Gelation of a Monoclonal Antibody at the Silicone Oil–Water Interface and Subsequent Rupture of the Interfacial Gel Results in Aggregation and Particle Formation

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ABSTRACT: The formation of viscoelastic gels by a monoclonal antibody (mAb) at the silicone oil–water interface was studied using interfacial shear rheology. At a concentration of 50 μg/mL, the mAb formed gels in less than 1 h, and the gelation time decreased with increasing protein concentration. To probe the effects of mechanical rupture of the interfacial gel layers, a layer of silicone oil was overlaid on the surface of aqueous solutions of mAb, and the interface was ruptured periodically with a needle. Rupture of the interfacial gel resulted in formation of subvisible particles and substantial losses of mAb monomer, which were detected by microflow imaging and quantified by size-exclusion chromatography, respectively. Resonance mass measurement showed that levels of both protein particles and silicone oil droplets increased as the gel was repeatedly ruptured with a needle. In contrast, in samples wherein the interfacial gels were not ruptured, much lower levels of aggregates and particles were detected. Addition of nonionic surfactants (polysorbate 20 or polysorbate 80) protected against aggregation and protein particle formation, with increased protection seen with increasing surfactant levels, and with the greatest inhibition observed in samples containing polysorbate 80. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1282–1290, 2015

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INTRODUCTION

Pharmaceutical proteins encounter various interfaces during manufacturing and storage as they go through purification, filling, freeze–thaw, transportation, storage, and delivery to patients.[1–6] Exposure of protein solutions to interfaces often results in protein aggregates and particles in the bulk solution.[1,3–5,7,8] Protein molecules readily adsorb to many interfaces,[6,8–11] such as the relatively hydrophobic, air–water, and silicone oil–water interfaces.[12–14] Upon adsorption to interfaces, proteins have potential to unfold,aggregate and form viscoelastic gel layers.[15,16] The protein gel formed can be stabilized by a variety of noncovalent interactions between protein molecules including hydrogen bonding and electrostatic interactions.[17]

Recently, Rudiuk et al.[18] showed that rupturing the gel layer of protein formed at the air–water interface of an IgG solution resulted in the release of protein aggregates from the interface into the bilk solution. This model is of interest because it suggests a mechanistic explanation of interface-induced aggregation related to perturbation of protein gel layers during mechanical stress such as agitation.[5,18] Furthermore, they showed that the presence of surfactants in the IgG formulation reduced the amount of aggregation that was detected. Our study is an extension of the work carried out by Rudiuk et al.,[18] wherein we are studying the behavior of a monoclonal antibody (mAb) at the silicone oil–water interface using new tools and techniques.

Nonionic surfactants, commonly polysorbate 20 and polysorbate 80, are often used as stabilizers in protein formulations in order to reduce aggregation.[9,19] One mechanism by which they may inhibit aggregation is through competitive adsorption to interfaces, because of their higher adsorption energies per unit area than protein molecules.[20] Above the critical micelle concentration (CMC), polysorbates can saturate hydrophobic interfaces and thus be most effective at inhibiting interfacial adsorption of protein molecules.[21,22] This observation is consistent with many biologics formulations containing surfactant concentrations above their CMC level. However, some formulations contain surfactants below their CMC level, and a few studies have shown polysorbates can confer significant protection to protein against surface-induced aggregation even below their CMC concentrations.[10,21,23] In some cases, this was through partial competitive adsorption. In other cases, surfactant molecules bound to the native protein molecules at stoichiometric ratios, and the resulting complexes were resistant to aggregation.[24–26]

The silicone oil–water interface is commonly encountered by therapeutic proteins during storage in drug product containers.[27] Silicone oil is widely used as a lubricant for the plunger in prefilled syringes, and for stoppers for glass vials.[27] In prefilled syringes, formation of protein particles has been linked to the presence of silicone oil,[3,27–29] and conformational changes have been observed in a number of monoclonal antibodies upon adsorption to the silicone oil–water interface.[3,30,31] Proteins have been shown to form gels at other oil–water (e.g., coconut oil–water) interfaces but this phenomenon has not yet been characterized at the silicone oil–water interface.[32] We hypothesize that formation of protein gels at the silicone oil–water

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interface occurs rapidly and that such gel formation contributes to protein aggregation and particle formation.

Interfacial gel formation by proteins can be monitored by interfacial shear rheology measurements.[33,34] The data obtained from these experiments can provide important insights into the formation of interfacial layers and can help characterize viscoelastic materials.[17] In one approach, an oscillatory electromagnetic force is applied to a magnetized rod located on an interface of interest, and the resulting frequency-dependent interfacial deformation is measured.[35] Interfacial gel formation by protein molecules adsorbed at the interface can be determined by comparing the shear elastic (storage) and viscous (loss) moduli.[5] In the current study, we used interfacial shear rheology to determine the concentration-dependent time required for a mAb to adsorb and form a viscoelastic gel at the silicone oil–water interface.

