Synopsis of the Thesis Entitled

"Solution Behaviour of Aqueous Mixed Surfactant Systems with and without Additives"

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By

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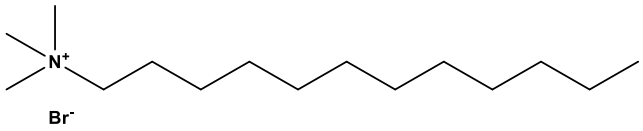
Surface chemistry explores how matter behaves and changes as a result of external forces acting on a surface or interface. When a liquid /solid meets up with a gas /air, the word "surface" is commonly employed. As a result, any surface may be referred to as a part of the interface. In the fields of pharmacy, healthcare, cosmetics, and other technologies, the interfacial phenomena are important [1]. In catalysis, chemical reactivity at the surface or contact plays a significant role. Surface chemistry may be the most multidisciplinary among all the fields of science and technology [2]. However, surface science has long been recognized for its significance. Surface-free energy changes as a consequence of the formation of a liquid surface [3]. The energy required for increasing a unit surface area is known as surface-free energy. A molecule is known as a surfactant that reduces a liquid's surface tension and enhances its spreading and wetting properties. One of the most versatile chemicals, the surfactant is utilized in numerous goods and technologies, such as those used in the biotech and healthcare fields, the fossil fuel industry, nutraceuticals, hygiene products, stain removers, fire prevention, fuel additives, foaming agents, dyes, varnishes, and epoxy resins [4]. In recent time, studies were conducted on the development of various morphological surfactant aggregates, especially those containing mixtures of various charged types. During mixing, various micro-structures resulted which can be controlled by compositional variation and can be used in numerous prospective fields. The present thesis embodied research work conducted in view of above perspective.

There are seven chapters in the thesis, including i) ***General Introduction***; ii) ***Materials and Methodologies;*** iii) ***Association behaviour and Interaction of Oppositely Charged Gemini Surfactants in Aqueous Solution;*** iv) ***Composition Triggered Morphologies of Mixed Oppositely Charged Geminis having different Chain-length and Spacers;*** v) ***Solubilization of Poly aromatic hydrocarbons (PAHs) in Individual and Mixed Geminis Systems;*** vi) ***Counter Charged Gemini mixtures for Solubilization/Release of Various Drugs and*** vii) ***Overall Conclusion.***

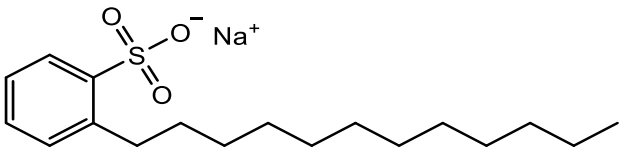
***Chapter 1*: GENERAL INTRODUCTION**

A broad review of the literature on the behaviour of surfactants in solutions is presented in this chapter. Surface-active agents are referred to collectively as surfactants. They are typically organic substances with a special molecular structure that has two distinct factions. The surface-active molecule has partially lipophilic ("tail") and partially hydrophilic ("head") part. Surfactants can be categorized into four categories depending on the charge of the head group, including,

Cationic surfactant: dodecyl trimethylammonium bromide (DTAB)



Anionic surfactant: Sodium dodecylbenzene sulfonate



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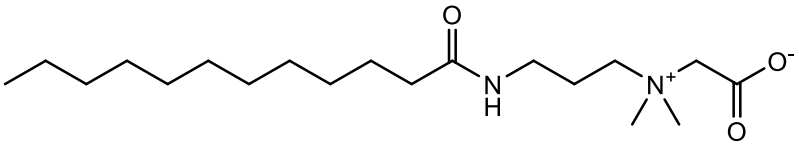
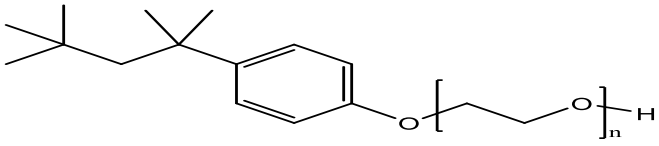
Zwitterionic surfactant: Cocamidopropyl betaine (CAPB)



Cocamidopropyl betaine (CAPB)

Nonionic surfactant: Triton X-100



Triton X-100

Surfactants are also categorized into several types based on their molecular architecture. Examples include gemini surfactants, bola form amphiphiles, block copolymers, and bio-surfactants. A spacer forms a chemical link between two conventional surfactant molecules to form a *gemini* surfactant (Figure 1). Gemini surfactants potentially aggregate at much lower concentrations and have superior surface property than ordinary surfactant.

