Surfactant Effects on Particle Generation in Antibody Formulations in Pre-filled Syringes

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ABSTRACT: Protein aggregation and particle formation have been observed when protein solutions contact hydrophobic interfaces, and it has been suggested that this undesirable phenomenon may be initiated by interfacial adsorption and subsequent gelation of the protein. The addition of surfactants, such as polysorbate 20, to protein formulations has been proposed as a way to reduce protein adsorption at silicone oil–water interfaces and mitigate the production of aggregates and particles. In an accelerated stability study, monoclonal antibody formulations containing varying concentrations of polysorbate 20 were incubated and agitated in pre-filled glass syringes (PFS), exposing the protein to silicone oil–water interfaces at the siliconized syringe walls, air–water interfaces, and agitation stress. Following agitation in siliconized syringes that contained an air bubble, lower particle concentrations were measured in the surfactant-containing antibody formulations than in surfactant-free formulations. Polysorbate 20 reduced particle formation when added at concentrations above or below the critical micelle concentration (CMC). The ability of polysorbate 20 to decrease particle generation in PFS corresponded with its ability to inhibit gelation of the adsorbed protein layer, which was assessed by measuring the interfacial diffusion of individual antibody molecules at the silicone oil–water interface using total internal reflectance fluorescence (TIRF) microscopy with single-molecule tracking. J Pharm Sci 104:4056–4064, 2015
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INTRODUCTION

Therapeutic protein molecules may encounter a variety of interfaces (air–liquid, solid–liquid, and liquid–liquid) during their manufacturing, transportation, and storage. Proteins are generally surface active and readily adsorb to many interfaces.[1] In some formulations, adsorbed proteins may undergo conformational changes at interfaces,[2–9] and they also may form viscoelastic interfacial protein gels.[10–13] In turn, formation of interfacial gels may be associated with agitation-induced formation of protein aggregates.[12,13]

Interfaces are a particular concern for protein therapeutics formulated in glass pre-filled syringes (PFS). In PFS, protein molecules may be exposed to air–water interfaces because of air bubbles that typically remain after syringe filling and stoppering. In addition, because silicone oil is often used as a lubricant on the syringe wall to provide low, smooth glide forces during injection, protein molecules may encounter silicone oil–water interfaces in PFS. Adsorption to air–water interfaces and silicone oil–water interfaces has been shown to foster protein aggregation and particle formation.[9,14–19]

A common strategy used by the biopharmaceutical industry to decrease the negative effects associated with protein adsorption to interfaces is to add nonionic surfactants such as polysorbate 20 (Tween 20®) or polysorbate 80 (Tween 80®) to protein formulations.[20,21] The addition of nonionic surfactants has been shown to decrease protein aggregation[22–27] and inhibit the formation of visible and sub-visible particles[25,28] in a number of protein formulations subjected to a variety of stress conditions. The protective effects of surfactants are commonly attributed to competitive adsorption of the surfactant to interfaces[12,23,29–31] or to the formation of surfactant–protein complexes.[26,27,32] Because of their strong affinity for interfaces, it has been proposed that surfactants may out-compete proteins for adsorption to interfaces, an effect that should correlate with the critical micelle concentration (CMC) of the surfactant.[29] Polysorbate 80 has been shown to decrease the amount of lysozyme and Factor VIII that adsorb on hydrophobic silica surfaces,[33,34] and the addition of polysorbate 20 decreased the adsorption of four different model proteins at the silicone oil–water interface.[31] Polysorbate 20 is also effective at displacing β-lactoglobulin from the *n*-hexadecane–water interface.[35] Some proteins also form surfactant–protein complexes that inhibit aggregation.[32] Polysorbate 20 binds to hydrophobic patches on the surface of recombinant human growth hormone and decreases aggregation at surfactant:protein molar ratios above 2.[32] Furthermore, at concentrations below their respective CMC's, polysorbate 20 and polysorbate 80 inhibit agitation-induced aggregation of albutropin and darbepoetin alfa because of the formation of surfactant–protein complexes.[26,27]

An additional effect of surfactants on proteins adsorbed to interfaces is the ability of surfactants to inhibit gelation of adsorbed protein layers. Polysorbate 20 prevented gelation of β-lactoglobulin at the air–water interface[10] and at the *n*-hexadecane–water interface.[35] Addition of polysorbate 20 to

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formulations of keratinocyte growth factor 2 (KGF-2) also prevented gelation at the air–water interface, and the addition of polysorbate 20 to a pre-formed KGF-2 gel caused the gel to break down.[12] Reversal of the gelation process was also observed when sodium dodecyl sulfate (SDS) was added to a pre-formed β-casein gel.[11]

Recently, several studies attributed agitation-induced aggregation and particle formation in protein formulations to mechanical rupture of the adsorbed protein gel layer at air–water interfaces and at oil–water interfaces.[13,16,17,36] Previously, we studied protein aggregation and particle formation in surfactant-free protein formulations in siliconized PFS. We observed that, especially in the presence of air–water interfaces, agitation induced extensive particle formation. We attributed this particle generation to agitation-induced rupture of a gelled protein layer at the silicone oil–water interface.[36] In the current study, we hypothesize that the addition of a nonionic surfactant to a protein formulation will inhibit interfacial gel formation at the silicone oil–water interface and thus reduce the number of particles generated in similarly agitated PFS.

