogous to the results of ionic+non-ionic mixed surfactant systems where non-ionic surfactant is the dominating part in the mixed micelle.[50] Due to the rigid structure of bile salts, their mole fraction in the mixed micelles is small in the whole mole fraction range.

The values of surface tension at the cmc (γ_{cmc}) of pure P123, NaC and their associated binary mixtures are also given in Table 1. γ_{cmc} value of pure P123 shows the lowest (39.8 mN/m) value while as pure NaC shows the highest value of 48.4 mN/m indicating P123 to be more surface active than NaC. The γ_{cmc} values of binary mixtures increase progressively with increasing mole fraction of NaC showing that NaC decreased the surface activity of P123.

In order to evaluate the effect of NaC on the solubilization capacity of P123, we used P123 and its binary combinations with NaC to solubilize two model hydrophobes-Naproxen and Pyrene. The absorption spectra of Naproxen and Pyrene and their solubilized concentrations in P123 and P123+NaC mixed systems are presented in Fig 2. The solubility of both naproxen and pyrene decreased with increase in the mole fraction of NaC with more decrease in solubility of latter compared to former. This could be because of the destabilization of P123 micelles by NaC owing to steric and electrostatic repulsions leading to formation of smaller sized mixed micelles as observed in other systems[46] with decreased micellar core volume, thereby decreasing the solubility of hydrophobic compounds within the micellar core. The effect of NaC on the hydrodynamic diameter of P123 also indicates the formation of smaller sized micelles. Also, the incorporation of NaC in a micelle increased the hydrophillicity of the mixed micelle[44] due to its significant charged interfacial area which could lead to the decreased solubilization of hydrophobic compounds. Since pyrene is more hydrophobic than naproxen, therefore it is expected to be solubilized in micellar core and hence a greater decrease in its extent of solubilization is observed. Naproxen, an acidic drug (pka = 4.8) is reported to be solubilized in the interfacial region in case of non-ionic surfactants wherein the interaction between carboxyl groups of naproxen and PEO assists in solubilization.[42] However, the incorporation of negative charge within mixed micelle could have little effect on the solubilization of naproxen due to electrostatic repulsion since the micellar mole fraction of NaC in mixed micelle is very small. Therefore, the observed decrease in its solubility could be because of formation of small sized mixed micelles. The solubilization results therefore suggest that the change in micellar characteristics of P123 with the addition of NaC i.e, decreased micellar size and increased hydrophillicity of mixed micelle is possibly responsible for reducing the solubility of Naproxen and Pyrene. Thus for the desired loading capacity, ratio of the two surfactants needs to be taken into consideration while formulating P123 based delivery vehicles.

Densitometric Results: The density of the binary mixtures decrease with increase in temperature with a more profound decrease around a temperature which can be related to their critical micellization temperature (CMT).[49] To probe this effect, apparent partial specific volume of binary mixtures were calculated using eqn (1).The variation of Apparent Partial Specific volume $v(c)$ of binary mixtures with temperature are presented in Fig 3. The $v(c)$ increases with temperature marking an abrupt increase at certain temperature called CMT which corresponds to the formation of micelles. The CMT of the binary mixtures increase with increase in the mole fraction of NaC. It increases from 18.97°C in case of pure P123 to 22.91°C in case of 1:9 P123: NaC indicating micellization of P123 is delayed by the addition of NaC similar to the tensiometric results wherein increase in CMC with the increase in mole fraction of NaC is observed. This can be ascribed to the formation of negatively charged mixed micelles due to the incorporation of NaC leading to electrostatic repulsion between the head groups. This consequently delays micellization and increases CMT of binary mixtures. A similar suppression of micellization of F127 and F68 by sodium taurodeoxycholate in aqueous solution was reported by Gomez et al.[2]

Micellar Characterization -DLS Results: In order to investigate the influence of addition of NaC on the aggregation state of P123, DLS measurements were carried out on pure P123 (25wt%) and its binary mixtures with varying concentration of NaC (0.1-10wt%) at 25°C. It is pertinent to mention that intensity fraction distribution of P123 is not affected much by increase in concentration from 5 to 25 wt% (Fig. S1). Fig. 4 shows the intensity weighted size distribution of P123 (25 wt%) micelles and its binary combinations with NaC. The hydrodynamic diameter (D_H) of pure P123 is found to be 14.3 nm which decreases to 10 nm with the addition of NaC upto concentration of 1.5wt%. The formation of negatively charged mixed micelles with the addition of NaC to the already existing non-ionic P123 micelles results in the increase in intra aggregate repulsive forces favouring demicellization and hence decreasing micellar size. [46] With further increase in concentration of NaC (from 2.5-10 wt%), DLS results evidenced the presence of two types of aggregates- smaller sized aggregates of D_H =2.6 nm and bigger aggregates with D_H varying from 14.3 nm to 28.9 nm. At higher concentrations of NaC, mixed micelle does not allow further incorporation of NaC owing to enhanced electrostatic repulsions. However, the NaC added will form NaC rich aggregate which may consist of cooperatively associated NaC interacting with copolymer unimers. This corresponds to the smaller sized aggregate of D_H = 2.6nm which is comparable to the size of pure NaC micelle[58]. The larger aggregates correspond to the presence of copolymer rich aggregates resembling the corresponding pure copolymer micelle. Due to decreased contribution of NaC to the mixed micelles, larger copolymer rich aggregates with a wider size distribution are formed indicative of increased polydispersity in their sizes. A similar observation has been found by Roy et al[44] in bile salt-P123 systems at different mole ratios of bile salt-P123. Upto bile salt-P123 mole ratio=1:3, size of P123 micelles decrease due to electrostatic repulsion in mixed micelle formation while as at higher mole ratios of bile salt-P123 (>1:3), two types of complexes-copolymer rich P123-bile salt complexes and bile salt rich bile salt-P123 complexes are reported. Moreover, Jansonn et al[59] also observed two types of copolymer-surfactant complexes in P123-SDS system at intermediate concentration of SDS. It is pertinent to mention that the time dependent micellar growth as observed by Ganguly et al.[8] could not be envisaged in this study due to limited time range of the experiment.

