Myelin Figures Formation from Oil Droplets in Surfactants: Insights for Wet Artificial Life

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Abstract

Self-organizing systems, such as aqueous surfactant solutions forming vesicles and other microscopic structures, provide insights into complex biological architectures. Myelin figures, formed by the self-assembly of amphiphilic molecules around oil droplets, resemble structures found in biological systems, specifically myelin sheaths around nerve fibers. This study investigates the formation of myelin figures in a decanol-sodium decanoate system using polarized optical microscopy. The system's simplicity allows for the exploration of emergent behaviour, akin to biological processes. By varying parameters such as pH and salt concentration, the morphology and growth of myelin figures and anisotropy evolution are examined. Understanding the mechanisms driving myelin figure formation provides fundamental knowledge applicable to wet artificial life research. Insights gained from this study contribute to our understanding of self-organization and emergent behaviour in complex systems, offering potential applications in drug delivery, materials science, and biomedical research. Furthermore, the study sheds light on the parallels between artificial systems and biological processes, enhancing our understanding of both. Through interdisciplinary approaches, such as those employed in this study, we can uncover the intricate interplay between chemical systems and biological phenomena, paving the way for innovative solutions in artificial life research and beyond.

Introduction

In wet artificial life, researchers often work with simple chemical systems that mimic some aspects of biological processes at the cellular level. Myelin figures are one such phenomenon that can be studied. These structures are formed when certain surfactant molecules self-assemble in water, typically in response to changes in environmental conditions such as pH, temperature, or the presence of other molecules. The term "myelin figures" is borrowed from neuroscience, where myelin is a fatty substance that forms a sheath around nerve fibres, allowing for faster transmission of nerve impulses (Boullerne, 2016). In the context of wet artificial life, however, myelin figures refer to visually striking patterns or structures that emerge from the self-assembly of molecules.

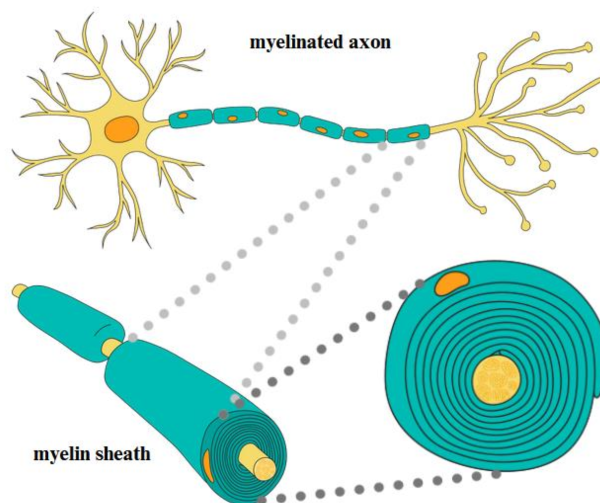

Figure 1: Scheme of neuron with the characteristic segments of myelinated axon.

Myelin figures were first observed by Rudolf Virchow in 1854 during his chemical experiments, which involved bringing lipid extracts from various organs into contact with water, from which myelin figures began to form (Stoeckenius, 1959). Based on the similarity of myelin figures to axon enveloping myelin, efforts are being made in a variety of systems to achieve the formation of myelin figures and to study them in detail, from which potential applications could emerge. These chemical systems are most composed of phospholipids. Among the simplest experiments in which myelin figures have been observed are experiments with egg yolk lecithin-water system (Sakurai et al., 1990). Lecithin is abundant in egg yolk and has also been extracted from brain, blood and various organic substances and is a component of cell membranes. Lecithin contains oils, free fatty acids, and moisture and forms myelin figures on contact with water (Figure 2A). Myelin figures formed from bilayers separated by water can break down into liposomes, which are vesicles with a single lipid bilayer (Kirk & Othmer, 1978).

