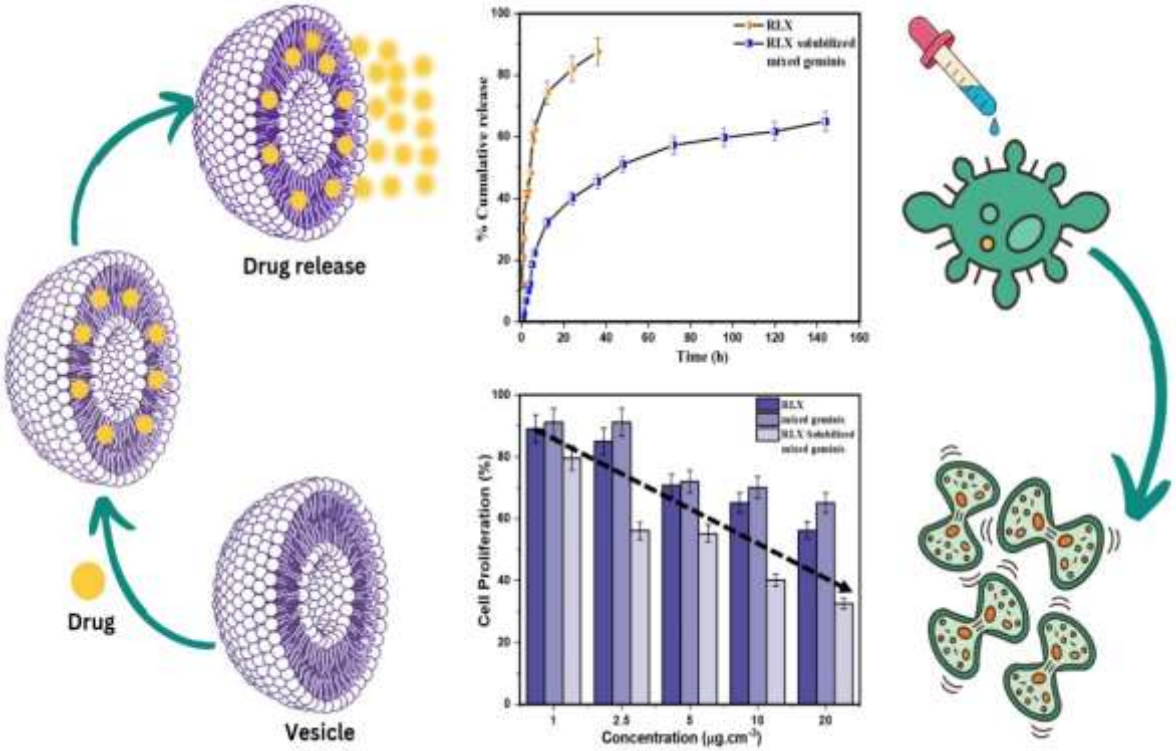
Chapter 5

Counter Charged Geminis Mixture for Solubilization/Release of Raloxifene Hydrochloride



5.1. Introduction

Studies embodied in chapters III and IV dictate the nature of system which can be used for water insoluble materials such as drugs, dyes, pesticides etc. [1–3]. This system can also find potential application dealing with delivery/targeting of some specific drugs e.g., anti-cancer drugs [4]. Drug delivery mostly preferred over surgical intervention/sterilizing treatments [5,6]. Micellar systems are preferable for increasing solubility/bio-availability/interactions involving drugs or drug intermediates and therefore crucial in the pharma industry and overall health sectors [7–9]. Few systems optimized from the studies of previous chapters can be used to achieve above mentioned goal(s). It may be mentioned that vesicular systems are potentially more beneficial for the process of drug delivery in comparison to other surfactant based self-assemblies [1,10,11].

There are numerous approaches that may be adopted in order to produce surfactant vesicles in an aqueous solution (as detailed in previous chapter related to morphology). It may be mentioned here that blending of surfactants (in particular counter charged surface active moieties) is a direct and convenient approach to obtain vesicles in aqueous environment [12]. Above mixing provides other surfactant-based morphologies (spherical or ellipsoidal micelle, rod or warm like micelle, bilayers etc.,) whose behaviour and performance can directly be compared with vesicular assemblies.

