Effect of the Siliconization Method on Particle Generation in a Monoclonal Antibody Formulation in Pre-filled Syringes

ALANA GERHARDT,1 BAO H. NGUYEN,1 RACHAEL LEWUS,2 JOHN F. CARPENTER,3 THEODORE W. RANDOLPH1

1Department of Chemical and Biological Engineering, University of Colorado-Boulder, Boulder, Colorado
2Formulation Sciences Department, MedImmune, Gaithersburg, Maryland
3Department of Pharmaceutical Sciences, University of Colorado-Denver, Aurora, Colorado

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ABSTRACT: Silicone oil is used as a lubricant in glass pre-filled syringes (PFS) but can contribute to the generation of particles within protein formulations in PFS. To mitigate the production of such particles, various silicone oil coating processes have been proposed. In this study, three siliconization methods (the “covalent” method, the “baked silicone oil” method, and the “liquid silicone oil” method) were used to coat glass syringes with silicone oil. Glide forces were determined for syringes coated by each method. Then, a monoclonal antibody formulation or a buffer solution were incubated in the coated syringes in either the presence or absence of an air bubble, and the syringes were rotated end-over-end to induce air bubble movement within the syringe. The particle concentrations were measured throughout the incubation period using flow microscopy. The coating method did not affect particle concentrations measured in buffer alone, nor did the coating method affect particle concentrations measured in protein formulations agitated in the presence of an air bubble. Particle concentrations were influenced by the syringe coating method in protein formulations agitated in the presence of an air bubble, with the most particles formed in syringes lubricated with liquid silicone oil. Fewer particles were produced in syringes lubricated with baked silicone oil, and the fewest particles were produced in syringes with covalently-attached silicone oil. However, the glide forces measured in syringes coated with silicone oil by each method are inversely correlated with the measured particle concentrations.

Keywords: PFS; silicone oil; microparticles; protein formulation; protein aggregation; image analysis; adsorption; monoclonal antibody; glide force

INTRODUCTION

Pre-filled syringes (PFS) are in widespread use as storage and delivery devices for protein therapeutics.1,2 In order to ensure smooth movement of the syringe plunger during administration of the product to patients, silicone oil is used as a lubricant on the inner walls of the syringe barrel.3 Previous studies have shown that proteins may adsorb to the silicone oil–water interface4–6 and that, as a result, proteins may experience conformational perturbations or form aggregates.6–9 Also, silicone oil droplets may leach from the syringe wall into the protein formulation.10–12 Despite these concerns, silicone oil in syringes is essential in order to obtain acceptable break-loose and glide forces for expelling solution from the syringe.11

Silicone oil, which is a linear polydimethylsiloxane (PDMS), is attractive as a lubricant for several reasons. It has a low surface tension (20.4 mN/m) that allows it to wet most surfaces,13 and the application of silicone oil to a surface makes the surface hydrophobic because of the sidechain methyl groups of PDMS.15 Below 150°C, silicone oil is chemically inert and resistant to decomposition.16 Over the last century, PDMS has been used in a number of medical applications, and it is approved by the US Pharmacopeia for use in pharmaceutical applications, including PFS.17

Two important parameters characterize the coating of syringes with liquid silicone oil: the amount of silicone oil applied to the syringe barrel and its distribution. There must be enough silicone oil to provide the required lubrication, but excess silicone oil can slough off of the barrel into the protein formulation.18 In addition, silicone oil must be applied homogeneously along the syringe barrel so that the entire length of the barrel has the same degree of lubrication. If there are dry spots, the plunger will “stall,” and the glide forces will fluctuate along the length of the syringe.19 In the commonly used method of “spray-on” siliconization, silicone oil is sprayed from a moving nozzle along the length of the barrel of the syringe to achieve the desired silicone oil amount and distribution.19 This process coats each syringe barrel with approximately 0.4–1.0 mg of silicone oil.1

An alternative method of siliconization is the “bake-on” method. In this process, the silicone oil is first sprayed onto the interior walls of the syringe barrel, and then the syringe is subsequently “baked” at high temperature. Baking temperatures range from 100°C to 320°C,18,19 although Dow Corning recommends baking below 250°C to minimize formaldehyde formation.18 During the baking process, the thin layer of silicone oil directly on top of the glass becomes covalently bonded to the surface.20 On top of this thin bonded layer is another thicker layer of silicone oil where cross-linking occurs between the PDMS chains.20 Both the covalent attachment and the polymerization of PDMS chains contribute to a silicone oil layer that is better adhered to the glass surface than an un-baked silicone oil layer. However, some silicone oil may potentially...
slough from the surface into the solution because the entire layer is not bonded to the glass.

