Formation and Transformation of the Subgel Phase in Dioctadecyldimethylammonium Bromide Aqueous Dispersions

Fu-Gen Wu,‡ Zhi-Wu Yu,* ‡ and Gang Ji‡

Abstract: We have characterized the structure and phase behavior of dioctadecyldimethylammonium bromide (DODAB) aqueous dispersions by using conventional and high-sensitivity nano-differential scanning calorimetry, microscopy, cryogenic transmission electron microscopy, attenuated total reflection Fourier transform infrared spectroscopy, and electric conductivity measurements. Special attention has been paid to the formation and transformation of the subgel phase. An almost pure subgel can be obtained in the concentrated region (above 6.7 wt %). We found that unilamellar vesicles were spontaneously formed in the subgel phase of a 5 mM DODAB dispersion. Infrared spectroscopic data reveal that the only significant change during the gel to subgel phase transition is the ordering in the lipid alkyl chain packing. That is, the head and tail parts of the DODAB molecules change nonsynchronously upon the gel to subgel transition, and the subgel phase is triggered only by the change of the lipid tail part. We propose that the morphological change (from curled membranes in the gel phase to unilamellar vesicles with faceted surface in the subgel phase) is coupled to the change of alkyl chain packing state during the gel to subgel transition. Finally, a full picture of the phase transition sequences for the dilute and concentrated DODAB dispersions is given.

1. Introduction

When dispersed in water, amphiphilic molecules can self-assemble into aggregates such as micelles, microemulsions, vesicles, and disks. The physical states of these dispersions are classified as crystalline, gel, liquid crystalline, hexagonal, and cubic phases, etc. Transformations between these phases may be accompanied by the changes in molecular conformation/packing states. Knowledge on the transformation mechanisms is fundamental to the design of materials with tailored properties and functions, which in turn lies in the core of the various applications of amphiphilic dispersions such as biomembrane models, sensors, drug delivery systems, and microreactors.

Dioctadecyldimethylammonium bromide (DODAB) is a simple-structured double-chained cationic surfactant and is frequently used as model systems for fundamental studies in the colloidal and interface science. It is a bilayer-forming lipid with its bilayer structure analogous to biological membranes. Therefore, our understanding of biological membranes can be deepened by investigating the physical properties of vesicles formed by DODAB and other natural or synthetic amphiphiles. Other than being used as a membrane-mimicking model molecule, DODAB can be used as antimicrobial agents and potential drug gene, or vaccine carriers.

The neat DODAB aqueous dispersions have been investigated from various aspects, including sample preparation methods, phase behaviors, and structural characterizations of vesicles or other aggregation forms. In particular, thermotropic phase behaviors of the system have been carefully examined. Coagel, subgel, gel, and liquid crystalline phases have been observed. The coagel and subgel phases are both crystalline phases. The coagel phase is often depicted as a hydrated multilamellar phase, and it transforms into the liquid crystalline phase at 54 °C upon heating. The subgel phase is only formed when cooling a dilute DODAB suspension in the gel phase to a temperature below 15 °C. When heating the sample of the subgel phase, a low-temperature transition (subgel to gel transition) centered at 36 °C occurs, followed by the main transition (gel to liquid crystalline transition) at 44 °C.

Phase behaviors of DODAB aggregates may have a close relationship with the properties of various binary or more complex systems such as DODAB—phospholipid, DODAB—surfactant, DODAB—sterol, DODAB—DNA, DODAB—protein, DODAB—polymer, DODAB—polymeric particle/latex, and DODAB—cell.

Although the phase behaviors of amphiphiles (especially the lipid systems) have been widely studied from various aspects, an in-depth understanding of the polymorphism and, in particular, the reversibility of the phase transitions of amphiphiles still needs...
great efforts. To address this question, we proposed that the “headgroups” (the polar region) and “tails” (the apolar region) of amphiphiles may change nonsynchronously (or non-cooperatively) in response to the changes of temperature or solute concentration. A few systems have been examined in the past two years.\textsuperscript{12,32–35} Investigations from the viewpoint of synchronicity or cooperativity would open a broad window for us to challenge important questions including the kinetics, polymorphism, metastability, and reversibility of phase transitions of amphiphiles. However, not enough attention has been paid to this issue so far.