The purpose of this investigation is to employ morphologies of mixed counter-charged geminis to facilitates the aqueous solubility, release profile, and anti-carcinogenic effect of an anticancer drug of a synthetic anti-cancer drug (RLX, raloxifene hydrochloride). RLX has been used in the prevention and treatment of osteoporosis and invasive breast cancer in advance aged females. Solubilization behaviour of RLX has been investigated in a variety of designed

mixed blended systems. Data related RLX solubility in mixed geminis are acquired from spectrophotometry. Such experiments helped in deciding the optimum system(s) for further studies related to *in-vitro* and cell proliferation activity. The experiment may address the problem of less bioavailability and poor systemic exposure. Further, controlling the release kinetics of RLX using above said delivery vehicle can another potential benefit of using an appropriate composition of the blend.

RLX release mechanism, under physiological environment, has been also been investigated considering a number of different kinetic models. A tetrazolium salt MTT test was employed to evaluate the cytotoxic effects of the optimized systems against MCF-7 (a breast cancer cell).

5.2. Results and Discussion

5.2.1. RLX Solubilization

RLX absorption spectra at various compositions of gemini are compiled in Figure 1. For the purpose, two combinations of geminis (14-Eda-14 + 12-4-12A and 12-Eda-12 + 12-4-12A) are used by taking various compositions (x = 0-1).

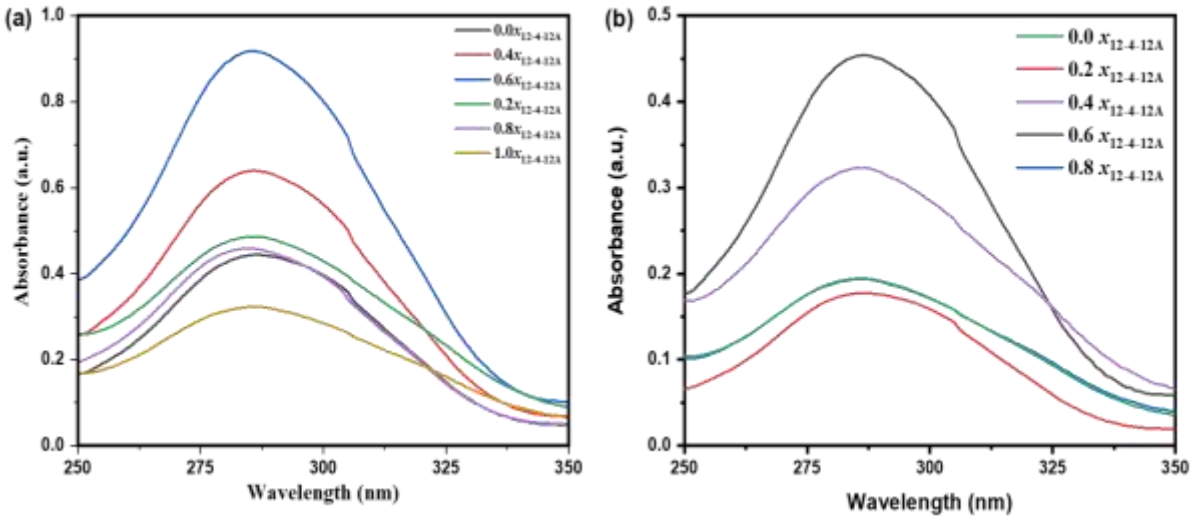


Figure 1. Representative UV-visible spectra of RLX solubilized in 10 mM aqueous mixed geminis at different mole fractions of anionic gemini ($x_{12\text{-}4\text{-}12A}$): (a) 14-Eda-12 and (b) 12-Eda-12.

Absorption spectra show that absorbance is dependent on composition of gemini mixture. However, absorbance values at the λ_{max} (wavelength of maximum absorbance) are higher when the two components are nearly equal (x = 0.4 or 0.6). Incidentally these compositions give vesicular aggregates as observed in Chapter IV. Therefore, vesicles can effectively use to get higher aqueous solubility of RLX (or other drugs in general). With similar gemini mixtures, aqueous solubilities for polycyclic aromatic hydrocarbons (PAHs) were found higher in similar composition range [13,14].

