STUDIES ON PARTICULATE GROWTH AND ORGANIZATION IN NON-EQUILIBRIUM MYELIN STRUCTURE

Project Report

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MASTER OF SCIENCE IN CHEMISTRY

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NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

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CERTIFICATE

This is to certify that the topic on "**Studies on particulate growth and organization in non-equilibrium myelin structure** " being carried out by **Miss Shanti Kar** for the degree of M.Sc in chemistry is an outcome of bonafied research work carried in the department of chemistry**, National Institute of Technology, Rourkela.**

Date: Signature of guide

Place: **Dr. Garudadhwaj Hota**

DECLARATION

I, **Shanti Kar,** do hereby declare that research work incorporated in the report entitled "**Studies on particulate growth and organization in non-equilibrium myelin structure"** is an authentic work carried out by me under the supervision of **Dr G .Hota**, Department of Chemistry, **National Institute of Technology Rourkela (NITR), Rourkela**. The present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree regarding to my belief.

Date: **(Ms. Shanti Kar)**

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(Ms. Shanti Kar)

ABSTRACT

The interesting phenomenon of myelin formation is observed when a surfactant lamellar phase is swollen in excess solvent. Myelins are essentially long, thin finger like cylindrical structures that rapidly grow when water is brought into contact with concentrate lamellar phase of sparingly water soluble surfactants. They are well known non-equilibrium microstructures. In the present study we have reported the formation & growth mechanism of myelin structure using anionic surfactant AOT (Aerosol-OT) in aqueous medium. The effect of surfactant concentrations on the formation, growth, shape and stability of myelin structures has also been investigated. Digital video microscopy has been used to investigate the swelling & dissolution dynamics of myelin growth.

Apart from this, attempt has also been made to synthesize ultra-fine particles using these non-equilibrium myelin structures. In this work, we have successfully prepared $Ca_3(PO_4)_2$ particles during the myelin formation by using AOT/water system. The formation of $Ca_3(PO_4)_2$ in these systems lead to organize a chain of particles enforced by water micro flow induced by myelin growth. This new phenomenon is absent in equilibrium systems such as micelles & reverse micelles. It is also observed that increase in particle concentration causes extensive coiling of myelin structure. It is supposed to play an important role in biological systems in the formation of bones during embryonic stages.

Keywords: Myelin, Aerosol-OT, Optical Microscopy, Modeling

INTRODUCTION

In past few years, the formation and other aspects of non-equilibrium structures have been studied (Buchanan, et al., 2000) and it is appropriate that these can be developed as a model system to carry out chemical reactions. The formation of myelin in surfactant system is one example of the non-equilibrium system. Dynamics of surfactant in contact with water shows many instabilities, classical myelin growth being one of them. Virchow in 1854 first observed this myelinic growth in lipids and since then many attempts have been made to provide theoretical explanation for these unusual phenomenon. Myelins are highly viscous, gel-like, microstructures consisting of elongated tubules composed of concentrically stacked multilamellae formed with water and insoluble surfactants.

The formation of particles in non-equilibrium surfactant aggregates such as myelin system is of both scientific and engineering interest. Non-equilibrium systems have the potential to mimic biological systems as well as serving as templates for making nanostructures. Carrying out chemical reactions in equilibrium structures like micelles, microemulsions, and other supramolecular assemblies has been an active area of research. Particular mention should be made of attempts to use these systems as templates for the preparation of nanoparticles to control the size and shape. The swelling and the dissolution dynamics of lamellar phase of nonionic surfactant in water has been qualitatively and quantitatively investigated by Buchanan et al. (2000). They have observed the classical myelinic instabilities at the interface of insoluble lamellar phase, which are absent in soluble case. The authors claimed that water enters at the root, rather than through the wall of the myelin. The close inspection of myelin shows the deep channel of water that penetrates towards the lamellar phase. The myelin growth has been quantitatively measured. In the present work, we have reported the preparation of $Ca_3(PO_4)_2$ particles during myelin formation in AOT/Water system.

