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Ionic Liquid Expedites Partition of Curcumin into Solid Gel Phase but Discourages Partition into Liquid Crystalline Phase of 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine Liposomes

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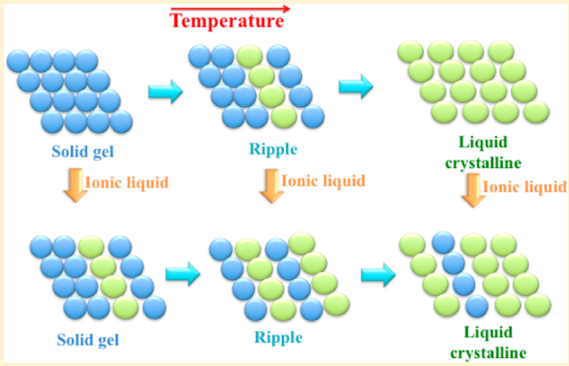
Supporting Information

ABSTRACT: The hydrolysis of curcumin in alkaline and neutral buffer conditions is of interest because of the therapeutic applicability of curcumin. We show that hydrolysis of curcumin can be remarkably suppressed in 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) liposomes. The fluorescence of curcumin sensitively detects the phase transition temperature of liposomes. However, at greater concentrations, curcumin affects the phase transition temperature, encouraging fusion of two membrane phases. The interaction of curcumin with DMPC is found to be strong, with a partition coefficient value of K_p = 2.78 × 10^5 in the solid gel phase, which dramatically increases in the liquid crystalline phase to K_p = 1.15 × 10^6. The importance of ionic liquids as green solvents has drawn interest because of their toxicological effect on human health; however, the impact of ionic liquids (ILs) on liposomes is not yet understood. The present study establishes that ILs such as 1-methyl-3-octylimidazolium chloride (moic) affect the permeability and fluidity of liposomes and thus influence parition of curcumin into DMPC liposomes, helping in the solid gel phase but diminishing in the liquid crystalline phase. The K_p value of curcumin does not change appreciably with moic concentration in the solid gel state but decreases with moic concentration in the liquid crystalline phase. Curcumin, a rotor sensitive to detect phase transition temperature, is applied to investigate the influence of ionic liquids such as 1-methyl-3-octylimidazolium chloride, 1-buytl-3-methyl imadazolium tetrafluoroborate, and 1-benzyl-3-methyl imidazolium tetrafluoroborate on DMPC liposome properties. 1-Methyl-3-octylimidazolium chloride lowers the phase transition temperature, but 1-buytl-3-methyl imidazolium tetrafluoroborate and 1-benzyl-3-methyl imidazolium tetrafluoroborate do not perceptibly modify the phase transition temperature; rather, they broaden the phase transition.



1. INTRODUCTION

Curcumin is a yellow pigment found in the rhizomes of *Curcuma longa* (tumeric). It is a well-known Indian spice and is used as a food-coloring agent;[1] in the Lebanese cake "sfoof", it is added to give the dessert its characteristic yellow color. Curcumin has long been used in Asian medicine for its healing and preventive properties. It protects the skin from sunburns and prevents other forms of skin eruptions and diseases. Medicinal applications of curcumin are becoming widely recognized.[2−5] Recently, there has been a growing interest in the anticancer,[5,6] antiamyloid,[7] antioxidant,[8,9] and anti-inflammatory[10,11] properties of curcumin. Various health benefits of curcumin have recently attracted interest in curcumin as a dietary supplement from the pharmaceutical market. However, two of the major challenges of curcumin are poor solubility and limited stability, which limit bioavaliability and oral delivery of curcumin. There is major interest in improving the water solubility and stability, with the goal of improving bioavailability. The aqueous solubility of curcumin can be improved by increasing the pH of the solution. Nevertheless, this approach leads to an undesirably high rate of degradation by alkaline hydrolysis.[12−14] An alternative approach to improving poor aqueous solubility is encapsulation of curcumin in micelle or surfactant solutions. Several studies have shown significantly higher solubility of curcumin in micelles,[15,16] but it is found that the nature of the surfactant plays a crucial role. For example, anionic micelles may not help suppress alkaline degradation, whereas cationic micelles might suppress alkaline hydrolysis.[17] On the other hand, the solubility of curcumin can be improved by applying nanotechnology.[18−21] Encapsulation of curcumin in liposomes offers a good alternative. Membrane-bound curcumin is now available as a dietary supplement under the name BCM-95 (Bio-Curcumin). The effect of curcumin on membrane properties was reported earlier.[22−27] To our knowledge, there is no literature reporting alkaline hydrolysis studies of curcumin in liposomes. We chose liposomes made from the zwitterionic lipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) for a case study for degradation of curcumin and investigate the interaction of curcumin with DMPC liposomes. It is established that hydrolysis of curcumin

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even in alkaline conditions can be suppressed dramatically in the DMPC liposome environment. The partition coefficient of curcumin into DMPC liposomes is substantially enhanced in the liquid crystalline phase compared to that of the solid gel phase because of the increase in fluidity. Although low concentrations of curcumin do not remarkably influence membrane properties, they do help probe membrane properties such as phase transition temperature and fluidity. Higher molar ratios of curcumin disorder DMPC membrane properties.