In order to corroborate the results of the experimental investigations on the solubility of RLX in a blended geminis, a few parameters were computed such as molar solubilization ratio (MSR), distribution coefficient (K_m) and standard Gibbs free of drug solubilization (ΔG_s^0). Equation 1 has been used to obtain MSR values from solubilities (acquired from Figure 1 using Beer-Lambert law) in mixed geminis [15].

$$MSR = \frac{S_T - S_{CMC}}{C_M} \quad (1)$$

Where S_T denotes total RLX solubility, S_{CMC} denotes drug solubility at the CMC (of mixed geminis) and C_M denotes aggregated [gemini(s)].

MSR values are used to compute magnitude of mole fraction of drug in aggregate (or micellar) phase (X_m) using the following relation.

$$X_m = \frac{MSR}{1+MSR} \quad (2)$$

K_m has been found to dependent on temperature and gemini/RLX nature. K_m can be obtained by the ratio of mole fraction of RLX in aggregate/micelle (X_m) and aqueous phase (X_a). The mathematical relation can be shown here as:

$$K_m = \frac{X_m}{X_a} \quad (3)$$

Here, X_a can be obtained using following equation (4).

$$X_a = S_{CMC} * V_m \quad (4)$$

Where, V_m is the molar volume of water, equal to 0.01805 l mol^{-1}. S_{CMC} can be considered as a drug's water solubility. Therefore, partition coefficient (K_m) for solubilization becomes:

$$K_m = \frac{MSR}{(S_{CMC})\, V_m\, (1+MSR)} \quad (5)$$

Exploring the thermodynamic factors that influence solubility is indeed crucial for providing a better understanding of the RLX solubilization process within the aqueous blended

geminis. ΔG_s^0 has been computed using above obtained K_m values (Table 1) by applying the following expression.

$$\Delta G_s^0 = -RTlnK_m \quad (6)$$

where R and T are gas constant and experimental temperature. The so obtained data related RLX solubilization in mixed geminis are also compiled in Table 1.

Table 1. Solubilization parameters (molar solubilization ratio, MSR; micelle-aqueous Phase partition coefficient, $\ln K_m$; Gibbs free energy, ΔG_s^0) of **RLX** in aqueous single and mixed gemini surfactants with varying mole fraction of 12-4-12A ($x_{12\text{-}4\text{-}12A}$) at 303 K.

$x_{12-4-12A}$	**Morphology**	**MSR**	**ln K_m**	$-\Delta G_s^0$ **(kJ mol^{-1})**
		12-Eda-12		
0.0	Ellipsoidal	0.119	8.681	21.868
0.2	Ellipsoidal	0.119	8.679	21.865
0.4	Rod	0.144	8.842	22.276
0.6	Vesicle	**0.201**	9.131	23.003
0.8	Ellipsoidal	0.116	8.656	21.806
1.0	Ellipsoidal	0.104	8.552	21.544
		14-Eda-14		
0.0	Ellipsoidal	0.121	8.693	21.898
0.2	Ellipsoidal	0.127	8.737	22.009
0.4	Vesicle	0.154	8.905	22.433
0.6	Vesicle	**0.204**	9.140	23.025
0.8	Ellipsoidal	0.125	8.724	21.977
1.0	Ellipsoidal	0.104	8.552	21.544

Data of Table 1 show that counter charged blended geminis give improved performance over the individual components of the blends. Higher MSR values and effective RLX solubilization attribute to voluminous aggregates with an effective higher hydrophobic volume than that of the individual gemini aggregates. The findings allow to say that vesicles are more efficient in RLX solubilization in comparison to other blended morphological structures as mentioned earlier (Chapter IV), vesicles are formed near equal mole fraction of the two components of a typical blend (x = 0.4 or 0.6). Therefore, as a corollary, the composition and architecture of aggregates play significant roles in the process of solubilizing hydrophobic drug e.g., RLX.

It might be possible to assert that a usual gemini aggregates may be suspected of as being comprised of distinct layers of varying polarities [16,17]. Such polarity alterations are influenced by the length of the hydrocarbon tail, the type of head group, the behaviour of the spacer together with composition of the blend. Figure 2 depicts the effect of varying mole fraction of geminis (12–4–12A + 12-Eda-12/ 14-Eda-14) on the D_{exp} and MSR of RLX.

