Extended time range modeling of myelin growth

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Abstract

When some poorly water-soluble solid surfactants are contacted with water, several microstructures are observed as part of the dissolution process of the surfactants in water. One such microstructure called “myelin,” which is observed when a surfactant like phosphatidylcholine is contacted with water, is the subject of this paper. In this study we have used video microscopy to investigate myelin growth over a wide time range, namely 0.25–700 s, and found that existing models do not correctly express myelin growth over extended time ranges. When studied over a wide time range, the myelin growth was found to evolve over three distinct regimes, namely ballistic, diffusional, and subdiffusional regimes. The underlying growth models are physically explained and mathematically expressed. A relationship is derived between the width of myelin and the growth rate at long times. The estimated width of myelin is consistent with experiments.

Keywords: Myelin; Phosphatidylcholine; Modeling; Ballistic; Diffusional; Subdiffusional

1. Introduction

Because solid surfactants are the basic components of most commercial cleaning products, the study of their dissolution in water holds great interest. A thorough understanding of the dissolution process will enable enhancement of their performance and hence their application. The study of dynamic microstructures observed during dissolution holds the key to understanding the dissolution process. One such nonequilibrium microstructure “myelins” is observed when the lamellar phase of surfactants like phosphatidylcholine (PC) is contacted with excess water. Myelins are highly viscous, gel-like, microstructures consisting of multilamellar tubules of an alternating amphiphile bilayer and a water layer with a core axis of water.

Buchanan et al. [1] suggested that myelin is a structure formed from immiscible backflow of a swollen surfactant phase established to counter the permeation of solvent into the lamellar phase. Thus monitoring the growth of myelins can give an idea of the flux of permeation of solvent into the lamellar phase.

Sakurai et al. [2] investigated the growth behavior and morphological features of myelin figures in egg-yolk lecithin by microscopic observation at room temperature quantitatively. They concluded that in terms of morphological evolution, the myelin growth process is a three-step process. However they restricted the study of the myelin growth process as a diffusion process to the first step which was in the first 10 min for water as solvent. They found that the average length of myelin figures is proportional to the square root of time, implying a diffusion-limited growth mechanism.

Mishima and Yoshiyama [3] showed by means of video tape recording that myelin growth is not diffusion limited during the beginning of myelin growth as a rearrangement of bilayers takes place but they have confirmed that the myelin length changes to the square root of time as myelin growth progresses in accordance with the following expression:

\[ l = -1 + (1 + 2v_0 k t)^{1/2}/k. \]  

Buchanan et al. [4], studying myelin growth in non-ionic surfactants with large miscibility gaps (viz. C_{12}E_3) with water, confirmed that myelin growth is diffusive in nature but
with a time lag, in which the rapid formation of myelin is not expected to be diffusive, in accordance with the following expression:

\[ \langle L(t) \rangle = k_m \sqrt{T - t_0} + L_0. \]  

(2)

In this paper, we investigate the dynamics of myelins formed by PC in aqueous medium over a large time range and explain the experimental observations with diffusion concepts. This will enable understanding of the growth of myelins as a continuous process, undergoing characteristic changes in its diffusive behavior as time progresses, rather than a series of diffusion steps with different diffusion coefficients.

2. Experimental

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

2.1. Materials

The surfactant we have used for this work is phosphatidylcholine (a natural surfactant). Phosphatidylcholine from Sigma Chemical Co. (St. Louis, MO) was used without further purification. Chloroform (purity 99.5%) from Sisco Research Lab. Pvt. Ltd., India, is used as a casting solvent.

2.2. Methodology of 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 and pressed to flatten out the surfactant lump to get a thin slice. This was to enable a 2D visualization of growth of a single myelin front along the circumference of the surfactant slice. The coverslip also ensures that the permeation flux of solvent around the lamellar phase is very high due to the high speed of solvent imparted by capillary action. The benefit of a high permeation flux is understood in the analysis of experimental results considered later. 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 video images (25 images/s) were obtained with a square pixel XC-77CE Sony video camera, Japan, feeding into an ATI Frame grabber, ATI Technologies Inc. A Pentium III-863 MHz PC served as a host for the grabber. The growth sequence was captured by means of video microscopy in MPEG2 format and then each frame of the particular movie was extracted by means of Frames (frame extraction software). After converting images into TIFF formats, all images were analyzed with the help of Scion Image (Release Beta 4.0.2) software, Scion Corporation. Images were calibrated with a standard calibration tool in which the 10× objective had a pixels to micrometer ratio of 0.1. 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 with water.