Ionic liquids (ILs) are neoteric solvents, i.e., new types of solvents that have recently been used as solvents for green chemistry. They are composed entirely of poorly coordinating ions and can therefore be highly polar yet noncoordinating.[28−30] ILs are immiscible with a number of organic solvents and thus provide nonaqueous polar alternatives for two-phase systems. They are of particular interest because of their environmentally friendly nature, their exciting features, and their economical convenience.[31−38] During recent years, ionic liquids have found increasing importance in applications spanning many fields of chemistry. They are molten salts and liquids below 100 °C and display exceptional properties such as chemical and thermal stability, low vapor pressure, and high ionic conductivity.[39] Besides their use as solvents for syntheses and extractions, ILs are applied as diluents in polymeric membranes to afford high conductivities for applications such as lithium batteries,[40,41] solar cells,[39,42,43] and electromechanical transducers.[44−48] Because of their increasingly widespread use, the effects of ionic liquids on human health, particularly their toxicity, remain crucial. Earlier we have observed[44] that ILs affect binding properties of curcumin with micellar systems and encourage micelle formation by reducing the critical micellar concentration of charged surfactants, whereas they discourage micelle formation of nonionic TX100. The impact of ionic liquids on membrane and liposome properties is not yet clearly understood.[27] The present goal is to investigate the modulation of DMPC membrane properties by ionic liquids by monitoring the fluorescence of curcumin. It is established that, depending on the state, ILs strongly influence partition of curcumin into DMPC liposomes. Long chain IL 1-methyl-3-octylimidazolium chloride (moic) shifts the phase transition temperature, whereas short chain ILs such as 1-buytl-3-methyl imidazolium tetrafluoroborate (bmit) and 1-benzyl-3-methyl imidazolium tetrafluoroborate (bzmit) broaden the phase transition. Membrane properties are disordered in the presence of three different kinds of ILs.

2. MATERIALS AND METHODS

2.1. Materials. DMPC, moic, bmit, and bzmit were obtained from Sigma-Aldrich and used as received. Curcumin was obtained from Acros Organics. The solvents used were of spectroscopic grade and obtained from Acros Organics or Sigma-Aldrich. 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 a 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 Membranes. DMPC liposomes were prepared by the solvent evaporation method.[45] The desired amount of DMPC phospholipid was dissolved in 15 mL of a chloroform/methanol mixture (2:1 volume 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 37 °C, about 14 °C above the phase transition temperatures for DMPC. 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 ILs. Stock solutions, ∼7.89 mM, of ILs such as moic, bmit, and bzmit were prepared in chloroform/ethanol. The solutions were prepared by adding the desired volume (a few microliters) of IL stock of appropriate concentration to the liposome (MLV) solution at 37 °C (liquid crystalline phase). The solutions were equilibrated for 30 min before analysis.

2.4. Spectroscopic Measurements. The absorption spectra were recorded at room temperature using a JASCO V-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. The excitation source was a 100 W xenon lamp, and the detector used was an R-928 operating at a voltage of 950 V. 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. The fluorescence lifetime measurements were done using the same instrument except with a 405 nm pulsed diode laser for excitation.

3. RESULTS AND DISCUSSION

3.1. Suppressing Hydrolysis of Curcumin in DMPC Liposomes. In the UV−visible spectral region, curcumin exhibited an intense absorption peak around 410 nm in neutral or acidic aqueous environments, which shifted to ∼425 nm in DMPC liposomes. The absorption spectra of curcumin in DMPC liposomes were structured with two additional peaks at 400 and 450 nm (Figure 1A). The prototropic forms of curcumin are given in Figure S1 (Supporting Information). In aqueous solution, there are three pK_a values (8.38, 9.88, and 10.51), corresponding to deprotonation of the three hydroxyl groups of curcumin;[13] thus, curcumin is completely deprotonated at pH 13 to form the highly negatively charged species Cur^{3-}. At pH 13, Cur^{3-} in water had an absorption maximum at 468 nm that shifted to 457 nm in DMPC liposomes, and the structure of the absorption peak of curcumin in DMPC was lost in the alkaline medium. Studies have shown that the degradation of curcumin in aqueous solution is linked to hydrolysis that occurs rapidly above neutral pH. High-performance liquid chromatography data have proven that curcumin degrades to form *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal as the main product, which further decomposes to vanilin, ferulic acid, and feruloyl methane.[12] It has been established that the contribution of condensation products to the total absorption signal of curcumin is relatively minor; thus, the decrease in the visible absorbance over time can be used as a measurement of degradation of curcumin.[17] Therefore, the absorbance change of curcumin and Cur^{3-} was monitored with time in the presence of DMPC liposomes and compared with the absorbance change when in a buffer solution

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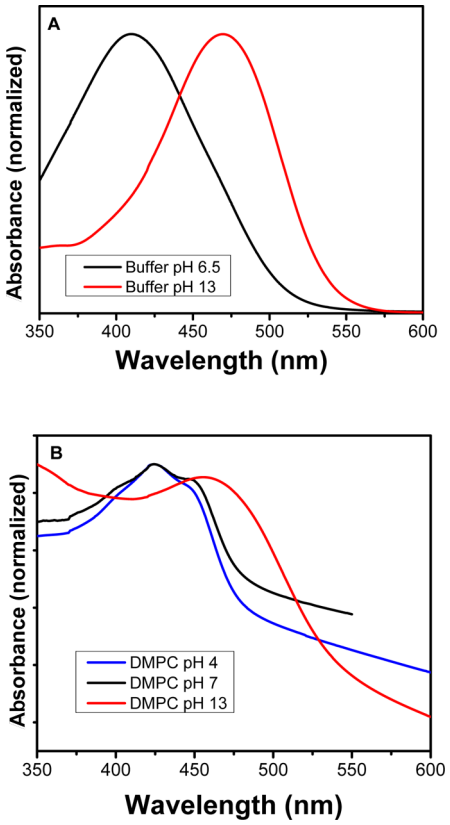

Figure 1. UV−vis absorption spectra of curcumin (pH <7) and Cur^{3-} (pH 13) in (A) buffer and (B) DMPC liposomes.

