Myelin growth and initial dynamics

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Abstract

During the dissolution of solid surfactants in water, various types of nonequilibrium microstructures have been observed. The most important one is the myelin growth which can be observed when some poorly water soluble surfactants such as phosphatidylcholine (PC), Aerosol-OT (AOT), etc. are contacted with water. In this study initial myelin growth for a period of 2–4 s is studied both qualitatively as well as quantitatively in all the directions with respect to water flow in a PC system using digital video microscopy. Further, overall myelin growth is studied by means of optical microscopy to understand the effect of distance between cover slip and glass slide on myelin growth. Experiments are also performed to study effect of additives (silica) to lamellar phase on diffusion coefficients. It has been observed that the presence of silica particles causes extensive coiling of myelin structures. The mechanism of water transport into the lamellar phase during myelin growth is investigated by using silica in a colloidal range as dopant material.

Keywords: Myelin; Digital video microscopy; Optical microscopy; Silica; Phosphatidylcholine; Slit width

1. Introduction

Surfactants in dilute solutions assemble into a variety of microstructures. Knowledge of the formation and behavior of these phases of surfactant systems is essential for the effective use of surfactants in their applications. Equilibrium studies of the surfactant–water systems have been studied extensively. However, initial kinetics of dissolution of surfactant systems is not still clear though it is important to enhance the performance of surfactant-based products. This study could be useful for potential applications such as encapsulation, drug delivery, and nanotechnology and also to tailor the performance of laundry detergent and a bar of soap. By means of digital video microscopy it is possible to capture a movie having 30 frames per second (fps). Therefore, by having a large amount of information in terms of images within a few seconds, it is very easy to have a lucid idea of initial dynamics of surfactant dissolution which is essential to enhance the performance of surfactant-based products.

Myelin growth in amphiphile systems is a classical example of dynamic interface instability. This phenomenon was first observed by Virchow in 1854. When an almost insoluble (in the solvent medium) amphiphile lamellar phase is placed next to the solvent, cylindrical rod-like microstructures known as myelin figures are developed at the amphiphile–solvent interface. Myelins are highly viscous gel-like microstructures consisting of concentrically arranged multilamellar tubules of alternating amphiphile bilayers and water layers with a core axis of water (Figs. 1 and 2).

Myelin figures in phosphatidylcholine and a water system have been studied in great detail [3–9]. The structural features of myelin figures are very similar to that of the nerve myelin sheath (from which the name myelin was taken) and bilayers are very similar to bio-membranes [8]. By changing the texture of the lamellar phase to the onion phase (consists of closely packed multilamellar vesicles) by shearing it is possible to suppress myelin growth [2].

Sakurai and Kawamura [7] studied the growth behavior of myelin figures in phosphatidylcholine (PC) quantitatively. But they restrict their study only to the first step growth. Growth behavior and morphological features of myelin figures of egg-yolk lecithin/hydrophilic liquid systems were investigated by optical microscopic observation at room temperature by Sakurai et al. [5].

Mishima and Yoshiyama [3] have shown by means of video tape recording that myelin growth is not diffusion-limited during the beginning of myelin growth as a rearrangement of bilayers take place.
One of the objectives of this study was to study the initial kinetics of myelin growth by means of digital video microscopy. Other objectives were to investigate the effect of additives such as silica particles on the growth rate of myelins and to get an idea of the mechanism of water transport into myelins during the swelling process using silica as dopant particles.

2. Experimental

This section provides an explanation of the experimental procedures followed for this work and materials used.

2.1. Materials

The surfactant we have used for this work is phosphatidylcholine (a natural surfactant). Phosphatidylcholine from Sigma Chemicals Co., USA, was used without further purification. Chloroform (purity: 99.5%) from Sisco Research Lab. Pvt. Ltd., India, is used as a casting solvent. Two types of silica samples, a fumed silica and Ultrasil-VN3 provided by ISRO, Trivandrum were used as additives and dopant material. Aqueous silica solutions were sonicated for about 2 min to get uniform dispersions.

2.2. Methodology for digital video microscopy

2.2.1. Penetration scan method

Penetration scan experiments provide a useful method for studying surfactant phase behavior and growth rate. In these experiments, a drop of surfactant–chloroform solution was taken on a slide and the solvent was allowed to evaporate. The solvent-cast surfactant lump was then covered with a coverslip maintaining a fixed distance between coverslip and slide using a spacer as shown in Fig. 3 and gently pressed to get a thin slice. The specimen was then mounted on a microscope stage and properly focused using the appropriate lens. A drop of water was introduced at the edge of the coverslip and growth was observed and captured as a movie.

