

## Research Article

Theme: Next Generation Formulation Design: Innovations in Material Selection and Functionality  
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# Particle Characterization for a Protein Drug Product Stored in Pre-Filled Syringes Using Micro-Flow Imaging, Archimedes, and Quartz Crystal Microbalance with Dissipation

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**Abstract.** Micro-flow imaging (MFI) has been used for formulation development for analyzing sub-visible particles. Archimedes, a novel technique for analyzing sub-micron particles, has been considered as an orthogonal method to currently existing techniques. This study utilized these two techniques to investigate the effectiveness of polysorbate (PS-80) in mitigating the particle formation of a therapeutic protein formulation stored in silicone oil-coated pre-filled syringes. The results indicated that PS-80 prevented the formation of both protein and silicone oil particles. In the case of protein particles, PS-80 might involve in the interactions with the hydrophobic patches of protein, air bubbles, and the stressed surfaces of silicone oil-coated pre-filled syringes. Such interactions played a role in mitigating the formation of protein particles. Subsequently, quartz crystal microbalance with dissipation (QCM-D) was utilized to characterize the interactions associated with silicone oil, protein, and PS-80 in the solutions. Based on QCM-D results, we proposed that PS-80 likely formed a layer on the interior surfaces of syringes. As a result, the adsorbed PS-80 might block the leakage of silicone oil from the surfaces to solution so that the silicone oil particles were mitigated at the presence of PS-80. Overall, this study demonstrated the necessary of utilizing these three techniques cooperatively in order to better understand the interfacial role of PS-80 in mitigating the formation of protein and silicone oil particles.

**KEY WORDS:** Archimedes; micro-flow imaging (MFI); pre-filled syringe; protein particles; quartz crystal microbalance with dissipation (QCM-D); silicone oil particles.

## INTRODUCTION

Conventionally, optical techniques such as light obscuration have been widely utilized for particle analysis (1). However, such techniques do not provide any information regarding shape of particles. In contrast, micro-flow imaging (MFI), a microscopy-based dynamic imaging system, measures sub-visible particles with sizes 2–100  $\mu\text{m}$  and categorizes their shapes (1). In this technique, images are captured as a continuous sample stream which is passed through a flow cell. During the operation, the flow cell is illuminated and differences in refractive index (RI) and light intensity of particles are captured relative to the background (2). The analytical software then extracts and classifies the images

utilizing mathematical filters based on particle morphology, size, intensity, and optical parameters (2, 3). One of the prominent applications of MFI has been in the differentiation of protein particles from non-protein particles such as silicone oil droplets, air bubbles, and extrinsic contaminants in prefilled syringes (3). For example, differentiating protein particles from silicone oil particles is based on protein particles that have a fiber-like shape, whereas silicone oil particles have a circular form.

The particles with small sizes have been of growing interests of research, development, quality control, and regulatory. Therefore, emerging new techniques suitable for the analysis of sub-micron particles in therapeutic protein drugs are highly desirable. Recently, the application of the Archimedes technology has allowed for the characterization of particles with sub-micron sizes (4, 5). The technique measures resonant frequency of an oscillating finger containing a microfluidics channel. Any given particle passing into the microchannel causes a change in the resonant frequency which is proportional to the buoyant mass. From the change in frequency, the buoyant dry mass and particle size can be calculated. While there are growing interests in the application of Archimedes instrument, its utility during biopharmaceutical development as

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well as the advantages of utilizing it in combination with MFI requires further exploration.

As reported in literatures, quartz crystal microbalance with dissipation (QCM-D) measurement is based on damping a resonating quartz sensor when molecules are adsorbed onto the surfaces of electrodes. It has been used to investigate the adsorption of molecules at various interfaces (6–8). The advantages of QCM-D lie in its nano-gram level sensitivity and the flexibility in the variety of electrode surfaces that can be prepared. The rheological properties of adsorbed layer, and thus the molecular structure and interactions between the interfacial layer and bulk solution, can be inferred. Since aggregation or particle formation in protein solution is often induced by interfacial interactions, QCM-D can be used to explore the mechanism of formation of aggregates.