We have recently found that during the liquid crystalline to gel phase transition upon cooling the head and tail parts of DODAB molecules change nonsynchronously.\textsuperscript{12} In this work, by using conventional and high-sensitivity nano-differential scanning calorimetry (DSC and nanoDSC), microscopy, cryogenic transmission electron microscopy (cryo-TEM), attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, and electric conductivity measurements, we have characterized the thermotropic phase behavior of DODAB aqueous dispersions over a wide concentration range. Special attention has been paid to the formation and transformation of the subgel phase.

2. EXPERIMENTAL SECTION

2.1. Sample Preparation. DODAB with purity greater than 99\% was purchased from Acros Organics (Belgium). Double deionized water with a resistivity of $18.2 \, \text{MΩ} \cdot \text{cm}$ was used for the aqueous hydration and suspension of the DODAB samples. Homogeneous dispersions of the low concentration (0.013 wt % (0.2 mM)–1.6 wt % (25 mM)) samples were prepared by stirring the samples at temperatures around 70 \degree C for 1 h. For high concentration samples (3.2–25 wt %), the samples were cycled at least four times between 20 and 70 \degree C, interspersed with incubation at 70 \degree C for 5 min.

2.2. Conventional DSC and NanoDSC. For the low concentration DODAB samples (0.013 wt % (0.2 mM)–1.6 wt % (25 mM)), the calorimetric measurements were performed on a CSC Model 6300 Nano III differential scanning calorimeter (Calorimetry Sciences Corp., Lindon, UT).

For the high concentration DODAB samples (3.2–25 wt %), the calorimetric data were obtained using a conventional differential scanning calorimeter DSC821\textsuperscript{*} equipped with the high-sensitivity sensor of HSS7 (Mettler-Toledo Co., Switzerland).

2.3. Microscopy. The appearance of the samples was examined under an inverted Olympus IX71 microscope (Olympus Optical, Tokyo, Japan) at 25 \degree C.

2.4. Cryo-TEM. For the cryo-TEM experiment, the specimen was prepared in the controlled environment vitrification system (CEVS) at 25 \degree C and 100% relative humidity to avoid loss of water. A 3.5 \mu L drop of solution was placed on a TEM copper grid covered with a perforated carbon film and blotted with filter paper (4 s) to form a thin liquid film of the sample. The thin film sample was plunged into liquid ethane at its freezing temperature ($-183 \degree C$) to get vitrified and then transferred to liquid nitrogen ($-196 \degree C$) for storage. The vitrified specimens were examined in an FEI Tecnai 20 TEM operating at an accelerating voltage of 120 kV. A Gatan 626 cryoholder that maintained the specimens below $-175 \degree C$ during sample transfer and observation was used.

2.5. ATR-FTIR Spectroscopy. Infrared spectra in the range from 4000 to 650 cm\textsuperscript{-1} were collected using a Nicolet 5700 Fourier transform infrared (FTIR) spectrometer, equipped with a liquid-nitrogen-cooled MCT detector. The attenuated total reflection (ATR) cell was made of trapezoidal ZnSe crystal with an incident angle of 45 \degree and 12 reflections. Spectra were recorded with a resolution of 2 cm\textsuperscript{-1} and 32 scans. DODAB dispersions with the concentration of 5 mM was used for the IR measurement, and IR spectra of pure water as the background collected under the same condition were subtracted to obtain the final IR spectra.

2.6. Electric Conductivity Measurements. Measurements of electric conductance, $\kappa$, of the 5 mM DODAB suspensions were carried out on a conductivity meter (Model DDS-307, Shanghai Leici Instrument Inc., Shanghai, China). To record the change of conductivity during the isothermal incubation at 10.7 or 13.2 \degree C, the sample at 25 \degree C was quickly transferred to a thermostatic water bath with the desired temperature (10.7 or 13.2 \degree C). The time required for dropping the temperature of the sample from 25 \degree C to 10.7 or 13.2 \degree C was $\sim$2 min. The conductivity of a solution is temperature-dependent. To better compare the results, all the conductivity data determined at various temperatures have been corrected to those at 25 \degree C.