Rheological Measurements:

Flow curve: Fig. 5 shows the shear rate dependence of viscosity of pure P123 (25wt%) and its binary mixtures with varying concentration of NaC (0.1-10wt%). Viscosity of the binary mixtures decrease with increase in concentration of NaC and the behaviour of the flow curve change to Newtonian from 5wt% of NaC in the whole shear rate range. 25wt% of P123 shows non-Newtonian behaviour of shear thinning followed by Newtonian plateau at higher shear rates while as the flow curve of binary mixtures containing NaC upto 2.5wt% shows shear thickening region first at lower shear rates followed by shear thinning and then Newtonian behaviour at higher shear rates. Although the viscosity at lower shear rates decrease with increase in NaC concentration but the critical shear rate i.e, the shear rate at which shear thinning begins shifts to higher values with increase in NaC concentration leading to wider shear thickening regions. P123 at 25wt% exhibits a clear soft gel phase[60], where the reversible entanglements between the corona of the neighboring micelles is responsible for the gelation of the polymer[61]. The gelation characteristics of the 25wt% P123 at 25^{o}C is also evidenced by its oscillatory frequency sweep where storage modulus G′ is greater than loss modulus G″ (Fig. 6).The shear thinning behaviour of the P123 micellar gel is due to the disruption of entanglements between the micelles by the applied shear which largely decreases the system viscosity till the copolymer is present predominantly as individual micelles or unimers which exhibits Newtonian behavior to the system at higher shear rates. With the addition of NaC to the non-ionic P123 micelles, negatively charged mixed micelles are formed with the hydrophobic convex face of cholate facing towards the PPO core and its hydrophilic concave face towards PEO corona. The incorporation of negative charge within nonionic micelle leads to the intra as well as inter-aggregate repulsions. The extent of repulsion and hence inter micellar distance being function of sodium cholate mole fraction. The inter-micellar electrostatic repulsion results in separation of negatively charged mixed micelles with consequent decrease in the viscosity of binary mixtures with increase in mole fraction of sodium cholate[1,46](Fig. 5). However the shear induced shear thickening may be due to the shear driven movement/alignment of negatively charged mixed micelles towards each other and the subsequent growth of micelles into large wormlike clusters leading to increase in viscosity upto shear rate which dominates over electrostatic repulsion. At higher mole fraction of NaC, mixed micelles could be more farther apart than at lower mole fraction of NaC because of larger electrostatic repulsion. As a result, they are driven towards each other upto higher shear rates and hence wider shear thickening region is observed in them. Beyond shear thickening region, shear thinning is observed which could be the consequence of alignment of larger micellar aggregates in the direction of the shear and the disruption of entanglements between them.[62] At 2.5, 5 and 10 wt% of NaC, two types of aggregates are present as revealed by DLS results-one being bile salt rich aggregate of the size of NaC micelle (which consist of cooperatively associated NaC molecules interacting with copolymer unimers) while other P123-rich complex. As a result, inter aggregate interactions between P123 rich aggregates are reduced by the presence of negatively charged NaC rich micelles between them which leads to abrupt decrease in viscosity and hence formation of complete Newtonian fluids from 5wt% NaC where the interactions between aggregates may be completely disrupted and aggregates present as individual moieties. These results show that the rheology of the block copolymer can be tuned by varying the concentration of such facial amphiphiles.

Oscillatory Frequency Sweep: Fig. 6 and 7 presents the oscillatory frequency sweep of 25wt% P123 and its binary mixtures with NaC ranging from 0.1wt% to 10wt% at 25°C. Upto NaC concentration of 1.5 wt%, gel like behaviour is observed as the storage modulus (G′) is dominating over the loss modulus (G″) in the whole frequency range and both moduli are weakly dependent on frequency. Gel stability, however, decrease with further increase in NaC concentration as shown in Fig. 7. At 2.5 and 5wt% of NaC, G′ is almost equal to G″ at lower frequencies indicating system tending to be in a sol state. However, with 10wt% NaC, initially it is G″ > G′ which confirms the system to be in sol state. In all the three cases, both moduli are lower in magnitude first and then sharply increases with increase in frequency upto crossover frequency where G′ overtakes G″ and then dominating further ascertaining gel like behaviour. However, the gel point occurs at higher frequency as the concentration of NaC is increased in the binary mixture. Thus the stability of the gel phase first remains same at lower concentrations of NaC but decreases progressively with increase in NaC concentration from 2.5wt% at 25^{o}C. The presence of charged species at higher concentration of NaC i.e, NaC rich micelles increase the fluidity of the sample due to intermicellar repulsion that makes the condition unfavourable for the growth of micelles leading to reduction in viscosity of system.[1] The results also indicate that the elastic structure formed by P123 is weakened by the addition of NaC. A similar decrease in P123 gel strength has been observed Ganguly et al[31] at higher concentrations of SDS due to decrease in micellar volume fraction.

Correlation between Micellization and Gelation-Temperature Ramp Results: The variation of dynamic moduli of 25wt% of P123 with temperature [Fig. 8(a)] could be divided into 3 regions. In the region I corresponding to temperature range of 5-18^{o}C, G′ is slightly greater than G″. In this temperature range, 25wt% P123 is found to be in clear sol phase which is indeed a micellar solution.[11] The interaction between P123 micelles in such solution would be sufficiently high due to their high concentration leading to greater value of G′ than G″. In the region II, there is first abrupt increase in the magnitude of both the moduli at around 19^{o}C, which could be taken as the gelation temperature as per Li et al.[63] Thereafter, a gradual increase in both the moduli upto 44^{o}C with G′ increasing more than G″ is observed. During the gelation process, the micelles begin to contact each other through overlapping of coronas forming highly ordered network which contributes high modulus to the system (Scheme 2). This could be related to the formation of cubic structures within this system under these conditions.[11,14] Thereafter, at higher temperatures in region III, decrease in the magnitude of both the moduli could be due to transition from hard gel to soft gel.[6] Since after gelation, temperature does not affect the concentration of micelles as almost all the polymer chains exist already as micelles, further increase in temperature decreases the solubility of PEO blocks causing a part of the PEO blocks to merge into the core of the micelle leading to increase in micellar core size and the consequent decrease in the overlapping degree of micellar corona and hence drop of modulus at higher temperatures (Scheme 2).[6]