In this study, we combined MFI and Archimedes to measure protein and silicone oil particles from formulation with or without PS-80 stored in silicone oil-coated pre-filled syringes. PS-80 is a nonionic surfactant with hydrophilic polyethers groups and hydrophobic alkanes tails, which has been used in biologics drug products as a stabilizer. We further used QCM-D to investigate the interfacial interaction associated with the role of PS-80 in mitigating particle formation.

## MATERIALS AND METHODS

### Materials

#### *Preparation of Protein Samples and Reagents*

The protein studied here is a therapeutic protein which was formulated in 20 mM histidine and 600 mM sucrose, pH 7. The protein solution was prepared at a protein concentration of 0.3 mg/mL and divided into two separate plastic containers. To one of these samples, a PS-80 stock solution (5% w/v) was added to bring a final PS-80 concentration to 0.05% w/v. All the reagents used in preparing the formulations were obtained from commercially available source (J.T. Baker, Center Valley, PA). The formulation solutions were filtered through a 0.2- $\mu$ m filter (Millipore, Billerica, MA) prior to store the formulations in silicone oil-coated pre-filled syringes, 27G stw 1/2" needle, BD Hypack SCF glass prefillable syringes (BD Frankline Lakes, NJ).

### Methods

#### *Agitation Stress Procedure*

The samples stored in pre-filled syringes were agitated using a wrist shaker (Burrel scientific, model number 75, Pittsburgh, PA) for up to 18 days at room temperature and shaking intensity of 150 rpm. Sample solutions were collected at initial 6, 12, and 18 days for analytical characterization.

#### *Micro-Flow Imaging (MFI)*

Sub-visible particles in collected samples were measured using an MFI 5200 Flow Microscope system (Protein Simple, Ottawa Ontario, Canada). The system was equipped with 100- $\mu$ m flow cell and the BAI sample handler and was controlled by the MFI View system Software (MVSS). The data acquisition and image analysis

used the Lumetecs analytical software (Protein Simple, Ottawa Ontario, Canada) for the particles with the sizes ranged from  $\geq 2$   $\mu$ m. Prior to sample analysis, the system was flushed by 10 mL of purified water at maximum flow rate and checked visually between measurements. Initial baseline was determined using purified water and buffer followed by actual measurement of the sample. Sample aliquots (300–400  $\mu$ L) were used to perform “optimize illumination” prior to sample measurement to ensure correct thresholding of the MFI system. The remaining portion of each sample (600  $\mu$ L) was then analyzed at a flow rate of 0.17 mL/min. Samples were measured in triplicate, and each sample measurement was followed by a water flush and buffer prime. The particles with a circularity  $< 0.95$  were categorized as potential protein particles, and those which did not meet this specified criteria were considered as potential silicone oil particles. It is a practical approach for classifying types of particles which provided a mechanistic understanding on the effectiveness of PS-80 in mitigating protein or silicone oil particles. Images of particles captured by MFI with the sizes  $\geq 5$   $\mu$ m were manually examined. Only two distinct populations were observed in the captured images. One set of images showed cycle ring shape, and another set of images showed ribbon-like in morphology. These two types of particles were classified as silicone oil particles and protein particles, respectively, based on literature (2). Since air bubbles were not evidenced in the images of particles with the sizes  $\geq 5$   $\mu$ m, it was assumed that the captured images of small particles with size  $< 5$   $\mu$ m did not cover air bubbles as well.