3. RESULTS AND DISCUSSION

3.1. DSC. Figure 1 shows the calorimetric results of the DODAB–H\textsubscript{2}O system over a wide DODAB concentration range. In the low concentration (0.2–25 mM or 0.013–1.6 wt %) region obtained by nanoDSC (Figure 1A), we can see evident concentration-dependent phase behavior. At very low concentrations, i.e., 0.2, 0.5, and 1.0 mM, two endothermic peaks residing at 34 and 44 \degree C were observed. The former peak corresponds to the subtransition process (subgel to gel transition), and the latter corresponds to the main transition process (gel to liquid crystalline transition).\textsuperscript{13} At 5.0 mM, a tiny peak at 51 \degree C starts to appear. This peak corresponds to the coagel to
liquid crystalline phase transition.\(^9,12\) Above 5.0 mM, this peak becomes more and more evident as the DODAB concentration increases.

From the thermograms obtained by conventional DSC (Figure 1B) in the high concentration (3.2–25.0 wt %) range, we can see similar phase behaviors of the samples at 3.2, 4.7, and 6.7 wt % as those of 10.0 and 25.0 mM in the nanoDSC results in Figure 1A. At 9.1, 14.3, and 25.0 wt %, only one large peak at 53–54 °C was observed, indicating that these samples are completely in the coagel phase before the main transition.

From the nanoDSC and conventional DSC data, we can see that two crystalline phases may exist in the DODAB samples: the subgel phase formed at low temperatures (usually below 15 °C) and low concentrations with a low transition temperature (around 34 °C) and the coagel phase formed at high concentrations (above 5.0 mM) with a high transition temperature (51–54 °C). The subgel and coagel phases coexist in the concentration range of 7.5 mM (0.47 wt %) to 6.7 wt % at temperatures below 30 °C. Thus, in order to examine the various properties of the subgel phase without the interference of the coagel phase, we have to use the dilute samples (0.2–5 mM).

Shown in Figure 2 are the cooling and reheating calorimetric results of the DODAB–H\(_2\)O system. (A) The low concentration DODAB (1 mM, 0.063 wt %) using nanoDSC. (B) The high concentration DODAB (14.3 wt %) using DSC. The scan rates are 0.5 °C/min.

![Figure 2.](Image)

**Figure 2.** Heating, cooling, and reheating calorimetric results of the DODAB–H\(_2\)O system. (A) The low concentration DODAB (1 mM, 0.063 wt %) using nanoDSC. (B) The high concentration DODAB (14.3 wt %) using DSC. The scan rates are 0.5 °C/min.

From Figure 2A, when the dilute sample in the liquid crystalline phase converts to the subgel phase at low temperatures, two exothermic peaks are seen. The first peak at 39.1 °C is assigned to the liquid crystalline to gel phase transition process. Upon further cooling to below 15 °C, the gel phase converts to the subgel phase. In the subsequent heating scan, the subgel phase first converts to the gel phase at 34 °C, and then the gel phase transforms into the liquid crystalline phase at 44 °C. In Figure 2B, the high concentration sample (14.3 wt %) cooled from 65 °C in the liquid crystalline phase first converts to the gel phase at 43.3 °C and then transforms into the coagel phase at 21.5 °C. Upon the subsequent heating, the coagel phase converts directly to the liquid crystalline phase at 53.3 °C.

The concentration-dependent phase behaviors of the DODAB dispersions can guide us to prepare pure subgel phase from the dilute samples (0.2, 0.5, and 1.0 mM) and pure coagel phase from the concentrated samples (9.1, 14.3, and 25.0 wt %). This allows us to determine the transition enthalpies for the gel to subgel (or subgel to gel), gel to liquid crystalline (or liquid crystalline to gel), gel to coagel, and coagel to liquid crystalline transitions. The results are presented in Table 1. We can see that the enthalpy of the gel to subgel transition (around ~30 kJ/mol) is much smaller in absolute value than that of the gel to coagel transition (around ~60 kJ/mol); this indicates that the coagel phase is more stable than the subgel phase.