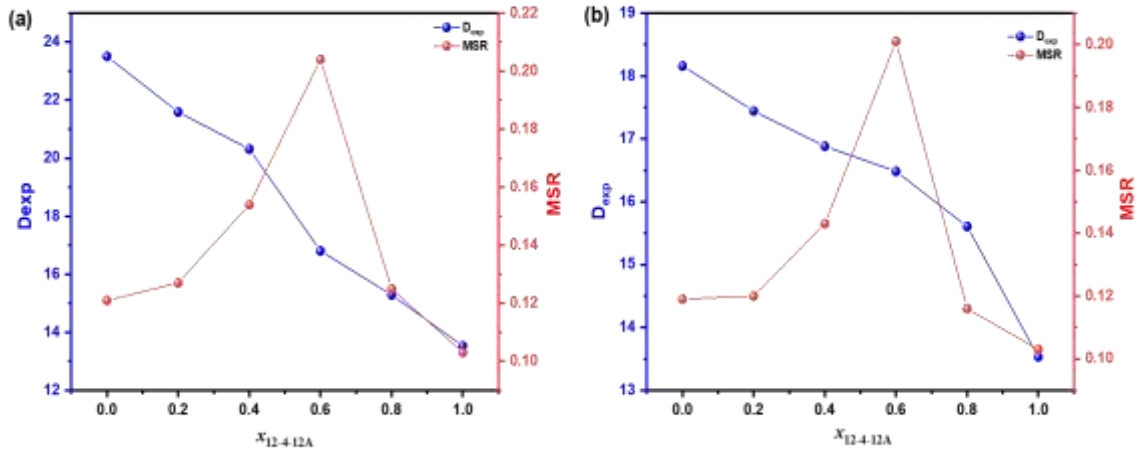


Figure 2. Variation of D_{exp}/MSR of RLX with varying $x_{12\text{-}4\text{-}12A}$:(a) 14-Eda-14 and (b) 12-Eda-12.

5.2.2. RLX Release Study

Based on Solubilization studies (previous section), 14-Eda-14 + 12-4-12A system has been chosen for further studies discussed here. RLX release profile (as detailed in Chapter II) has been obtained (Figure 3) in gemini mixture containing vesicles (0.6 *x*12-4-12A + 0.4 *x* 14-Eda-14) under physiological conditions (the system shows highest MSR (Table 1) at this composition together with vesicular assemblies). The release was evaluated up to 120 h with different-different time intervals based on trial-and-error methodology. RLX release is governed by the presence of vesicles in the RLX release mixture. The rate of release of drug has been found to be dependent on the site of solubilization within the gemini(s) aggregates [18]. As vesicles has environments of varying polarity, RLX may partition in those regions and bind with different interactive forces [19]. If this is correct then release profile should follow non-monotonous behaviour. This indeed was observed from the release profile of RLX shown in Figure 3. The head group region of the mixed aggregates is quite mobile (medium polarity) and RLX may release speedily from this region as can be seen from the initial part of release profile of RLX (Figure 3). However, RLX partitioning from vesicle interior may show slow release which is responsible for the result of later part of release profile (Figure 3).

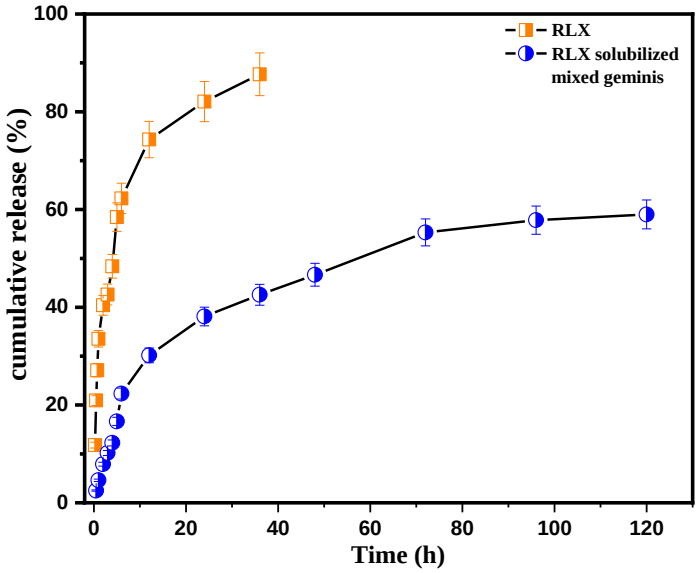


Figure 3. Cumulative release profile of RLX from micellar formulation at physiological conditions.

