

インターンシップ報告書

Internship Report

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Research project title: Study of the solid–liquid interfacial properties in concentric separation

Hosting research company: RIKEN; Period of stay: 2022/09/05 ~ 2022/10/05

1. Introduction

Although separating substances is entropically unfavorable, it is often essential for life and any activity occurring in our environment. In this regard, a grand challenge is to instantly separate substances with nearly identical structures at a low cost. I have discovered a serendipitous finding that a mixture of molecules if linked to polyethylene glycol (PEG), can be instantly separated into concentric circles, even though their structures are very similar to one another. This intriguing phenomenon occurs in an aqueous medium with a very high $(\text{NH}_4)_2\text{SO}_4$ -to-PEG weight ratio ($\sim 10^6$), where PEG-enriched droplets initially form via primary liquid–liquid phase separation (LLPS), and the resulting aqueous two-phase system subsequently undergoes salt layer-assisted secondary LLPS on a glass plate spontaneously covered with a crystalline layer of ammonium sulfate, to furnish micrometer-scale concentric circles. The terminally distinct PEGs thus separated via double LLPS (DLLPS) can be selectively extracted by elaborate rinsing. Here, due to the limitation of the methods to observe this phenomenon, I utilized the cutting-edge resource from the hosting organization, RIKEN, to study this phenomenon in-depth.

2. Structural analysis of the salt layer at the solid–liquid interface

The apparent orders of PEGs from the periphery to the core of the concentric patterns appear to be perfectly correlated with their individual phase diagrams in aqueous $(\text{NH}_4)_2\text{SO}_4$, where a PEG with a wider two-phase region in the phase diagram has a larger tendency to localize in the core region of the concentric pattern. It is known that a PEG with a higher molecular weight has a wider two-phase region and is therefore more susceptible to LLPS^{1–3}. However, so far, the effects of PEG termini on LLPS have been poorly understood. Next, the spatial distribution of chemical species on a solid surface can be determined using time-of-flight secondary ion mass spectrometry (TOF-SIMS). Accordingly, I evaluated the surface of a glass plate with concentric circles generated by DLLPS of a 1:3 aqueous mixture of $^{\text{Mc}}\text{PEG}_{5\text{K}}^{\text{RhB}}$ and $^{\text{H}}\text{PEG}_{35\text{K}}^{\text{OH}}$. Although both the peripheral (orange-fluorescent $^{\text{Mc}}\text{PEG}_{5\text{K}}^{\text{RhB}}$) and core (non-fluorescent $^{\text{H}}\text{PEG}_{35\text{K}}^{\text{OH}}$) regions of the concentric pattern in confocal laser scanning microscopy (CLSM) showed, as observed by TOF-SIMS, a $\text{C}_2\text{H}_5\text{O}^+$ ion originating from PEG, the glass surface was turned out to be completely covered with NH_4^+ ions and SO_4^{2-} ions. About this observation, quartz crystal microbalance (QCM-D) and X-ray diffraction analyses further supported the claim that $(\text{NH}_4)_2\text{SO}_4$ spontaneously accumulated on the glass plate surface under DLLPS conditions to form a crystalline salt layer (**Fig. 1**). I assume that this crystalline layer is essential for the instant concentric separation of a mixture of terminally distinct PEGs.