From Figure 1A, we can see that 5 mM DODAB sample can form almost “pure” subgel phase at low temperatures. Besides, as compared with the 0.2–1 mM sample, it has a relatively high concentration, which is suitable for further morphological and structural characterizations. Thus, we carried out a detailed DSC investigation for this sample. The nanoDSC results of the heating, cooling, and subsequent reheating scans of the DO-DAB–H\(_2\)O system (5 mM, 0.32 wt %) are shown in Figure 3. The first heating scan (Figure 3a), starting from 25 °C (the initial 25–30 °C curve is not shown since the duration for the adjustment of the nanoDSC instrument is around 5 °C at a heating rate of 0.5 °C/min), shows only one peak at 43.7 °C, and this is the gel to liquid crystalline phase transition. Upon cooling (Figure 3b) and subsequent reheating (Figure 3c), the phase behaviors are similar to those for the 1 mM sample in Figure 2A.

We found through nanoDSC experiments that the subgel phase of the 1 and 5 mM samples obtained by cooling the sample to 0 °C (ice-free) are stable at room temperature for at least 3 days. To test whether the subgel phase can transform into the more stable coagel phase, the 5 mM DODAB dispersion in the subgel phase was stored at −20 °C for 24 h, and after that, the sample was incubated at room temperature for 30 min. The initial heating scan (Figure 3d) reveals that the sample state before heating was the coagel phase. This means that the −20 °C incubation procedure, which involves the ice formation and ice

### Table 1. Calorimetric Data of the DODAB–H\(_2\)O Systems

<table>
<thead>
<tr>
<th>T(_{\text{peak}}) (°C)</th>
<th>(\Delta H) (kJ/mol)</th>
<th>assignment</th>
</tr>
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<tbody>
<tr>
<td>cooling(^a)</td>
<td>39.1 ± 0.4</td>
<td>−39.7 ± 2.3</td>
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<tr>
<td></td>
<td>4.3 ± 3.2</td>
<td>−30.5 ± 1.2</td>
</tr>
<tr>
<td>heating(^a)</td>
<td>33.8 ± 0.5</td>
<td>31.5 ± 0.7</td>
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<tr>
<td></td>
<td>43.8 ± 0.2</td>
<td>40.2 ± 1.8</td>
</tr>
<tr>
<td>cooling(^b)</td>
<td>43.3 ± 0.3</td>
<td>−39.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>23.8 ± 4.3</td>
<td>−60.2 ± 3.1</td>
</tr>
<tr>
<td>heating(^b)</td>
<td>53.6 ± 0.6</td>
<td>105.2 ± 1.5</td>
</tr>
</tbody>
</table>

\(^{a}\) Data obtained from the dilute samples (0.2, 0.5, 1.0 mM). \(^{b}\) Data obtained from the concentrated samples (9.1, 14.3, 25.0 wt %).
melting processes, promotes the nucleation and growth of the coagel phase from the subgel phase. The promotion mechanism is in fact similar to the acceleration of the coagel phase at higher DODAB concentrations: As at $-20^\circ C$, we found that the whole sample was composed of transparent ice, interspersed with some white patches containing DODAB aggregates. In these white patches the concentration of DODAB significantly increases. This largely increases the vesicle-vesicle interaction and accelerates the formation of the coagel phase. The formed coagel phase is stable at room temperature for several months.

3.2. Photographs, Microscopy, and Cryo-TEM. Shown in Figure 4 are the photographs of the aqueous dispersions in three different phases (gel, subgel, and coagel) for the 5 mM sample at $25^\circ C$. The dispersion of the gel phase is bluish and translucent (Figure 4A), indicating the formation of self-assembled structures (e.g., vesicles). For the dispersion of the subgel phase, their appearance is only slightly different as compared with that of the gel phase (Figure 4B). While the dispersion of the coagel phase (Figure 4C) is opaque, and after centrifugation at 15 000 rpm for 3 min, sediments are observed at the bottom of the tube (Figure 4D). The microscopy result of one drop of the coagel phase dispersion revealed the formation of needlelike aggregates with lengths up to several hundred micrometers (Figure 4E).