While recording a run of myelin growth, before adding water, it was ensured that the edge of the surfactant slice was maintained parallel to the horizontal edge of the screen and very close to either of the horizontal edges. This made measurements possible and easy for extended time ranges, as the entire screen area was available for viewing myelin growth. On measurements using Scion Image software, first the area of screen occupied by surfactant slice \( (A_s) \), before the introduction of water, was measured. For images showing myelin growth, the area of the screen occupied by myelins and surfactant slice was measured \( (A_m) \) and the area measured in the image before the introduction of water was deducted from it and the result was divided by the breadth of the screen measured along the interface (Fig. 1). This gave an average distance of myelin front from the interface and accounted for the variations in the distances of tips of myelins from interface. The initial area deduction procedure was adopted to account for the uncertainty that arises while determining the positions of the roots of the myelins in later

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Fig. 1. (a) Initial area measurement from a micrograph. Scale bar indicates 50 µm. (b) Measurement of area occupied by surfactant slice and myelins from a micrograph. Scale bar indicates 50 µm.
stages which seem to be indivisible from the dilute lamellar phase, which builds up in the surfactant slice near the roots of the myelins. A minimum of three different runs was carried out and after measurement of the average myelin growth length for each run, the overall average myelin growth length was determined. The experiments were carried out at room temperature. The error bars in the plots indicate variations in $L$ obtained over these measurements in terms of standard deviations, which could be attributed to the erratic nature of the myelin growth process. However, it was observed that the initial area deduction procedure helped to reduce the values of standard deviations associated with the experimental data. Finally the square of the overall average myelin growth length was plotted against time and appropriate trend curves were drawn to characterize the data pattern.

3. Experimental observations

As explained in the experimental methodology of digital video microscopy, penetration scan experiments were carried out and images were extracted from the captured movies. The overall average myelin growth lengths ($L$) were then calculated from different experiments. When the squares of these lengths ($L^2$) were plotted against time on a logarithmic scale, it was observed that the models proposed by Mishima and Yoshiyama ($k = 0.21 \mu m^{-1}, v_0 = 13.5 \mu m/\text{s}$) [3] and Buchanan et al. ($k_m = 10.4 \mu m/\text{s}^{1/2}$) [4] based on diffusional concepts could not explain the behavior over all time ranges. As seen in Fig. 2, in initial seconds, $L^2$ increased with time $t$ in a nonlinear manner, indicating that initial seconds cannot be expressed with the diffusional equation. Similarly in an extended time range (after 100 s), the rate of increase of $L^2$ with time $t$ reduced, indicating that a deviation from the line represents a diffusional process. The following observations clearly indicated the need for a model that took into consideration the differences in the rate of increase of $L^2$ with time $t$ over different time ranges.

4. Proposed model

We propose that the myelin growth can be split up into three distinct regimes in different time intervals with curves of different equations fitting the experimental points in the different regimes. These regimes were termed as ballistic, diffusional, and subdiffusional regimes based on the nature of dependence of $L^2$ on $t$.

4.1. Ballistic regime

When a solvent, like water, contacts a surfactant interface and permeates into the bilayer organization (regular organization or stack of bilayer membranes) in the lamellar phase, it gives rise to thermally excited undulations of membranes. Lipowsky and Leibler [5] suggest that for a value of Hamaker constant lower than a critical value, these thermally driven fluctuations overcome the attractive microscopic forces and the separation of membranes (peel off of membranes) from the stack starts. The bending fluctuations induced in the membranes have been visualized in a hat configuration by Helfrich [6] with a spherical cap and a logarithmically falling rim from the hat’s center (Fig. 3). Buchanan [7], interpreting observations of polymer gel swelling in excess solvent, suggested that the permeation flux of solvent, when large enough, induces a stress that influences local order in the lamellar phase. This could be interpreted as a side pressure acting on the membrane rim due to the permeation flux of solvent, thereby producing tubular myelins. The cap

![Fig. 2. Plot of square of average myelin growth against time in a time range of 1–700 s at room temperature on a logarithmic scale. The deviations in Regimes 1 and 3, though on a logarithmic scale seem to be small, are actually of a large magnitude.](image)

![Fig. 3. Schematic of evolution of myelin from membrane fluctuations. U stands for region of unbinding transition while C denotes region for period without collisions.](image)
in the hat configuration forming the tip of myelin performs a random walk as the membrane peels away from the stack.