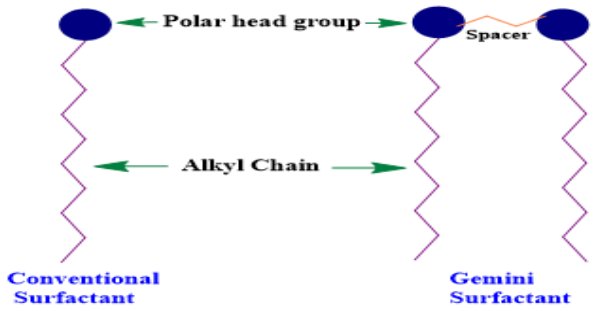


Figure 1: Representative structure of conventional and gemini surfactant

Surfactants assemble into micelles and other aggregates in the bulk aqueous phase. A surfactant's critical micelle concentration (CMC) is the concentration at which micellar aggregates begin to form. Numerous physicochemical techniques are used to determine CMC.

When two surfactants are dissolved in an aqueous environment, generate mixed micelles (Figure 2). Multiple physicochemical parameters of the mixed aggregate system differ from those of the single surfactant when more than one surfactant is introduced to water. The synergistic or antagonistic interactions in different surfactant components are shown by the negative or positive departures from the calculated CMC (ideal CMC) (calculated from regular solution theory) [5].

Figure 2: Representative structure of mixed micelle.

Chapter 2: MATERIALS AND METHODOLOGIES

2.1.Materials

Various gemini surfactants are synthesized and characterized by FT-IR, NMR and ESI-mass [6]. Various samples of individual and mixed surfactants are prepared and morphological changes have been studied using various methodologies.

2.2. Methodology

2.2.1. Electric Conductivity measurements

A conductivity meter (EUTECH Cyberscan CON510, cell constant 1 cm^{-1}) with an inbuilt temperature sensor was used to measure the conductivity of the aqueous gemini mixture (of different mole fractions) as a function of [surfactant or mixture]. Specific conductance (κ) has been measured at each concentration using a pre-calibrated cell (thermo-stated at 303 + 0.1 K using a SCHOTT CT1650 bath). In a plot of κ versus [surfactant or mixture], the CMC value was calculated from the junction of two straight lines.

2.2.2. Steady-state Fluorescence measurements

Using pyrene (2 μM) as a probe, the CMC of single and mixed geminis was evaluated using a spectrofluorometer (RF-6000) at 303 K. Fluorescence values were acquired using 337 nm constant excitation. Excitation and emission slit widths were constant at 1.5 and 3 nm,

respectively. Between 350 and 410 nm, emission spectra were scanned at a 60 nm/minute scan rate. The ratio of first and third vibronic peaks (I_1/I_3) of the fluorescence spectra of pyrene, were utilized to compute CMC (using Boltzmann Sigmoidal Fitting) and micellar apparent dielectric constant (Dexp).

2.2.3. Dynamic light scattering (DLS) / Zeta (ζ)-Potential measurements

Average hydrodynamic diameter (Dh) and Zeta (ζ) - potential measurements were obtained from the Malvern Zeta sizer (Nano ZS ZEN3600). This instrument is equipped with a green (5320Å) laser and photomultiplier tube detectors. Nearly 0.5 ml sample (filtered through nylon filter of 0.22 μm) taken into dipped electrode plastic cuvette and placed in a sample chamber. Values are an average of 5 decay cycles (each cycle is 11 runs with 10s interval).

2.2.4. Transmission Electron Microscopy

Transmission electron microscope (TEM) image was obtained with a JEOL JEM 2100 transmission electron microscope accelerating at a working voltage of 120 kV. A drop of mixed gemini solution was placed on the carbon-coated copper grid (200 mesh) followed by drying for a few minutes (~298 K).

2.2.5. NMR Measurement

^{1}H NMR spectra were obtained with a Bruker NMR spectrometer with a proton resonance frequency of 400.15 MHz at 298 K.