RESEARCH OBJECTIVE

- To study the formation and growth myelin structure by using AOT/$CHCl_3$ system.
- To study the growth and stability of myelin by varying the AOT concentration.
- Preparation of AgCl & $Ca_3(PO_4)_2$ particles in myelin structure.

EXPERIMENTAL PROCEDURE

The stock solution of AOT in chloroform was prepared. A drop of this solution was taken on the glass slide. After the evaporation of solvent, a round cover slip was gently pressed onto the dry surfactant droplet & myelin growth was observed when surfactant phase was contacted with a drop of water. Myelin growth kinetics was observed using an optical microscope.

Particle synthesis in myelin structure

For particle synthesis in myelin structure, microemulsions were prepared by mixing the aqueous solution of calcium chloride of different concentrations to the AOT/$CHCl_3$ stock solutions. Water to AOT molar ratio (R) was kept constant and is equal to 10 in all cases. A drop of the above microemulsion containing the $CaCl_2$ solution was placed on the glass slide and the solvents, chloroform and water, were allowed to evaporate leaving a dry droplet of AOT containing $CaCl_2$. A cover slip was gently pressed onto the sample forming a film of the sample of thickness about 50 μm on a glass slide. The surfactant phase was then contacted with water containing Na_3PO_4, by introducing a drop of this solution at the edge of cover slip close to surfactant phases. After contact with aqueous sodium phosphate solution, through capillary action, calcium phosphate particles are formed by precipitation reaction.

RESULT& DISCUSSION

Olympus BX51 microscope fitted with a CCD camera connected to a frame grabber card & personal computer is used in this experiment. The camera is alternatively connected to a video-television set-up for real-time video capture and analysis of the experiments at very short time intervals. All experiments have been carried out at room temperature. Image analysis software has been used for time acquired of images at different time intervals.

Myelin growth kinetics:

There are several literature reported by various group regarding the formation of myelin structure. Myelins are essentially formed by back flow of water and have attributed their growth to dissolution process (Buchanan et al.2000). We have also observed same mechanism in our experiment during the formation of myelin structure using AOT/water system. Fig 1 shows growth of myelin at different time intervals. Fig 1-a represent pure surfactant phase in the absence of water and Fig 1b-d, represents the growth of myelin, after surfactant phase is brought

in contact with water at 1min, 2min and 3 min time interval respectively. It is observed from these images that myelin subsequently grew in to the water phase with increase in time.