With the addition of NaC to 25wt% P123, gelation is delayed to higher temperatures and the sol-gel transition temperature (Tg) increases with increase in NaC concentration [Fig. 8(b)]. Since gelation is not a process independent of micellization- depending on size of micelles, number of micelles and the possible bridges formed among micelles. Therefore, any factor which affects micellization is likely to affect gelation in a similar fashion. As mentioned earlier, addition of NaC delayed micellization of P123 or lead to drop of viscosity of the system, so does it lead to the dispersion of gel phase of P123 because of disruption of entanglements between the corona of mixed micelles due to intermicellar repulsion (Scheme 2). An increase of CMT of P123-NaC binary mixtures with increase in the mole fraction of NaC as observed from densiometric measurements for 1mM concentration might have subsequently resulted in the increase of Tg which again corroborates the fact that the micellization and gelation are interdependent or directly related to each other. At the CMT, the micellar volume fraction is small as only a fraction of unimers form micelles. However, with increasing temperature above CMT, more and more unimers are converted to micelles leading to increase of micellar volume fraction which become sufficiently high for a gel to form at Critical Gelation Temperature (CGT). Also, the literature[3-5] reports the increase of size or the aggregation number of the Pluronic based micelles with increasing temperature. At higher temperatures, the hydrophobicity of the P123 increases due to breaking of H-bonds of PPO unit which subsequently drives micellization and increases aggregation number leading to gelation. In presence of NaC, such micellar growth is suppressed due to increased charge repulsion, thus decreasing the temperature range for the stability of the gel phase. Also an increase in the size polydispersity of the micelles at higher mole fractions of NaC as revealed by DLS results would lead to lattice disruption and suppression of gelation as already speculated by Malmsten and Lindman.[64]

Conclusion:

In Conclusion, the micellar and gelation characteristics of Pluronic P123+NaC system has been investigated using Tensiometry, Rheology, Densitometry and DLS measurements with the aim of obtaining useful information on the interaction and self-assembling behavior of facial amphiphile with the Pluronics. The addition of NaC to P123 profoundly increased CMC, CMT and CGT which clearly indicates the suppression of micellization as well as gelation of Pluronic P123. Since micellization and gelation are affected in a similar way, therefore, it confirms the interdependence of two processes. Also, the rheological studies show that the viscosity of P123 decreases dramatically with the addition of NaC and can be tuned to a specific value by the appropriate combination of two components. The rheological results were supported by DLS measurements. DLS measurements used to characterise the mixed micellar system confirm the size of mixed micellar aggregates first decreased with the addition of NaC upto 1.5wt% and then two types of aggregates viz, small sized aggregates and larger aggregates are formed at higher concentration of NaC (2.5-10wt%). The change in aggregation characteristics of P123 micelles by NaC resulted in decreasing their encapsulation capacity. Thus, from the results it is evident that the aggregation and transport characteristics of P123 formulations are significantly influenced by the presence of biological amphiphiles. Therefore, it is imperative to take such effects into consideration while designing Pluronic based formulations.

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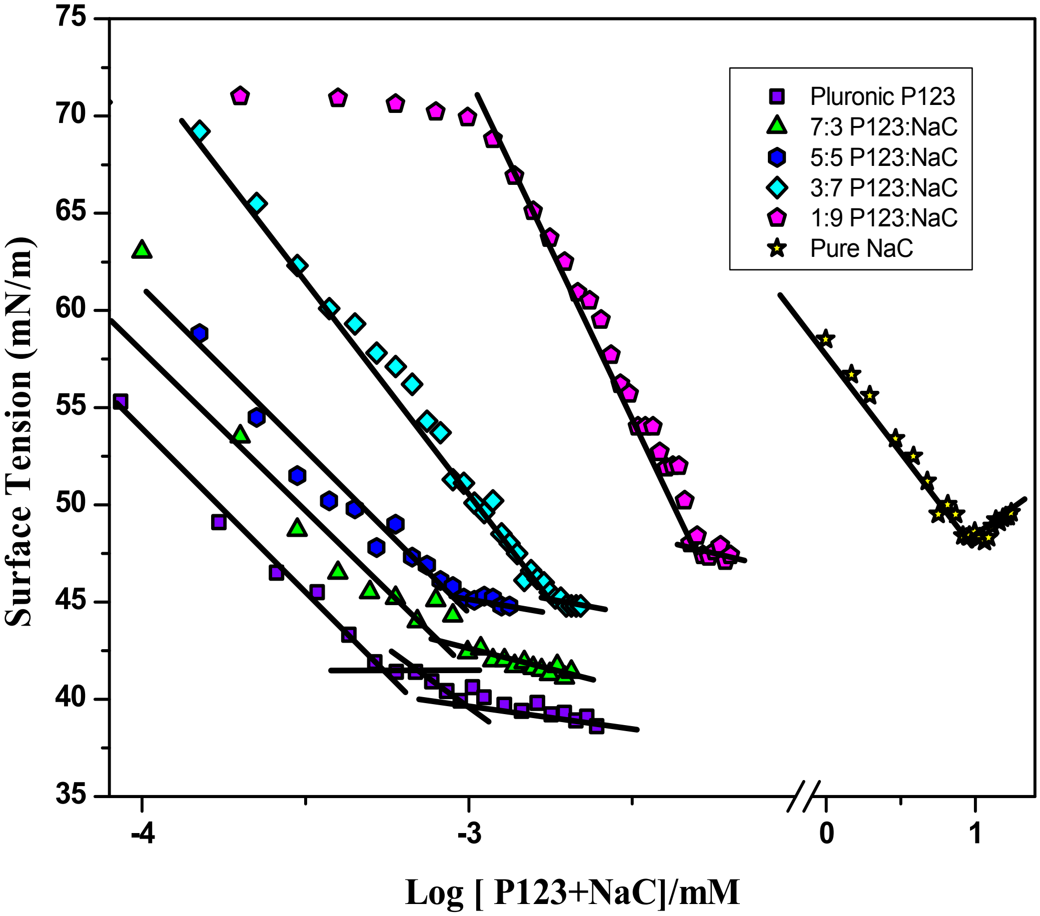