2.2.2. Growth rate study

The microstructures were observed with an optical microscope from Carl Zeiss, Inc., Germany with a 10× objective lens and the microscope was connected to the digital CCD camera WAT-202D, Japan, which directly converts the two-dimensional optical image into a digital signal and finally using different software the images were captured in terms of MPEG or AVI files. The initial growth movie was captured by means of video microscopy in terms of AVI or MPEG and then each frame of the particular movie is extracted by means of AVIquick or AVIedit software. After converting images into TIFF formats, all images were analyzed with the help of Scion Image software. Images were calibrated with a standard calibration tool in which the 10× objective has a pixels to µm ratio of 1.6. The myelin growth was analyzed by measuring the distance of myelin front from the myelin root, \( L \), as a function of time after the contact of the surfactant lump front with water.

In each run of myelin growth, a minimum of two individual myelin length was measured. Similarly, a minimum of four different runs were carried out and after measuring the average myelin growth length for each run the overall average myelin growth length was determined. As shown in Fig. 4, two to three myelins were analyzed to get the average myelin growth length for that particular run. The experiments were carried out at room temperature and the slit width between the cover slip and glass slide as given by the spacer thickness was kept at 60 µm by means of adhesive tape. The error bars in the plots indicate the variation in \( L \) obtained over these measurements in terms of standard deviation which could be attributed to the erratic nature of the myelin growth process. Finally, overall average myelin growth was plotted against the square root of the time, and based on the slope of the best fit line, the diffusivity value.
Fig. 4. Myelin growth after contacting pure surfactant with water. Several lengths of individual myelins $L(t)$ are measured from myelin roots to myelin front at a particular time.

Fig. 5. Schematic diagram indicating growth direction of myelin with respect to water flow direction.

was calculated. Myelin growth is measured in a different direction with respect to water flow as shown in Fig. 5.

2.3. Methodology for optical microscopy

2.3.1. Penetration scan method

As mentioned in the digital video microscopy section, a penetration scan method was carried out. However, images were directly stored and analyzed by Image Pro-Plus (version 3.1) software from Media Cybernetics.

2.3.2. Spacer thickness

Initially, penetration scan experiments were carried out without any spacer between the slide and coverslip, which was leading to inconsistent results. So the effect of spacer thickness was studied by varying the distance between the cover slip and glass slide from 50 to 200 µm.

2.3.3. Growth rate study

For the growth rate study, time series microscopy of myelins was done by capturing the optical micrographs by OLYMPUS B60 type at regular intervals. A CCD Sony digital camera (XC-75 CE model) was attached to the microscope and the image is sensed by the CCD camera, which sends the analog signal to the grabber card, which in turn converts analog signal to digital signal so that the image can be stored for subsequent recovery and analysis. The average growth length of myelins was measured using the Image Pro-Plus software.

The effect of additives on myelin growth rate was studied by adding 1% (w/w) silica to the surfactant phase and carrying out penetration scan experiments. Table 1 lists the various system combinations of PC and silica used for investigation on the effect of additives on diffusion coefficient.

The myelin growth was analyzed by measuring the distance of myelin front from the myelin root, $L$, as a function of time after contact of the surfactant lump with water. Several lengths were averaged to get the average growth of the myelin front as shown in Fig. 6.

<table>
<thead>
<tr>
<th>System</th>
<th>Surfactant phase</th>
<th>Contacting liquid phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC/water</td>
<td>PC</td>
<td>Water</td>
</tr>
<tr>
<td>PC-silica/water</td>
<td>PC containing 1% (w/w) fumed silica</td>
<td>Water</td>
</tr>
<tr>
<td>PC-silica/water</td>
<td>PC containing 1% (w/w) Ultrasil-VN$_3$</td>
<td>Water</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. Digital video microscopy

As explained in the experimental methodology of digital video microscopy, penetration scan experiments were carried out and images were extracted from the captured movie. As shown in Fig. 4, overall averaged myelin growth length $L$ was calculated from individual lengths of myelins of different experiments. During initial 2–4 seconds of myelin growth, it is very easy to make clear distinction of individual myelins which are bulging from the lamellar phase. There-
fore, we have used individual myelins length measurements to calculate diffusion coefficients (Fig. 7).