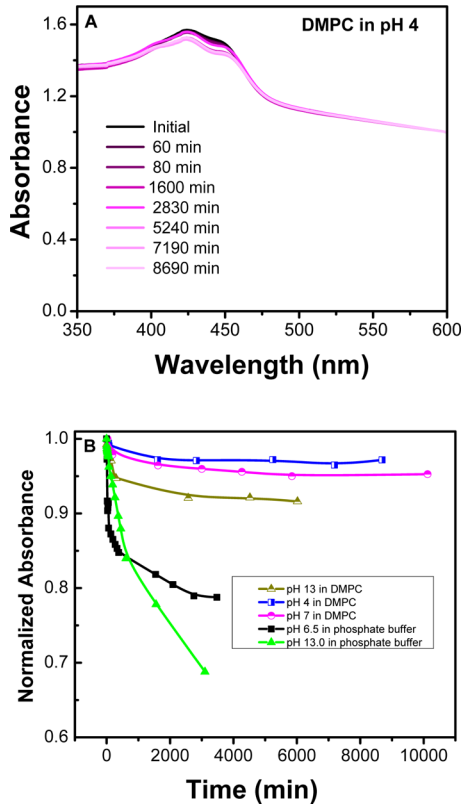

Figure 2. (A) UV−vis absorption spectra of curcumin in DMPC liposomes at pH 4. (B) Comparison of decays of curcumin absorption maxima due to degradation in the buffer and DMPC liposomes.

(Figure 2). The absorption maximum of curcumin at pH 6 decayed to approximately 68% of the original value in 60 h, whereas it took just 50 h to decay the same amount for Cur^{3-} at pH 13. However, in the presence of DMPC liposomes at pH 4, the absorption maximum decayed to just 97% of the original value even after 145 h, whereas it decayed to 96% of the original value at pH 7. The degradation of Cur^{3-} at pH 13 was relatively faster (92% decay of the original value in 100 h), but it is still much more stable than curcumin or Cur^{3-} in buffer solution. This value is much lower than that reported for SDS micelles and even lower than that for other cationic micelles reported after 20 h.[17] Thus, the rate of degradation of curcumin and Cur^{3-} is significantly suppressed in DMPC liposomes.

3.2. Encapsulation of Curcumin into DMPC Liposomes. The suppression of degradation of curcumin and Cur^{3-} in DMPC liposomes could be due to efficient encapsulation inside the DMPC liposomes. This is also reflected in the ∼12 nm red shift in the absorption maximum of curcumin in DMPC liposomes compared to that of curcumin in the aqueous solution. In contrast, Cur^{3-} showed an ∼11 nm blue shift in DMPC liposomes compared to that in aqueous solution. This could be due to association of Cur^{3-} with DMPC. The detailed analysis of the interaction of curcumin with DMPC liposmes was carried out by fluorescence titration because of its sensitivity and simplicity. The fluorescence spectra of curcumin in various concetrations of DMPC liposomes in the solid gel and liquid crystalline phases are given in Figure 3A,B. The fluorescence intensity of DMPC appreciably increased in the presence of DMPC liposomes in both phases, which proves a strong association of curcumin with DMPC liposomes. It is well-established that the poor fluorescence of curcumin in water improves in acetonitrile and methanol[46] and also while associating with micelles[44] and membranes.[27] The rate of increase of fluorescence intensity in the liquid crystalline phase was higher than that in the solid gel phase of the liposome; however, in both cases the change in fluorescence intensity reached saturation level at a greater DMPC concentration (Figure 3C). An ∼50 nm shift to the lower wavelength was observed in the emission maximum of curcumin in the presence of DMPC liposomes compared to that in the buffer solution. This blue shift further confirms that curcumin is partitioning into DMPC liposomes. The fluorescence lifetime of curcumin in the DMPC liposomes was found to be biexponetial, and the data are presented in Table 1. These values are similar to those in a nonpolar solvent[46] and less than those in the buffer solution, further supporting incorporation of curcumin into DMPC liposomes. The percentage of the long component in the fluorescence lifetime dramatically decreased in the presence of the membrane, whereas the percentage of the short component in the fluorescence lifetime, which is due to bonding to the membrane, increased. These results are inconsistent with the trend earlier observed for DPPC membranes.[27]

3.3. Partition Coefficient of Curcumin in Solid Gel versus Liquid Crystalline Phases. The lipophilicity of the compound can be estimated by comparison with the partition coefficient (K_p), which gives information about the fraction of the associated compound with the lipid. The difference between the fluorescence intensity of curcumin in the aqueous phase and after incorporation into the liposomes could be a good tool to evaluate K_p. The partition coefficient of curcumin in DMPC liposomes can be expressed as[47]