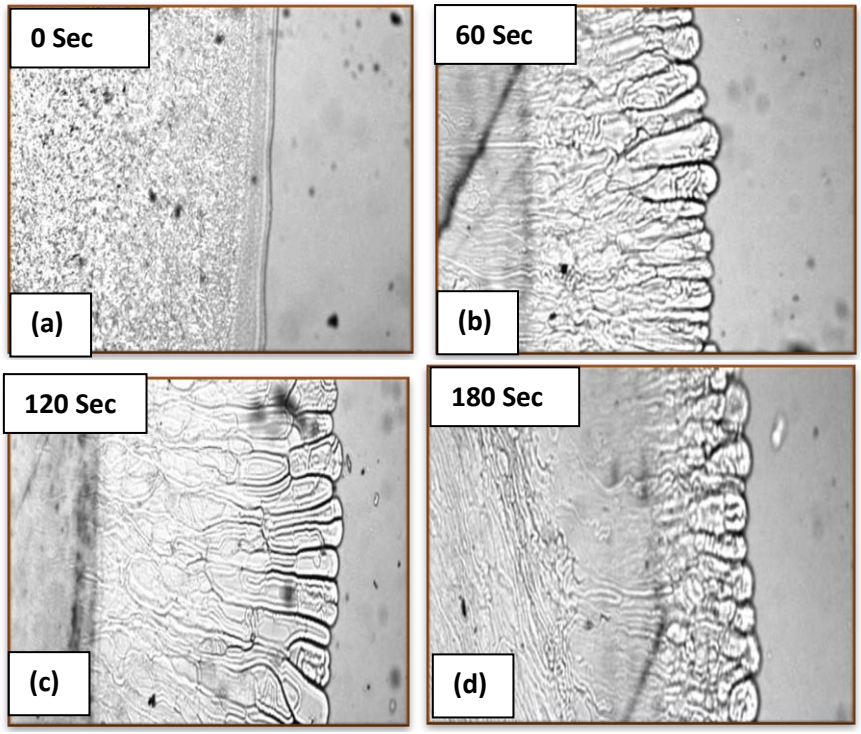

Fig.1: Optical micrograph of myelin growth at different time intervals

The rate of myelin growth was analyzed by measuring the distance between myelin edge & myelin root, *L*, with time after contact of the surfactant phase with water. Several lengths were averaged to get the average growth of the myelin as shown in Figure 2. Myelin growth follows a diffusive law (Fig 2), where the square of average length $< L^2 >$ has been plotted with time. The curve is approximately linear. The linear dependence is not fully understood but this diffusive growth is a characteristic of myelin growth. It is our belief that the myelin growth is controlled by individual surfactant diffusion. However, the exact mechanism is not known but it appears through the formation of budding vesicles, that are distorted due to confinement enforced by the neighboring vesicles and the glass plates and finally these develop into the appearance of myelin.

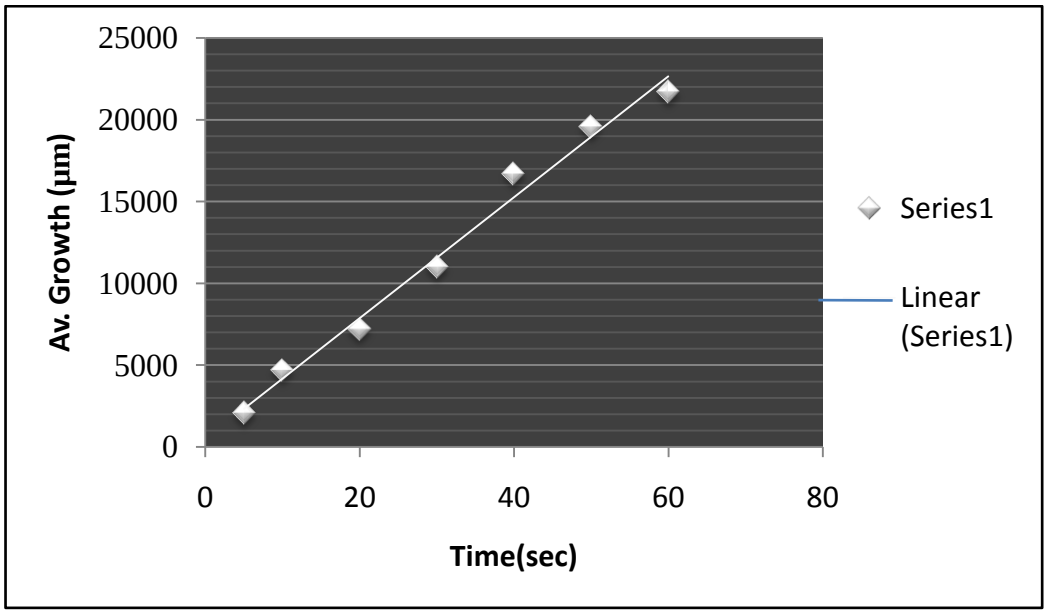

Fig.2: Plot of L^2 vs. t for AOT/Water system

Effect of AOT concentration on myelin growth:

We have also studied here the effect of AOT concentration on the formation, shape and growth of myelin structure. We have varies the AOT concentration 0.1 to 0.6M. Fig 3 represents the growth of myelin at different surfactant concentration.