We have also carried out cryo-TEM experiments of the 5 mM DODAB sample, and the results are shown in Figure 5. From Figure 5A, we can see that curled membranes with sizes ranging from 50 nm to $>1 \mu m$ are present. The cryo-TEM picture for the gel phase does not show typical unilamellar character. In the subgel phase (Figure 5B), the cryo-TEM picture shows the formations of unilamellar vesicles with sizes of around 300–700 nm and large unilamellar vesicles with sizes above 1 $\mu m$. The sizes of the aggregates also agree with our dynamic light scattering results (data not shown). Another very interesting difference between the gel and subgel phase is that the latter is consists of faceted or polygonal. Similar angular geometries were also found in the cryo-TEM picture of a 10 mM extruded DODAB vesicles.16 The origin of the formation of these irregular vesicles has not yet been fully clarified, but it was proposed the

Figure 3. NanoDSC results of the first heating (a), cooling (b), and subsequent reheating (c) scans of the DODAB–H$_2$O system (5 mM, 0.32 wt %). The subgel phase obtained by cooling the sample to 0 $^\circ C$ was stored at $-24^\circ C$ for 24 h, and after that, the sample was incubated at room temperature for 30 min. NanoDSC measurement was employed for this sample (d). The scan rates for all the samples are 0.5 $^\circ C$/min.

Figure 4. Photographs of the 5 mM DODAB–H$_2$O system at 25 $^\circ C$. (A) The gel phase. (B) The subgel phase. (C) The coagel phase. (D) The coagel phase was centrifuged at 15 000 rpm for 3 min. (E) The microscopy result of one drop of the coagel phase dispersion (scale bar = 200 $\mu m$).

relatively high bending rigidity of DODAB bilayers and crystallization defects are important factors.16

3.3. FTIR. FTIR spectroscopy was used to provide a submolecular understanding of the three phases of DODAB dispersions (gel, subgel, and coagel), and the results are shown in Figure 6. The asymmetric and symmetric stretching vibrations of the methylene groups ($\nu_\text{as}\text{CH}_2$ and $\nu_\text{sym}\text{CH}_2$) shown in Figure 6A are indicators of the change in the conformations of lipid alkyl tails.36 The almost conserved band positions of $\nu_\text{as}\text{CH}_2$ ($\sim 2916 \text{ cm}^{-1}$) and $\nu_\text{sym}\text{CH}_2$ ($\sim 2850 \text{ cm}^{-1}$) for the three phases show that the all-trans conformations of the lipid alkyl chains do not have significant changes during the phase transformations. In Figure 6B, there are two important IR bands. The band centered at 1487–1490 cm$^{-1}$ is ascribed to the asymmetric deformation vibration of the methyl groups attached to the N$^+$ atom ($\delta_\text{as}\text{CH}_3$–N$^+$) in the hydrophilic part of the amphiphilic molecule, which is known to be sensitive to the extent of disorder and the packing of the headgroups.39,40 For the subgel and coagel phases, this band residing at 1487.3 cm$^{-1}$ can be related to a relatively disordered, hydrated headgroups.12 The higher wavenumber and sharper shape of the band at 1489.0 cm$^{-1}$ in the coagel phase suggest the ordering and dehydration of polar headgroups. The other band in this region is from the CH$_2$ scissoring vibration ($\delta\text{CH}_2$). This band is very sensitive to the intermolecular forces and can be served as a key band for examining the state of packing of the hydrocarbon chains in various phases.12,34,36,41 For the subgel and coagel phases, this band is centered at 1470.3 and 1470.8 cm$^{-1}$, respectively. This single peak indicates that the alkyl chains of the two phases are both packed in a close-to-triclinic 2D lattice. However, the alkyl chain packing in the coagel phase is tighter and more ordered than that in the gel phase as its $\delta\text{CH}_2$ band is sharper. While in the gel phase, the band at 1466.3 cm$^{-1}$ suggests that the alkyl chains form a close-to-hexagonal 2D lattice.