#### *Archimedes*

The formulations with and without PS-80 stored in pre-filled syringes and stressed by shaking condition were also analyzed by Archimedes instrument (Malvern Instrument, Malvern, UK) with a Hi-Q nanosensor. Determination of particle size in Archimedes is calculated from the buoyant mass of a particle, which is proportional to the change in frequency of the MEMS sensor as a particle passes through the detection channel. The buoyant mass  $M_B$  of a particle is calculated using Eq. 1

$$M_B = \Delta f_c / S \quad (1)$$

where,  $\Delta f_c$  is designated as the frequency change and  $S$  is designated as the sensitivity (units [mHz/fg]), an instrument calibration constant which is determined for each sensor. The actual particle mass  $M$  (or dry mass) is calculated from buoyant mass  $M_B$  as shown in Eq. 2

$$M = M_B / (1 - \rho_f / \rho_p) \quad (2)$$

where,  $\rho_f$  and  $\rho_p$  are designated as the densities of the fluid and particle, respectively. The equivalent sphere diameter  $D$  can then be calculated from the actual particle mass  $M$  as described by Eq. 3

$$D = \left[ 6M / (\pi \rho_p) \right]^{1/3} \quad (3)$$

One of the key features of Archimedes system is its ability to differentiate particles detected in a sample based on

in their buoyant mass, and therefore density, relative to that of a suspending fluid. Proteinaceous particles and silicone oil particles contained in a sample solution can be quantified by the fact that they are negatively and positively buoyant, respectively, compared with the suspending fluid.

For this study, the sensor was flushed for 10 min with deionized water prior to instrument calibration. In order to confirm that Archimedes was operating within acceptable parameters, the micro sensor was first used to measure the sizes of particles in a polystyrene standard (Sigma, Saint Louis, Missouri). The size of polystyrene was calculated using the density of polystyrene at 1.05 g/mL. The sizes of protein particles and silicone oil particles were calculated with the densities at the values of 1.30 g/mL and 0.97 mg/mL (9, 10), respectively. Between sample runs, the fluidics were rinsed with water to prevent any cross-contamination between samples and the instrument was set up to accumulate data with one measurement for each sample with the condition detecting 600 particles for each run.

#### Quartz Crystal Microbalance with Dissipation (QCM-D)

**Procedure for Sensor Surface Preparation.** Silicone oil-coated Au sensor surfaces were produced using spin coating. Silicone oil was diluted to a concentration of 10 mg/mL in acetone. The solutions were then added to a bare Au sensor surface and spun at 4000 rpm for 30 s in the spin coater. The solvent was allowed to evaporate overnight before use of the silicone oil-coated Au sensors.

**Procedure for QCM-D Measurements and Data Analysis.** All QCM-D measurements were performed using the Q-Sense Analyzer (previously known as Q-Sense E4 Biolin Scientific Inc., NJ 07652), which allowed simultaneous monitoring of frequency and dissipation changes of four individual sensors. The sensors coated with silicone oil were used for all measurements. In general, the following procedures were used for experiments including control measurements. The buffer solution with absence of PS-80 was flown over the sensors to equilibrate the system. After a stable baseline was obtained, the buffer as well as protein solutions with and without PS-80 was introduced on the sensors and the adsorption was measured in real-time. All experiments were carried out at a flow rate of 50  $\mu$ L/min. Voigt viscoelastic model was applied in data analysis based on positive change in dissipation,  $\Delta D > 0$ . The modeling involved fitting-simulated frequency change,  $\Delta f$  and  $\Delta D$  to the actual  $\Delta f$  and  $\Delta D$  profiles until a satisfactory fit was obtained.