The release kinetics has been studied by considering various kinetics models (zero-order, first-order and Higuchi) and fitted results are given in Table 2. Based on the analysis of the results (square of correlation coefficient, R^2), it can be seen that RLX release can be best described by Higuchi model which is the indication of the release of RLX via diffusion mechanism. Therefore, present vesicular systems can be used for sustained RLX release from the resultant medium.

Table 2. Different kinetic models depicting the Raloxifene hydrochloride **(RLX)** release pattern from mixed gemini micelles **(14-Eda-14 + 12-4-12A)**.

	Mathematical models for drug release kinetics			
	Higuchi	Zero-Order	First-Order	K-peppas
R square	**0.983**	0.862	0.713	0.901
Slope	11.844	2.777	0.105	1.093
Intercept	-8.911	1.669	0.512	0.474

5.2.3. Cell Proliferation Activity

Cell proliferation activity (on MCF-7 cells) of RLX, mixed geminis and RLX solubilized mixed geminis (12-4-12A + 14-Eda-14, x = 0.6) was obtained by MTT assay (Figure 4). 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. IC_{50} ([RLX] needed to attain 50% cell proliferation) values for pure RLX, vesicles and RLX solubilized vesicles were computed and compiled in Table 3. The order of IC_{50} value is found 9.06, 21.75 and 28.37 for RLX solubilized gemini mixture, pure RLX and pure gemini mixture, respectively. IC_{50} data clearly suggest that RLX solubilized vesicular system provides increase cell proliferation in comparison to pure RLX.

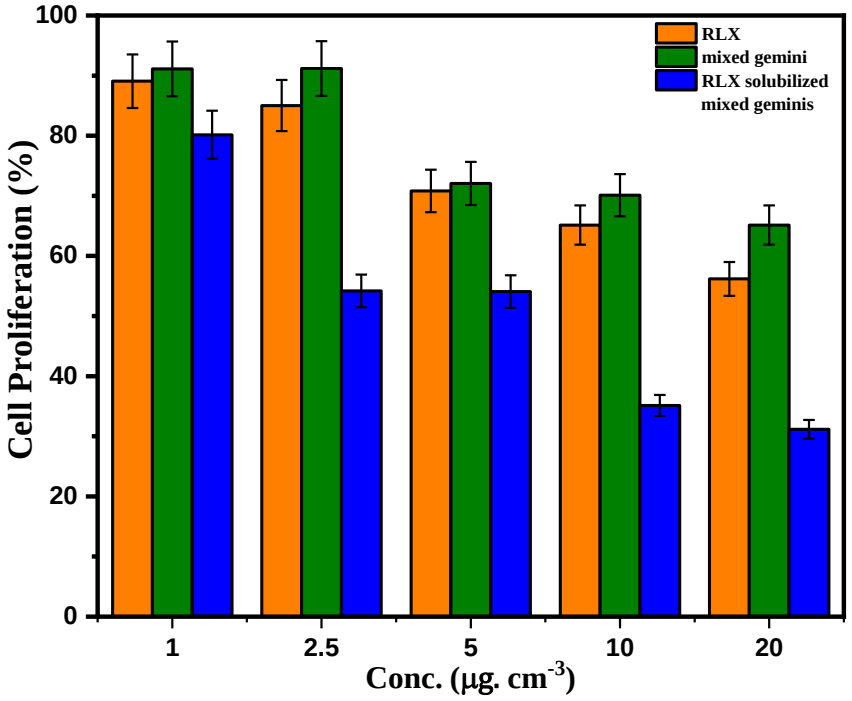


Figure 4. Cell proliferation activity of pure RLX, mixed geminis and RLX solubilized in mixed geminis towards MCF-7 cells.

Table 3. The half maximal inhibitory concentration (IC_{50}) of raloxifene hydrochloride (RLX), mixed geminis and RLX solubilized in mixed geminis in MCF-7 cells.

System	**IC_{50} (µg/mL)**
RLX	21.75
Mixed geminis	28.37
RLX solubilized mixed geminis	9.06

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