For a plot of the square of average myelin growth length against time for initial seconds, we expect the average myelin growth length to be quadratic in time. This observation is in line with the observed short time solution of the Langevin equation [8] that for a particle performing Brownian motion, i.e., the mean squared displacement of a particle performing Brownian motion at short times is quadratic in time. Mathematically expressed as

\[ \langle x^2 \rangle = \frac{3kT}{m} t^2. \]  

Conventionally this regime is called the ballistic regime, since it corresponds to ballistic motion (motion without collisions) for a particle with velocity equal to \((3kT/m)^{1/2}\). In this case, the analysis has been extended to a myelin tip moving away from its root. With side pressure acting on the rim of the membrane and the continuous stacking of membranes near the interface, the myelin tips have a tendency to move swiftly away from the interface without swaying and colliding with the neighboring myelins, a situation corresponding to the ballistic regime for particles observing Brownian motion. The random membrane fluctuations, which stabilize and take concrete cylindrical shapes, are the ones that grow into myelins. Favoring these, the fluid drag reduces as the speed of solvent permeation reduces [4]. As this happens, the myelin tubes explore angular freedoms of small magnitudes (small enough to neglect for analysis of radial diffusion), which result in a few collisions with neighboring myelins and therefore the ballistic regime shifts to diffusional regime.

4.2. Diffusional regime

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

\[ L^2 \approx 2Dt, \]  

where \(D\) is the relevant diffusion coefficient. Thus one expects the square of average myelin growth length to be linear with time in a diffusional regime, which will follow the ballistic regime.

4.3. Subdiffusional regime

Experiments have revealed that myelin growth slows down in an extended time range (above 100 s). Also in this time range, fitting a line of diffusion equation \((L^2 \approx 2Dt)\) to experimental data in this regime gives a negative time intercept, which is not expected in a diffusion equation. In an extended time range, it has been observed that myelins do not stick to radial motion only but also exhibit motion like coiling and curling. This could be thought of as myelins using an added dimension for motion: angular dimension.

In analysis with spherical coordinates, angular coordinates along with radial coordinates would need to be considered. In this time range, for fundamental analysis of the random motion exhibited by a myelin, Random Walk Analysis can be considered with Central Limit Theorem [9,10], that

"As the number of steps become large, the Central Limit Theorem states that the probability density function of particle displacement becomes Gaussian."

For a simplified analysis of myelin growth, we consider a 2-dimensional random walk (the third dimension, height of cell, can be considered to be constant and would not be available for movement as we flatten out surfactant drop with a coverslip) with constraints in one dimension. Thus the independent variables are distance of myelin front from interface \(L\) and angle \(\theta\) subtended at the center of drop. Myelin front is essentially where most of the myelin tips are collectively present or where most of the coiling takes place (it has been observed that myelins show superficial coiling, near the myelin front). For 2-dimensional random walk in a cartesian coordinate system the representation is

\[ P(x, y) \, dx \, dy = \left(2\pi l^2 n^2 \right)^{-2/2} \exp \left(-\frac{2L^2}{2nl^2}\right) \, dx \, dy, \]

here \(L^2 = x^2 + y^2 + z^2\), \(n\) is number of steps, and \(l\) is infinitesimally small step size.

To find the probability of finding the free end of a single myelin at distance \(L\) from its point of origin, consider the movement of myelin to be restricted to angle \(\theta\) subtended at the center of the surfactant drop (Fig. 4a).

For representation in a spherical coordinate system

\[ dx \, dy \rightarrow \theta(R + L) \, dL, \]

\[ P(L) \, dL = \theta(R + L) \left(2\pi l^2 n^2 \right)^{-2/2} \exp \left(-\frac{L^2}{nl^2}\right) \, dL, \]

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![Fig. 4. (a) Schematic of angular freedom exploited by a myelin in an extended time range for a circular interface. \(\theta\) indicates angular freedom enjoyed by the myelin. (b) Schematic of angular freedom exploited by myelins in an extended time range for a flat interface. Here \(\theta_1, \theta_2, \) and \(\theta_3\) indicate individual angular freedoms enjoyed by the myelins.](image)
where \( R \) is the radius of a surfactant drop (distance of origin from center of drop).