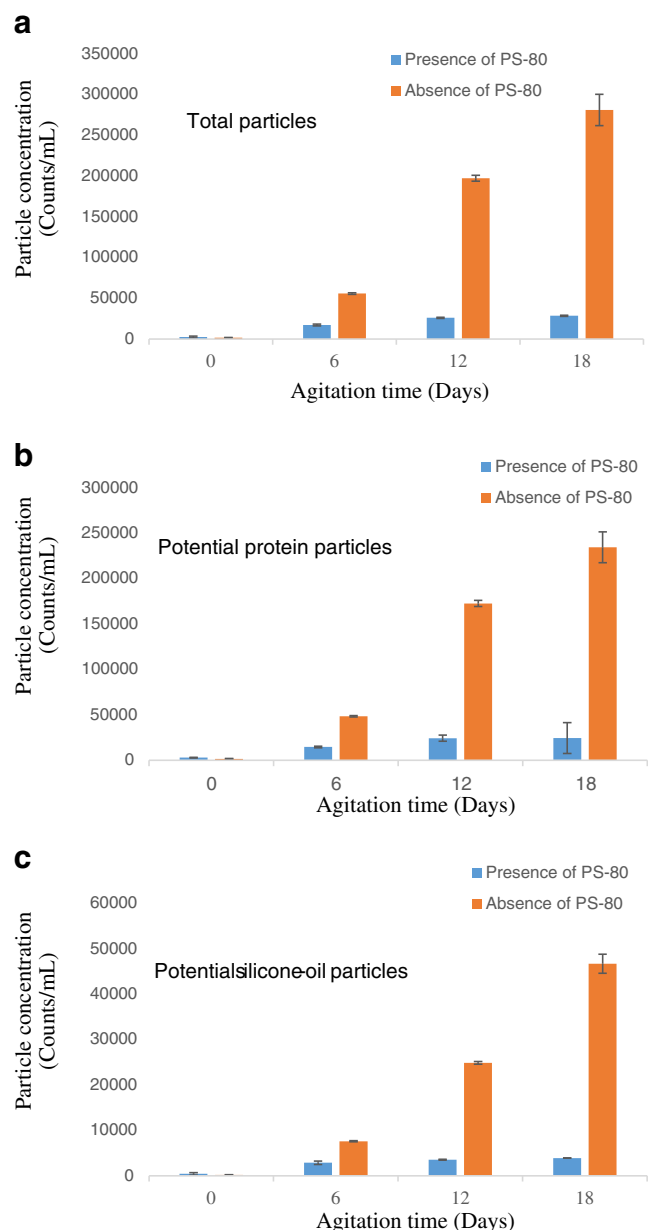
## RESULTS

### Particle Formation with and Without PS-80

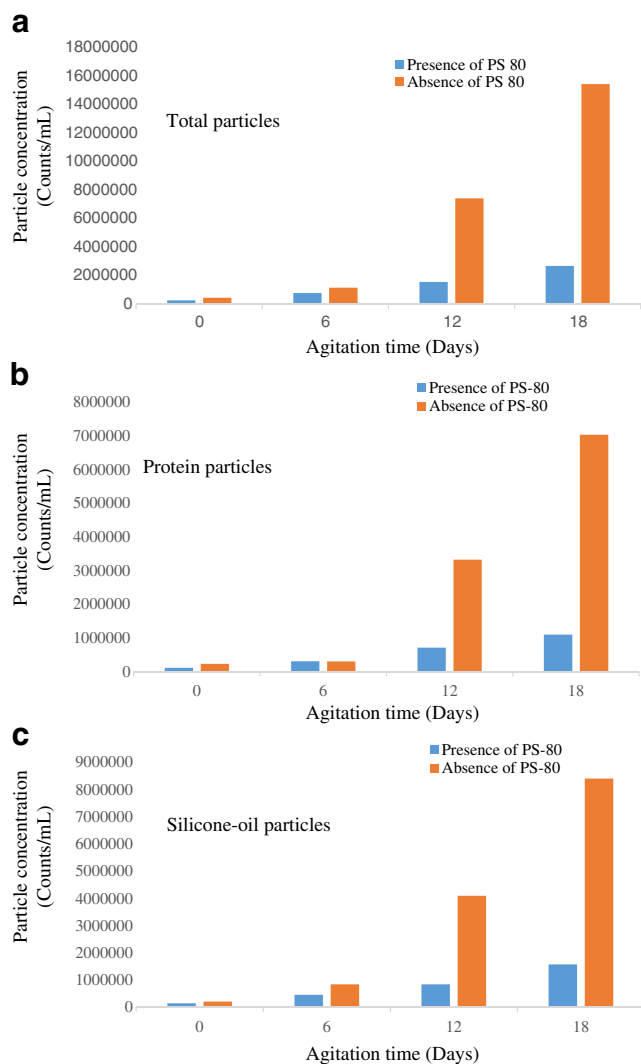
As shown in Fig. 1a, there were few particles in initial samples. In comparison to the initial samples, there were essentially no changes for the particles in both samples with and without PS-80 stored for 18 days without any agitation (data not shown here). The particles increased slightly in the sample with the presence of PS-80 as increasing agitation time. Conversely, the particles increased dramatically for the