Other commonly used systems capable of forming myelin figures are similar in chemical structure to lecithin as they are also composed of phospholipids. Experiments are typically

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conducted ranging from variously acidic stock solutions to strongly basic ones to study at which environment myelin figures grow more in each system and whether any differences are observable. For example, acidic solutions are treated with hydrochloric acid and basic solutions with sodium chloride (Panahi et al., 2020). Such systems are synthetically prepared as opposed to simple experiments with egg-yolk lecithin. Such phospholipid-aqueous systems contain, e.g. a mixture of dimyristoyl-sn-glycero-3-phosphocholine (DMPC), dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), distearoyl-sn-glycero-3-phosphocholine (DSPC), dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) and dimyristoyl-sn-glycero-3-phosphatidic acid (DMPA) dispersed in aqueous solution (Mishima et al., 1984), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC, Figure 2B) or di-myristol phosphaditylcholine (DMPC) in aqueous solution (Sakurai et al., 1985; Zou & Nagel, 2006), poorly water soluble surfactants as phosphatidylcholine (PC) or Aerosol-OT in contact with water (Dave et al., 2003) and many others. Such an arrangement of surfactant or lipid with the aqueous phase is generally sufficient for the formation of myelin figures. Depending on the experimental arrangement, for example, polymers such as gelatine or collagen can be added to the surfactant portion of the system to observe their effect on the response and growth of myelin figures.

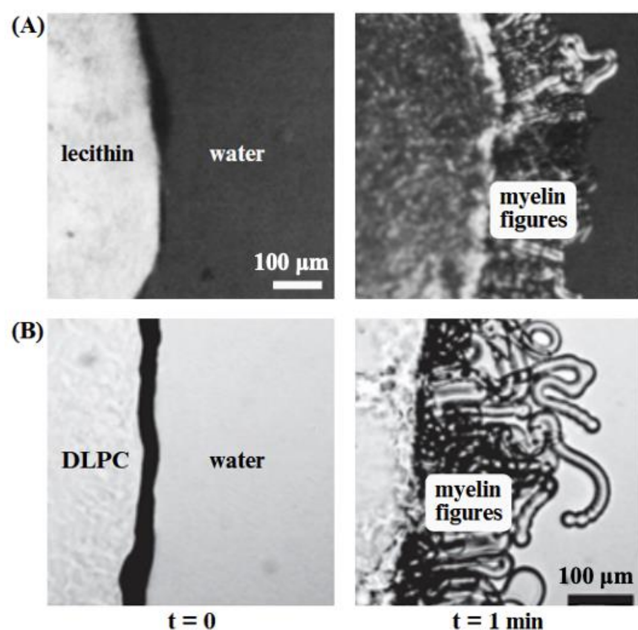

Figure 2: Examples of myelin figures. (A) Egg yolk lecithin-water (Sakurai et al., 1985) and (B) DLPC-water (Zou & Nagel, 2006) systems just after contact with waater (left), and myelin figures formed 1 min later (right).

In present work, we focus on myelin figures formation when decanol droplets are dispersed in a sodium decanoate solution. This system has been previously studied by others, who focused, for instance, on artificial chemotaxis (Čejková et al., 2014; Holler et al., 2018) or collective behaviour of droplet swarms (Čejková et al., 2019). While these phenomena were observable to the naked eye, this work focuses on the microscopic structures of myelin figures. Although the chemical composition of decanol-sodium decanoate systems bears no resemblance to that of living systems, the formation of myelin figures is still relevant to understanding biological membranes.

For our experiments, a customized polarizing microscope was built to be able to hold almost the entire 1 cm diameter circular cover slip in the field of view. Such setup allows us to study the evolution of anisotropy and myelin figures growth in larger scale than by using a conventional microscope. Anisotropy refers to the property of being directionally dependent, meaning that certain properties can vary depending on the direction. In the context of myelin figures and living systems, anisotropy can indeed be observed and has important implications for the function and behaviour of these systems. In biological systems, myelin sheaths are naturally anisotropic structures. The myelin layers wrap around nerve fibers (axons) in a specific direction, forming a cylindrical structure with distinct properties along the axis of the nerve fiber compared to perpendicular directions. This anisotropic structure is critical for the efficient propagation of nerve impulses along axons. Myelin figures can also exhibit anisotropic properties.