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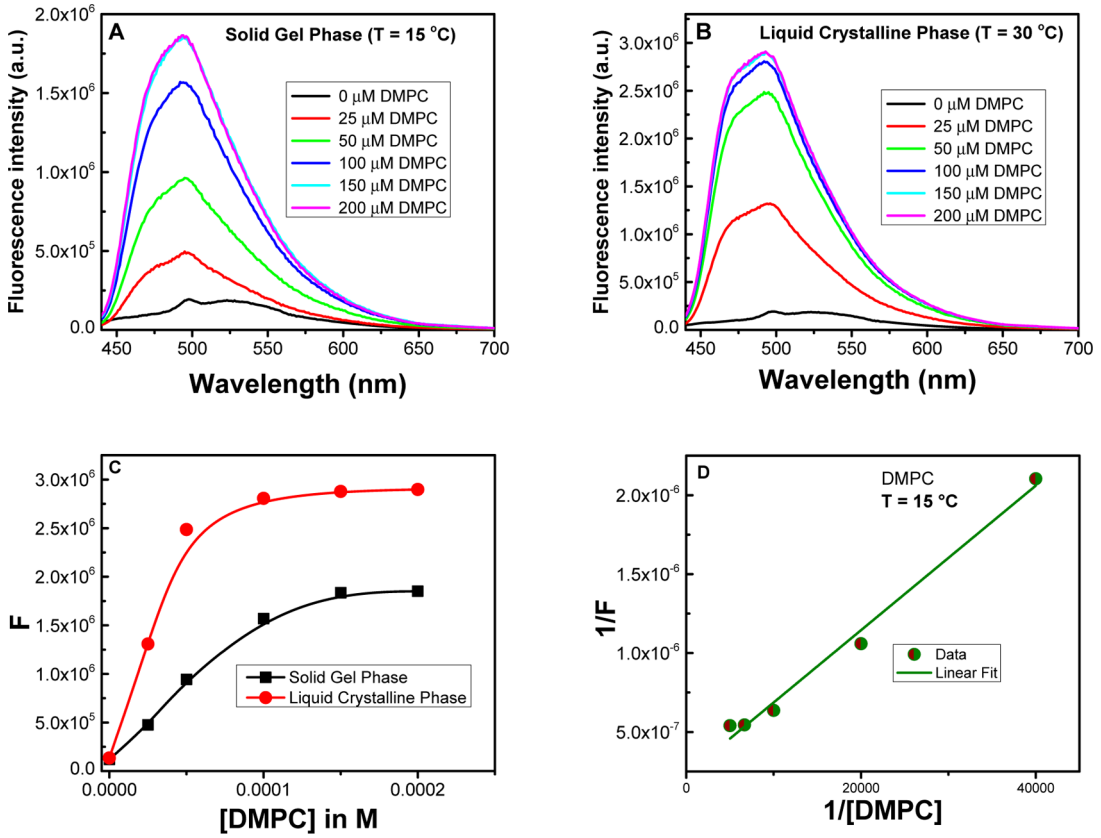

Figure 3. Fluorescence emission spectra of curcumin in various concentrations of DMPC liposomes in (A) solid gel phase and (B) liquid crystalline phase. (C) Variation of fluorescence intensity of curcumin vs [DMPC] in solid gel and liquid crystalline phases at excitation wavelength 425 nm and emission wavelength 492 nm. (D) Plot of $1/F$ vs $1/[\mathrm{DMPC}]$ from the data given in (C) for solid gel phase.

Table 1. Fluorescence Lifetime Analysis of Curcumin in Buffer and DMPC Liposomes

sample	τ_1 (B_1) (ps)	τ_2 (B_2) (ps)
buffer	674 (52%)	5167 (58%)
DMPC	588 (79%)	3.16 (21%)

$$K_p = (\mathrm{CUR_b}/\mathrm{DMPC})/(\mathrm{CUR_f}/\mathrm{W})$$

where $\mathrm{CUR_b}$, $\mathrm{CUR_f}$, DMPC, and W refer to moles or molar concentrations of the membrane-associated curcumin, free curcumin in aqueous phase, phospholipid membrane, and water, respectively. Because curcumin is essentially very weakly fluorescent unless incorporated into membranes, the fluorescence, F, is proportional to the concentration of membrane-associated curcumin

$$F = a\mathrm{CUR_b}$$

where a is a proportionality constant.

When the total curcumin concentration is $\mathrm{CUR} = \mathrm{CUR_b} + \mathrm{CUR_f}$, we could write

$$F = F_0 \times \mathrm{DMPC}/(\mathrm{W}/K_p + \mathrm{DMPC})$$

where $F_0 = a\mathrm{CUR}$ is the maximum fluorescence resulting from total incorporation of curcumin into the membrane. The molar concentration of the membrane in the present work is less than 0.2% of the total volume, so the molar concentration of water can be treated as that of pure water

$$F = F_0 \times \mathrm{DMPC}/(55.6/K_p + [\mathrm{DMPC}])$$

Or

$$1/F = [55.6/(K_pF_0)](1/\mathrm{DMPC}) + 1/F_0$$

The above equation indicates that the double-reciprocal plot of the fluorescence and the lipid concentration should give a linear curve and that K_p could be estimated from the slope and intercept. The double-reciprocal plot of $1/F$ against $1/[\mathrm{DMPC}]$ was found to be linear. A representative plot is depicted in Figure 3D. K_p was estimated from the slope and intercept according to the equation. The partition coefficients of curcumin in the solid gel and liquid crystalline states of DMPC liposomes were evaluated at 15 and 30 °C, respectively. Table 2 summarizes the results. The partition coefficient of curcumin into the solid gel phase was found to be 2.78×10^5, which is in a range similar to that of fisetin[48] and 3-hydroxyflavone[49] in a DMPC liposome membrane. However, the partition coefficient of curcumin remarkably increased to 1.15×10^6 in the liquid crystalline phase of DMPC. This value is higher than that reported for fisetin[48] but similar to the

Table 2. Partition Coefficients of Curcumin into DMPC Liposomes

	partition coefficients	
curcumin in DPPC	solid gel phase	liquid crystalline phase
buffer pH 7.0	2.78×10^5	1.15×10^6
5 μM moic	6.38×10^5	8.36×10^5
10 μM moic	5.00×10^5	7.21×10^5
25 μM moic	5.94×10^5	3.45×10^5