The average end-to-end distance of the myelin tip from the origin can be given as

\[
\bar{L}^2 = \int_0^\infty L^2 P(L) \, dL. \tag{7}
\]

Substituting (6) in (7) gives

\[
\bar{L}^2 = \int_0^\infty \theta (R + L)(\pi nl^2)^{-1} L^2 \exp\left(-\frac{L^2}{nl^2}\right) \, dL. \tag{8}
\]

Using the following identities

\[
\int_0^\infty x^n \exp(-ax^2) \, dx = \frac{1}{4a} \sqrt{\frac{\pi}{a}} \rightarrow n = 2,
\]

\[
\int_0^\infty x^n \exp(-ax^2) \, dx = \frac{1}{2a^2} \rightarrow n = 3.
\]

This gives

\[
\bar{L}^2 = \frac{\theta R}{4\sqrt{\pi}} \sqrt{nl^2} + \frac{\theta}{2\pi} (nl^2). \tag{9}
\]

Since

\[
nl^2 = \alpha_1 t, \tag{10}
\]

where \( \alpha_1 \) is the curvilinear diffusion coefficient

\[
\bar{L}^2 = \frac{\theta R}{4\sqrt{\pi}} \sqrt{\alpha_1} \sqrt{t} + \frac{\theta \alpha_1}{2\pi} t. \tag{11}
\]

This can be represented as

\[
\bar{L}^2 = \alpha t + \beta \sqrt{t}, \tag{12}
\]

where

\[
\alpha = \frac{\theta \alpha_1}{2\pi}, \tag{13}
\]

\[
\beta = \frac{(\theta R)}{4\sqrt{\pi}} \sqrt{\alpha_1}. \tag{14}
\]

Equation (12) represents the average distance of myelin front from point of origin. \( \alpha \) represents unconstrained curvilinear diffusion coefficient restricted to angle \( \theta \), while \( \beta \) represents the angular freedom available to a myelin. \( \beta \) is in joint proportion with \( \theta \) and \( R \) such that as \( R \rightarrow \infty, \theta \rightarrow 0 \).

This would appear to imply that for \( R = \infty \) (flat surface) an alternate analysis might be required. However, the product of \( \theta \) and \( R \) gives the diameter of myelins for closely packed myelins or distance between the central axis of adjacent myelins or spacing between myelins. Thus \( \beta \) for the subdiffusional regime is dependent on myelin spacing and is relatively insensitive to the curvature of the surfactant drop. As a result, the analysis can also be extended to a flat surface where there will be a definite myelin spacing \( S \), corresponding to \((\theta R)\) in Fig. 4a. \( \alpha \) containing \( \theta \) will signify the average angular freedom which each myelin enjoys (Fig. 4b). However for a flat interface, the angular freedom for myelins will vary individually about an average angular freedom and like a circular drop where the angular freedoms add up to 360° rendering an almost constant average angular freedom, the average angular freedom in this case might be variable. Buchanan et al. [11] also suggested the presence of a subdiffusional regime which sets in a few minutes after contact.

The analysis presented above is when a single myelin is considered. However, the analysis can be extended to a network of myelins, which are adjacent to each other along the circumference. \( \theta \) is the average angular freedom available to each myelin. The angular freedom exploited by a myelin can fluctuate about the average value. In other words, if a myelin exploits an angular freedom larger/lesser than \( \theta \), the angular freedom of the adjacent myelins will be affected. However, the entire network fills up the 360° space (2-dimensional). In other words, the summation of angular freedom will add up to almost 360°. A myelin will choose to exploit its angular freedom to satisfy its coiling tendency (tendency of each myelin to decrease the surface area in contact with surrounding fluid) or when it experiences resistance in fluid during growth (including fluctuations due to thermal kicks by fluid molecules or even drag due to fluid flow).

5. Results and discussions

The observation for first 6 s, as shown in Fig. 5a, was that the square of average myelin growth length was quadratic in time \((L^2 = 4.8742t^2 + 66.911t - 6.9695)\), this being in conjunction with the proposed model of an initial ballistic regime. From the quadratic equation and using Eq. (3), it can be calculated that ballistic motion will correspond to a velocity of 2.207 \( \mu \)m/s. The diffusion constant given by \((L^2/\tau)\) is 66.911 \( \mu\)m²/s. The negative constant in the expression accounts for the fact that the ballistic regime sets in after an initial time lag during which membranes are fluctuated by the water flowing into the membrane layers. If \( L \) is plotted \( v/s \ t \), behavior for less than 1 s is nonlinear (Fig. 5b). However from 1 to 6 s, \( L \) increases with \( t \) in a linear fashion adhering to Eq. (3) \((L = 3.1318t + 5.6357)\). The constant of increase is very near in value to the ballistic constant given by the previous quadratic equation.