2.2.6. Solubilization experiment

Solubility of PAH/drug has been determined in aqueous gemini systems (single or mixed), at different mole fractions (x = 0 – 1, total [gemini] = 10mM) by adding an excess amount of PAHs or Drugs. Aqueous gemini(s) + PAH/Drug mixture has been equilibrated for 48h before centrifugation to remove excess PAH/Drug. The solubilization of PAH/Drug in micellar solutions, containing different morphologies, has been analyzed, at respective λ_{max}, by UV-visible spectrophotometer (Shimadzu, UV-1900) having a quartz cell (path length 1 cm) at 303 K. The composition of gemini mixture was the same in both reference and measurement cuvettes to eliminate its effect on the UV absorbance.

2.2.7. *In-vitro* drug release study

Release profile of RLX from gemini mixture was investigated at physiological conditions in alcoholic phosphate buffer (phosphate buffer: methanol (80:20), 310 K and pH 7.4) by adopting dialysis bag method. 3 mL RLX solubilized mixed gemini solution and pure drug solution (in water) were kept in two separate dialysis bags under identical conditions. 1 mL samples were taken from the medium at fixed time periods and compensated by the same volume of fresh

medium. This maintains good sink conditions. The release [RLX] from the above bags was analyses by UV spectrophotometry (UV-1900) at λmax 286 nm. Triplicate measurements have been performed to ensure reproducibility.

2.2.8. *In-vitro* Antioxidant Activity

Drug and drug-solubilized mixed gemins were tested for their antioxidant activity by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. To assess the antioxidant activity of drugs and drugs solubilized mixed geminis with a range of concentrations (1.0- 10 g/mL), a set quantity of DPPH (0.1 mmol/L, 2.0 mL) was added as an influencing free radical. Prior to measurements, the sample solutions were thoroughly mixed, and the mixtures were incubated at room temperature for 15 minutes in a darkened area. The samples were then examined using UV-visible spectroscopy at 517 nm. Three replicates of each experiment were performed. The following equation was used to get the percentage of inhibition.

2.2.9. *In-vitro* cell Proliferation Activity

The cytotoxicity of the optimized RLX, mixed geminis (without RLX), and RLX solubilized mixed geminis against MCF-7 breast cancer cells has been obtained by a tetrazolium salt MTT assay (5.0 mg.cm-3, 24h). IC50 (half-maximal inhibitory content) has been acquired by a well-known MTT assay procedure [7]. After incubation, MTT has been removed followed by DMSO addition in each well. Absorbance data have been recorded (570 nm) by a multimode microplate recorder (spectramax M2e, molecular device, USA). Statistical analysis has been performed using the student's t-test by employing sigma state 2.0 software. Shapiro-Wilk test has been used to ensure the normality of data before performing the above test. Two-tailed p values of <0.01 are regarded as statistically significant differences.

Chapter 3: ASSOCIATION BEHAVIOUR AND INTERACTION OF OPPOSITELY CHARGED GEMINI SURFACTANTS IN AQUEOUS SOLUTION

CMC of all single and counter-charged mixed geminis have been determined by fluorometry and conductometry. Fluorometry results of a probe (pyrene) can be utilized to understand micelle formation and its internal polarity. A few typical plots related to intensity vs wavelength are shown in Figure 3(a) (0.2 $x_{12\text{-}4\text{-}12A}$ + 0.8 $x_{14\text{-}4\text{-}14}$). The ratio of the intensities of solvent responsive peak to the solvent irresponsive peak (I_1/I_3) gives an idea of internal environment (polarity) of the micelle. Various such graphs (Figure 3 (a)) are used to obtain I_1/I_3

vs [mixed geminis] sigmoid type curves for gemini mixture at different compositions (x = 0-1) (Figure 3 (b)).