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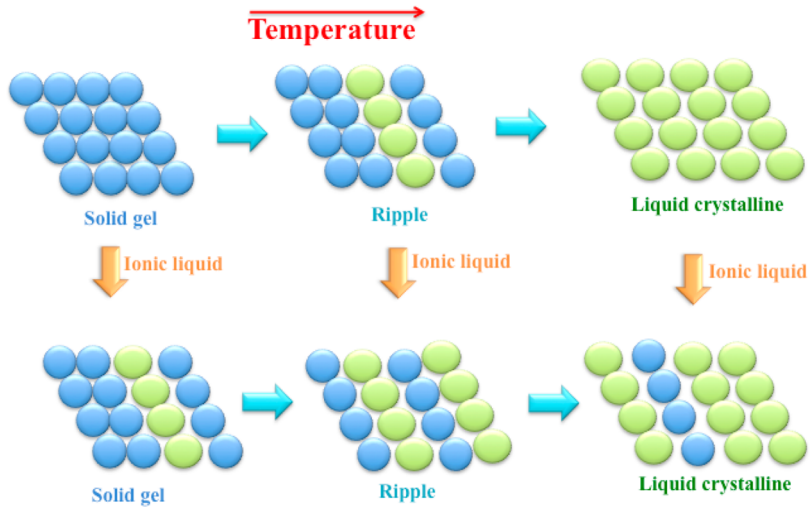

Figure 4. Schematic two-dimensional view of the DMPC membrane during melting showing the two main phases, solid gel and liquid crystalline, as well as the intermediate pretransition ripple phase in the absence and presence of ionic liquid (moic).

reported value for DPH in the liquid crystalline phase.[50] Thus, the partition coefficient of curcumin in the liquid crystalline phase is ~4-fold greater than that in the solid gel phase; coincidentally, this result reflects the same trend we observed earlier for curcumin in DPPC membranes.[27] The solid gel phase is known to be more compact and rigid, whereas the liquid crystalline phase is more flexible.[45] This encourages permeability of the membrane; thus, the incorporation of curcumin into the membrane is increased. Therefore, the 4-fold increase in the K_p value in the liquid crystalline phase compared to the solid gel phase is logical.

3.4. Effect of Curcumin on the Phase Transition of DMPC Liposomes. The fluorescence of curcumin was evaluated to determine the phase transition of the DMPC liposomes. This study is usually carried out using differential scanning calorimetry, which is a very precise technique. However, fluorescence has the advantage of being highly sensitive to very low concentrations, requiring smaller samples. Numerous vital membrane functions in biological membranes are affected by this phase change; therefore, we monitored the phase transition of the DMPC liposomes in the presence of curcumin. In our experiments, the concentration of curcumin in the samples was fixed at 1 μM, while that of the membrane was 100 μM. The first phase transition, usually termed pretransition, when DMPC liposomes undergo a change from the solid gel phase to rippled gel phase, occurs at ~10 °C[51] (Figure 4). The main phase transition, associated with the melting of acyl chains in the hydrophobic core of the membrane, from the rigid gel phase to the more fluid liquid crystalline phase of DMPC liposomes, occurs near 23 °C.[52] As Figure 5 shows, when the temperature was increased, the maximum fluorescence intensity of curcumin in DMPC liposomes increased until it reached a maximum at the phase transition temperature, and then it decreased as the system regained homogeneity in the new phase. This trend in fluorescence intensity of curcumin in DMPC liposomes with temperature can be reasoned based on the permeability of DMPC liposomes and/or fluidity of the DMPC liposomes. When the rigid gel phase transforms into a more fluid liquid crystalline phase, the possibility of curcumin permeating the DMPC membrane is increased; this is further supported by earlier observations of an increase in the partition coefficient in

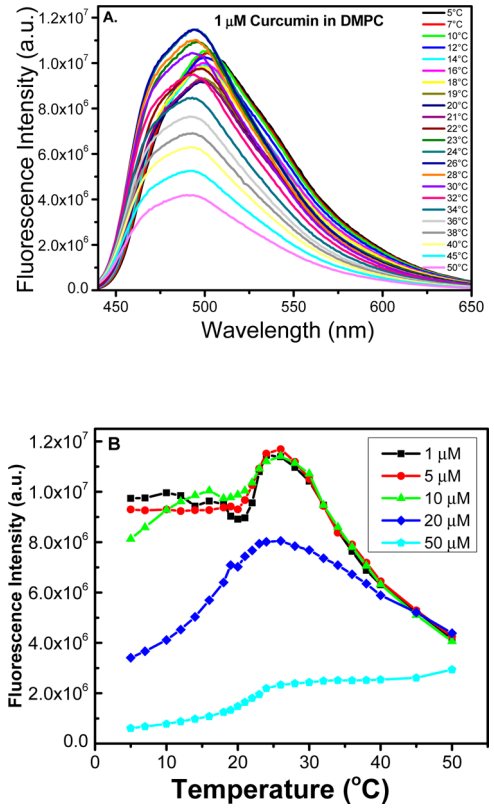

Figure 5. (A) Fluorescence spectra of curcumin in DMPC liposomes at various temperatures and (B) profile of temperature–fluorescence intensity of curcumin at various molar ratios of curcumin concentration in DMPC liposomes. Here 1, 20, and 50 μM CUR correspond to 1:50, 2:5, and 1:1 curcumin:DMPC molar ratios, respectively.

the liquid crystalline phase. As the system transforms from the rigid gel phase into a more fluid liquid crystalline phase, the permeability of the membrane to curcumin is enhanced; hence, an increase in the fluorescence intensity is observed. Moreover, the observed blue shift in the fluorescence emission maxima of curcumin with temperature, from 504 to 494 nm, for DMPC liposomes confirms the partition of curcumin into a deeper

