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Interaction of curcumin with 1,2-dioctadecanoyl-sn-*glycero*-3-phosphocholine liposomes: Intercalation of rhamnolipids enhances membrane fluidity, permeability and stability of drug molecule

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ABSTRACT

Stability of curcumin in neutral and alkaline buffer conditions has been a serious concern for its medicinal applications. We demonstrate that the stability of curucmin can be improved in 1,2-Dioctadecanoyl-sn-*glycero*-3-phosphocholine (DSPC) liposomes. Curcumin strongly partition into liquid crystalline phase compared to solid gel phase of DSPC liposomes. Variation of fluorescence intensity of curcumin associated with liposomes with temperature successfully determines phase transition temperature of DSPC liposomes. However, at higher molar ratio curcumin can influence phase transition temperature by intercalating into deep hydrophobic layer of liposomes and facilitating fusion of two membrane phases. Rhamnolipids (RLs) are recently being applied for various biomedical applications. Here, we have explored new insight on intercalation of rhamnolipids with DSPC liposomes. Intercalation of rhamnolipids exceptionally increases partition of curcumin into solid gel phase of DSPC liposomes, whereas this increase is moderate in liquid crystalline phase. Fluorescence quenching study establishes that permeability and fluidity of the DSPC liposomes are enhanced in the presence of RLs. Membrane permeability and fluidity can be improved further by increasing the percentage of RLs in DSPC liposomes. The phase transition temperature of DSPC liposomes decreases with increase in percentage of RLs in DSPC liposomes by encouraging fusion between solid gel and liquid crystalline phases. Intercalation of RLs is found to further boost stability of drug, curcumin, in DSPC liposomes. Thus, mixing RLs with DSPC liposomes could potentially serve as a good candidate for drug delivery application.

1. Introduction

Curcumin (see Scheme 1A) is extracted from the roots of the perennial herb Curcuma longa Linn [1]. Curcumin is well known for its wide range of biological activities [2]; it is effective against many inflammatory diseases [3–6]. The potential of curcumin against different autoimmune diseases has been reported [7–10]. Curcumin as a potential drug for anti-cancer activities [11–13] and anti-amyloid behavior [14] is widely getting realized. Moreover, interaction of curcumin with heterogeneous media can tune shape and size during nanorods synthesis [15]. However, its poor solubility [16] and poor stability especially in neutral/alkaline medium [17] remain important concerns. Several studies have targeted the enhancement of curcumin bioavailability and its delivery. These studies include incorporation into liposomes and phospholipids [18], surfactants [19], encapsulation as polymer nanoparticles [20], polyethylene glycol [21], and several cyclodextran inclusion complexes [22].

Encapsulation of curcumin in liposome offers another good alternative. Liposomes are of important interest since they can trap and release two compounds of different solubility [23,24]. Moreover, due to their biocompatibility and low toxicity, food and pharmaceutical industries have extensively investigated liposomal systems and their applications [25–27]. The most abundant lipid in animal cell membrane is phospatidylcholine, which gives structural framework. Phospatidylcholine primarily functions as part of the permeability barrier in the outer leaflet. It is also primary substrate of phospholipase D enzymes that produce the signaling lipids-phosphatidic acid and lysophosphatidic acid. 1,2-Dioctadecanoyl-sn-*glycero*-3-phosphocholine (DSPC) (see Scheme 1B) is an 18-carbon chain phospatidylcholine.

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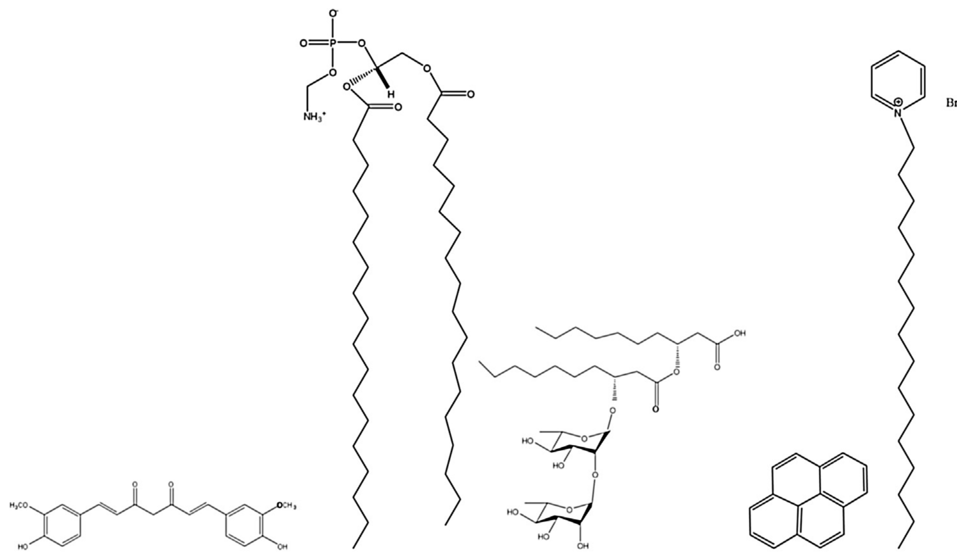


(A) Curcumin (B) DSPC (C) RLs (D) Pyrene (E) CPB

Scheme 1. Structure of (A) Curcumin; (B) DSPC; (C) RLs; (D) Pyrene; and (E) CPB.