A major concern in the development of protein formulations in PFS is the generation of particles (such as silicone oil) in the protein solution during product transportation and storage. USP <788> enumerates the number of particles of sizes greater than 10 μm and greater than 25 μm that are acceptable in an injectable drug product. In addition, particles in the 0.1–10 μm range are receiving increasing attention because of studies suggesting that particles in this size range may be immunogenic.

Silicone oil–water interfaces, air–water interfaces, and agitation have been observed to work synergistically to induce protein aggregation and to generate particles in protein formulations. In an excipient-free albinferon formulation, simultaneous exposure to both silicone oil microdroplets and agitation induced greater monomer loss than exposure to silicone oil microdroplets or agitation alone. Likewise, in two different excipient-free monoclonal antibody formulations, agitation in the presence of silicone oil led to more aggregation than exposure to silicone oil alone or agitation alone. The excipient-free albinferon formulation also showed significantly greater particle formation when it was agitated in the presence of siliconized glass beads than when it was agitated in the absence of siliconized beads or incubated quiescently in the presence or absence of siliconized beads. Furthermore, an excipient-free antibody formulation exhibited greater monomer loss and higher particle concentrations when it was agitated on an orbital shaker in the presence of siliconized glass beads than when it was agitated without siliconized beads or incubated quiescently with siliconized beads. That same antibody formulation also showed greater monomer loss and higher particle concentrations when it was rotated end-over-end in the presence of both siliconized beads and an air–water interface than when it was rotated in the presence of siliconized beads but without an air–water interface. In PFS, this synergism was also apparent in our previous study of an antibody formulation (the same as is used in the current study) and a lysozyme formulation, where the “worst case” levels of particles in an accelerated stability study were observed when the formulations were agitated with an air bubble in siliconized syringes. In that study, protein aggregates, silicone oil droplets, and agglomerates of protein aggregates and silicone oil were all observed.

How might particle generation in siliconized glass syringes be avoided? Consistent with the mechanism proposed in the previous study, we hypothesize that fewer particles will be generated in siliconized syringes, even in the presence of an air bubble, if the silicone oil coating is better adhered to the glass surface because it will be unable to be removed with gelled protein by capillary forces at the three-phase contact line. To investigate how the degree to which the silicone oil coating adheres to the surface of glass syringes influences the number of particles generated, we developed three different siliconization procedures. The “liquid silicone oil” method results in a silicone oil coating that resembles the “spray-on” method typically used by syringe manufacturers. Our previous study showed that silicone oil droplets are easily removed from the “spray-on” coating. The “baked silicone oil” method is similar to the commercial “bake-on” method described above, and it produces a silicone oil coating that is more strongly adhered to the glass surface than the liquid silicone oil coating. The “covalent” method uses a commercially available preparation (SurfaSil®) that contains PDMS modified so as to contain reactive groups on each end of the polymer chain. Upon reaction with the glass surface, the SurfaSil® PDMS molecules become covalently attached to the glass surface.

To test the effects of the three coatings on particle generation in PFS, a monoclonal antibody formulation and its buffer solution were agitated in syringes that had been coated with silicone oil using each of the methods. Some of the syringes were filled so as to avoid the presence of an air bubble, whereas other syringes contained an air bubble, and all syringes were rotated end-over-end. Particle concentrations within the syringes were measured at various time points during the agitation study using flow microscopy, which was also used to record images of the particles. In addition, the break-loose and glide forces were measured to evaluate if the different coatings were able to provide the necessary lubrication to the syringe barrels.