From 6 to 80 s (Fig. 6), we found that the square of average myelin growth length was linear in time, indicating diffusional growth \((L^2 = 108.14 - 13.641)\). The slope of the best fit line and Eq. (4) gave the value of a conventional diffusion coefficient as 54.07 \( \mu\)m²/s. The negative \( y \) intercept in the best fit line equation accounts for the fact that the diffusional regime sets in after an initial time lag of 6 s in which the ballistic regime is active. To compare with the ballistic regime, the parameter under consideration is the diffusion constant, which for the diffusional regime, for this case, has been found to be 108.14 \( \mu\)m²/s, a clear indication that the diffusional velocity of myelins has increased. Diffu-
Fig. 5. (a) Plot of square of average myelin growth against time in time range of 0–6 s at room temperature. Vertical bars are the standard deviation in the results. (b) Plot of average myelin growth against time in the time range of 0–6 s at room temperature. Vertical bars are the standard deviation in the results.

Fig. 6. Plot of square of average myelin growth against time in time range of 7–80 s at room temperature. Vertical bars are the standard deviation in the results.

The diffusion constant is the constant of dependence of $L^2$ on $t$ and is found by knowing the slope of best fit line to the experimental data in the diffusional regime. This appears to be because of a directed flow of surfactant molecules into the solvent in form of cylindrical myelins in the diffusional regime, in contrast to the random fluctuating motion of the molecules in the ballistic motion.

The plot of experimental points for time range greater than 80 s shown in Fig. 7 clearly shows the deviation of experimental points from the line of diffusion equation, applicable to a time range of 6–80 s, after about 100 s. When a curve of the form of Eq. (12) was fitted to the experimental data points, we obtained a restricted curvilinear diffusion coefficient or diffusion constant $\alpha$ as 63.67 $\mu$m$^2$/s and $\beta$ as 381.44 $\mu$m$^2/s^{1/2}$ ($L^2 = 63.67t + 381.44\sqrt{t}$). The diffusion constant has noticeably dropped in this regime as the myelins explore directions other than strictly radial.

These gave values of curvilinear diffusion coefficient $\alpha_1$ as $350.128 \times 10^3$ $\mu$m$^2$/s and angular freedom $\theta$ as 0.00114$^\circ$. The average diameter of a myelin could then be calculated as 4.57 $\mu$m (considering myelins to be closely packed cylinders). Experimentally the diameters of myelins were found to be in the size range of 3–6 $\mu$m.

The analysis involved restricting motion in one dimension (height of cell). Under these conditions, the myelin growth was represented by Eq. (12) in the subdiffusional regime. The behavior would change if the cell also allows vertical motion. To ensure 2D analysis, the surfactant lump was pressed with the coverslip to produce a thin surfactant slice. The resulting myelins were 3–6 $\mu$m in diameter while previously myelins of 20 $\mu$m have been reported by Saku-rai et al. [2]. This clearly shows that myelin formation and growth adapt to the available environment such as height of cell.

6. Conclusions

The swelling and dissolution process of lamellar surfactant phase is characterized by interesting nonlinear dynamics. We have shown that the myelin growth process undergoes characteristic changes with the advent in time which is reflected in the speed of dissolution of the surfactant aggregates and earlier models are unable to model this speed of dissolution over all time ranges. In the proposed model, the growth process was observed to undergo transition through three regimes: ballistic, diffusional, and subdiffusional based on the nature of the dependence of square of average myelin growth length on time. The model predictions found perfect fit with the experimental points (Fig. 8). For comparison between the different regimes, the parameter “diffusion constant” was monitored and it was concluded that myelin growth speeds up as it undergoes transition from the ballistic regime to the diffusional regime and slows down as the subdiffusional regime sets in.
Fig. 8. Plot of square of average myelin growth against time in time range of 1–700 s at room temperature on a logarithmic scale with inclusion of proposed model predictions. The deviations of earlier models in Regimes 1 and 3, though on a logarithmic scale seem to be small, are actually of a large magnitude. The proposed model line, being superimposed on the experimental data because of the perfect fit, may not be visibly distinguished.

References