On the other hand, biosurfactants [28] are surface-active biomolecules of microbial origin among which are Rhamnolipids (RLs) [29] (see Scheme 1C). RLs are glycolipid biosurfactants produced mainly by Pseudomonas aeruginosa [30] and can be described as glycosides that are composed, of a rhamnose moieties and lipid part [31]. RLs are characterized by unique physiochemical properties namely surface activity, wetting ability, detergency, and other amphipathic- related qualities [32–35]. These properties render RLs physiological functions that are highly important for the producing organism as well as many applications in industry [36]. Studies targeted the topological changes of liposomes that are induced upon interactions with surfactants [37], in addition to effect of anionic surfactants on both the disintegration of liposomes [38] and mixed liposome solubilization [39]. Moreover, few reports assessed the thermodynamics of liposome-surfactant interactions [40] and other work that aimed at understanding the effect of physiochemical interaction of cationic surfactants with model membranes on the antimicrobial effect of the former [41]. To our knowledge there is little report on influence of RLs on liposomes properties, which is aim in this work.

Curcumin is a fluorescent molecule and its photo-physical properties are greatly reliant on the polarity of the environment and the pH of the medium [42,43]. We have successfully established curcumin as a molecular probe to investigate liposome characteristics [44–46]. In this study, we have applied curcumin as a molecular probe to investigate the properties of DSPC liposomes that is not yet explored. It is found that curcumin strongly partition into DSPC liposomes and can effectively determine the phase transition temperature of DSPC liposomes. However, at higher concentrations, curcumin can influence phase transition temperature of DSPC liposomes. This probing concept has been further extended to examine the impact of RLs on DSPC membrane properties. This is the first study that applies curcumin as a probe to study the interaction between a biosurfactant and a phospholipid membrane and brings new insight on modulation of liposomes properties induced by RLs. The phase transition temperature of DSPC liposomes is influenced by RLs and it decreases with increase in percentage of RLs in DSPC liposomes. The study proves that intercalation of RLs with DSPC liposomes improves stability of curcumin by enhancing permeability and fluidity of the liposomes opening the possibility of using such system for drug delivery.

2. Materials and methods

2.1. Materials

DSPC was obtained from Avanti. Rhamnolipids, curcumin, pyrene and cetylpyridinium bromide (CPB) were obtained from Sigma-Aldrich and used as received. The stock solution of membrane was prepared in 50 mM mono- and dibasic phosphate buffer at pH 7.0. Further dilution was made in 50 mM mono- and dibasic phosphate buffer at pH 7.0. Because of poor solubility, the stock solution of curcumin was made in spectroscopic grade ethanol. A few microliters of the stock sample of curcumin (~1 mM) was taken in a vial and added to a final sample for measurement in buffer. Steps were taken to ensure that the final concentration of ethanol was negligible, less than 0.1% in the measurement sample, to avoid affecting the sample.

2.2. Preparation of liposomes

DSPC liposomes were prepared by the solvent evaporation method [47]. The desired amount of DSPC phospholipid was dissolved in 15 mL of a chloroform/methanol mixture (1:1 vol ratio). The solvents were evaporated using a rotary evaporator at 57–60 °C. The membrane formed was dried under vacuum for 10 min. Then glass beads were added, and enough phosphate buffer at pH 7.0 was used to form liposomes at a concentration of 1 mM. The mixture was vortexed rigorously for 15 min and finally heated for 30 min at 65 °C, about 14 °C above the phase transition temperatures for DSPC. When required, further dilution was made in 50 mM mono- and dibasic phosphate buffer at pH 7.0. The prepared liposomes were multilamellar vesicles (MLVs).

2.3. Incorporation of RLs

Stock solutions, ~1 g/L was prepared in double distilled water. The solutions were prepared by adding the desired volume (a few microliters) of RLs stock of appropriate concentration to the liposome (MLV) solution at 37 °C.

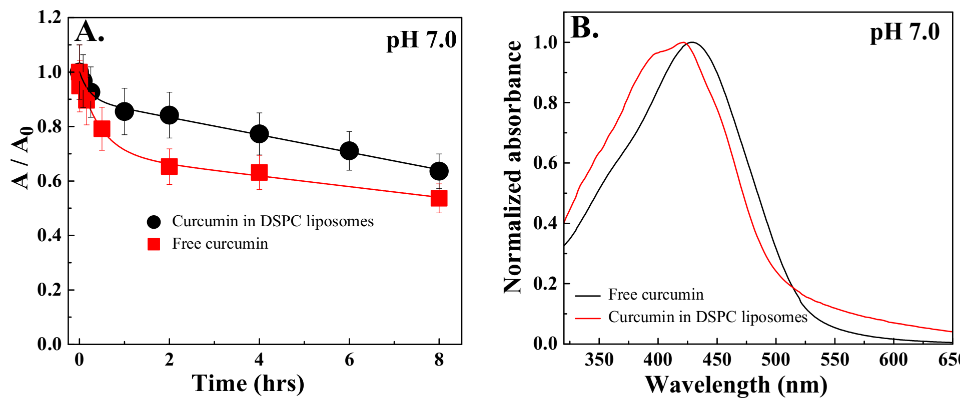


Fig. 1. (A) Rate of degradation of curcumin at pH 7.0 buffer solution in the absence and presence of DSPC liposomes, error bars are given in SD (n = 3); (B) Normalized UV–vis absorption spectra of curcumin in the absence and presence of DSPC liposomes at pH 7.0 buffer solution.