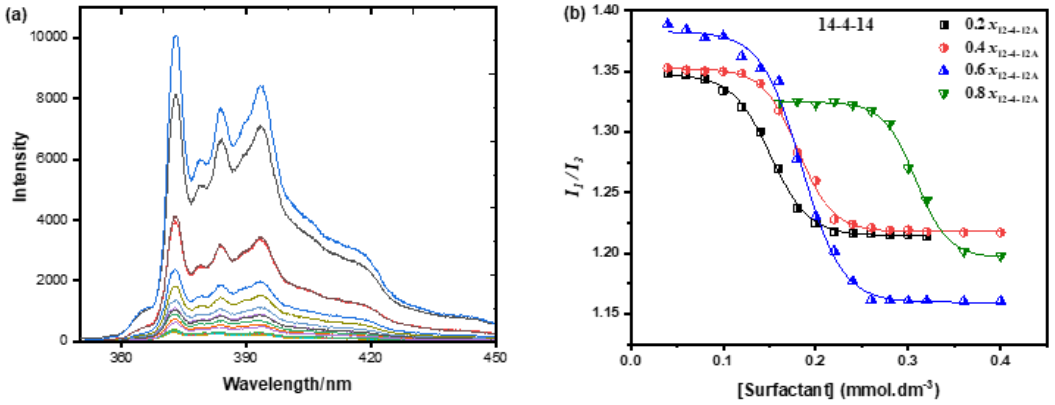


Figure 3. (*a*). Representative Intensity vs Wavelength plots for pyrene in 0.2 $x_{12\text{-}4\text{-}12A}$ + 0.8 $x_{14\text{-}4\text{-}14}$ at 303 K and (b) Variation of I_1/I_3 *vs* [mixed geminis] in aqueous solution at 303 K.

One of the most used techniques for determining a change in micellization is conductivity measurements. Conductance grew steadily up to a particular concentration when [surfactant] was added, and then it abruptly reduced. The critical micelle concentration (CMC) can be accurately determined at the sharp/narrow breakpoint established at that point. Figure 4 shows the variation of κ with [mixed geminis] for 14-*s*-14 / 14-Eg-14 or 14-Isb-14 + 12-4-12A combinations.

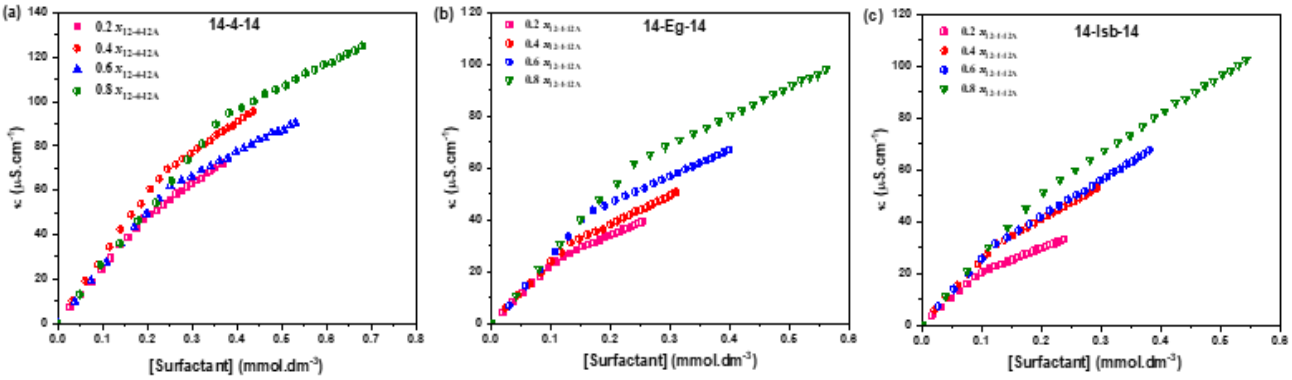


Figure 4: Plots of specific conductance (κ) *vs* [Surfactant] of aqueous mixed geminis (a) 14-4-14 (b) 14-Eg-14 and (c) 14-Isb-14 at different $x_{12\text{-}4\text{-}12A}$ at 303 K.

A pseudo phase separation model has been applied to evaluate how the CMCs of binary mixtures (12-4-12A + cationic gemini) deviate from the ideal mixing [8]. The CMC values of the mixture (CMC_{exp}) are found lower than the individual components of the mixture. β^m values have

been found negative in all the samples of mixed oppositely charged gemini surfactants. This behavior of β^m tells that all the geminis interact synergistically at each mixing composition (x = 0.2 – 0.8).