We also hypothesized that rupture of the interfacial protein gel formed at the silicone oil–water interface would result in mAb aggregates in the bulk aqueous phase, similar to the results observed by Rudiuk et al.[18] We used microflow imaging (MFI) and resonance mass measurement (RMM) to characterize the concentrations and sizes of subvisible particles. We also used size-exclusion chromatography (SEC) to observe loss of mAb monomers and formation of soluble aggregates. Further, we tested the effectiveness of polysorbate 20 and polysorbate 80 at inhibiting aggregation induced by exposure of the mAb to the silicone oil–water interface.

The model of interfacial gel rupture presented here is applicable to therapeutic proteins that are stored in prefilled syringes or vials with siliconized stoppers and are exposed to the silicone oil–water interface during their shelf-life. Perturbation of the protein gel layer has been shown as the major cause of protein aggregation in various studies such as rotation of prefilled syringes[29] and agitation of protein formulations.[5] However, the complexity of those models makes a mechanistic understanding difficult. The major benefit of this model is the direct study of mechanical rupture of the interfacial gel formed at the silicone oil–water interface. Conversely, the simplistic experimental setup is a significant limitation of this study. The mechanical impact on the protein solution is unlikely to directly relate to real-world stresses. Therefore, this model should be used in conjunction with other models to fully understand the phenomena of aggregate formation resulting from the interaction of protein, interfaces, and agitation.

MATERIALS AND METHODS

Materials

Purified mAb was provided by MedImmune (Gaithersburg, Maryland) in a lyophilized formulation. The lyophilized material was reconstituted with 2.2 mL of water for injection to obtain 50 mg/mL mAb in 10 mM histidine, 6% (w/v) trehalose, 2% (w/v) arginine, and 0.025% (w/v) polysorbate 80 at pH 6.0. Following reconstitution, the mAb solution was dialyzed against 10 mM histidine buffer at pH 6.0. The stock protein solution was then diluted by a factor of 50 to obtain the 1-mg/mL solution used in the studies. Dialysis is not a robust method to remove polysorbate 80 from solution; however, the dilution step resulted in a maximum polysorbate 80 concentration of 0.0005% (w/v). It is assumed that this low concentration of PS has a minimal impact. This was the starting material for the experiments carried out below and used as the control during the polysorbate study.

USP grade reagents such as L-histidine, silicone oil (50 cst) were purchased from Fisher Scientific (Fair Lawn, New Jersey). Silicone oil (1000 cst) used to study rupture of interfacial gels was of medical grade and purchased from Dow Corning (Midland, Michigan). Unless otherwise indicated, deionized MilliQ® water was used to prepare all solutions. Lyophilization vials (2 mL) and caps were purchased from West Pharmaceutical (Lionville, Pennsylvania). The rotating mixer was purchased from Appropriate Technical Resources (Laurel, Maryland).

Interfacial Shear Rheology Measurement

To study gelation of the mAb at the silicone oil–water interface, a custom-built interfacial shear rheometer was used as previously described.[5,17,35] In this experiment, mAb solution was placed in a glass channel (length × width = 15 × 1 cm^2), which was placed in a glass container. A magnetic rod (diameter × length = 0.06 × 2.54 cm^2), with anodized black and white stripes was inserted in the middle of a 5-cm polytetrafluoethylene (PTFE) tube (Small-parts.com). The inner diameter of the PTFE tubing was 0.0635 cm. Both ends of the PTFE tubing were sealed with paraffin wax. The magnetic rod assembly was aligned in the middle of a glass channel that contained 40 mL of mAb solution. An aliquot (6 mL) of silicone oil (50 cst) was layered on top, in order to cover the entire area of the protein solution in the glass channel. The magnetic rod assembly remained suspended at the silicone oil–water interface.

As previously described,[5] oscillatory forces were applied on the rod by electromagnetic coils placed on each side of the glass channel. Because of the applied force, the magnetic rod moved back and forth, and sheared the silicone oil–water interface. The applied forces were proportional to the difference in currents between the two electromagnetic coils. Further, this was used to determine the applied stress. A charge-coupled device (CCD) camera was used to track the rod's motion. The motion was later used to determine the resulting strain. The rheological parameters were calculated using the following equations[34,35]:

$$G' = \frac{|\sigma|}{\gamma}\cos\varphi \tag{1}$$

and

$$G'' = \frac{|\sigma|}{|\gamma|}\sin\varphi \tag{2}$$

here, G' and G'' are the elastic (storage or solid like) and viscous (loss or liquid like) moduli, respectively. In these equations, σ, γ, and ϕ represent stress, strain, and phase angle (the difference between the rod response and the applied force), respectively.

For buffer in the absence of protein, the elastic modulus (G') is smaller than the viscous modulus (G''). If the added mAb forms a gel at the interface, the initially smaller G' will surpass G''.[34] Therefore, the interfacial gel transition time can be determined by the cross-over time between G' and G''.

Before each measurement, the glass channel was soaked for 1 h in a mixture of sulfuric acid and hydrogen peroxide (v:v = 2:1) to remove any surface contaminants.[34] Also, this procedure helped to maximize the hydrophilicity of the glass surface and thus ensured that the magnetic rod assembly remained at the