Studying anisotropy of myelin figures and similar phenomena in wet artificial life can provide insights into the principles underlying self-organization and emergent behaviour in complex systems. Researchers can use these insights to design and manipulate artificial systems for various applications, such as drug delivery, materials science, and nanotechnology. Furthermore, understanding anisotropy and the processes involved in myelin figure formation can contribute to research on neurological disorders, such as multiple sclerosis, which is associated with damage to myelin sheaths.

Experimental

For the experiments, various chemicals and devices were employed. Decanoic acid, sodium hydroxide, sodium decanoate, 1-decanol, Sudan Black B and Oil Red O were acquired from Sigma-Aldrich. Additionally, sodium bromide, sodium chloride, sodium iodide and potassium chloride were sourced from Lach-Ner. Water purification was conducted using a Millipore Milli-Q system.

Three types of solutions were prepared: acidic, basic and neutral sodium decanoate solutions. Basic solutions, ranging in concentrations from 5mM to 80mM, were obtained by dissolving decanoic acid in demineralized water and adjusting the pH to 11-12 using 5M sodium hydroxide solution. Neutral solutions of identical concentrations were prepared by dissolving sodium decanoate in demineralized water at pH 7. Acidic solutions were prepared by adding hydrochloric acid to neutral solutions of sodium decanoate, adjusting the pH to approximately 2. Decanol was used either without staining or stained with Oil Red O or Sudan Black B.

Devices utilized in the experiments included a Nikon Polarizing Microscope ECLIPSE E400 POL, and a customized polarizing microscope – attached macro-optics Navitar 12X

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Zoom Lens System with a heat plate to the stand. Glass slides sized 26 × 76 mm from Menzel-Gläser, 1 cm diameter circular cover slips from P-LAB.

Experiments were performed as follows. A 1cm circular cover slip was coated with 76 µl of aqueous phase containing sodium decanoate and salt. Subsequently, 0.72 µl of a small droplet of decanol was added. Experiments involved varying parameters such as the pH of the sodium decanoate (acidic, basic or neutral), concentration of sodium decanoate (ranging from 0 to 80mM), type of salt (NaBr, NaCl, NaI, KCl), and its concentration (ranging from 0 to 100mM). The volumes of aqueous solutions and decanol remained constant across all experiments. The slip was left to spontaneously evaporate in a quiet place on a heat plate maintaining a constant laboratory temperature (22.5 °C).

Results and Discussion

In this study, we investigate the system of decanol in an aqueous solution of sodium decanoate under spontaneous evaporation at steady-state laboratory temperature using polarized optical microscopy. This technique is valuable because it allows us to study the anisotropy and visualize molecular structures and orientations within the system.

By employing polarized optical microscopy, we can observe that when a small droplet of decanol is placed on water alone, no anisotropy is detected. This absence of anisotropy can be explained by the isotropic nature of the decanol liquid, where no preferential orientation or alignment of molecules is present due to the absence of specific organizing forces or interactions. The same is observed when placing a droplet of decanol on a salt solution. There is no reason for the molecules to self-organize and form an anisotropic phase in this case.

However, when we substitute pure water with a basic or neutral 5mM aqueous solution of sodium decanoate, interestingly, we observe anisotropy at the interface between the aqueous phase and the decanol. Decanol, a nearly insoluble long-chain fatty alcohol, has a hydrophilic hydroxyl group and a hydrophobic hydrocarbon tail that interacts favorably with surfactant molecules. Sodium decanoate has a hydrophilic carboxylate head and a hydrophobic tail (Figure 3A).

Due to their amphiphilic nature, surfactant molecules can adsorb at interfaces between polar and non-polar substances, reduce interfacial tension and self-organize into aggregates in bulk media due to the hydrophobic effect. In our experiments, sodium decanoate molecules spontaneously assemble on the surface of the aqueous phase with hydrophobic tails facing outward. The addition of decanol leads to a spontaneous rearrangement of molecules due to the affinity for polar and non-polar environments, and decanol molecules were only slightly present in the aqueous phase. The sodium decanoate molecules arrange themselves at the decanol-water interface with hydrophobic tails toward decanol and hydrophilic heads toward water (Figure 3B and Figure 3C).