2.4. Spectroscopic measurements

The absorption spectra were recorded at room temperature using a JASCOV-570 UV–vis–NIR spectrophotometer. The steady-state fluorescence measurements were recorded with a resolution increment of 1 nm, slit 5 using a HORIBA Jobin Yvon Fluorolog-3 fluorometer and the FluorEssence program. To regulate the temperature, a thermostat was coupled with the sample holder. The final temperature was noted in the sample compartment using a thermometer rather than thermostat.

3. Results and discussion

3.1. Interaction and stabilization of curcumin in DSPC liposomes

Degradation of curcumin in physiological buffer solution is well reported [48] and for its applicability, stabilization of curcumin in neutral buffer condition is significant [49]. The degradation products of curcumin have different visible absorption than curcumin, thus, effect of the degradation products on the main absorption maximum of curcumin is negligible. Thus, UV–vis absorption spectrum of curcumin has been used to understand the degradation process of curcumin [50]. In the present case, the degradation of curcumin was studied at physiologically significant buffer, pH 7.0, in the presence and absence of DSPC. In neutral condition, 47% of curcumin degraded in 8 h in buffer solution compared to 37% degradation when associated with DSPC (see Fig. 1A), thus, presence of DSPC liposomes significantly stabilizes curcumin in neutral pH conditions. The suppression of the degradation of curcumin in this condition is possibly caused by interaction of curcumin with the DSPC liposomes. An evidence of this interaction is shift in the absorption spectrum of curcumin observed in DSPC liposomes. In neutral buffer condition (pH ~7) we found a 10 nm blue shift in the absorption maximum of curcumin associated with DSPC (see Fig. 1B). In non-polar environment curcumin absorbs in shorter wavelength compared to polar environment [45]. The blue shift in the absorption maximum of curcumin in DSPC suggests that curcumin is incorporated in hydrophobic pocket of DSPC. Moreover, the peak of curcumin in DSPC liposomes broadened with a relatively small shoulder at 397 nm. Such structure in absorption spectrum has also been noted for curcumin in Gemini [51] and neutral [52] surfactant solutions, thus, indicating association of curcumin with DSPC liposomes. Similarly, at pH 4.0, the UV–vis spectrum of curcumin revealed a strong absorption peak at ~432 nm, which was shifted to ~423 nm in DSPC liposomes at pH 4.0 suggesting acidic environment does not affect association of curcumin with DSPC liposomes. However, at pH 13 the absorption maximum of curcumin in DSPC liposomes was found to be same as that of free curcumin with maximum at ~468 nm. Curcumin possess 3 pKa values: 8.38, 9.88, and 10.5 [53]. At pH 13, curcumin is de-protonated to give a negatively charged species Cur^{3-}. Since there is little influence by DSPC liposomes on deprotonated form of curcumin, Cur^{3-}, compared to protonated form of curcumin (cur), association of curcumin with liposomes is pH dependent. The fluorescence intensity of curcumin was significantly enhanced in the presence of DSPC irrespective of solid-gel (shown in Fig. 2A) or liquid crystalline phases. Heightening of curcumin fluorescence intensity in water has been reported with micelles, membranes and methanol [42,54]. The rate of increase in the intensity of fluorescence in solid-gel was greater than that in liquid-crystalline phase; however, in both there is a blue shift of ~57 nm magnitude in the emission spectra of curcumin in the presence of DSPC compared to curcumin in buffer solution. This >50 nm shift further reconfirms the association of curcumin with DSPC liposomes.

3.2. Partition coefficient of curcumin in DSPC liposomes

The partition coefficient (K_p) indicates the fraction of the lipophilic molecule associated with the lipid. The change in the fluorescence intensity of curcumin in the aqueous phase and after association with the liposomes presents a potential tool to evaluate Kp. The partition coefficient of curcumin into DSPC liposomes can be expressed as [55]:

$$\frac{1}{F} = \left[55.6/KpF_0\right]\left(1/DSPC\right) + \frac{1}{F_0}$$

where F is fluorescence at a given liposome concentration and F_0 is the fluorescence obtained from total curcumin incorporated into the liposomes. K_p was calculated by 1/F vs. 1/[DSPC] as depicted in Fig. 2B. The partition coefficient of curcumin into DSPC at the solid gel phase was found to be 8.38×10^4. The partition coefficient increased by a factor of 3.6 in the liquid crystalline phase ($K_p = 3.08 \times 10^5$) suggesting that the partition of curcumin into DSPC at the liquid crystalline phase is more than that at the solid gel phase. This trend is similar to earlier observation for DMPC [45] and DPPC [46] liposomes and logical due to the fact that the permeability of the liposome at the liquid crystalline phase is higher than that at the solid gel phase where the former is characterized by flexibility compared to the rigidity and the latter is known to be dense form. As the permeability increases, the incorporation of curcumin into the membrane is expected to enhance.