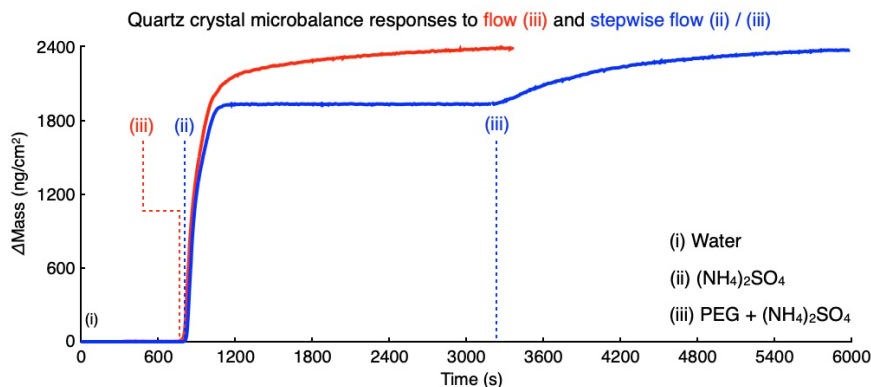


Figure. 1. QCM profiles of a mass change on a quartz plate under the all-at-once flow of an aqueous $(\text{NH}_4)_2\text{SO}_4$ solution of $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{OH}}$ (red) and the stepwise flow of an aqueous $(\text{NH}_4)_2\text{SO}_4$ solution of $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{OH}}$ followed by an aqueous solution of $(\text{NH}_4)_2\text{SO}_4$ (blue).

All the above results allow us to propose a possible mechanism for the DLLPS-induced concentric separation of terminally distinct PEGs. Initially, the addition of $(\text{NH}_4)_2\text{SO}_4$ to an aqueous mixture of PEG-A and PEG-B results in the formation of PEG-enriched droplets via primary LLPS containing a homogenous mixture of PEG-A and PEG-B. Then, these droplets deposit onto a crystalline salt layer spontaneously formed on the surface of a glass plate and undergo salt layer-assisted secondary LLPS, where PEG-B, which is more susceptible to LLPS than PEG-A, forms a PEG-B-dominating phase to occupy the core region of the concentric pattern upon surface wetting, thereby excluding the PEG-A-dominating phase to the periphery to form an open circle. Here, it must be noted again that, when individual PEGs are subjected to DLLPS, they only form a filled circle. Hence, the concentric separation that I highlight in the present work is a competitive phase behavior that occurs only when two or more structurally distinct PEGs are combined. In this regard, the principle of concentric separation is different from those of coffee-ring effect⁴⁻⁵ and chromatographic separation⁶⁻⁸, where the behaviors of individual components are unaffected by their companions.

3. Morphology of the solid–liquid interface

Since the interface was at the solid–liquid interface, it became very difficult to monitor its surface in situ. Environmental scanning electron microscopy (ESEM) is a powerful technology to observe the surface at its interface under solution at room temperature. Unfortunately, I was not able to see the clear surface pattern of the concentric separation. On the other hand, a coupled technology with ESEM is the solution scanning transmission electron microscope (Solution-STEM) can observe the droplet undergo a surface-wetting process (**Fig. 2**). According to the mechanism, when a PEG-enriched droplet was attached to the Si surface, it undergoes secondary LLPS and surface wetting. Initially, as observed from the solution-STEM, the PEG-enriched droplet is homogenous initially, once it was immobilized by the surface, secondary LLPS was clearly observed in the internal part of the droplet. Within the droplet, many low-contrast small droplets emerged, indicating that the density is lower than the original droplet, as expected. These droplets later coalesced and merged into a bigger droplet in the center. However, I did not understand why the whole droplet was shrinking against time and disappearing in the end. This may correspond to the surface wetting of the droplet, and also indicate that the newly formed surface pattern was very thin, namely, insufficiently providing contrast for the observation under solution-STEM. In fact, this result is consistent with the bright-field microscopy result that I observed for the same process. Next, I measured the substrate containing the