As water evaporates, further rearrangement of molecules occurs, forming micelles, vesicles, and myelin structures. Micelles are circular formations with hydrophobic tails facing inward and hydrophilic heads facing outward. No anisotropy was present at CMC of 20 mM decanol and sodium decanoate. When the concentration was further increased due to water evaporation, more complex structures and anisotropic phases appeared, forming hexagonal or lamellar phases and myelinated figures (Figure 3D).

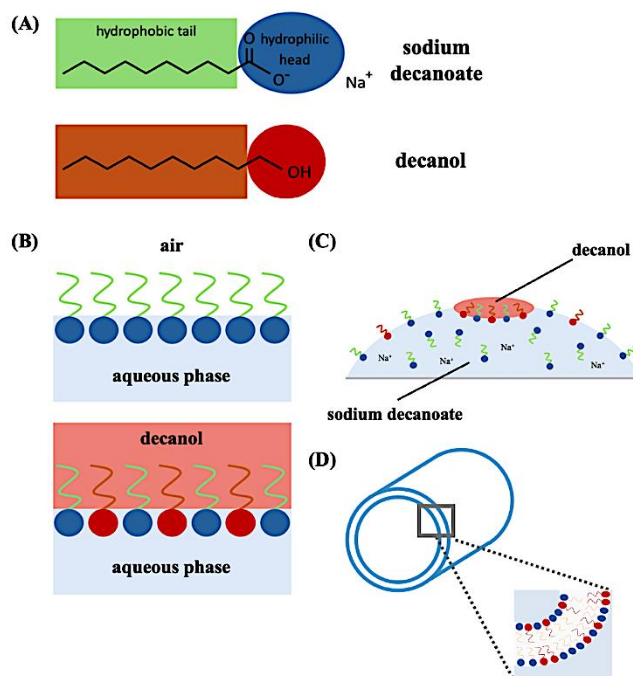

Figure 3: (A) Chemical formulas of sodium decanoate and decanol. (B) Schematic arrangement of sodium decanoate molecules at the air/water interface and decanol and sodium decanoate molecules at the decanol/water interface. (C) Side view of schematic arrangement of decanol-sodium decanoate system on a circular cover slip. (D) Scheme of cylindrical myelin figure and cross-sectional arrangement of decanol and decanoate molecules into the double layer separating the surrounding and inner aqueous phases.

Interestingly, for the strongly acidic environment of aqueous sodium decanoate solution, this behaviour is not observed and the decanol droplet does not exhibit anisotropy or any morphological changes as in pure aqueous and salt solutions. For basic and neutral solutions, the time of anisotropy and droplet behaviour varies for each chemical arrangement of the system depending on the given concentration and pH. Far from equilibrium, molecules organize themselves into stable configurations based on their chemical arrangement. However, during evaporation, local changes in concentration or pH, controlled diffusion of surfactants and osmotic stress lead to rearrangement of molecules, which affects the properties of the decanol. As the aqueous phase evaporates, the concentration of sodium decanoate on the cover slip increases. Over time, the decanol droplets form into different shapes depending on the concentration and environmental factors. In some cases, the molecules arrange themselves into myelin figures, liquid

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crystalline metastable phases characterized by long-range periodicity and anisotropic behaviour.

Further the experiments for both neutral and basic pH were performed with the aim of finding the lowest concentration of sodium decanoate, when the molecules are arranged in the system in such a way that anisotropy occurs. For concentrations between 0 – 5mM, several experiments were made, and it has been found that for the basic environment of sodium decanoate, the system was shown to be capable of anisotropic behaviour in the smallest possible concentration of decanoate in the range of 0.25 to 0.5mM. In a neutral environment, the system can rearrange the molecules to such properties at much lower concentrations. The limit where the system is capable of anisotropy is around 0.001mM.