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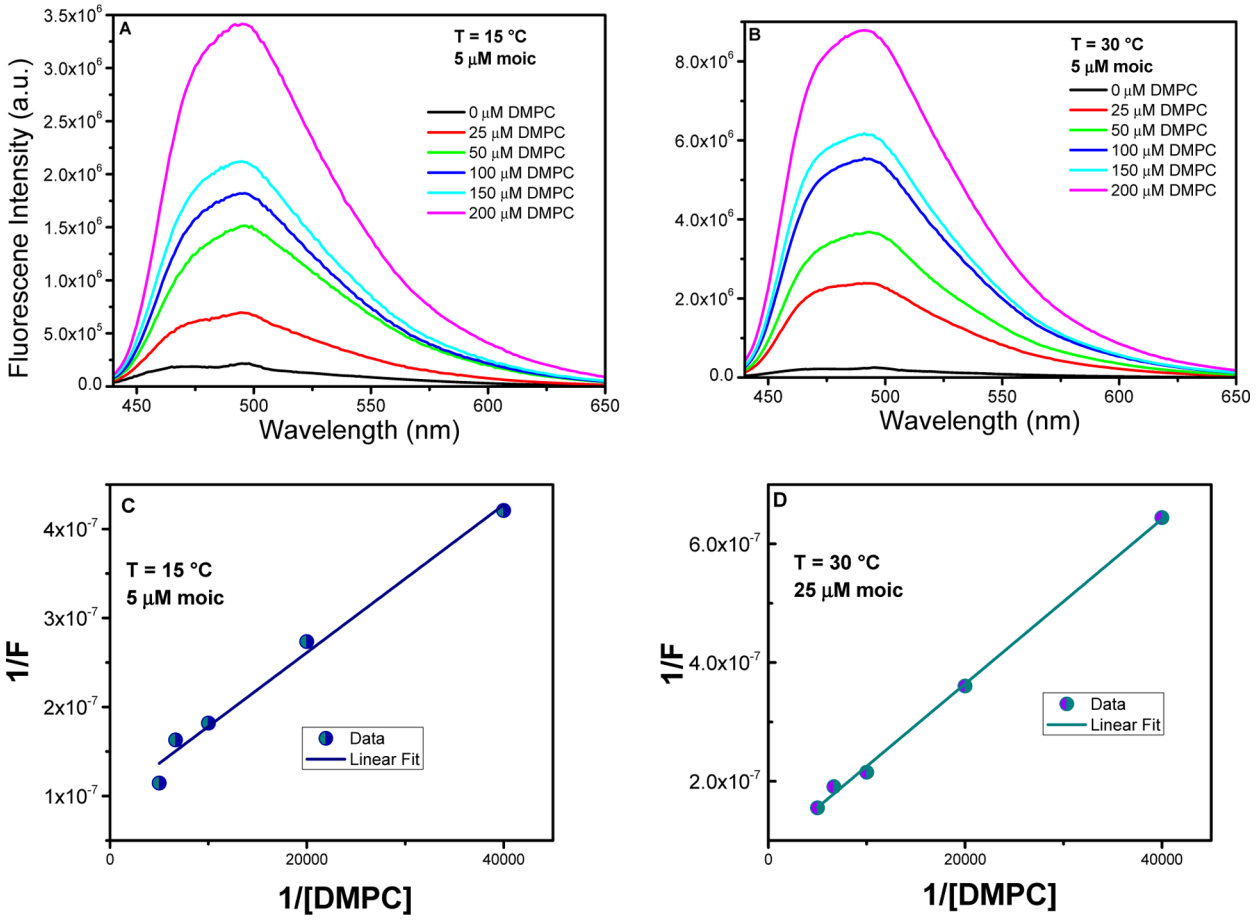

Figure 6. Fluorescence emission spectra of curcumin in the presence of 5 μM moic in various concentrations of DMPC liposomes in solid gel (A) and liquid crystalline (B) phases. Plots of $1/F$ vs $1/[\text{DMPC}]$ for curcumin in the presence of 5 μM moic (C) in the solid gel phase and 25 μM moic (D) in the liquid crystalline phase of DMPC liposomes.

hydrophobic layer of the membrane. If permeability were the only case to be considered, the fluorescence intensity would have continuously increased after the phase transition until saturation. This was not observed in our experiments. On the other hand, the fluorescence intensity of curcumin is also influenced by the microviscosity and fluidity of the environment. In such situations, the transformation to a more flexible liquid crystalline phase from a rigid gel state would diminish the fluorescence intensity. Therefore, the fluorescence intensity of curcumin was dominated by the permeability of curcumin until the phase transition was attained. Afterward, it decreased gradually because of the decrease in the microviscosity or the increase in the fluidity of the membrane. On the basis of this we found 23 °C to be the phase transition temperatures for DMPC membranes, which is in accordance with values reported in the literature.[45] The pretransition temperature for DMPC is found to be ∼10 °C below the main phase transition temperature. We observed a shoulder at ∼14 °C, which is similar to values found in the literature.[52] When the molar concentration of curcumin in DMPC liposomes was increased from 1:50 to 1:1, there was no major change in the phase transition temperature (Figure 5B); instead, the phase transition temperature broadened. Furthermore, the hump that marks the pretransition phase in the temperature−fluorescence intensity profile faded when the concentration of curcumin became equal to that of the phospholipid. This result indicated interposition of curcumin molecules between the acyl chains of DMPC that disrupts the regular packing in both phases. Barry et al. suggested that at high concentrations (>1:100 molar ratio) curcumin molecules form oligomers that occupy the inner core of the membrane and that the mismatch between them and the thickness of the bilayer causes the thinning of the membrane to match the length of the curcumin complex.[22]