**MATERIALS AND METHODS**

**Materials**

Humanized IgG1 monoclonal antibody (molecular weight 146 kDa), here denoted as “3M,” was provided by MedImmune (Gaithersburg, Maryland). Because of its propensity to aggregate at silicone oil–water interfaces, 3M was previously used to examine the mechanism of protein aggregation at silicone oil–water interfaces. The antibody was obtained at a stock concentration of 150 mg/mL in 10 mM L-histidine at pH 6. For this study, 3M was formulated at 1 mg/mL in 10 mM L-histidine pH 5. Although addition of nonionic surfactant may mitigate some of the tendency for proteins to aggregate at silicone oil–water interfaces, no surfactant was added in order to more clearly delineate the effect of the various surface coatings that we tested. All buffer salts were of ACS grade or higher. All solvents were of ACS grade. All solutions were prepared in de-ionized (DI) water filtered with a 0.22 μm Millipore filter (Billerica, Massachusetts). The syringes used in the agitation studies were BD Hypak SCF 1 mL long 27G1/2 (BD Medical-Pharmaceutical Systems, Franklin Lakes, New Jersey).

**Removal of Silicone Oil from Syringes**

Siliconized glass syringes were cleaned to remove their original silicone oil coating so that the new silicone oil coatings could be applied to a bare glass surface. A 1% solution of Micro-90 (International Products Corporation, Burlington, New Jersey) was pipetted in and out of the syringes four times. This was followed by a rinse with DI water. Then, hexane was pipetted in and out of the syringes five times, and the syringes were left to air dry. Finally, the syringes were submerged in piranha solution (70% sulfuric acid:30% hydrogen peroxide) for 1 h (with the needle facing up and out of the solution) and then rinsed with DI water and dried with nitrogen. This method was previously shown to be effective in removing silicone oil from glass syringes.

**Siliconization Methods**

Cleaned syringes were coated with silicone oil using one of the three different siliconization methods: the “covalent” method, the “baked silicone oil” method, or the “liquid silicone oil” method. For the covalent method, a 1% SurfaSil Siliconizing Fluid® (Thermo Scientific, Rockford, Illinois) in acetone solution was prepared. This solution was pipetted into the...
syringes, and the syringes were incubated at room temperature overnight. Then, the SurfaSil\textsuperscript{®} solution was removed, the syringes were rinsed three times with acetone and one time with methanol, and the syringes were dried at 100°C for 1 h. For the baked silicone oil method, a 3% PDMS (DC 360, 1000 cSt; Dow Corning, Midland, Michigan) in toluene solution was incubated in the syringes overnight at room temperature. Then, the PDMS solution was removed, and the syringes were rinsed with acetone three times and dried at 100°C for 1.5 h. For the liquid silicone oil method, a 3% PDMS in toluene solution was incubated in the syringes overnight at room temperature. After incubation, the solution was removed, and the syringes were air-dried at room temperature for at least 1.5 h before use. Images of the menisci that were obtained after the coated syringes were filled with 1 mL of DI water are shown in Figure 1. As can be seen, the syringes coated with silicone oil have hydrophobic surfaces.

Agitation Studies of Protein Formulations in PFS

A protein formulation of 1 mg/mL 3M in 10 mM L-histidine pH 5 was prepared. For syringes with an air bubble, 1.26 mL of the formulation was pipetted into the syringe, and the syringe was stoppered, creating a headspace containing approximately 30 µL of air. For conditions with no air bubble, the syringes were stoppered such that no air bubbles remained. Triplicate syringes were prepared for each incubation condition at each time point. All syringes were rotated end-over-end at 1.5 rpm at room temperature. As controls, syringes were agitated with buffer only for conditions with or without an air bubble.

Counting of Particles in Agitated Protein Formulations

In the same way described previously,\textsuperscript{32} at each time point during the agitation study, syringes were un-stoppered, and the formulation was removed from the flanged end using a transfer pipet. The protein formulation was not ejected using the syringe needle to avoid the generation of particles because of plunger movement along the syringe barrel. For each sample, particles between 2 µm and 2 mm (equivalent spherical diameter) were counted using a Fluid Imaging Technologies Benchtop FlowCAM\textsuperscript{®} (Scarborough, Maine). The FlowCAM was fitted with a FC100 flow cell, a 10X objective and collimator, and a 0.5 mL syringe. The gain and flash duration were set such that the average intensity mean of the image was consistently between 180 and 200. A sample volume of 0.2 mL was analyzed for each sample at a flow rate of 0.145 mL/min. Particle counts were normalized by dividing the number of particles per sample by the total volume imaged per sample to obtain the particle concentration (#/mL). In addition to the samples agitated in syringes, samples of buffer solution and samples of the protein solution prior to filling in syringes were also analyzed by FlowCAM.