In conclusion, the IR results show the largest difference between the subgel and coagel phases lies in the hydration degree of the lipid headgroups, while the only significant difference between the gel phase and subgel phase is the change of the lipid alkyl chain packing state. This means that upon cooling to below 15 $^\circ C$, during the gel to subgel phase transition, the head and tail parts of the amphiphile change nonsynchronously, with only the ordering in the packing of the lipid alkyl chains. The results clearly show that the nucleation of the crystallization process (the formation of subgel phase) occurs only in the lipid tail region, which deepens our understanding on the crystallization mechanism of DODAB molecules.

3.4. Electric Conductivity Measurements. To gain further information on the packing state of DODAB molecules in the
membranes and to study the gel to subgel transition kinetics, we carried out time-dependent electric conductivity ($\kappa$) measurements for the 5 mM DODAB sample, and the results are shown in Figure 7. Before measurement, the 5 mM DODAB dispersion was stirred at around 70°C for 1 h and then cooled to 25°C. As shown in Figure 3, the thus-obtained sample was in the gel phase. For the convenience of comparison, all the $\kappa$ values have been corrected to the values at 25°C. The gel-state DODAB suspension has an electric conductivity of 19.4 μS/cm at 25°C. Before measurement, the sample at 25°C was dropped to 10.7 or 13.2°C. Time was recorded after the sample reached the desired temperature (10.7 or 13.2°C) after ~2 min.

Figure 5. Cryo-TEM results of the DODAB–H2O sample (5 mM) at 25°C. (A) The gel phase. (B) The subgel phase.

Figure 6. FTIR absorbance spectra of DODAB–H2O (5 mM) in the gel, subgel, and coagel phases at 25°C: (A) 3000–2800 cm⁻¹; (B) 1510–1400 cm⁻¹.

Figure 7. Dependency of electric conductivity of 5 mM DODAB dispersion on time: (A) 10.7°C; (B) 13.2°C. Before measurement, the sample at 25°C was dropped to 10.7 or 13.2°C. Time was recorded after the sample reached the desired temperature (10.7 or 13.2°C) after ~2 min.
Our electric conductivity measurements revealed that the conductivity value decreases during the isothermal gel to subgel transition process. The decrease in the electric conductivity of the dispersion indicates a reduced concentration of the dissociated counterions (Br\(^-\)) in the solution. The result means that the surface of the subgel phase has a stronger ability to “catch” counterions as compared with that of the gel phase. As concluded in the literature,\(^{42-46}\) a membrane with a larger surface charge density has a stronger counterion binding ability (or a weaker counterion release ability). Thus, our electric conductivity results suggest that the subgel phase has a larger membrane surface charge density as compared with the gel phase. This is related with the tighter packing of the lipid molecules in the subgel phase, which has been confirmed by our FTIR data in Figure 6. Besides, the tighter packing of the DODAB molecules in the subgel phase as revealed by our electric conductivity measurement can lead to an increased membrane bending rigidity, which may account for the observation of the faceted vesicular surfaces in Figure 5B.

3.5. Formation and Transformation of the Subgel Phase. Saveyn et al.\(^13\) once carried out wide-angle X-ray diffraction (WAXS) experiments on dilute DODAB dispersions and found a broad diffuse peak centered around 0.461 nm in the liquid crystalline state, a single sharp peak at 0.423 nm in the gel phase, and three other peaks appeared at 0.461, 0.400, and 0.383 nm in the subgel phase. The reflections observed for the subgel phase corresponds to a triclinic subcell in which the zigzag planes of the alkyl chains are all parallel.\(^13\) The alkyl chains are more tightly packed in the subgel phase than in the gel phase.\(^13\) The triclinic packing revealed by WAXS for the subgel phase is in line with our FTIR analysis on the CH\(_2\) scissoring band.