3.5. Effect of Ionic Liquid on Partition of Curcumin into DMPC Liposomes. To understand the influence of ionic liquids on DMPC liposome properties, the fluorescence response of curcumin in DMPC liposomes in the presence of an ionic liquid was monitored. In this case, the concentration of curcumin was kept at 1 μM, which was less than 2 molar percentage of the phospholipid concentration, or molar ratio 1:50, to minimize the effect of curcumin on DMPC phospholipids as per preceding findings. The ionic liquid concentration was varied from 1:10 to 1:2 molar ratio (IL:DMPC). Similar to the results obtained in the absence of the ionic liquid, the fluorescence of curcumin in the presence of ILs, e.g., moic, increased with the increase in DMPC liposome concentration. The emission spectra of curcumin in the presence of 5 μM moic with various DMPC liposome concentrations in the solid gel and liquid crystalline phases are given in Figure 6A,B, respectively. The results suggest that in the presence of moic, curcumin is incorporated in the hydrophobic center of the membrane. The membrane-modulating effect of moic as an external additive was further investigated by estimating the partition coefficient of curcumin

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in the DMPC membrane in the presence of moic in both the solid gel and liquid crystalline phases. Two representative plots for determination of the partition coefficient in the solid gel and liquid crystalline states are given in Figure 6C,D. The partition coefficients of curcumin into DMPC liposomes in the presence of moic are summarized in Table 2. The partition coefficient of curcumin into DMPC in the solid gel phase in the presence of 5 μM moic increased 2-fold compared to that in the absence of moic; however, subsequent increase of moic concentration to 10 or 25 μM did not alter remarkably the partition coefficient value. A slight decrease was observed in the presence of 10 μM moic, which could be due to saturation. In the liquid crystalline phase, on the other hand, addition of 10 μM moic appreciably decreased the partition coefficient of curcumin in DMPC liposomes, and it continued to decrease with increasing moic concentration. The results are in agreement with our earlier discussion. In the solid gel phase, the liposomes are packed tightly; partition of curcumin is initially encouraged by the moic, but it reaches saturation at higher moic concentration. At the same time, the ability of curcumin to go deep inside the membrane increases in the liquid crystalline phase, but in the presence of moic, the hydrophobic tail of moic makes it less flexible and obstructs the deep permeability of curcumin by decreasing the partition coefficient.

3.6. Influence of Ionic Liquid on the Phase Transition of DMPC Liposomes. To understand the influence of ionic liquids on DMPC liposome properties, three different kinds of ionic liquids were selected and compared. The phase transition properties of DMPC liposomes in the presence of (i) long hydrophobic tail (moic), (ii) short butyl group (bmit), and (iii) bulky benzyl group (bzmit) ionic liquids were investigated by monitoring the fluorescence of curcumin. As shown in Figure 7A, the phase transition temperature in the presence of 1:10 moic:DMPC was found to be 24 °C, compared to 23 °C in the absence of moic. The phase transition temperature shifted to the lower-temperature range by increasing the moic molar ratio and reached 16 °C for a 1:2 ratio. The correlation of phase transition temperature with moic concentration is given in Figure 7B. When incorporated into the membrane, the long chain amphiphilic compound tends to decrease the membrane fluidity. It is reasonable to propose that the hydrophilic headgroup of moic is anchored at the DMPC liposome surface and the hydrophobic chain intercalates between the acyl chains of the phospholipid (Figure 8, left panel). This may enhance the packing density of the hydrocarbon moiety in the lipid bilayer. On the other hand, the pretransition temperature was not detected in the presence of moic. We also found that as the moic molar ratio increased to 1:2 in DMPC membranes, the temperature-dependent change broadened in the solid gel phase; the sharpness of the phase transition was lost, indicating that the separation of the solid gel and liquid crystalline states of the membrane is decreased by encouraging the fusion of the two states (Figure 4).

The effect of two other different kinds of ILs on the membrane fluidity was compared under the same concentration range of the ionic liquid in the DMPC membrane phases. Figure 9 shows the temperature−fluorescence intensity profile of curcumin bound to DMPC in the presence of bzmit. In the presence of bzmit, there was no appreciable change in the phase transition temperature; however, unlike what was observed for moic, as the molar ratio of bzmit:membrane changed from 1:10 to 1:2, the appearance of the temperature−fluorescence intensity profile curve slightly sharpened. This could be due

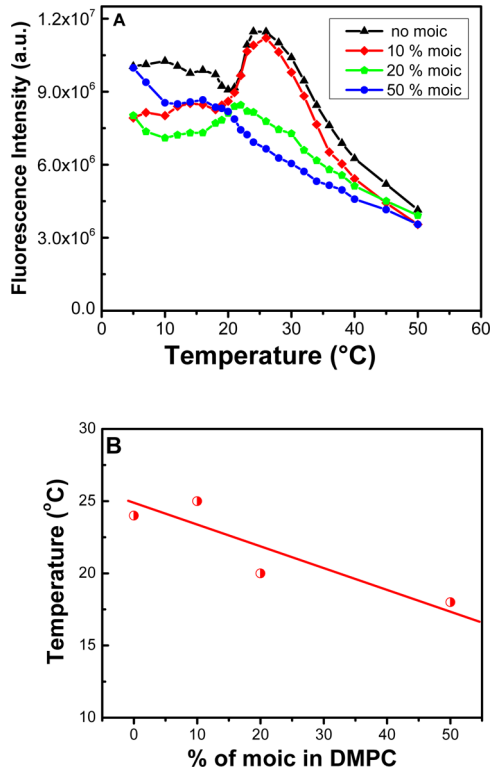

Figure 7. (A) Profile of temperature−fluorescence intensity of curcumin at various molar ratios of moic in DMPC liposomes. (B) Correlation of phase transition temperature with moic concentration.