When the square of this length \( l^2 \) is plotted against corresponding time, a straight line is obtained. So it can be said that \( l \) varies as \( t^{1/2} \); i.e., myelin growth is a diffusion-like process. Because of the concentration gradient at the PC and liquid medium interface and the inability of the PC molecules to dissolve molecularly into the medium, there is an aggregative transport of the lecithin molecules from the less hydrated regions in the lump toward the more hydrated myelin figures. Using the conventional theory of diffusion processes, the governing equation for myelin growth becomes \( l \approx (2Dt)^{1/2} \), where \( D \) is the relevant diffusion coefficient. Thus the value of \( D \) is calculated from the slope of the \( l \) vs \( t^{1/2} \) plot. The myelin growth in counter-current direction with respect to water flow is shown in Figs. 8 and 9. It shows that the diffusion coefficient during initial growth of 2 s is \( 2.252 \times 10^{-6} \text{ cm}^2/\text{s} \), which is higher than the diffusion coefficient \( 1.245 \times 10^{-6} \text{ cm}^2/\text{s} \) for 2 min growth. It is also clear from Fig. 9 that approximately after 2 s myelin growth decreases compared to the initial growth of 2 s.

From Fig. 8, it is clear that myelin growth is having a time delay of approximately 0.1 s after the water comes in contact with the surfactant lump.

From Tables 2 and 3, the diffusion coefficient values in all the directions lie between 2 and \( 3 \times 10^{-6} \text{ cm}^2/\text{s} \).

The diffusion coefficient for a 2 wt% PC–water system in the co-current direction was found to be \( 2.136 \times 10^{-6} \text{ cm}^2/\text{s} \), which is 2 to 12% less compared to other directions of myelin growth. Also, the diffusion coefficient for a 5 wt% PC–water system in the co-current direction was found to be \( 2.036 \times 10^{-6} \text{ cm}^2/\text{s} \), which is 22 to 35% less compared to other directions of myelin growth. Based on these data, one can infer that the diffusion coefficient in the co-current direction is marginally less compared to other
directions of myelin growth. One reason could be a lower water velocity at the co-current myelin growth region compared to counter-current growth and cross-current growth region. From the experiments, water velocity in the co-current growth region was found to be approximately half compared to the counter-current growth region.

3.2. Optical microscopy

As explained in the methodology of optical microscopy, penetration scan experiments were carried out. The average growth of myelin was measured taking an average of different lengths measured as shown in Fig. 6. Similarly various experiments were carried out and overall average myelin growth length was measured, which was plotted against the square root of corresponding time. The plot gives a straight line and that indicates myelin growth is a diffusional process. Moreover, as shown in the Fig. 6, the number of growing myelins is large and a root to front method is used for length measurements.

3.2.1. Effect of spacer thickness

We have studied the effect of varying the width between the cover slip and slide by means of changing the thickness of spacers from 50 to 200 µm. The results are shown in Table 4, where \( n \) indicates the number of experiments that have been carried out for the particular study. From this table it can be concluded that the thickness of the spacer which in turn controls the height of the surfactant–solvent interface does not affect the growth rate much as long as other experimental conditions are unchanged. It has been observed that when the spacer thickness is increased beyond 150 µm, the contacting liquid flown over the surfactant lump and the myelin growth was observed at the interface as well as on the surface of the surfactant lump, making it difficult to measure the growth length. Also, at a higher spacer thickness the myelin front grew in layers one above other, giving two to three growth fronts, which made the length measurements ambiguous.

3.2.2. Effect of silica as additives

Figure 10 shows some of the myelin structures observed when silica was added to the surfactant phase. It has been observed that the addition of silica results in an extensive increase in the number of helical structures compared with that in a pure surfactant–water system.

It can be seen from Tables 5 and 6 that the addition of silica to the surfactant phase reduces the diffusion coefficient. Since the silica concentration in the surfactant phase is about 20%, the silica particles entrapped in the bilayers may offer a hindrance to bilayer rearrangement, which can lead to retardation in the diffusion coefficients.

3.2.3. Mechanism of myelin formation

The mechanism of water transport into the lamellar phase can be understood by doping the liquid medium with tracer particles. We have used silica particles of 0 to 4 µm as dopant particles.