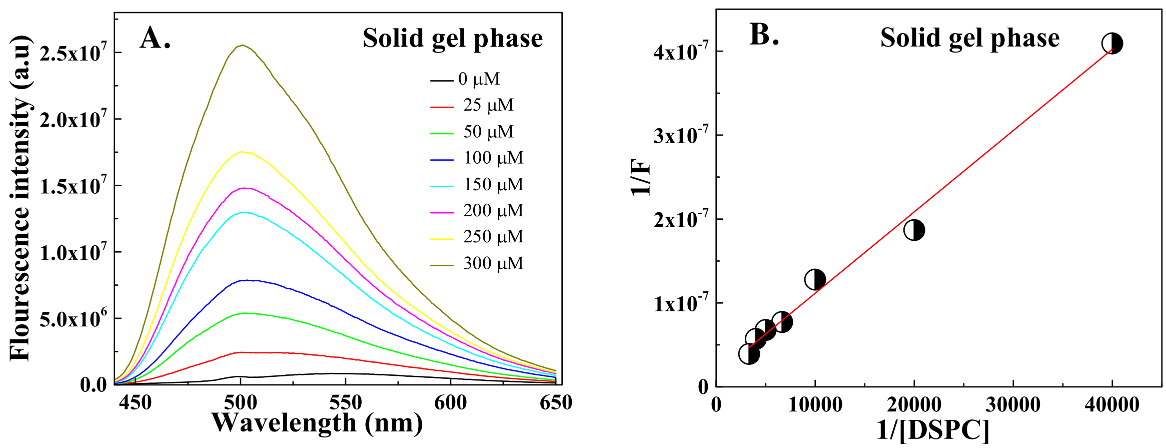


Fig. 2. (A) Fluorescence emission spectra of curcumin in various concentration of DSPC liposomes in solid gel phase; (B) Plot of 1/F vs 1/[DSPC] in solid gel phase. Fluorescence intensity was measured at excitation wavelength 425 nm and emission wavelength 492 nm.

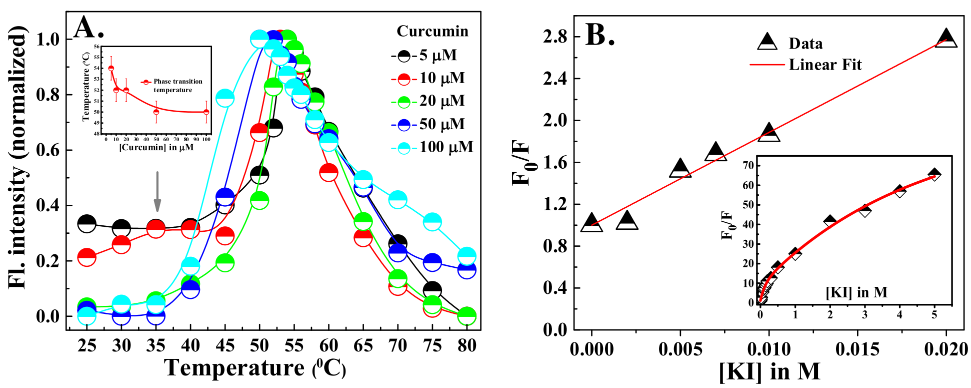


Fig. 3. (A) Profile of fluorescence intensity of curcumin vs. temperature at various concentration of curcumin concentration in DSPC liposomes, inset shows variation in phase transition temperature of DSPC liposomes with curcumin concentration, error bars are given in SD (n = 3); (B) Stern-Volmer plot for fluorescence quenching of pyrene by KI (0 to 20 mM) in DSPC liposomes, inset shown the same plot for KI from 0 to 5 M.

3.3. Determination of phase transition temperature of DSPC liposomes

Phase transition is an important aspect of the liposomes that affects various important functions of membranes. DSPC liposomes undergo a transition from solid gel to a rippled gel phase and it occurs around 35°, known as pre-transition [56]. The major phase change occurs along with the melting of the acyl chains in the hydrophobic part of the membrane. This transition into the liquid crystalline phase of DSPC occurs near 54 °C [56]. As shown in Fig. 3A, the fluorescence intensity of curcumin in DSPC liposomes increased with temperature to a maximum at the phase transition temperature (Tm), after Tm, the intensity decreased as the system became homogeneous at the liquid phase. Such a behavior is related to the changes in the permeability and the fluidity of the DSPC liposomes. Before the Tm, as the system is shifting from the dense solid gel phase to the less compacted liquid crystalline phase, the chance of curcumin permeating the DSPC membrane is enhanced, as it has been shown with the ~3.7 times increase in the partitioning of curcumin into DSPC at the liquid phase compared to the solid gel phase before. This increase in the permeability results in an enhancement in the fluorescence intensity of curcumin as more molecules enter the hydrophobic phase. In addition, the observed blue shift in the emission spectrum of curcumin from 512 nm to 492 nm occurring along with the increase in the temperature confirms the partitioning of curcumin into the hydrophobic core of the membrane. This observation is consistent with earlier work [45]. The fluorescence of curcumin is not only dependent on the permeability rather it is affected by the fluidity of the medium, which accounts for the decrease in the emission intensity after the phase transition temperature. Therefore, the fluorescence of curcumin was dominated by the permeability factor prior to the Tm and by the fluidity factor after attaining phase transition temperature. Thus, a maximum was obtained at the phase transition temperature, the phase transition temperature measured for DSPC liposomes in this case was found to be at 54 °C as depicted in Fig. 3A, which agrees with the values reported in literature [56].