The formation of anisotropic phases is not immediate upon contact of decanol with sodium decanoate but takes some time depending on experimental conditions (particularly the concentration of decanoate, pH, and presence or absence of salt). The droplet does not remain spherical but typically changes shape (forming irregular or finger-like structures), which are observable to the naked eye. Additionally, at a microscopic level, further anisotropic structures are formed, with a particular focus in this study on myelin figures.

A large number of experiments were conducted in this study where the concentration of decanoate was varied (from 0 to 80 mM), and the presence or absence of salt, as well as the type and concentration of the salt, were changed. Video records of most of the experiments with the sodium decanol-decanoate system presented in this work are available on the website of laboratory: https://droplets.vscht.cz/people/novakova. Further, we will introduce the results of experiments involving NaCl in a decanoate solution as an example.

The time course of events on the coverslip for 10mM sodium decanoate in the presence of 1mM sodium chloride is shown in Figure 4. We have developed a custom polarized darkfield microscope in our laboratory, which enables high-resolution imaging across the entire field of view, including details on a 1 cm diameter cover slip. This microscope's unique capabilities enhance contrast and visibility, particularly for observing anisotropic structures like myelin figures. This technology is invaluable for our research, allowing precise characterization and analysis of anisotropic structures at a microscopic level. Figure 4A represents the results from this microscope aiming to highlight the anisotropic phase. At the beginning of the experiment, nothing is visible in the field of view because not enough time has passed for the molecules to rearrange themselves into double layers; within 15 minutes, a continuous anisotropic layer has already formed around the decanol. Although it does not appear in the figure, the layer is indeed continuous, with different positions of the polarizer and polarizing filter forming a cross in the image to represent the positions of the quenching and the microscope configuration. Dramatic changes in shape of a decanol droplet occur in the following minutes, where deposits of myelin figures can be observed as early as minute 30. Myelin figures most frequently grow at the sites of energy maxima formed by the tearing of the decanol. At such sites, the molecules theoretically self-assemble to equilibrate the energy and thus form the deposits for the myelin figures. These begin to grow over the next few minutes, and around the 80th minute they retract back into the decanol, which tries to return to its original stable position. Around the 95th minute, the substrate dries up completely.