It has been observed that during the swelling process, silica particles continually accumulate at myelin roots. The particles are not observed to accumulate at myelin tips. This implies that the water transport into the lamellar phase occurs at myelin roots and not through the tips. These obser-

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Growth direction w.r.t. water flow</th>
<th>Best fit line equation ( D \times 10^6 ) (cm²/s)</th>
<th>Variation in ( D ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cross-current right side</td>
<td>( y = 20.89x - 2.513 ) 2.182 ±24</td>
<td>2.182 ±24</td>
</tr>
<tr>
<td>2</td>
<td>Cross-current left side</td>
<td>( y = 21.93x - 4.034 ) 2.406 ±26</td>
<td>2.406 ±26</td>
</tr>
<tr>
<td>3</td>
<td>Counter-current</td>
<td>( y = 21.22x - 3.142 ) 2.252 ±34</td>
<td>2.252 ±34</td>
</tr>
<tr>
<td>4</td>
<td>Co-current</td>
<td>( y = 20.67x - 3.945 ) 2.136 ±4</td>
<td>2.136 ±4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Surfactant Spacer thickness system (µm)</th>
<th>Best fit line equation ( D \times 10^6 ) (cm²/s)</th>
<th>Variation in ( D ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC/water Without spacer</td>
<td>( y = 394.7x - 53.137 ) 5 1.97</td>
<td>5 1.97</td>
</tr>
<tr>
<td>2</td>
<td>PC/water 50</td>
<td>( y = 258.13x + 60.981 ) 5 1.29</td>
<td>5 1.29</td>
</tr>
<tr>
<td>3</td>
<td>PC/water 100</td>
<td>( y = 328.29x + 24.432 ) 3 1.64</td>
<td>3 1.64</td>
</tr>
<tr>
<td>4</td>
<td>PC/water 150</td>
<td>( y = 298.21x + 1099.2 ) 3 1.49</td>
<td>3 1.49</td>
</tr>
</tbody>
</table>
Table 5
Summary of results of myelin growth rate study for PC (5%) with fumed silica as additive

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Surfactant Liquid medium</th>
<th>Best fit equation</th>
<th>( n )</th>
<th>( D \times 10^6 ) (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC Water</td>
<td>( y = 258.13x + 69.981 )</td>
<td>3</td>
<td>1.29</td>
</tr>
<tr>
<td>2</td>
<td>PC-silica (1%) Water</td>
<td>( y = 165.88x - 661.26 )</td>
<td>3</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Table 6
Summary of results of myelin growth rate study for PC (5%) with Ultrasil-VN₃ as additive

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Surfactant Liquid medium</th>
<th>Best fit equation</th>
<th>( n )</th>
<th>( D \times 10^6 ) (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC Water</td>
<td>( y = 258.13x + 69.981 )</td>
<td>3</td>
<td>1.29</td>
</tr>
<tr>
<td>2</td>
<td>PC-silica (1%) Water</td>
<td>( y = 189.55x - 2048.7 )</td>
<td>3</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Observations support the idea of a surfactant backflow mechanism of myelin growth introduced by Buchanan et al. [1]. Water entering the lamellar phase at the roots feeds the myelin structures, which grow backward into the surrounding water phase. Thus the essential mechanism of myelin growth is the backflow of surfactant to make way for water entering the lamellar phase as it swells to equilibrium.

4. Conclusions

Digital video microscopy has been found to be very useful to give an idea of the initial dynamics (2 to 4 s) of myelin growth. Myelin growth takes place approximately after 0.1 s when a surfactant lump comes in contact with water and it obeys the relation \( l^2 \approx 2Dt \) and therefore, it can be concluded that initial myelin growth is also a diffusional process after 0.1 s. During the initial period, the myelin growth rate is higher than overall myelin growth as from the evidence of a higher diffusion coefficient value for the initial myelin growth. Also, the diffusion coefficient in the co-current direction of myelin growth was marginally less compared to other directions which could be due to the decreased water velocity in the co-current growth region.

Optical microscopy is also being used to provide an idea of overall myelin growth but there is a limitation of lucid visualization of dynamics during the initial 1–2 s of myelin growth due to the insufficient speed in capturing images. The slit width (distance between cover slip and glass slide) does not affect the diffusion coefficient values of myelin growth. In the presence of silica as an additive, an extensive amount of coiling has been observed at a concentration of 1% (w/w). Silica doping suggests that water transport into the lamellar phase during swelling occurs at the roots of myelins and not at the tips. This supports the surfactant backflow mechanism of myelin formation wherein water entering the lamellar phase at the roots feeds the myelin structures which grow backward into the surrounding water phase.

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References