Chapter 4. COMPOSITION TRIGGERED MORPHOLOGIES OF MIXED OPPOSITELY CHARGED GEMINIS HAVING DIFFERENT CHAIN-LENGTH AND SPACERS

Among surfactant morphologies (spherical micelle, ellipsoidal micelle, rod shape micelle, worm like micelle, vesicle, helical structure, or tubular shape) that have been obtained so far, vesicle has attracted much attention because they can serve different purposes such as biological model membranes[9], containers for encapsulation and eventual release of drugs [10] and microreactor for the formation of a range of inorganic nanoparticles[11]. The present chapter contains studies on the effect of composition variation on morphological changes of aqueous mixed gemini surfactant systems. Such morphological studies are performed using scattering techniques (Dynamic light scattering (DLS) and Transmission Electron microscopy (TEM)). Zeta-potential measurements have also been performed to acquire information related to the variation of the magnitude of charge by compositional variation.

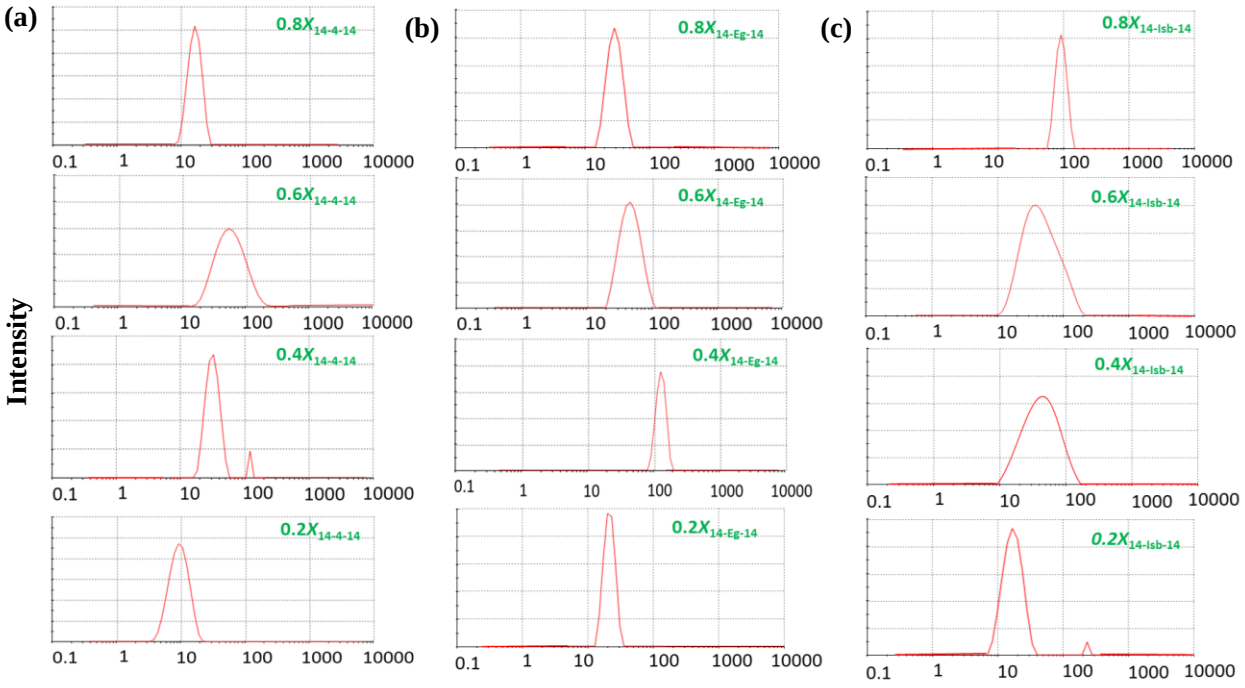


Figure 5. DLS spectra of 10 mM mixed aqueous gemini surfactant systems at different mole fraction of anionic gemini surfactant ($x_{12\text{-}4\text{-}12A}$) system at 303 K (a) 14-4-14 (b) 14-Eg-14 and (c) 14-Isb-14.