Fig. 1: *Plot of Surface tension (γ) vs logarithm of surfactant concentration for Single and Binary mixtures of P123 and NaC.*

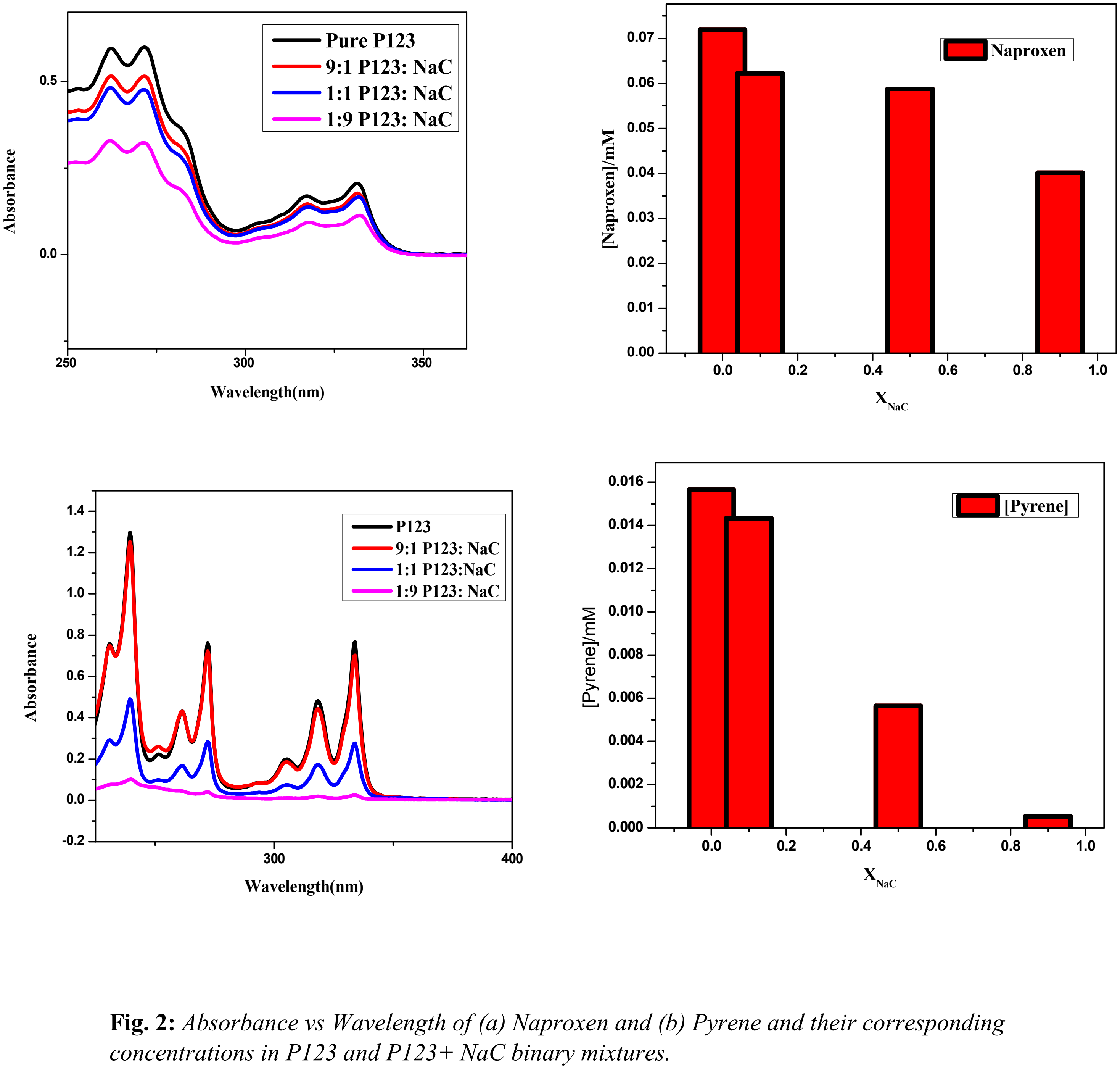


Fig. 2: *Absorbance vs Wavelength of (a) Naproxen and (b) Pyrene and their corresponding concentrations in P123 and P123+ NaC binary mixtures.*