In this work, we found that the formation of subgel phase or coagel phase depends largely on the DODAB concentration. From the DSC results, we can see that high DODAB concentration facilitates the formation of the coagel phase. The coagel phase was reported to be a multilamellar crystalline phase with a repeat distance of 3.7 nm.\(^12\) This work also reveals that the subgel phase is formed preferably at low DODAB concentrations, and our results imply that high concentration increases the intervesicle or interlayer interactions and promotes the formation of coagel phase. As shown in Figure 2, the gel to subgel transition in the dilute sample (1 mM, 0.063 wt %) is more sluggish than the gel to coagel transition in the concentrated sample (14.3 wt %) upon cooling. From the transition temperatures, we can see that the nucelation process for the dilute samples is more difficult than for the concentrated samples. These differences indicate that the formation mechanisms of subgel and coagel phases are different. We have previously demonstrated that the gel to coagel transition process involves the change of the alkyl chain packing from hexagonal to triclinic and significant dehydration of the polar headgroups.\(^12\) This is different from the gel to subgel transition process where only the alkyl chain packing state changes from hexagonal to triclinic.

In the concentrated sample, the higher DODAB concentration increases the vesicle—vesicle interactions. The accelerated formation of the coagel phase by incubating the subgel phase at −20 °C for 24 h has a similar mechanism: The formation of ice increases the local concentration of the DODAB and thus largely increases the vesicle—vesicle interaction and accelerates the formation of the coagel phase.

The IR results show that the only significant change during the subgel to gel transition process of the DODAB molecules is the rearrangement of lipid alkyl chain packing state (from triclinic to hexagonal). This is very similar to the cases of the low-temperature phase L\(_{RI}\) to gel (L\(_{rβ}\)) transition of phosphatidylethanolamines (PEs) and the low-temperature phase SGG to gel (L\(_{rβ}\)) transition of phosphatidylcholines (PCs) upon heating processes, which also only involve the packing rearrangements of the lipid alkyl chains from orthorhombic to hexagonal.\(^45\)

On the basis of the above results and analyses and our previous work,\(^12\) we can obtain a full picture of the phase transitions of DODAB aqueous dispersions at a wide concentration region (0.013—25 wt %). This is presented as two schematic models for the dilute and concentrated samples given in parts A and B of Figure 8, respectively. For samples in both of the two concentration ranges, the lipids at high temperatures are in the liquid crystalline phase with well-hydrated headgroups, and translational and flip-flop motions can occur and alkyl chain conformational disorder predominates.\(^13\) Upon cooling, the liquid crystalline phase first transforms into the gel phase, which

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**Figure 8.** Two schematic models showing the phase transition sequences of the (A) dilute (e.g., 0.2—5 mM or 0.013—0.32 wt %) and (B) concentrated (e.g., 9.1—25 wt %) DODAB aqueous dispersions. In these models, we gave only DODAB bilayer fragments for simple representations. The straight lines and the zigzag lines in the tail parts of the gel and subgel/coagel phases respectively represent the different packing states of the alkyl chains.
involves only the conformational change and packing state rearrangement of the lipid alkyl tails but does not cause the hydration state change of the lipid polar headgroups. In the formed gel phase, the molecules possess more solidlike alkyl chains with restricted molecular motions, and the chain packing is still somewhat disordered.\textsuperscript{13} The transformation between the liquid crystalline phase and the gel phase in both kinds of samples is reversible upon cooling and heating.

For the dilute samples (Figure 8A), upon further cooling to below 15 °C, gel to subgel phase transition occurs, and this phase transition is sluggish; the complete formation of the subgel phase takes a relatively longer time as compared with the main transition process. From gel to subgel, no evident change on the hydration level was observed for the lipid headgroups, nor was the change on the conformational state of the lipid alkyl chains. The only rearrangement of the lipid molecules is the lipid alkyl chain packing state (from hexagonal to triclinic), and the subgel phase has an increased alkyl chain packing density. Hence, the slow gel to subgel transition kinetics is mainly due to the difficulty in the rearrangement of the packing state of the lipid alkyl chain.