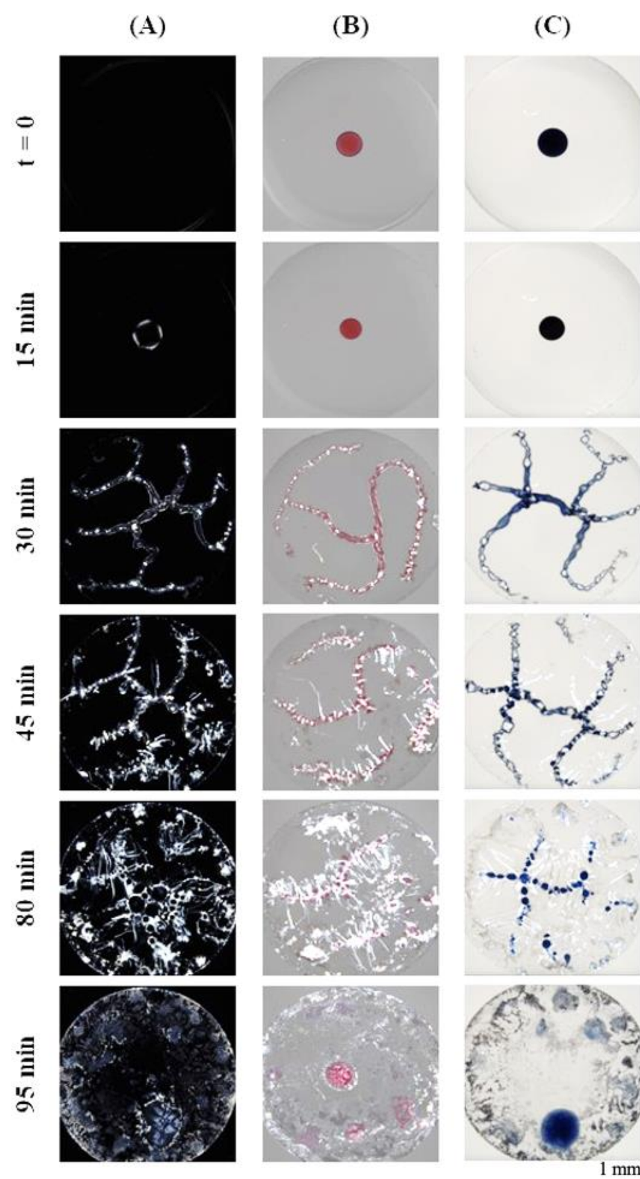

Figure 4: The progression of the experiment over time. Initial conditions: decanol droplet placed over an aqueous solution of 10mM sodium decanoate and 1mM NaCl at basic pH. (A) Uncoloured decanol, (B) decanol coloured by Oil Red O, (C) decanol coloured by Sudan Black B. (A) differs from (B) and (C) because a different rotation of the polarization filter was used.

We conducted experiments using both pure decanol and decanol stained with Oil Red O and Sudan Black B. The results showed that the added dye had no influence on the course of the experiments; in all cases, similar morphological changes in the decanol droplets occurred, and myelin figures developed to a similar extent. This is demonstrated in Figure 4 by (B), where the Oil Red O dye was used, and (C), where Sudan Black B was employed.

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In previous studies, it was found that the use of dye in droplets can significantly influence the behaviour of the entire system. For example, adding Sudan Black B to droplets dispersed in an aqueous phase of sodium dodecyl sulphate resulted in different behaviour compared to droplets stained by Oil Red O (Löffler, 2021). Other study has shown how droplets of paraffin can perform self-motion when powered by camphor and how such a motion can be affected by varying concentrations of the dye Oil Red O (Löffler et al., 2018). However, in this study, we did not observe a similar effect or alteration of system behaviour influenced by the dye. In our experiments, for the use of our custom darkfield polarizing microscope, we typically examined droplets without dye (as shown in Figure 4A). However, in other experiments, we employed Oil Red O for improved visualization (as shown in Figure 4B) to enhance contrast and facilitate clearer observations.

Myelin figures growing from uncoloured decanol droplets can appear differently coloured under darkfield polarized light due to varying thicknesses of the surfactant layers within the structure. This thickness variation affects the interference and scattering of light passing through, resulting in colour variations observed under polarized optical microscopy. From Figure 5 it is obvious that some myelin figures have thicker double layers as they light up in different colours. Therefore, it is possible that not all myelin figures have the same internal morphology.

Figure 5: Examples of myelin figures observed by polarized optical microscopy. Initial conditions: decanol droplet placed over an aqueous solution of 10mM sodium decanoate and 1mM NaCl at basic pH.

For the myelin figures in the decanol-sodium decanoate system, even though no lipid was present, it was verified by polarized optical microscopy that these are indeed cylindrical structures that typically grow in complex chemical lipid systems. Such verification is done by illuminating the figures in all rotational positions of the polarizer or polarizing filters. In our system setup, short tubes with lengths of tens of micrometers to tubes reaching lengths of several millimeters grow over a large part of the cover slip on which the experiment is performed. Similarly, there are various thicknesses in the system, from a few units to tens of micrometers. Myelin figures of different shapes and sizes are observed at different concentrations of both sodium decanoate and added salt. Both tubes and helices grow in this arrangement. The shapes of figures twist differently during the experiment outside of their growth in length, which is caused by local pressure changes, as they are water-core structures. Figure 6 shows the example of helices which were observed in the system where KCl was added.

Figure 6: Examples of helical myelin figures observed by optical microscopy. Initial conditions: decanol droplet placed over an aqueous solution of 10mM sodium decanoate and 1mM KCl at basic pH.