Break-Loose Force and Glide Force Measurements

Syringes were filled with 1.26 mL water for injection and stoppered. The force required to plunge each syringe was measured using an MTS Insight 2 material testing system instrument (Eden Prairie, Maine) fitted with a 250 N load cell. The crosshead was moved a distance of 35 mm at a speed of 260 mm/min. In addition to measuring the glide forces for syringes coated with silicone oil using the three methods described above, glide forces were also measured for bare glass syringes (with the silicone oil coating removed by the method described above) and for commercially available syringes siliconized by the manufacturer (“commercially-siliconized syringes”).

RESULTS

Particle Concentrations in Buffer Solutions and Protein Formulations in Siliconized Syringes

Particles (≥2 µm) were detected in a buffer solution agitated in syringes coated by the liquid silicone oil method, the baked silicone oil method, and the covalent method (Fig. 2). In syringes prepared by each of the coating methods, there was not a notable difference between the particle concentrations measured in buffer solutions agitated in the presence of an air bubble (Fig. 2a) and those measured in the absence of an air bubble (Fig. 2b).

However, in an agitated protein formulation, the number of particles generated varied depending on the coating method used to siliconize the syringe and was influenced by the presence of an air bubble (Fig. 3). In syringes coated by any of the three methods, protein formulations agitated in the presence of an air bubble (Fig. 3) had one to two orders of magnitude...
Figure 2. Particle concentrations as a function of agitation time in 10 mM L-histidine pH 5 buffer solutions agitated in syringes with different coatings in the presence (a) and absence (b) of an air bubble. The particle concentration for a buffer solution not agitated in syringes is also shown (solid black line). For comparison, the data for buffer solutions agitated in commercially-siliconized syringes are reproduced from Gerhardt et al.32

Figure 3. Particle concentrations as a function of agitation time in 3M formulations agitated in syringes with different coatings in the presence (a) and absence of an air bubble (b). The particle concentrations in a buffer solution (solid black line) and a protein solution (dashed black line) prior to filling in syringes are also shown. For comparison, the data for protein formulations agitated in commercially-siliconized syringes and in un-siliconized syringes are reproduced from Gerhardt et al.32

greater particle concentrations than did protein formulations agitated without an air bubble (Fig. 3). Without an air bubble, the particle concentration remained below approximately 10,000 particles/mL in a protein formulation agitated in syringes siliconized by any of the three coating methods.

The greatest differences between the three coating methods were seen in the particle concentrations of a protein formulation agitated with an air bubble. For protein formulations agitated in the presence of an air bubble in syringes with the covalent coating, the particle concentration was approximately 10,000–20,000 particles/mL and did not change appreciably with time. The particle concentrations were similar and also did not change with time in protein formulations agitated with an air bubble in syringes with the baked silicone oil coating. The particle concentrations observed in syringes with the covalent coating and the baked silicone oil coating are similar
to those observed in un-siliconized syringes. The highest concentrations of particles were observed in protein formulations that had been agitated with an air bubble in syringes with the liquid silicone oil coating. In this case, the particle concentration increased with time and reached an average value of approximately 400,000 particles/mL after 10 days of agitation. In summary, for protein formulations agitated in syringes in the presence an air bubble, the particle concentrations increased depending on the coating method, as follows: covalent method < baked silicone oil method < liquid silicone oil method. Flow-CAM images of particles detected in protein formulations that had been agitated in the presence of an air bubble in syringes coated using each of the three methods are shown in Figure 4.

**Break-Loose and Glide Forces in PFS**

The magnitude of the force necessary to expel solution from a syringe depended on the coating method used to siliconize the syringe. BD Hypak SCF 1 mL long 27G/2 syringes siliconized by the manufacturer and used without any modifications (commercially-siliconized syringes) had the lowest glide forces (Fig. 5). In addition, the force remained smooth and constant along the length of the syringe barrel. The glide forces increased slightly when the liquid silicone oil coating was used, and the force was not as smooth along the length of the barrel. The glide forces increased further in the syringes siliconized by the baked silicone oil method, and glide forces for syringes with the covalent coating were higher still. As expected, bare glass syringes exhibited the highest break-loose and glide forces because there was no lubrication on the syringe barrel.