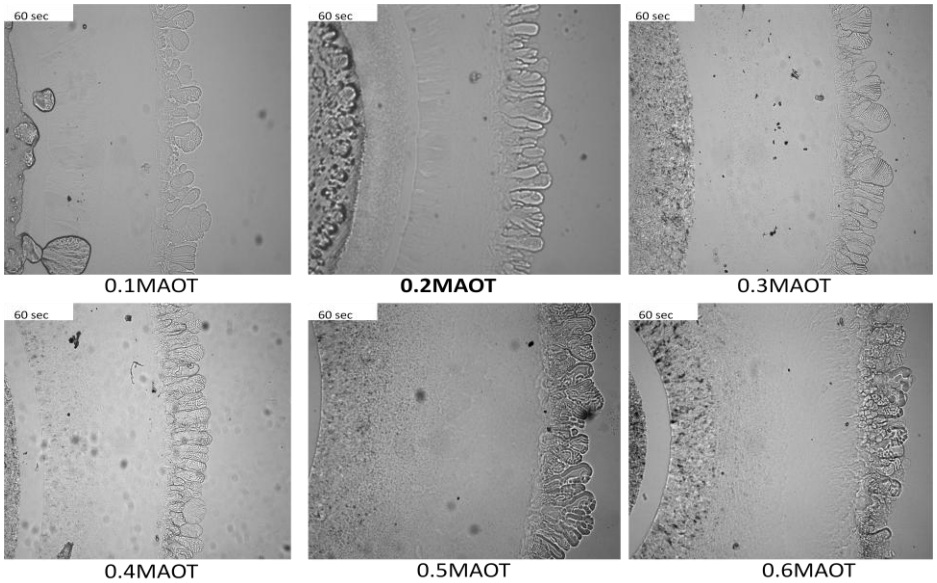

Fig.3: Effect of AOT concentrations on myelin growth

It is observed from the above figure that at low surfactant concentration, myelin formation is not clear, however with increase in AOT concentration prominent myelin structure are observed. It is also observed that change in AOT concentration influenced the rate of myelin growth.

Synthesis of $Ca_3 (PO_4)_2$ Particles in Myelin structure

We have carried out the chemical reactions in surfactant lamellar phase to understand the growth of myelin and the diffusion mechanism of water. When the surfactant phases (AOT) containing reactant $CaCl_2$ were contacted with a drop of water containing Na_3PO_4 (0.1 M) at room temperature, after an initial delay of a few seconds myelin growth was observed and soon the formation of a chain of $Ca_3 (PO_4)_2$ particles was observed at the root of myelin as shown in figure 4. These particles are formed through the precipitation reaction, i.e.

$$\mathbf{3CaCl_2 + 2Na_3PO_4 \rightarrow Ca_3 (PO_4)_2 \downarrow + 6\ NaCl}$$

Fig 4-f represents the formation of $Ca_3 (PO_4)_2$ particles chain with the well grown myelin. After some period the myelin structures vanishes leaving behind a chain of particulate materials.