samples with the absence of PS-80 as increasing agitation time. The growing trends of protein particles and silicone oil particles were similar for the sample without PS-80 as compared with Fig. 1b, c.

The results obtained from the Archimedes measurements are shown in Fig. 2. Similarly, the particles increased slightly for the sample with the presence of PS-80 as increasing agitation time. In contrast, the particles increased dramatically in the sample with the absence of PS-80. The growing trends of sub-micron particles were in a good agreement with that of sub-visible particles measured by MFI.



**Fig. 1.** The concentrations of sub-visible particles measured with MFI for the samples with and without PS-80 as a function of agitation time. **a** Represents total particles. **b** Represents potential protein particles. **c** Represents potential silicone oil particles



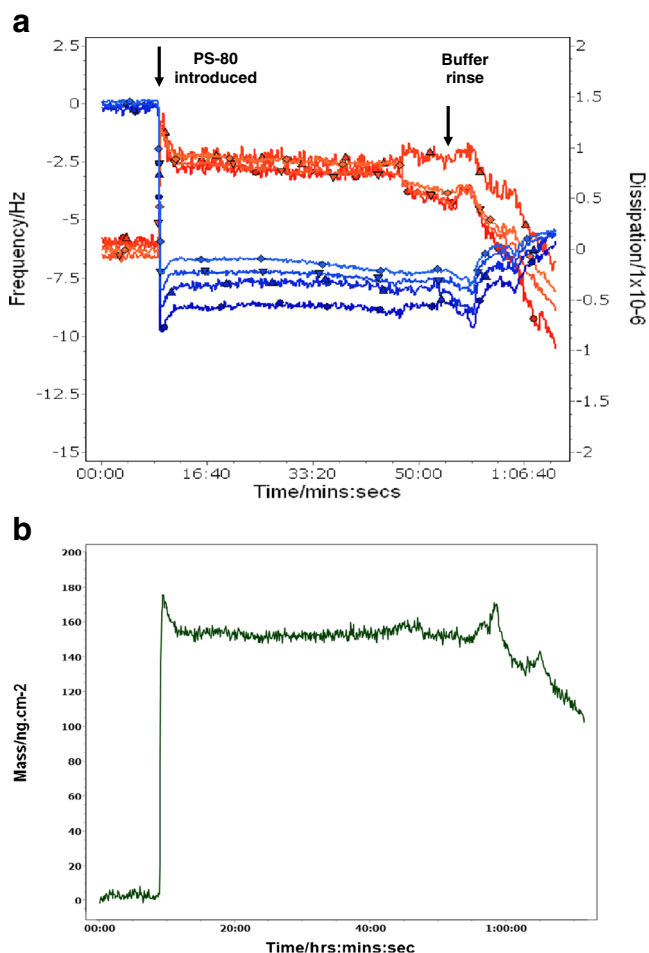
**Fig. 2.** The concentrations of sub-micron particles measured with Archimedes for the samples with and without PS-80 as a function of agitation time. **a** Represents total particles. **b** Represents protein particles. **c** Represents silicone oil particles