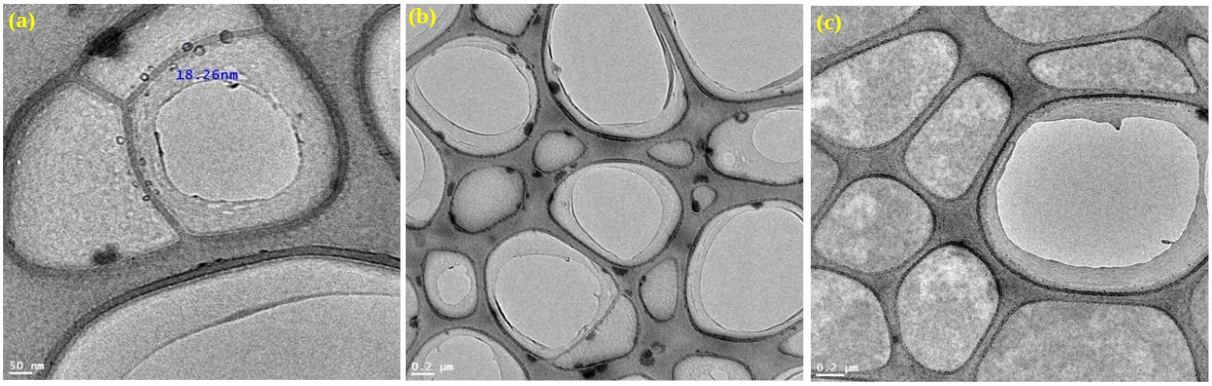


Figure 6. TEM images of 10 mmol.dm^{-3} aqueous mixed gemini surfactant system of (*a*) 0.4 $x_{12\text{-eda-}12}$ (*b*) 0.4 $x_{14\text{-Eda-}14}$ and (c) 0.6 $x_{14\text{-Eda-}14}$

Chapter 5. SOLUBILIZATION OF POLY AROMATIC HYDROCARBONS (PAHs) IN INDIVIDUAL AND MIXED GEMINIS SYSTEMS

Cationic gemini surfactant has recently been a common addition to mixed micelles in order to enhance solubilization, pollutant removal, or surface activity [12,13]. The solubilization of organic solutes rises significantly in aqueous surfactant aggregates (micelles or vesicles), which is the basis for numerous applications based on surfactants [14,15].

Increased solubilization of polycyclic aromatic hydrocarbons (PAHs) has been seen when cationic gemini and oppositely charged anionic gemini surfactant are combined [16,17]. Gemini combinations have a higher potential for solubilization than their separate constituents. Large aggregates with higher hydrophobic volume than the individual gemini surfactant micelles, are responsible for the effective solubilization of PAH and higher MSR values. Over rod- or ellipsoidal micellar systems, vesicular aggregates were shown to be more effective in solubilizing PAHs. Therefore, composition/morphology plays a decisive role in solubilizing the PAHs.

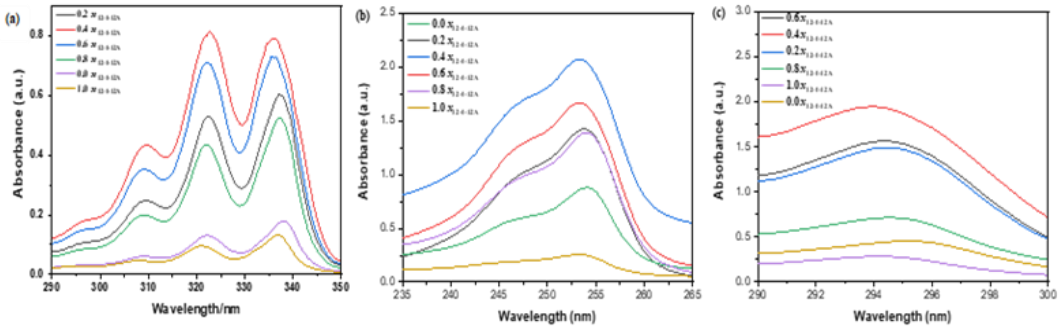


Figure 7. Representative UV-Visible spectra of (a) pyrene (b) anthracene and (c) phenanthrene in 10 mM aqueous pure and mixed gemini surfactant (14-4-14 + 12-4-12A) solution at different mole fractions of anionic gemini ($x_{12\text{-}4\text{-}12A}$).