For DMPC [45] and DPPC [46] liposomes, curcumin does influence the phase transition temperature at high molar ratio, though at low concentration it has no effect. Therefore, the molar concentration of curcumin was increased and the phase transition was measured at the following proportions between curcumin concentration and [DSPC]: 1:20, 1:10, 1:5, 1:2 and 1:1. Interestingly, when the curcumin concentration was increased to 50 μM ([curcumin]: [DSPC] = 1:2); and 100 μM ([curcumin]: [DSPC] = 1:1) in DSPC liposomes, the fluorescence emission maximum was found to be red shifted to ~550 nm (~545 nm in the presence of 50 μM of curcumin) compared to ~512 nm obtained in the presence of 5 μM of curcumin suggesting at this concentration (>50 μM) of curcumin large population is exposed to aqueous medium, thus, penetration of curcumin gets saturated at higher concentration. With increase in temperature of curcumin embedded DSPC liposomes, the fluorescence emission maximum blue shifted to ~492 nm and ~525 nm in the presence of 50 μM and 100 μM of curcumin respectively. This is a clear indication that most of the curcumin gets buried into the DSPC liposomes in liquid crystalline phase at 50 μM of curcumin

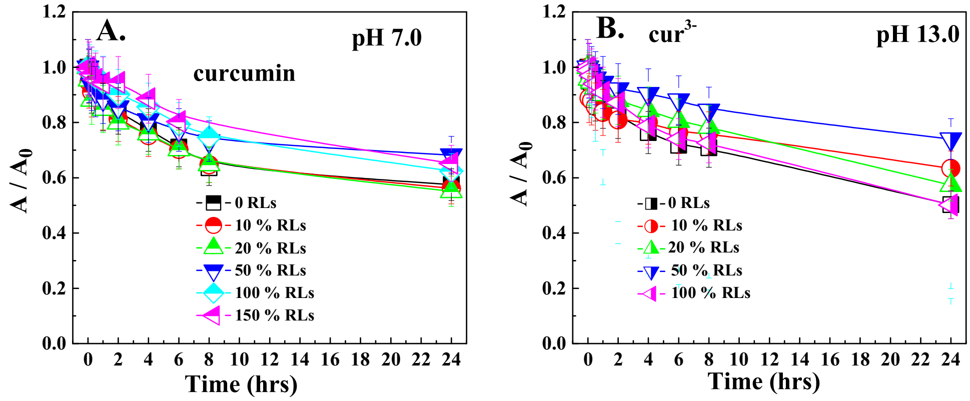


Fig. 4. Rate of degradation of curcumin at pH 7.0 (A) and pH 13.0 (B) buffer solution in the absence and presence of different % of RLs in DSPC liposomes. Error bars are given in SD (n = 3).

whereas majority amount of curcumin is still partially exposed to aqueous media in the liquid crystalline phase at 100 μM of curcumin. However, the variation of curcumin population in different depth of liposomes did not affect during estimation of the phase transition temperature [45]. As the concentration of curcumin increased, the phase transition temperature broadened (shown in Fig. 3A) with a shift from 54 °C at 5 μM of curcumin to 50 °C at 100 μM of curcumin. Moreover, the shoulder that marks the pre-transition stage faded as the concentration of curcumin increased to become at an equal proportion with that of the phospholipid. This outcome indicates the interposition of curcumin molecules between the acyl chains of DSPC thus disrupting the regular packing in both phases. It has been displayed in early studies that at high curcumin concentrations, curcumin molecules tend to form oligomers that occupy the deep core of the membrane and that diverge between them and the thickness of the bilayers results in thinning of the membrane to compensate the length of the curcumin oligomers [57].

3.4. Fluorescence quenching study in DSPC liposomes

Lipophilicity is one of the significant parameters usually used to predict membrane permeability and is roughly associated with the passive transport across cell membranes and the capability of a compound to partition and intercalate into a membrane. Changes in the membrane permeability are reflected in changes in the rate constant of the quenching reaction or ease of entry of the quencher to the probe, which is usually incorporated within the membrane hydrophobic core. Initially pyrene (see Scheme 1D) and curcumin were selected as external fluorescence probes for liposomes and KI was used as quencher. The fluorescence intensity of pyrene was quenched by KI in the absence and presence of DSPC liposomes. Under steady state conditions the relationship derived by Stern and Volmer describes quenching as