Further, image analysis of darkfield polarizing microscopy images was used to assess the presence of anisotropic phases in systems and track their evolution over time. The principle involves using images from darkfield polarized optical microscopy to visualize anisotropic structures exhibiting a polarization effect (appearing bright) compared to surrounding non-anisotropic structures (appearing dark) in the images. First, we identify the areas of anisotropic phase, and next, we quantify the total area of anisotropy. This analysis is repeated over time to monitor changes in the area of anisotropic regions. The results of this analysis can be presented using graphs depicting the dependence of the anisotropic area on time. This approach allows the quantification and visualization of the dynamic evolution of the anisotropic phase in our experimental systems.

As previously mentioned, a parametric study was conducted involving a comprehensive series of experiments, and this analysis was performed across all experiments. In this full text, Figure 7 serves as an example to illustrate the results obtained from experiments involving decanol in basic and neutral sodium decanoate at varying concentrations of decanoate.

The course of the experiment can be divided into the following stages: *(i)* lag phase, where no anisotropy is observed and the anisotropic area is almost zero, *(ii)* initiation phase,

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when the decanoate molecules begin to self-assemble around a droplet of decanol and thus the area lights up as the anisotropic phase is created, *(iii)* log phase, a rapid increase in anisotropic regions and the increase in surface area due to deformations of the decanol droplet, and *(iv)* stationary phase, where neither increase nor decrease of anisotropic phase occurred. In some experiments, namely in which various salts are additionally added to the system, the growth phase of myelin figures is also noticeable in certain graphs, from which their growth can be evaluated. The last phase *(v)* can be referred to as the dead phase, during which there is anisotropic extinction due to the arrangement of amphiphilic molecules in the drying system, accompanied by crystallization also characterized by anisotropy. Not all phases were present in all experiments.

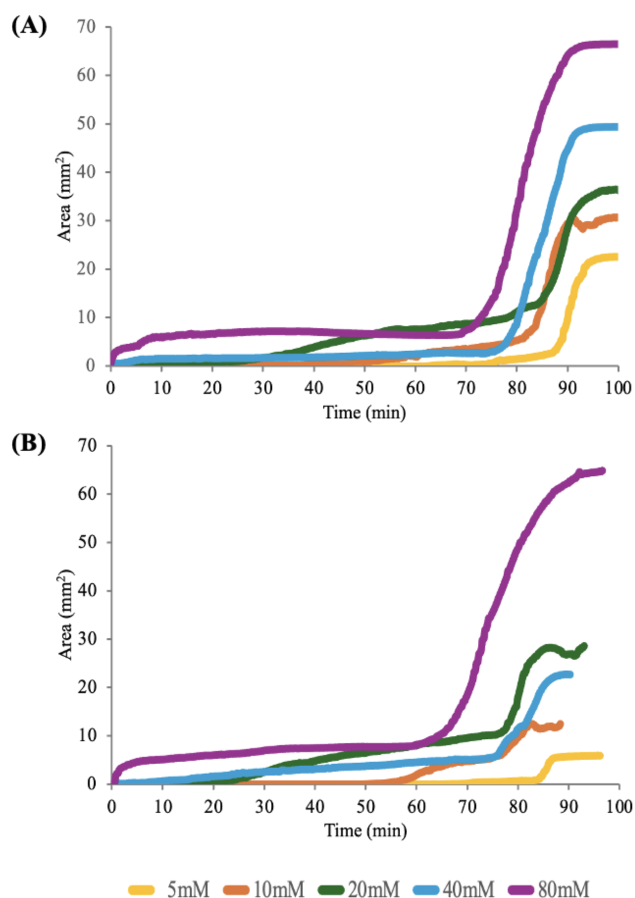

Figure 7: The evolution of the anisotropic phase over time for various concentrations of sodium decanoate (from 5 to 80mM) under (A) basic and (B) neutral pH conditions.

It is evident that the onset of anisotropic regions significantly depends on the initial concentration of sodium decanoate. For the lowest concentrations of decanoate, the system behaves isotropically for over an hour before any increase in anisotropy becomes apparent, whereas for the highest concentration, the increase in the anisotropic phase is immediate and significant. Furthermore, it is apparent how pH influences the system behaviour; under basic conditions, the formation of the anisotropic phase occurs more rapidly than under neutral.