Chapter 6. COUNTER CHARGED GEMINI MIXTURES FOR SOLUBILIZATION / RELEASE OF VARIOUS DRUGS

Pharmacology, detergency, microemulsion, increased oil recovery, and fabric dyeing all rely heavily on the aqueous solubilization of water-insoluble compounds. Micelles/vesicles are potent solubilizers amongst hydrodynamic self-assembled aggregates, however, the solubilization capabilities of vesicle bilayers are higher. The objective of the current work is to improve the aqueous solubility of the amphiphilic drugs by using aggregates of oppositely charged mixed geminis. UV-visible measurements were used to quantify the drug's solubility in a mixed gemini surfactant system. Molar solubilization ratio (MSR) and partition coefficient (Km) are two attributes which been calculated to corroborate experimental findings of drug solubilization in a mixed geminis. Solubilization plots wavelength *vs* absorbance for drug (Raloxifene) solubility in mixed geminis are shown in figure 8. Maximum solubilization of RLX was observed at compositions ($x_{12\text{-}4\text{-}12A}$ = 0.4 or 0.6) where higher order aggregates were present.

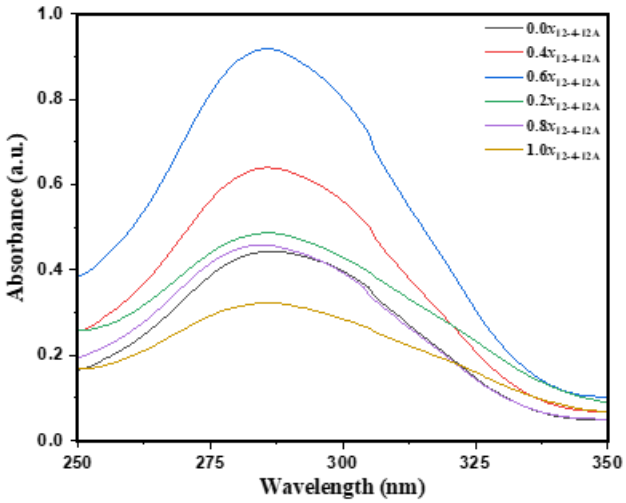


Figure 8: Representative UV-visible spectra of RLX in 10 mM aqueous pure and mixed geminis (12-Eda-12 + 12-4-12A) at different $x_{12\text{-}4\text{-}12A}$.

Drug release profile has been obtained in gemini mixture containing vesicles under physiological conditions [18]. Drug release is governed by the presence of vesicles in the release mixture. The rate of release of drug has been found to be dependent on the site of the solubilization within the surfactant aggregates. The release kinetics has been studied by considering various kinetics models (zero-order, first-order and Higuchi).

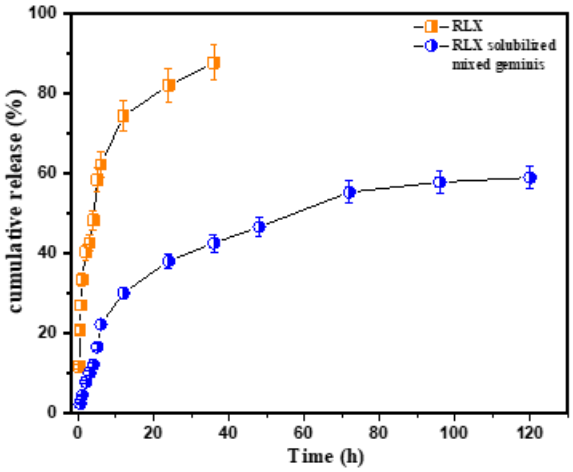


Figure 9: Cumulative release profile of Raloxifene hydrochloride from mixed micellar formulation at physiological conditions.

Cell proliferation activity (on MCF-7 cells) of anti-cancer drug RLX, mixed geminis and RLX solubilized mixed geminis (12-4-12A + 14-Eda-14, x = 0.6) was obtained by MTT assay (Figure 12). It can be seen that no apparent toxicity is observed when RLX solubilized in chosen vesicular system, indicating that the system is nearly safe and non-toxic and can be used in place of highly unsafe excipients.