$$F_0/F = 1 + K_{SV}\,[Q]$$

where F_0 and F are the fluorescence intensities in the absence and presence of different quencher concentration ([Q]), respectively, and Ksv is the Stern-Volmer quenching constant. The Stern-Volmer plot for fluorescence quenching of pyrene by KI in DSPC liposomes was found to be linear till 20 mM (see Fig. 3B) and at higher concentration of KI it is saturated as expected due to quenching sphere action (see inset of Fig. 3B). The K_{SV} value estimated for pyrene in aqueous environment was found to be ~102 M^{-1} [58] whereas in the presence of 100 μM DSPC liposomes was ~88 M^{-1}. This ~13% decrease in quenching rate is expected, though not substantial, because pyrene is expected to locate at the interface of liposomes, thus partially exposing to aqueous environment. Fluorescence quenching of curcumin by KI in DSPC liposomes followed two different patterns. At low concentrations of KI (below 60 mM), the florescence intensity of curcumin was quenched by KI but further increase in KI concentration recovered the lost fluorescence and above 1 M of KI an enhancement in fluorescence intensity of curcumin was observed in DSPC liposomes. In contrast a single trend was obtained for fluorescence quenching of curcumin by KI in buffer solution without DSPC liposomes. In aqueous environment curcumin and I^- are in close contact to cause quenching of curcumin fluorescence, however, in liposomes curcumin is largely present in hydrophobic environment whereas I^- in aqueous medium discouraging close contact between curcumin and I^-. Moreover, the difference in the fluorescence quenching by KI between pyrene and curcumin in DSPC liposomes can be related to the difference in their structures. Due to entropic reasons, pyrene is positioned close to the head groups of the bilayer, which is confirmed by fluorescence experimental approaches [59–61]. This position partially increases close contact between pyrene and I^- whereas curcumin intercalates parallel to the lipid bilayers of the liposome [44] and largely reduces contact between curcumin and I^-. At higher concentration of KI, more curcumin molecules are helped and pushed by KI into the deep lipid bilayers due to salting out effect, which rather enhance overall fluorescence intensity of curcumin.

3.5. Effect of rhamnolipids on stability of curcumin in DSPC liposomes

The absorption spectra of curcumin at pH 7.0 did not change in the absence and presence of 10 to 150% of RLs in DSPC liposomes. At pH 7, the absorption maximum was unaltered by rhamnolipids in DSPC liposomes but with time the absorbance decreased due to degradation. Interestingly, the stability of curcumin was further enhanced with increasing concentrations of RLs in DSPC liposomes till 8 h (degradation of curcumin in DSPC liposomes decreased from 37% in absence of RLs to 20% in presence of 150% RLs after 8 h), however, after 24 h the most stable condition was found to be 50% RLs where degradation was about 32% compared to 43% in the absence of RLs and 35% in the presence of 150% of RLs in DSPC liposomes as shown in Fig. 4A. Since degradation of curcumin in alkaline pH is often reported [50], degradation of curcumin in de-protonated form, Cur^{3-}, was investigated. Like in pH 7.0, the decrease in absorbance of Cur^{3-} at ~468 nm was monitored in this case for testing the stability. As expected after 24 h degradation of Cur^{3-} in DSPC liposomes was 50% in pH 13.0 (see Fig. 4B) compared to 43% for curcumin in pH 7.0. However, in the presence of 50% RLs

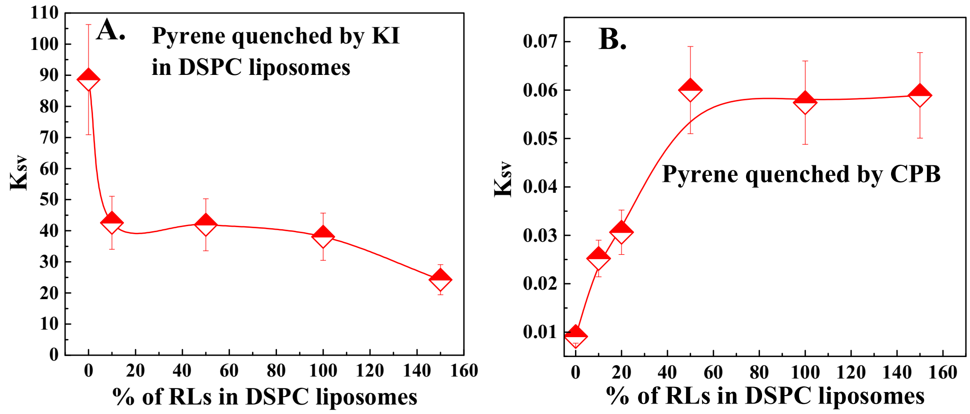


Fig. 5. (A) Plot of Ksv vs.% of RLs in DSPC liposomes during fluorescence quenching of pyrene by KI; (B) Plot of Ksv vs.% of RLs in DSPC liposomes during fluorescence quenching of pyrene by CPB. Error bars are given in SD (n = 3).