### Absorption of PS-80 on the Surface Coated with Silicone Oil

To help understand how PS-80 interacted with the sensor coated with silicone oil, a control experiment was performed in the buffer solution with the presence of PS-80. The negative shift in frequency and positive dissipation shift after the buffer solution containing PS-80 was introduced to the sensor as shown in Fig. 3a, suggesting that PS-80 was adsorbed rapidly to the silicone oil surface. The adsorbed PS-80 mass on the sensor coated with silicone oil is shown in Fig. 3b, where approximate 150 ng/cm<sup>2</sup> on the plateau equivalents to the thickness of 1.193 nm by dividing the corresponding mass by a PS-80 density value of 1.06 g/ml.

### Adsorption of Protein to the Surface Coated with Silicone Oil

$\Delta f$  and  $\Delta D$  versus time plots for protein samples with PS-80 and without PS-80 on the sensor coated with



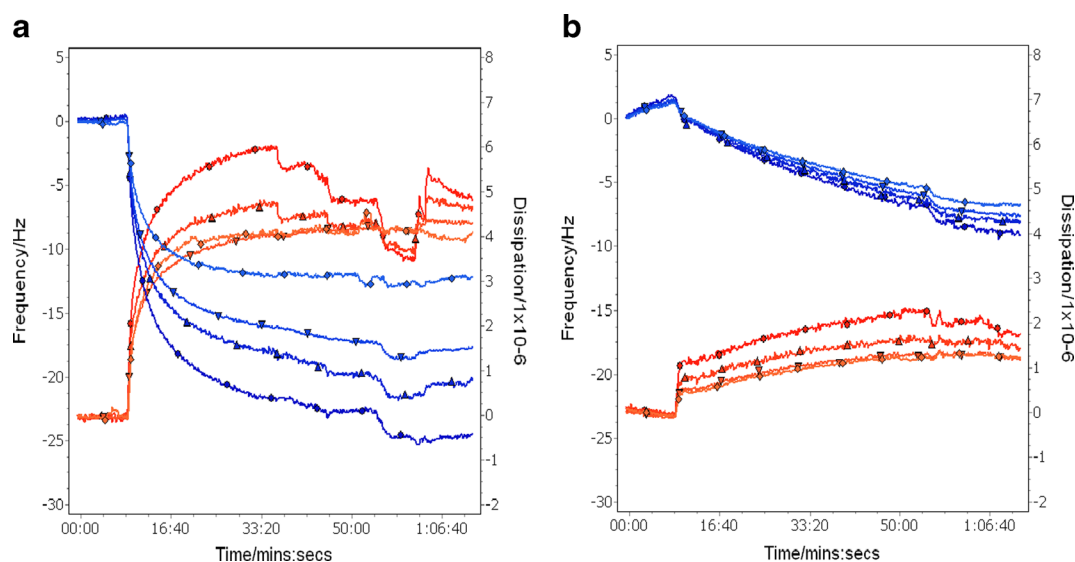
**Fig. 3.** **a** Displays the plots of  $\Delta f$  and  $\Delta D$  as a function of time for a control experiment for the buffer with the presence of PS-80. *Blue curve* represents frequency and *red curve* represents dissipation in **(a)**. **b** Displays the adsorbed PS-80 mass versus time on the sensor surface coated with silicone oil in the control experiment

silicone oil are shown in Fig. 4. Qualitatively, the presence of PS-80 resulted the smaller  $\Delta f$  decrease compared with the plot with PS-80 absent. Also, the layer formed in the absence of PS-80 was more viscoelastic than the layer formed in the presence of PS-80, as evidenced by the higher final dissipation in Fig. 4a compared with Fig. 4b. Proteins adsorbed at the oil surface, which might have stretched out to solution causing the layer more viscoelastic and damping the sensor vibration quickly, causing higher final dissipation. The comparison of the resulting plot of mass versus time is shown in Fig. 5, suggesting the smaller quantity of protein was adsorbed on the sensor coated with silicone oil with the presence of PS-80. In the plateau range of Fig. 5 for the condition without PS-80, 500 ng/cm<sup>2</sup> equivalents to the thickness of 3.85 nm by dividing the corresponding mass by a protein density value of 1.3 g/ml.