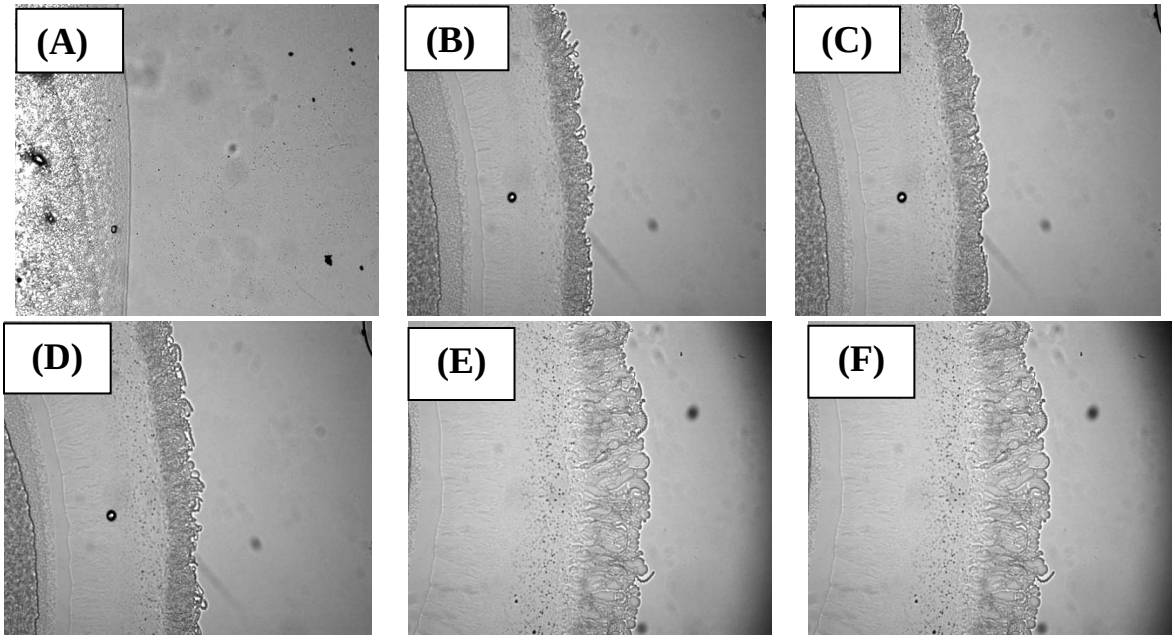

Fig.4 : Micrographs of organized $Ca_3 (PO_4)_2$ particles in myelin structure.

Coiling Structure of Myelin:

We have also observed that with increase in $CaCl_2$ concentration coiling like structure of myelin are formed. It may be because of larger size particles are formed in surfactant lamellar phase that can affect the diffusion process. This interesting observation of coiling structure has also been investigated by Haran et al. 2003 on addition of additive like PTS to the surfactant phase. However more in details study needed to gain insight the mechanism of formation of coiling structure and myelin growth.

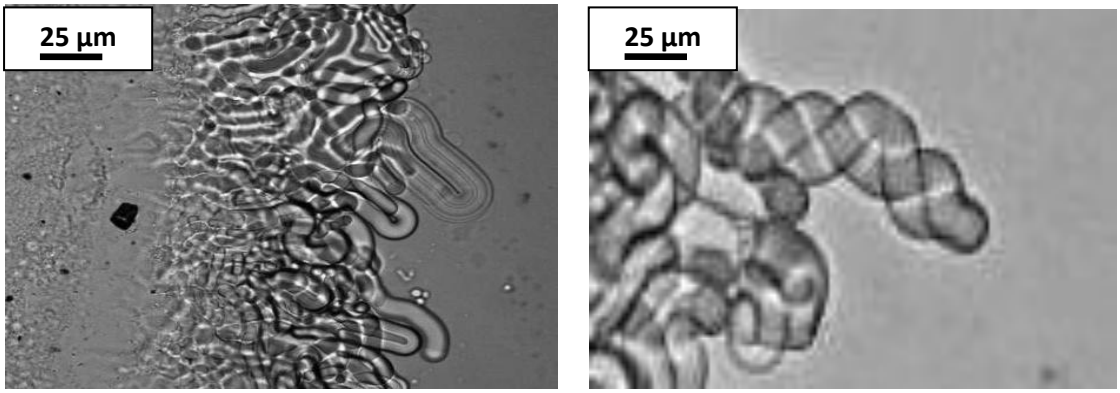

Fig.5: Myelin showing coiling instabilities.

CONCLUSION

Present studies on myelin growth in AOT systems can be summarized as below:

- We have studied the growth of non-equilibrium myelin structure using AOT/$CHCl_3$ system.
- Molecular diffusion of the surfactant from the surface of the lamellar phase plays an important role in the growth of myelin.
- It is observed that the rate of myelin growth and the structure depends on the concentration of surfactant.
- Formation and deposition of Ca_3 $(PO_4)_2$ particles indicates organized water flow on the length scales of several hundred microns.

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