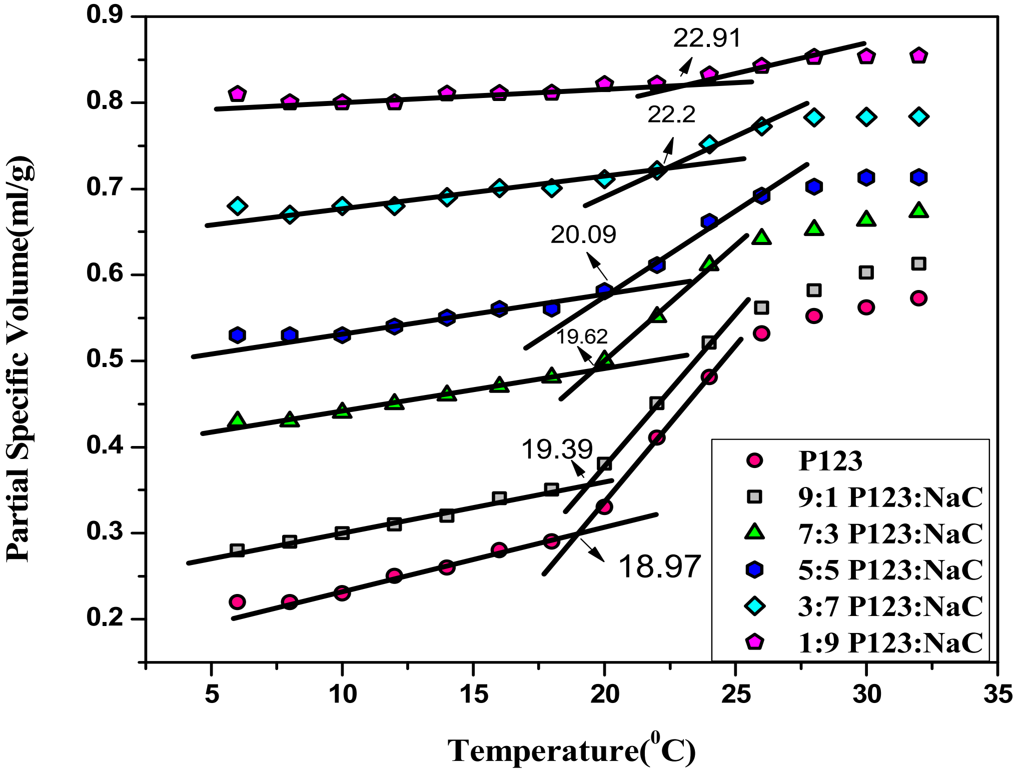


Fig. 3: *Plot of Partial Specific Volume vs Temperature for Pure P123 and its binary mixtures with NaC.*

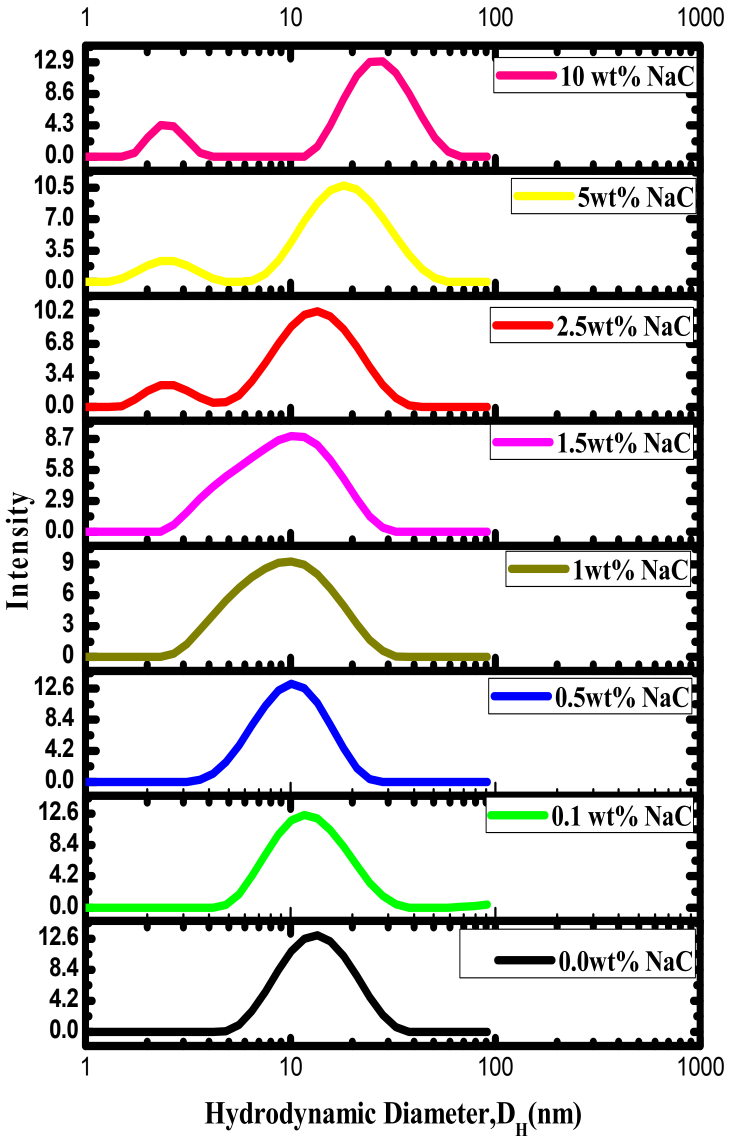


Fig. 4: *Intensity weighted Size distribution plots of 25wt% P123 and its binary mixtures with NaC.*