In general, pH and sodium decanoate concentration have a significant effect on the overall behaviour of the system and the reassembly of the molecules into a regular arrangement. Understanding of the self-assembly of amphiphilic molecules in aqueous solutions of sodium decanoate appears to be an essential step in understanding the formation of myelin figures in the system. Although the decanol-sodium decanoate system appears simple at first glance, the molecular rearrangement within it is quite complex. During spontaneous drying, various phenomena and changes occurrs, affecting both molecular assembly and droplet drying. These changes are influenced by the system's chemical composition, including pH, concentration, salt presence, the Marangoni effect, viscosity, and the interaction between decanol and the aqueous environment. The pH level influences ionization, impacting surface tension and viscosity, which are crucial for determining the drying rate. Surfactants generally slow down evaporation, while higher sodium decanoate concentrations increase viscosity, further affecting the drying process.

During the experiments, water is evaporated from the aqueous phase, increasing the concentration of sodium decanoate. After reaching a certain concentration, anisotropic behaviour is observed. By knowing the evaporation time and the moment when the anisotropic regions first appear, it is possible to estimate the concentration at which such rearrangement of the amphiphilic molecules occurs. The average evaporation time was 80 minutes for the basic aqueous phase and 90 minutes for the neutral solution. The evaporation rate was 0.95 µl/min for the basic solution and 0.84 µl/min for the neutral solution. Using the initial number of moles and the evaporation rate, the actual concentration was plotted. Although this simplified model assumes constant evaporation and all decanoate molecules in the aqueous phase, it is sufficient for further estimates.

Based on experimental records and graphical data, the occurrence of anisotropy was evaluated. For sodium decanoate concentrations from 5 to 20mM, anisotropy appears after a certain time. In basic medium, anisotropy appeared in 60 minutes for 5mM solutions, in 40 minutes for 10mM and within 1 minute for 20mM. In the neutral environment, anisotropy appeared in 70 minutes for 5mM, in 50 minutes for 10mM and in 10 minutes for 20mM. The concentration required for anisotropy was calculated using the evaporation rate. Under basic conditions, approximately 20mM is required, while under neutral conditions, approximately 22.5mM is required. This agrees with previous studies that showed a critical micelle concentration of 20mM for sodium decanoate in the presence of decanol. Future studies will determine if 22.5mM is the critical micelle concentration for neutral solution.

Let us summarize what occurs at the molecular level during the experiment. Molecular interactions and arrangements at the interface between decanol and sodium decanoate result in the formation of anisotropic structures. Decanol, being hydrophobic, clusters with other hydrophobic molecules, while sodium decanoate, an amphiphilic molecule,

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positions its hydrophilic head toward the aqueous phase and its hydrophobic tail away from it.

When decanol is introduced into the sodium decanoate environment, the hydrophobic tails of sodium decanoate interact with decanol molecules. This causes sodium decanoate molecules to align at the interface, with hydrophilic heads facing the aqueous phase and hydrophobic tails toward decanol.

As water evaporates from the aqueous phase, molecules further rearrange, leading to the formation of micelles, vesicles, and myelin structures. Micelles are circular aggregates with hydrophobic tails inward and hydrophilic heads outward. Myelin figures, a type of micellar structure, have a cylindrical shape composed of surfactant double layers.

Conclusions

In this work, we demonstrated how polarized optical microscopy can be useful in the study of myelin figures and other anisotropic structures in systems designed to mimic living systems. In this case, polarized opticalmicroscopy revealed crucial insights into the behaviour of decanol in the presence of sodium decanoate in aqueous solutions. It demonstrated how molecular interactions and self-assembly processes at interfaces can result in the formation of anisotropic structures, highlighting the role of surfactants in controlling molecular organization and orientation within such systems.

In the future, insights from studies involving decanol and decanoate may inform research on demyelinating diseases, where the integrity of myelin is compromised, such as multiple sclerosis. While these compounds may not directly translate to therapeutic interventions, understanding the principles of myelin assembly and stability can contribute to the development of strategies for promoting myelin repair and regeneration in neurological disorders.

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