## DISCUSSION

### Formation and Mitigation of Protein Particles

There were few protein particles in both initial samples with and without PS-80, as shown in Figs. 1b and 2b. The

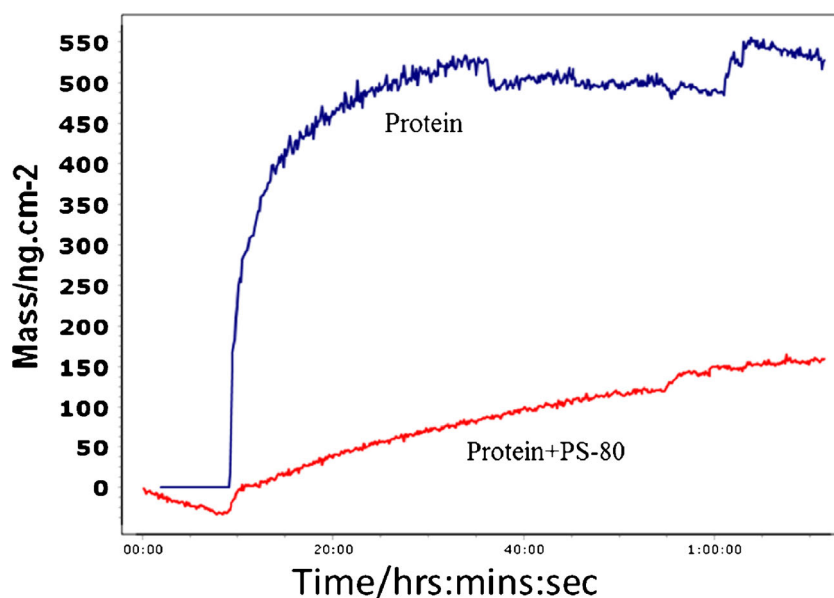


**Fig. 4.** **a** Is the plots of  $\Delta f$  and  $\Delta D$  versus time for the protein adsorption from the buffer to the surface coated with silicone oil in the absence of PS-80. **b** Is the plots of  $\Delta f$  and  $\Delta D$  versus time for the protein adsorption from the buffer to the surface coated with silicone oil in the presence of PS-80. Blue curve represents frequency and red curve represents dissipation in panels **a** and **b**

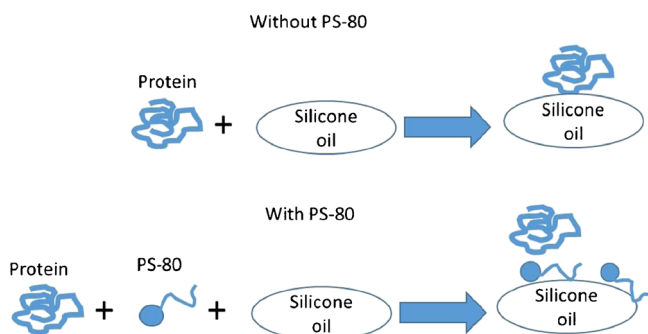
similar results were observed for the samples stored for 18 days without any agitation (data is not shown in here). Conversely, there were many protein particles in the agitated samples as shown in the same figures. Agitation plays a role in enhancing formation of air bubbles and sheer force. Previous studies suggest that air bubbles and interior surfaces of pre-filled syringes induce protein particles (11–13). Therefore, the observed correlation of increased protein particles and agitation time was likely due to the air bubbles and the sheer force that induced particle formation. In addition to the increase of protein particles, the silicone oil particles were increased as increasing agitation time, as shown in Figs. 1c

and 2c. The literatures from separated studies indicate that the presence of silicone oil particles induces protein particles (14, 15). Therefore, it was expected that the silicone oil induced protein particles as well.