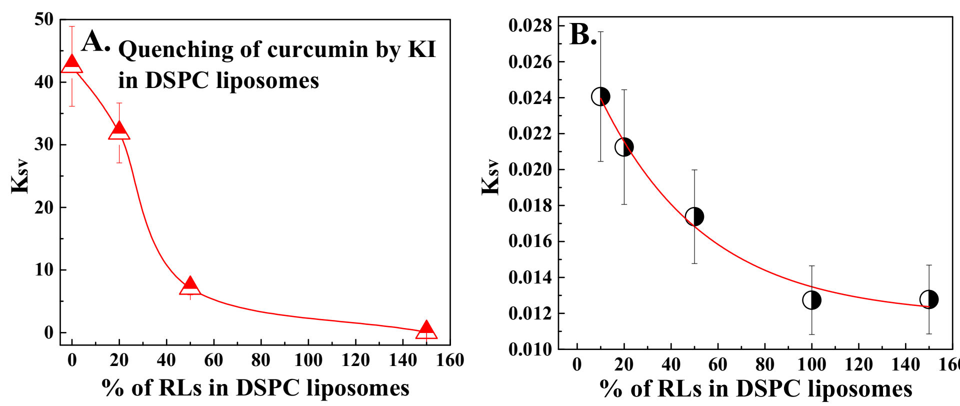


Fig. 6. (A) Plot of Ksv vs.% of RLs in DSPC liposomes during fluorescence quenching of curcumin by KI; (B) Plot of Ksv vs.% of RLs in DSPC liposomes during fluorescence quenching of curcumin by CPB. Error bars are given in SD (n = 3).

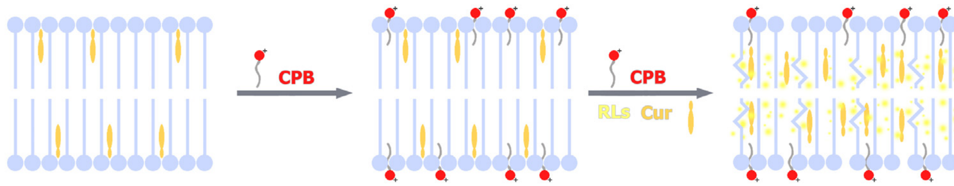
in liposomes, stability of Cur^{3-} improved and the degradation rate remained 26%, which is even better than for curcumin at pH 7.0. Nevertheless, further increase in RLs concentration did not help rather increased the degradation rate of Cur^{3-} (see Fig. 4B). Thus a different type of interaction occurs with the completely deprotontaed form compared to that of the protonated form of curcumin in DSPC liposomes.

3.6. Effect of rhamnolipids on partition coefficient of curcumin into DSPC liposomes

The partition of curcumin into DSPC liposomes in the presence of 100% RLs was estimated following earlier procedure. The partition coefficient values clearly establish that the partition coefficient of curcumin into DSPC in the solid gel phase was enhanced by ~22 folds in the presence of RLs ($Kp = 1.9 \times 10^6$) compared to that in the absence of RLs ($Kp = 8.38 \times 10^4$), this indicates presence of RLs increases permeability of DSPC liposomes in solid gel phase. This increase permeability of curcumin is also in an agreement with the enhanced stability of curcumin in the presence of RLs as seen at two different pHs earlier. However, in liquid crystalline phase ($Kp = 5.9 \times 10^5$), the increase was mild, the partition was enhanced only by a factor of ~1.9 compared to that at the solid gel phase where enhancement was ~22 fold. This can be explained based on the fact that in the solid gel phase the liposomes are closely packed, thus the interaction between the liposome and the glycolipids molecules alters the permeability and fluidity of membranes thereby pushing the curcumin into the hydrophobic region. But in the liquid crystalline phase the interaction between RLs and the liposomes is weakened due to the change in the membrane state, thus not helping in the partitioning of curcumin, which maintains a close value to that in the absence of RLs.

3.7. Effect of RLs on fluidity of DSPC liposomes

Fluidity of DSPC liposomes was compared in the presence of various concentrations of RLs in DSPC liposomes. Stern-Volmer constant (K_{SV}) remarkably decreased in the presence of 10% of RLs in DSPC liposomes, however, with further increase in % of RLs the reduction was reasonable as depicted in Fig. 5A. To complement this result, instead of a hydrophilic KI, a hydrophobic CPB (see Scheme 1E) was applied as quencher. CPB quenches the fluorescence of pyrene by electron transfer process, where electron in the excited state is transferred from the aromatic ring of pyrene to electron deficient N-atom of CPB [62]. In the meantime, in liposomes CPB intercalates within the hydrophobic part of the membrane with its charged moiety exposed at the surface, thus, liposome encourages contact between CPB and hydrophobic probe like pyrene. The Stern-Volmer constant for fluorescence quenching of pyrene by CPB increased linearly in the beginning with RLs concentration and at higher RLs concentration it saturated (see Fig. 5B). This is logical as increase in fluidity of liposomes in the presence of RLs facilitates more pyrene to enter the interface of liposomes and help in bringing N-atom of CPB and pyrene moiety come together. When pyrene was replaced with curcumin as external probe and KI was used as quencher, similar results were found (see Fig. 6A). However, when CPB was used as quencher for curcumin, the Stern-Volmer constant for curcumin as external probe molecule decreased exponentially



Scheme 2. Illustration of contact between curcumin and cetylpyridinium bromide (CPB) in Dioctadecanoyl-sn-*glycero*-3-phosphocholine (DSPC) liposomes in the absence and presence of rhamnolipids.