to the steric hindrance of the benzene ring in bzmit instead of the long octyl tail in moic. The increasing effect of the hydrophobic benzene ring competes with the repulsive electrical charge (positive) on the headgroup of bzmit and the ionic phosphatidylcholine group of DMPC as bzmit tends to locate near the headgroup of the liposomes (Figure 8, middle panel). At the bzmit:liposome molar ratio equals or exceeds 1:5, the hydrophobic benzene ring of bzmit overcomes the repulsive forces between headgroups of the phospholipid and the IL itself and pushes itself toward the hydrophobic tail of the membrane (Figure 8, right panel). However, in the case of moic, the repulsive force was irrelevant even at a 1:10 molar ratio, as the octyl tail of moic could easily align along the hydrophobic tail of DMPC without facing any bulky steric effect as is the case with the benzene group. Figure 10 shows the temperature−fluorescence intensity profile of curcumin bound to DMPC in the presence of bmit. The butyl group in bmit is less hydrophobic than the octyl group in moic; therefore, the polar positive charge on the headgroup of the ionic liquid does not favor bmit to go deeper into the membrane. At the same time, bmit is less bulky than bzmit; therefore, unlike the bzmit case, the sharpness of the temperature−fluorescence intensity profile for the 1:10 molar ratio was not observed. The shorter tails of DMPC make the ionic liquid close to curcumin most probably in the hydrophobic area of the membrane.

4. CONCLUSION

Hydrolysis of curcumin was remarkably suppressed in 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine liposomes. Curcumin was strongly associated with DMPC liposomes, and the partition of curcumin in the liquid crystalline phase was

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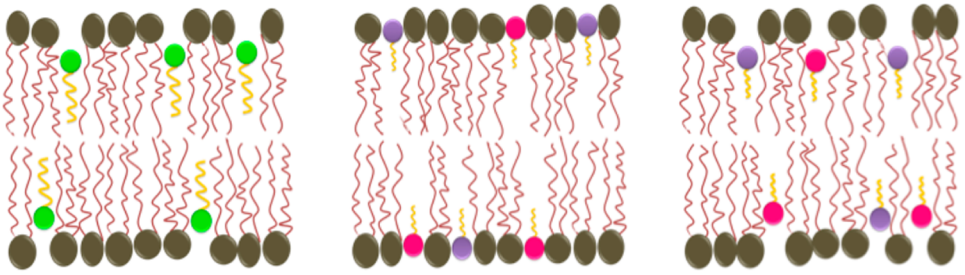

Figure 8. (Left panel) Cartoon schematic of the physical changes induced by moic in DMPC liposomes; moic is oriented with its long axis along the membrane. (Middle panel) Cartoon schematic of the physical changes induced by bzmit or bmit in DMPC liposomes at low concentration (10% molar ratio); bzmit or bmit is oriented near the headgroup of the membrane. (Right panel) Cartoon schematic of the physical changes induced by bzmit or bmit in the membrane at high concentration (50% molar ratio); bzmit or bmit is oriented with its long axis along the membrane. Cartoon images are not to scale.

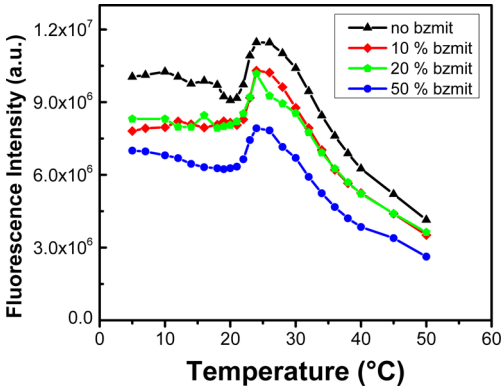

Figure 9. Profile of temperature−fluorescence intensity of curcumin at various molar ratios of bzmit in DMPC liposomes.

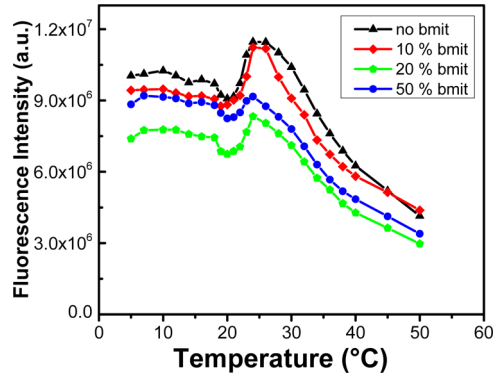

Figure 10. Profile of temperature−fluorescence intensity of curcumin at various molar ratios of bmit in DMPC liposomes.

found to be 4-fold higher than in the solid gel phase because of the increase in membrane fluidity. The spectroscopic property of curcumin successfully revealed the pretransition as well as phase transition temperature of DMPC liposomes; however, increase in curcumin concentration modulated membrane properties by encouraging fusing of the solid gel and liquid crystalline states of DMPC liposomes. Widespread use of ionic liquids as green solvents necessitates evaluation of its toxicological effects on human health; thus, it is important to understand modulation of membrane properties by ionic liquids. The influence of ILs, such as 1-methyl-3-octylimidazolium chloride, 1-buytl-3-methyl imadazolium tetrafluoroborate, and 1-benzyl-3-methyl imidazolium tetrafluoroborate, on DMPC liposome properties was remarkable. Permeability and fluidity of DMPC liposomes were affected by ionic liquids. Partition of curcumin increased in the solid gel state regardless of ionic liquid concentration, but it decreased in the liquid crystalline phase with ionic liquid concentration. The long chain ionic liquid 1-methyl-3-octylimidazolium chloride lowered the phase transition temperature, but short chain ILs 1-buytl-3-methyl imidazolium tetrafluoroborate and 1-benzyl-3-methyl imidazolium tetrafluoroborate did not perceptibly modify phase transition temperature; rather, they broadened the phase transition.

■ ASSOCIATED CONTENT

Supporting Information

Prototropic forms of curcumin and complete author list for refs 5, 7, and 18. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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