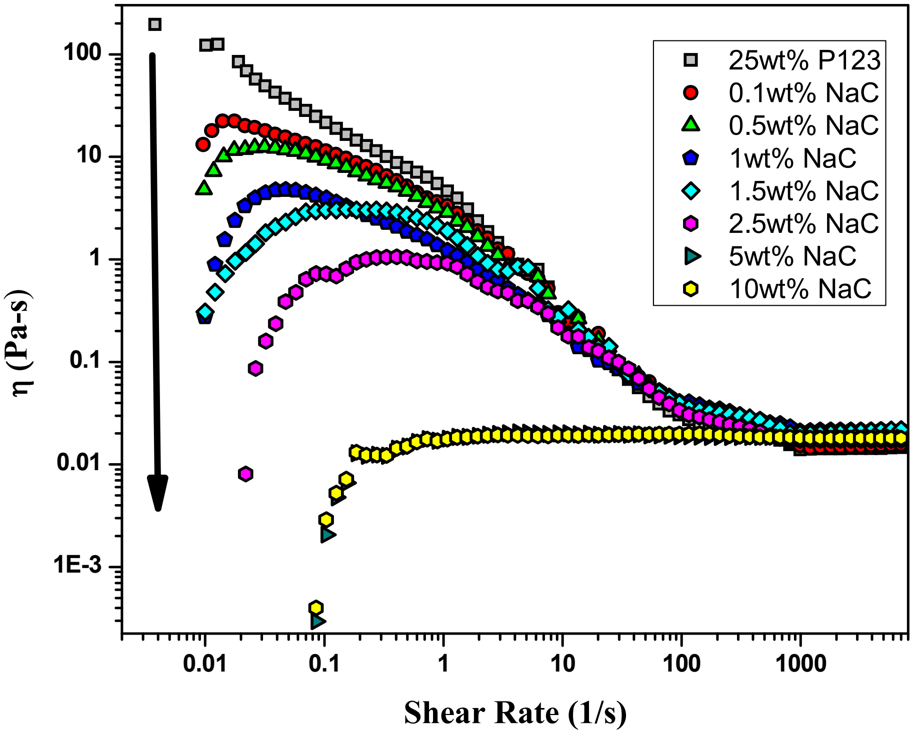


Fig. 5: *Viscosity vs Shear rate of P123 and its binary mixtures with NaC .*

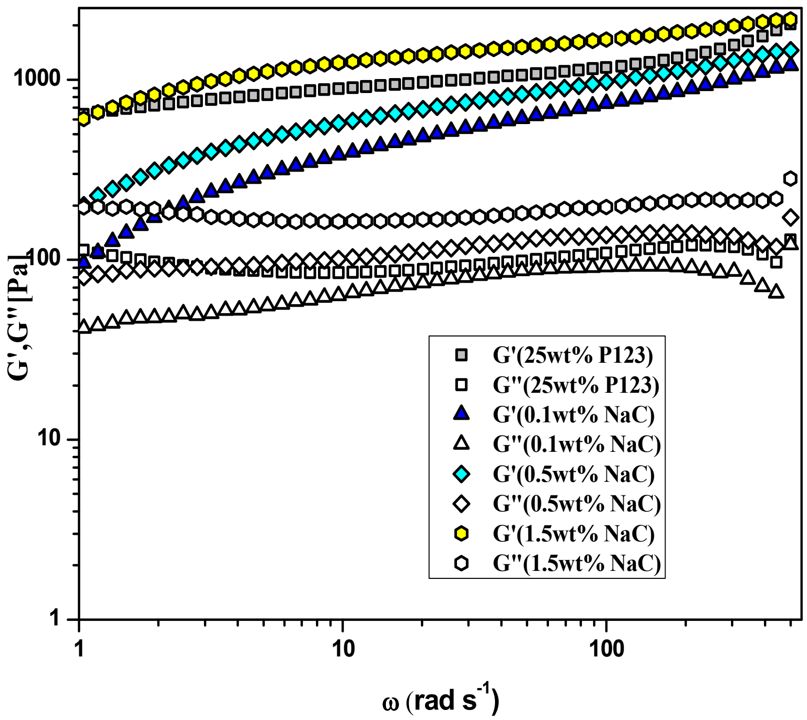


Fig. 6: *Oscillatory Frequency Sweep of 25wt% P123 and its binary mixture with NaC (upto 1.5 wt%)*