More interestingly, we have demonstrated that the morphological changes from curled membrane in the gel phase to faceted or polygonal surface with typical unilamellar feature in the subgel phase are coupled to the change of alkyl chain packing state. The increased membrane bending rigidity induced by the tightening of the lipid alkyl chain packing state may be responsible for the formation of faceted unilamellar vesicles in the subgel phase. The difficulty in the rearrangement of the packing state of the lipid alkyl chains may be due to the fact that the change of the alkyl chain packing state is accompanied by the change of the morphology of the vesicles.

The subgel phase can further convert to the coagel phase at conditions such as the incubation of the subgel phase at −20 °C for a certain period of time. The coagel phase has an even tighter alkyl chain packing than the subgel phase (reflected by the sharper χ\textsubscript{CH} band in Figure 6B); besides, the headgroups of DODAB in the coagel phase are partially dehydrated.\textsuperscript{12} Upon heating, the coagel phase directly transforms into the liquid crystalline phase.

While for the concentrated samples, as shown in Figure 8B, further cooling the gel phase results in the formation of the coagel phase, and upon heating, the coagel phase directly converts to the liquid crystalline phase.

Our previous studies have shown that during the phase transition processes the different parts of amphiphilic molecules can undergo nonsynchronous changes.\textsuperscript{12,32–35} Especially we have observed nonsynchronous change of the head and tail parts of DODAB molecules during the liquid crystalline to gel phase transition process, while our present work gives another example of nonsynchronicity phenomenon where the DODAB molecules only have a significant change in the lipid alkyl chain packing state during the gel to subgel phase transition process. This means that the ordering in the packing of the lipid alkyl chains is the bottleneck controlling the subgel formation process, and it provides a deeper understanding on the crystallization mechanism of the DODAB aggregates from the submolecular viewpoint. The origin of the nonsynchronous change of the lipid head and tail parts can be attributed to the lack of special intermolecular attractive forces (hydrogen bonding and electrostatic attraction) between the neighboring polar headgroups of DODAB molecules. This can explain the retention of the hydration degree of the lipid head part during the rearrangement of the lipid tail part.

Finally, we would like to mention that our current study mainly focuses on the lipid concentration effect on the phase behavior of DODAB. However, the phase behavior of DODAB can also be changed upon the addition of another compound. One very recent example\textsuperscript{46} is that the addition of an oligonucleotide changes the structure and stability of DODAB bilayer fragments. Oligonucleotide can increase bilayer packing due to bilayer fragment fusion, and a nonmonotonic behavior of colloid stability as a function of poly(dA) concentration was observed for the oligonucleotide.\textsuperscript{46}

4. CONCLUSIONS

We studied the formation and transformation processes of two crystalline phases (subgel and coagel) of the DODAB aqueous dispersions by the combined use of various techniques and gave a full picture of the phase transition sequences for the dilute and concentrated DODAB dispersions. On the basis of our above studies, we can see that the gel and subgel phases are both metastable phases, while the coagel phase is the stable phase at low temperatures and the liquid crystalline phase is the stable phase at high temperatures.

The combination of both conventional DSC and nanoDSC makes us possible to characterize the wide concentration coverage from the dilute to the concentrated samples. The subgel phase is formed preferably at low DODAB concentrations, while the coagel phase is formed preferably at high DODAB concentrations. An almost pure subgel phase was obtained in the dilute region (below 7.5 mM), while an almost pure coagel phase was obtained in the concentrated region (above 6.7 wt %). Besides, the finding that unilamellar vesicles can be spontaneously formed at the DODAB concentration of 5 mM in the subgel phase is very interesting, since the formation of unilamellar vesicles was usually achieved by sonication or extrusion methods.

FTIR reveals the submolecular details of the phase transformation processes, and it was found that the only significant change in the gel to subgel phase transition is the ordering in the lipid alkyl chain packing. The slow gel to subgel transition kinetics is mainly due to the difficulty in the rearrangement of the packing state of the lipid alkyl chains. The nonsynchronous change of the head and tail parts of the DODAB molecules during the gel to subgel transition shows that it is the lipid tail part that triggers the gel to subgel phase transformation. The ordering in the lipid alkyl chain packing during the gel to subgel transition may be responsible for the spontaneous formation of unilamellar vesicles in the subgel phase.

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REFERENCES


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