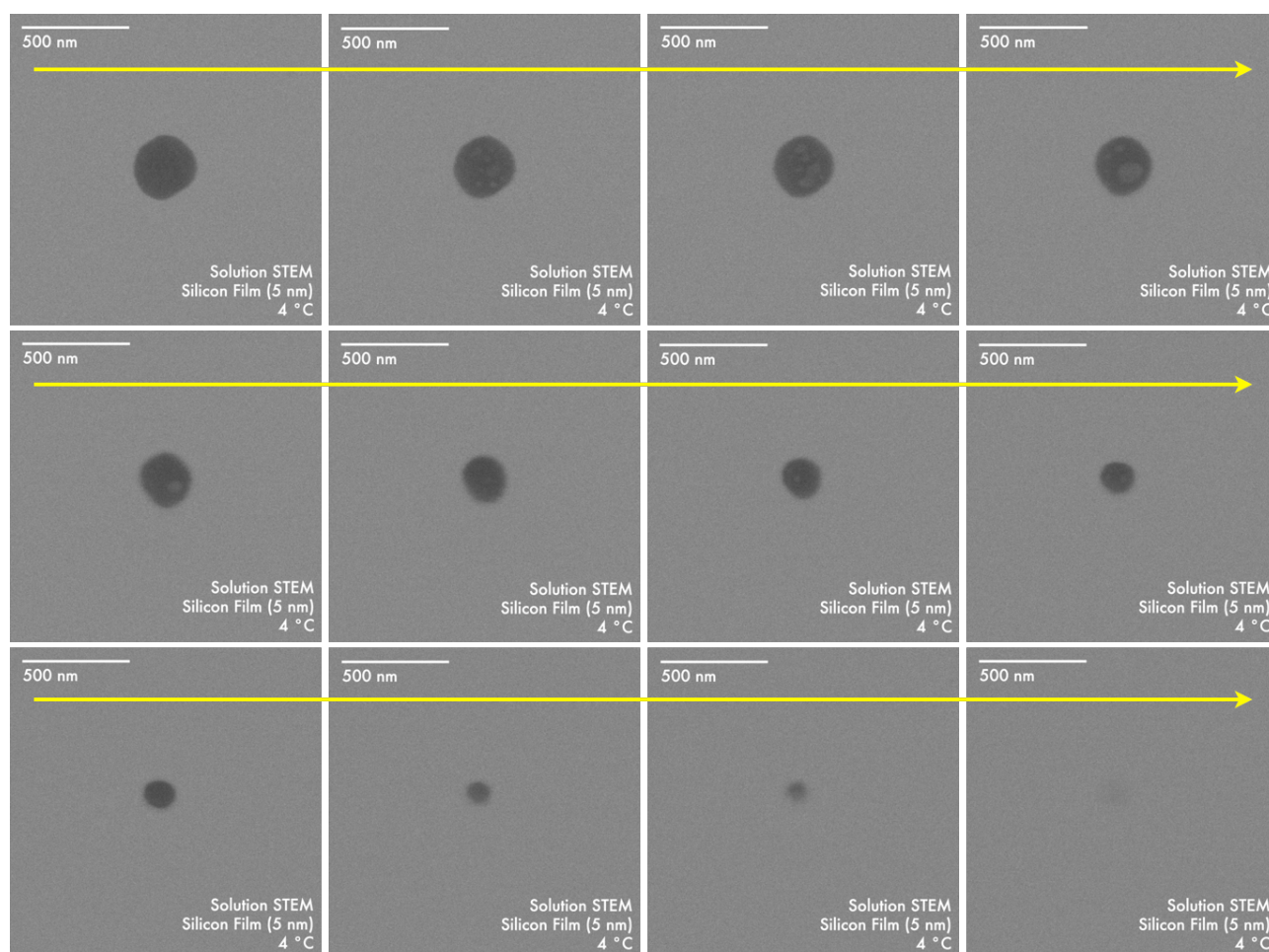


Figure 2. Time-dependent ESEM of the concentric separation, where the PEG-enriched droplet undergoes secondary LLPS and surface wetting. The time interval is 4 seconds per frame.

concentric pattern by atomic force microscope (AFM), and again, unfortunately, I was not able to visualize the pattern, which may indicate that the cantilever may be difficult to distinguish the interface of liquid and air. However, I was able to measure the force curve of the surface, and the result indicated that the surface contained both salt and PEG since the force curve on each substrate showed a different force curve.

4. Conclusion and acknowledgment

From the experience I have had in this internship, I was able to facilitate the understanding of my project and the communication skill with other researchers. Here, I acknowledge Dr. Yasuhiro Ishida who accepted me as an internship student. And I also thanks Dr. Daisuke Hashizume, Mr. Daishi Inoue, and Mr. Kiyohiro Adachi for the experimental support for the ESEM and XRD measurement.

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