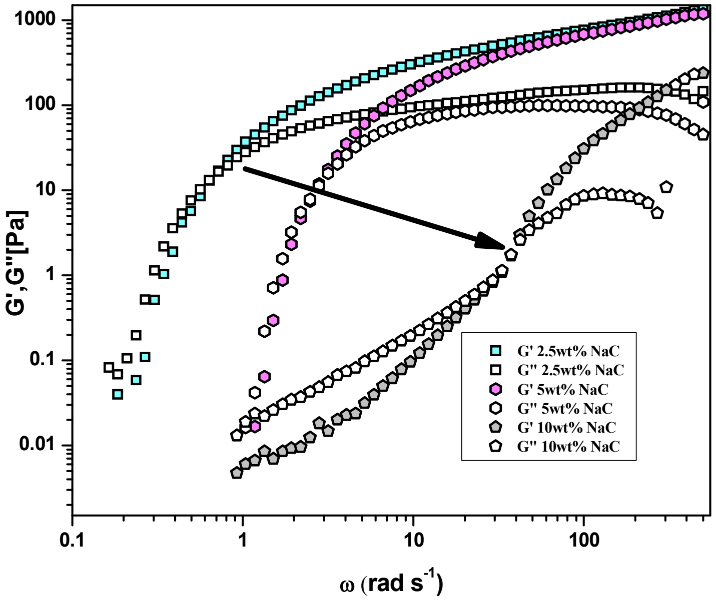


Fig.7: *Frequency Sweep of binary mixture of 25wt% P123 and NaC (2.5-10wt%)*

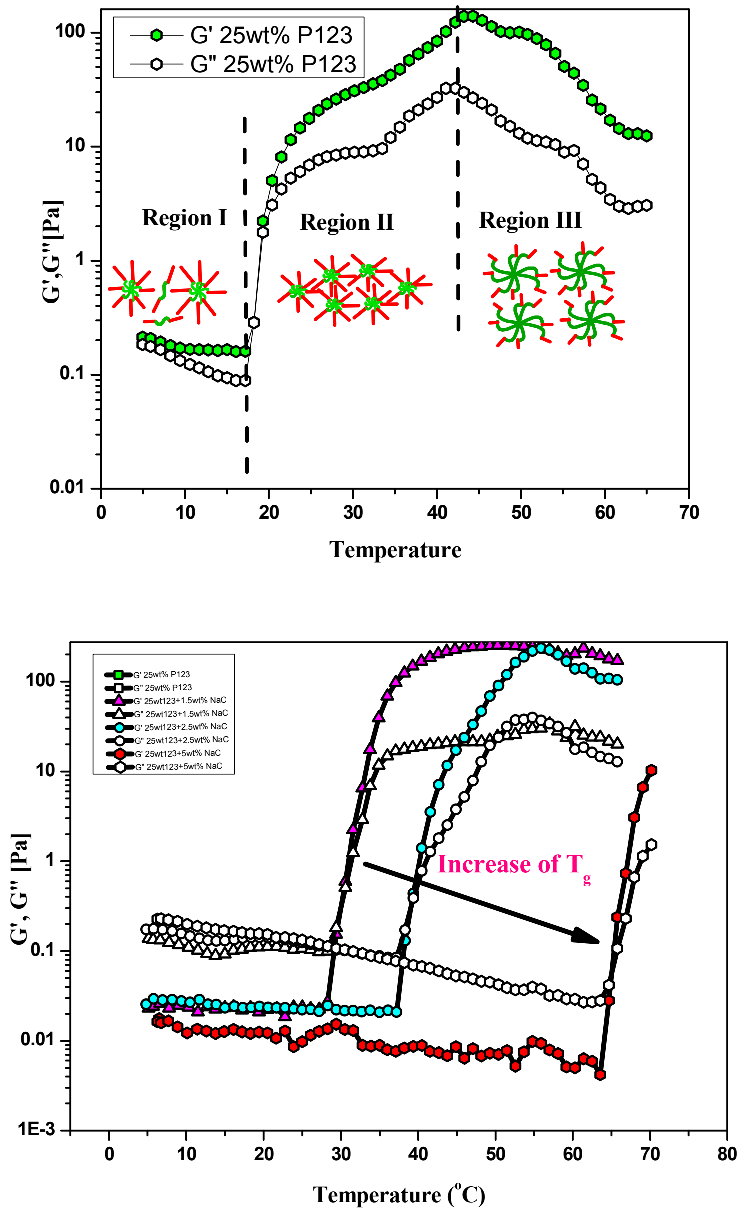


Fig. 8: *Variation of Dynamic Moduli of (a) 25wt% P123 (b) P123+NaC binary mixtures with Temperature.*

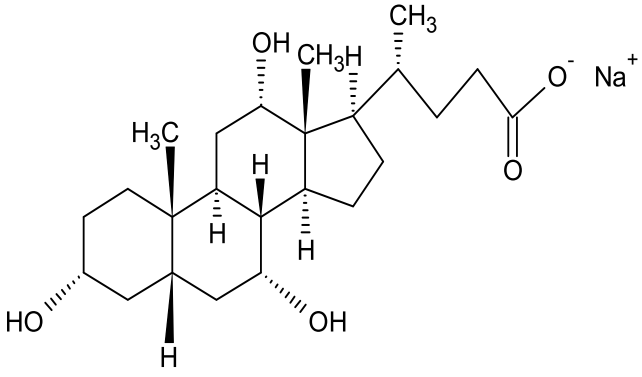
Table 1: Surface tension at the cmc (γ_{cmc}), experimental (cmc_{exp}) and ideal (cmc_{ideal}) critical micelle concentrations of single/binary surfactant mixtures alongwith the micellar mole fraction (X_i^M), interaction parameter (β), and activity coefficients (f_i) for binary systems calculated using Rubinghs model.

α_1	$cmc_{exp}(cmc_{ideal})/(mM)$	γ_{cmc} (mN/m)	β	X_1^M/X_2^M	f_1/f_2
P123-NaC					
1	0.00097	39.83			
0.8	0.001(0.0012)	41.26	-10.32	0.9051/0.0949	0.9112/0.0002
0.7	0.0011 (0.0013)	41.96	-10.14	0.8925/0.1075	0.8894/0.0003
0.5	0.00097 (0.0019)	44.92	-12.74	0.8063/0.1937	0.6201/0.0003
0.3	0.0019 (0.0032)	44.99	-10.63	0.8222/0.1778	0.7147/0.0008
0.1	0.005 (0.0096)	47.5	-9.79	0.791/0.209	0.6517/0.0022
0	9.9	48.4			

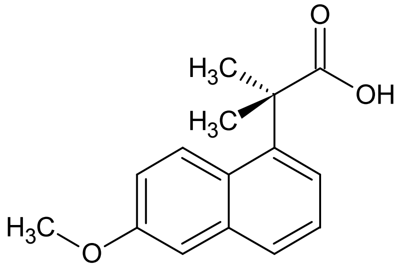
$cmc_{ideal} = \alpha_1/cmc_1 + \alpha_2/cmc_2$ for the ideal mixing as per the Clint equation where 1 and 2 represent two amphiphilic species.