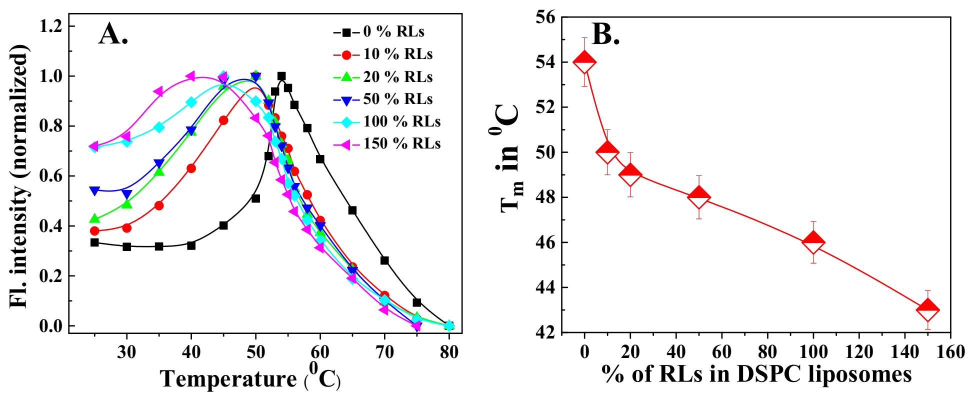


Fig. 7. (A) Profile of fluorescence intensity of curcumin vs. temperature at various% of RLs in DSPC liposomes; (B) Variation in phase transition temperature of DSPC liposomes with% of RLs in DSPC liposomes, error bars are given in SD (n = 3).

with increase in RLs percentage in DSPC liposomes (see Fig. 6B). This trend is quite opposite to that observed for pyrene as external probe molecule. Nonetheless, this difference is due to the difference in the structure of pyrene and curcumin and their relative position with respect to the phospholipid membrane. Increase in permeability of the DSPC liposomes in the presence of RLs allows curcumin to penetrate deeper within the membrane because curcumin intercalates and exist parallel to lipid bilayer unlike bulky pyrene that stays at the interface. More curcumin penetrates deeply into the lipid bilayer greater becomes the distance between curcumin and N^+-atom of CPB, and larger distance reduces the quenching rate (see Scheme 2). However, these results support the conclusion that rhamnolipids increase the permeability and fluidity of the liposomes. Liposomes have been successfully used for delivery of curcumin to test anti-cancer activities [63,64]. However, there is little information on stability of curcumin in such systems. It is known that membrane fluidity is affected by different kinds of liposomes. Hasan et al. has reported variation of membrane fluidity for salmon liposomes, rapeseed liposomes and soya liposomes and found that presence of curcumin decreases the membrane fluidity [65]. Although they have not studied degradation of curcumin in these systems, reduction in membrane fluidity is expected to decrease encapsulation of curcumin into such liposomes. However, in our present case, RLs helps in increasing membrane fluidity, thus, makes more curcumin to enter liposomes system, this, helps in stabilizing curcumin.

3.8. Effect of RLs on the phase transition of DSPC liposomes

To further understand the effect of RLs on modulation of membrane properties, the phase transition of DSPC was monitored with increasing% of rhamnolipids. In the presence of 10% RLs a remarkable broadening in the shape of fluorescence intensity of curcumin vs. temperature profile of DSPC liposomes was obtained. The shift in the phase transition temperature was also appreciable, which is similar to the results obtained for 100% of curcumin in DSPC liposomes. Further increase in percentage of RLs in DSPC liposomes, enhanced the broadness in the shape of fluorescence intensity of curcumin vs. temperature profile of DSPC liposomes by shifting the phase transition temperature further to the lower end as shown in Fig. 7A. Similar shift (to lower temperature) and broadening in phase transition temperature has been observed in DSC measurement [66]. The lowest phase transition temperature value was obtained at 150% of RLs in DSPC liposomes. The correlation between phase transition temperatures and the% of RLs in DSPC liposomes is shown in Fig. 7B. When associated with the membrane, the lipid moiety of the glycolipidic biosurfactants is most likely to disrupt the close packing of the phospholipid membrane thereby decreasing the temperature at which this solid packing changes to a liquid-crystalline nature. This change in the packing is accompanied with an increase in the permeability of the liposome, which is so far reflected in the gross enhancement of the partition coefficient at solid gel phase as well as the enhanced stabilization of curcumin at different pHs. Moreover, the sharpness in the phase transition was lost with the peak broadening with increasing rhamnolipids concentrations, hence, the fusion between the solid gel phase and the liquid crystalline state is encouraged with increasing percentage of rhamnolipids to liposome concentration.

4. Conclusion

Hydrolysis of curcumin was significantly reduced with DSPC. Curcumin was remarkably associated with DSPC liposomes and the partitioning of curcumin into the hydrophobic core of DSPC was enhanced by a factor of 3.6 in the liquid crystalline phase compared to that in the solid gel phase mainly due to the increase in membrane fluidity. The spectroscopic characteristics of curcumin were successfully shown in the pre-transition as well as phase transition temperature of DSPC liposome. The influence of rhamnolipids on DSPC liposome properties was remarkable. Permeability and fluidity of DSPC liposomes were affected by RLs. The permeability of the membrane increased with increasing concentrations of RLs as revealed by the enhanced partitioning of curcumin into DSPC at both phases in the presence of RLs. RLs lowered the phase transition temperature, and broadened it as an indication of fusion of solid gel and liquid crystalline phases. Moreover, increase fluidity of DSPC liposomes was reflected by reducing the degradation of curcumin further when DSPC liposomes were intercalated with RLs, thus, the present study opens up the possibility of using such mix system for curcumin and other unstable drug delivery application.

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