To test our hypothesis, we added the nonionic surfactant polysorbate 20 at concentrations that spanned a range above and below the CMC to formulations of a model monoclonal antibody. These formulations were filled into glass syringes that were subsequently agitated by end-over-end rotation. After agitation, the concentrations of particles in the formulations were measured. In addition, particle generation was monitored in formulations wherein the polysorbate 20:monoclonal antibody molar ratio was varied in order to probe whether protective effects were related to the CMC of polysorbate 20 or to specific binding of polysorbate 20 to the monoclonal antibody. Finally, to assess the ability of polysorbate 20 to inhibit formation of interfacial protein gels, we used total internal reflectance fluorescence (TIRF) microscopy with single-molecule tracking to measure the effect of various bulk concentrations of polysorbate 20 on the interfacial diffusion of single fluorescently-labeled monoclonal antibody molecules adsorbed to silicone oil–water interfaces.

MATERIALS AND METHODS

Materials

Humanized IgG1 monoclonal antibody (molecular weight 146 kDa), here denoted as "3M", was provided by MedImmune (Gaithersburg, Maryland).[37] The antibody was obtained at a stock concentration of 150 mg/mL in 10 mM L-histidine at pH 6. The antibody 3M is a human IgG1 with three mutations (S239D/A330L/I332E) in the C_H2 portion of the Fc. These mutations reduce the thermal stability of 3M,[37] which was chosen for the current work because of previous studies[36] that showed it to be prone to aggregation when exposed to silicone oil–water interfaces. Polysorbate 20 (>97% purity; Fisher BioReagents) was obtained from Fisher Scientific (Pittsburgh, Pennsylvania). All buffer salts were of reagent grade or higher, and all solutions were prepared in de-ionized water filtered through a 0.22 μm Millipore filter (Billerica, Massachusetts). Silicone oil (Dow Corning 360, 100 cSt) was of medical grade and purchased from Nexeo Solutions (Denver, Colorado). The syringes used in the incubation studies were BD Hypak SCF 1 mL long 27G1/2 (BD Medical-Pharmaceutical Systems, Franklin Lakes, New Jersey). Glass coverslips, Micro-90, and isopropyl alcohol were obtained from Fisher Scientific (Waltham, Massachusetts). Nickel transmission electron microscopy (TEM) grids (EMS G100-Ni) were obtained from Electron Microscopy Sciences (Hatfield, Pennsylvania). Teflon® rings were fabricated in-house at the University of Colorado-Boulder.

Table 1. 3M Concentrations and Polysorbate 20 Concentrations Corresponding to the Polysorbate 20:3M Molar Ratios Used in the Formulations Tested*

Polysorbate 20:3M Molar Ratio	Polysorbate 20 Concentration (% v/v)	3M Concentration (mg/mL)
0.0	0.0000	1.0
0.1	0.0005	7.6
0.3	0.0005	2.2
0.7	0.0005	1.0
1.3	0.0010	1.0
2.6	0.0020	1.0
6.5	0.0050	1.0
13.1	0.0100	1.0
13.1	0.0800	7.6

*The polysorbate 20 CMC is 0.007% v/v (0.06 mM).[38]

Incubation of 3M Formulation with Polysorbate 20 (Above CMC) in PFS

A formulation containing 1 mg/mL 3M with 0.01% (v/v) polysorbate 20 in 10 mM L-histidine pH 5 was prepared using the 3M stock (described above) and a 1% (v/v) stock solution of polysorbate 20 in 10 mM L-histidine pH 5. This 3M formulation was used to fill glass syringes. Prior to filling, the silicone oil coating on some of the syringes was removed, as previously described.[36] To prepare syringes containing an air bubble, 1.26 mL of the formulation was pipetted into the syringe, and the syringe was stoppered, creating a headspace containing 30 μL of air. The air–water interfacial area associated with this bubble was approximately 0.5 cm^2, or about 5% of the wetted silicone oil–water interfacial area to which protein was exposed to in each syringe. For incubation conditions without headspace, the syringes were stoppered such that no air bubbles remained. Triplicate syringes were prepared for each incubation condition at each time point. For incubation conditions with agitation, the syringes were rotated end-over-end at 1.5 rpm at room temperature. For quiescent incubation conditions, the syringes were incubated horizontally on the bench top at room temperature. In addition, solutions containing 10 mM L-histidine buffer only (no protein) were incubated in siliconized syringes either with or without headspace.

Agitation of 3M Formulations with Varying Surfactant:Protein Ratios in PFS

To evaluate how the surfactant:protein molar ratio in the formulation affects the number of particles generated by agitation in PFS, protein formulations containing polysorbate 20 at surfactant:protein molar ratios ranging from 0 to 13.1 were prepared by varying the polysorbate 20 concentration and the 3M concentration (Table 1). A volume of 1.26 mL of each formulation was pipetted into siliconized syringes, and the syringes were stoppered such that a headspace containing 30 μL of air remained in the syringe. Triplicate syringes were prepared for each surfactant:protein molar ratio, and the syringes were rotated end-over-end at 1.5 rpm for 24 hours at room temperature.