Previous studies indicate that PS-80 is typically adsorbed at various surfaces (7.8). It has been well known that PS-80 stabilizes proteins against interfacial stress by minimizing adsorption at liquid–container surfaces and liquid–air interfaces and reduces antibody self-association and aggregation (11, 12, 16). Protein particles were minimized effectively in the samples with the presence of PS-80 as shown in Figs. 1b and 2b, which were likely due to that PS-80 involved in the



**Fig. 5.** Comparison of the mass of protein adsorbed to the surface coated with silicone oil in the buffers with the absence and the presence of PS-80



**Fig. 6.** A proposed mechanism of the interactions of silicone oil with protein and PS-80

interactions with the hydrophobic patches of protein, air bubbles, and surface of syringes. Such interactions played a role to prevent protein self-assembly so that the formation of protein particles was mitigated.

### Mitigation of Silicone Oil Particles

As shown in Fig. 4a, a large shift in frequency (up to 25 Hz) was observed when protein solution without PS-80 was introduced to the sensor coated with silicone oil. In addition, the results derived by the fitting approach based on Voigt viscoelastic model described in QCM-D method session shows that larger amount of mass was adsorbed at the water/oil interface as compared with the sample with the presence of PS-80 as seen in Fig. 5. In this case, the adsorption was possibly driven by the hydrophobic interaction of the protein molecules with silicone oil. In addition, Fig. 4a shows that the energy dissipation increased with time, along with adsorption, suggesting that the adsorption of protein molecules at the water/oil interface rendered the interface phase very viscous, where a stretched out structure might be formed by the protein molecules. On the other hand, in the presence of PS-80 in protein solution, the shift of frequency was substantially reduced as shown in Fig. 4b. Furthermore, a significant reduction of dissipation was also observed. PS-80 is known to form a compact layer at the water/silicone oil interface and its adsorption decreased the overall dissipation (8). The control experiment confirmed that PS-80 molecules were adsorbed rapidly on the sensor coated with silicone oil as shown in Fig. 3b. Taken together, it was proposed that the smaller quantify of mass adsorbing to the surface in the sample with the presence of PS-80 compared with that in the absence of PS-80 was due to the adsorption of PS-80 on the sensor coated with silicone oil, indicating either a competitive adsorption of PS-80 or a co-adsorption of surfactant with protein on the surface of the sensor. Figure 6 shows a proposed mechanism, suggesting that the adsorption of PS-80 on the sensor coated with silicone oil was more favorable than that of protein. Based on this proposal, the reduction of silicone oil particles in the protein solution containing PS-80 was likely due to that PS-80 adsorbed on the surface of silicone oil-coated pre-filled syringes, which blocked the leakage of silicone oil into solution. As a result, the formation of silicone oil particles was inhibited in the sample containing PS-80.

### CONCLUSION

The results obtained from both the MFI and Archimedes studies demonstrated that PS-80 mitigated the formation of both protein and silicone oil particles. It is possible that PS-80 was involved in interactions with the hydrophobic patches of protein, air bubbles, and the stressed surface of silicone oil-coated syringes. As a result, PS-80 mitigated the formation of protein particles. The proposed hypothesis based on QCM-D results further demonstrated that PS-80 might block the leakage of the silicone oil from surfaces of syringes into solution so that the formation of silicone oil particles was minimized.

Biopharmaceutical industry has been challenged to comprehensively characterize the sub-visible and sub-micron particles in protein-based drug products due to the limitation on the analytical method suitable for analyzing sub-micron particles previously. This study showed the advantages of combining MFI and Archimedes to characterize sub-visible and sub-micron particles. In addition, QCM-D helped to understand the mechanism of PS-80 in mitigating the leakage of silicone oil particles from silicone oil-coated syringes.

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