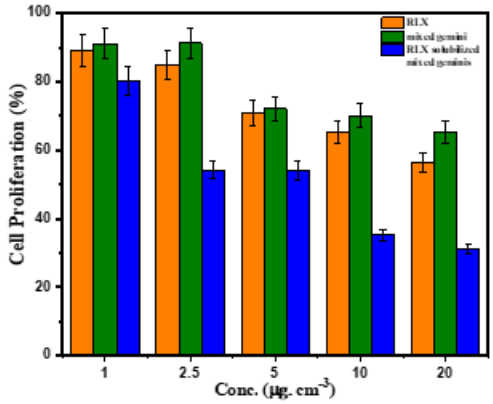


Figure 10: Cell proliferation activity of pure RLX, mixed geminis and RLX solubilized mixed geminis towards MCF-7 cells.

IC_{50} ([RLX] needed to attain 50% cell proliferation) data suggest that RLX solubilized vesicular system provides increase cell proliferation in comparison to pure RLX.

Chapter 7. OVERALL CONCLUSION

Surfactants' adaptability results from their amphiphilic nature, and the molecules' dual possession of non-polar, water-insoluble moieties and polar, water-soluble portions. To improve the aqueous surfactant systems' productivity on different fronts, numerous compositional changes have been made. Different aggregation and surface characteristics are determined using various physico-chemical techniques. The properties of mixed surfactant systems are superior to those of their constituent. Various regular solution theories (Clint, Rubingh and Motomura) are used to treat the CMC data. The synergistic interaction is clearly demonstrated by the negative values of interaction parameter (β) for all the systems. An alluring way to adjust the micellar shape and produce vesicles in the solution appears to be through above approach. It has been observed that a solution having vesicular aggregates is more effective than the other morphologies for solubilizing PAHs. Similarly, solubilization of hydrophobic drugs have also been found more in vesicles than any other morphologies. *In vitro* release study revealed sustained delivery of drugs from the mixed micelles (Higuchi model). It can be seen that hydrophobic micelles release of drug from the core region. The drug solubilized mixed micelle improved the neutralization of the DPPH radicals as well as the antioxidant tendency of the drug. Cell proliferation assay suggested improved cytotoxicity and IC_{50} values of drugs in the mixed micelle (co-micelles).

❖ **Presentations:**

(1). Brijesh Patel, Sneha Singh and Sanjeev Kumar, poster presentation in National Conference on Current trends and advantages in chemical sciences ,12 January,2021, held at B.K.M Science College, Valsad.

(2). Brijesh Patel, Sneha Singh and Sanjeev Kumar, poster presentation in national conference at PPSU, Surat.

(3). Brijesh Patel, Sneha Singh and Sanjeev Kumar, presented a paper in 'WORLD CHEMISTRY CONFERENCE 2021' organized by Department of Chemistry, Wilson College, Mumbai, on 3rd and 4th May,2021.

(4). Brijesh Patel, Sneha Singh, Debes Ray, Vinod K. Aswal and Sanjeev Kumar, oral presentation in Molecules to Materials (MTM – 2021) organized by Department of Chemistry, Sardar Vallabhbhai National Institute of Technology (SVNIT), Surat-07, Gujarat on 17-18th December, 2021.

(5). Brijesh Patel, Sneha Singh and Sanjeev Kumar, poster presentation in international seminar on Advanced material and applications (ISAMA 2022) organized by Applied Physics and Applied Chemistry Department, Faculty of Technology and Engineering, MSU Baroda, Vadodara on 18th July,2022.

❖ **Publications:**

1. Composition triggered Aggregation/Solubilization behaviour of mixed counter charged gemini Surfactants: A Multi-technique investigation.
   Brijesh Patel, Sneha Singh, Kushan Parikh, Vishwajit Chavda, Darshna Hirpara, Debes Ray, Vinod K. Aswal and Sanjeev Kumar. Journal of Molecular Liquids, 359, p.119242.
2. Micro-Environment Mapping of Mole Fraction Inspired Contrasting Charged Aqueous Gemini Micelles: A Drug Solubilization/Release study.
   Brijesh Patel, Sneha Singh, Kushan Parikh, Vishwajit Chavda, Debes Ray, Vinod K. Aswal, and Sanjeev Kumar. Journal of Molecular Liquids, p.119885.
3. Amplification of Curcumin entrapment in aqueous bio-amphiphile mixed with different counter-charged cationic surfactants: Influence of alkyl chain length and mixing composition.
   Brijesh Patel, Harsh Mahera and Sanjeev Kumar. Food Chemistry, (manuscript in preparation).

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