OH[-CH$_2$ CH$_2$ O-]$_{20}$ [CH(CH$_3$)CH$_2$O-]$_{70}$ [-CH2 CH2 O-]$_{20}$ H

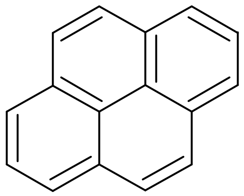
Pluronic P123



Sodium Cholate

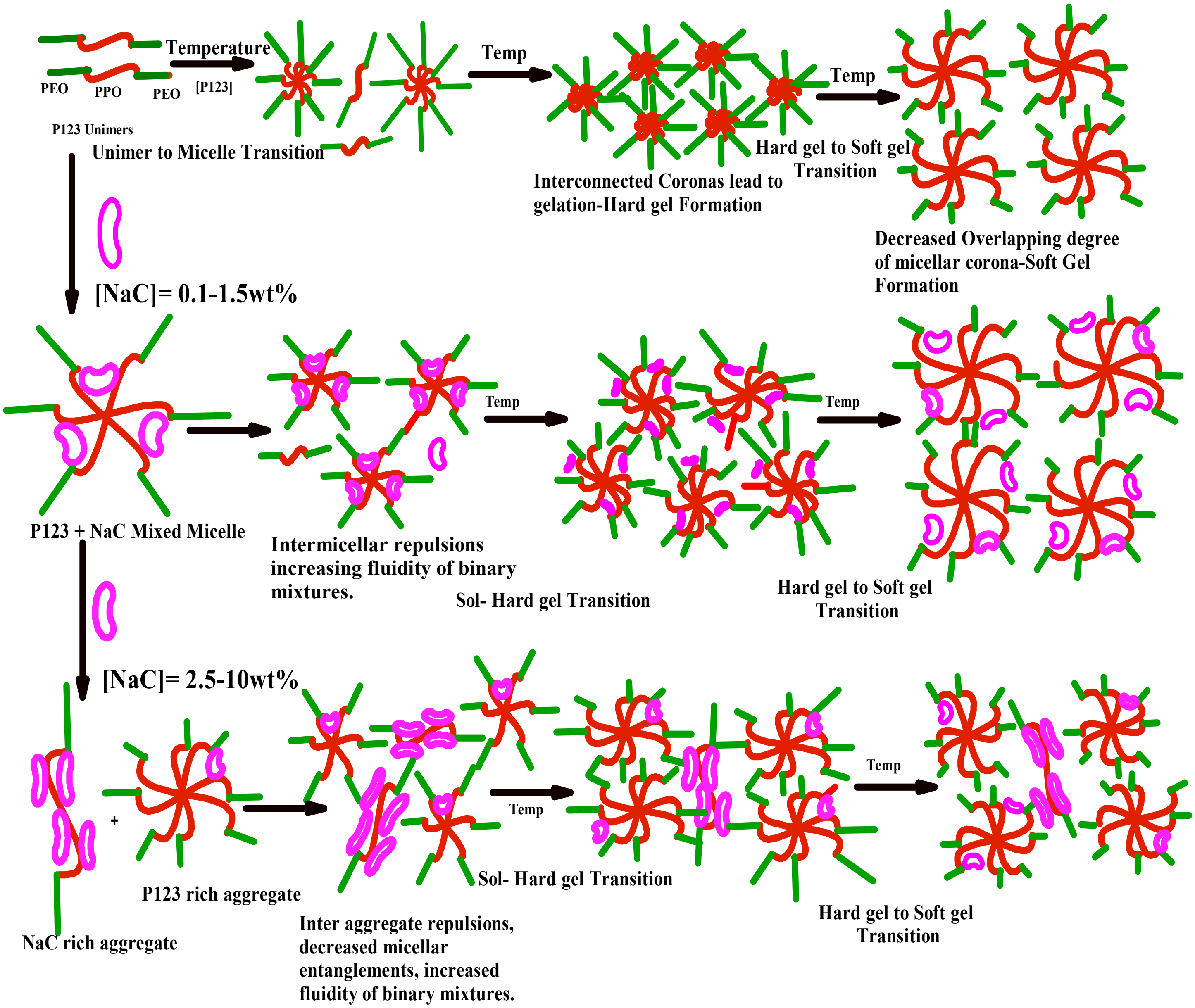


Naproxen



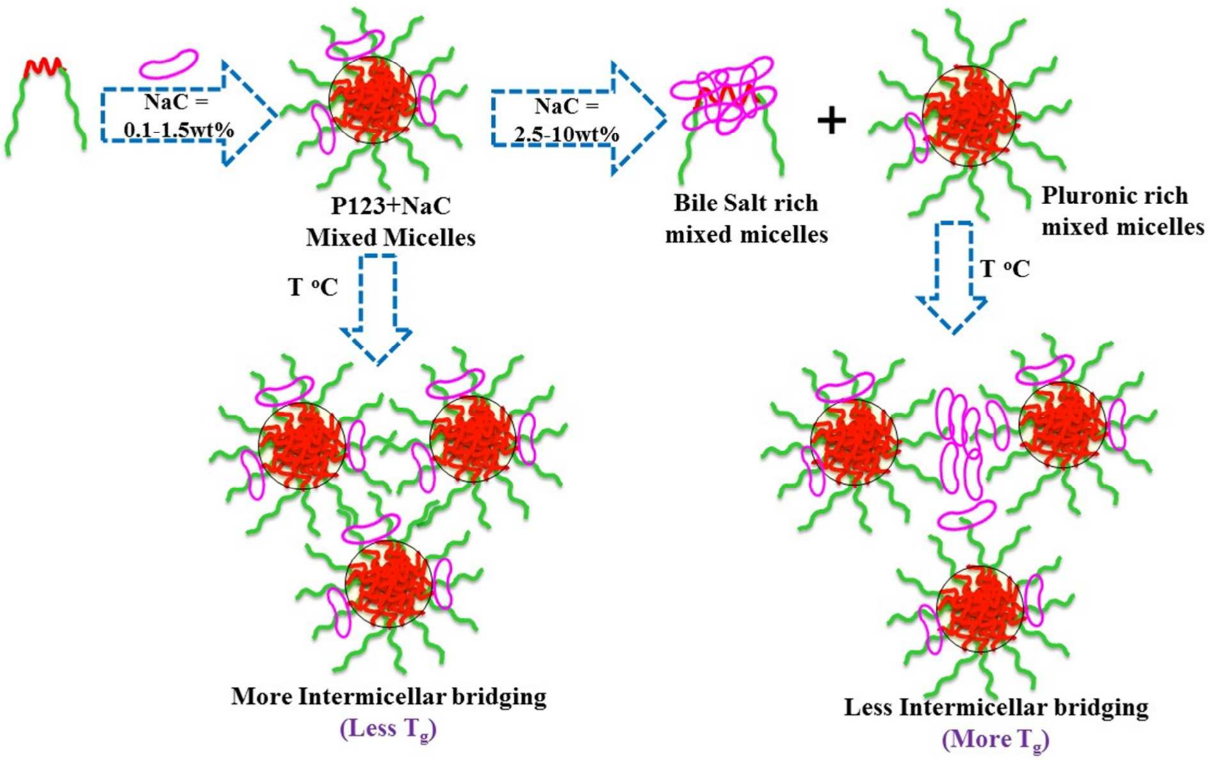
Pyrene

Scheme 1: Structure of Pluronic P123, Sodium Cholate, Naproxen and Pyrene.



Scheme 2: *Schematic diagram representing effect of temperature and NaC on gelation characteristics of P123.*

Graphical Abstract



We report impact of Sodium Cholate on micellization and gelation of P123.