**DISCUSSION**

**Mechanism of Particle Generation in Agitated, Siliconized Syringes**

Proteins have been shown to adsorb and gel at a variety of hydrophobic interfaces, including the silicone oil–water interface. Thus, it is likely that an interfacial protein gel layer is formed on the syringe surfaces coated by each of the three coating methods used in this study. In a previous study, we observed significant particle generation in two different protein formulations (containing either lysozyme or 3M monoclonal antibody) agitated with an air bubble in siliconized glass syringes, and we showed how silicone oil–water interfaces, air–water interfaces, and agitation contributed synergistically to create large numbers of particles in PFS. We proposed an interfacial mechanism of particle generation to explain these results, which can be summarized as follows: at even moderate concentrations, protein molecules adsorb rapidly (i.e., within seconds) to silicone oil–water interfaces, such as those present at the syringe wall, where they form gels. If a syringe is agitated, any air bubbles present in the syringe (typical filling and stopping processes leave a small amount of headspace within syringes) move along the siliconized syringe wall. At the three-phase contact line formed by the intersection of the silicone oil–water interface, the air–water interface, and the silicone oil–air interface, capillary forces fragment the gelled protein layer, generating mixed aggregates of gelled protein and silicone oil at the interface.

In the current study, when buffer solutions were agitated in siliconized syringes with an air bubble, there was no protein gel layer at the silicone oil–water interface to be disrupted, and thus, no differences in particle concentration were observed between the syringes coated by the different siliconization methods (Fig. 2). In addition, because there was no protein gel layer to disrupt in buffer solutions, the presence of an air bubble in agitated syringes had minimal effect on the number of particles generated, consistent with the previously proposed mechanism. However, for all of the coating methods tested, in syringes containing protein formulations, addition of an air bubble caused at least an order of magnitude increase in the particle concentrations observed after agitation (Fig. 3). This difference in particle concentration was attributed to the disruption of the protein gel layer and was not just because of shear forces induced by the movement of the air bubble in the syringe. In agitated protein formulations, the gelled protein layer at the silicone oil–water interface was ruptured when the air bubble moved along the syringe wall. Without the air bubble, this layer was not disrupted, and thus, fewer particles were observed in the bulk.

This silicone oil–water, air–water, and agitation synergism was also observed for an excipient-free antibody formulation and an excipient-free interferon e formulation agitated in the presence of covalently siliconized glass beads and an air–water interface. Similarly, in a study by Kiese et al. agitation and the presence of an air–water interface caused the formation of soluble aggregates, subvisible particles, and visible particles in a surfactant-free IgG1 formulation, but in the absence of the air–water interface, agitation alone did not cause protein aggregation or particle formation. For the same surfactant-free IgG1 formulation, stirring with a Teflon-coated stir bar in the absence of an air–water interface also caused the formation of particles. It is likely that stirring disrupted the gelled protein layer adsorbed at the Teflon–water interface causing particle formation. Furthermore, mechanical rupture and repeated compression/dilution of the air–water interface generated particles in two different antibody formulations, and this particle generation was attributed to disruption of the adsorbed protein layer.

**Coating Effects on Particle Generation in Agitated, Siliconized Syringes**

Protein formulations agitated in the presence of an air bubble in syringes coated by the liquid silicone oil method exhibited the highest particle concentrations of the three siliconization methods tested because the silicone oil layer adhered only weakly to the glass surface and could be easily removed with gelled protein by capillary forces at the three-phase contact line. This is consistent with observations that silicone oil coatings migrate along the syringe barrel and slough off the syringe wall because they are not adhered to the glass surface.

Covalent attachment of the PDMS to the glass surface reduced the number of particles, presumably because capillary forces at the three-phase contact line were insufficient to remove the covalently attached silicone oil. Particle concentrations in un-siliconized syringes were similar because there was no silicone oil layer that could be detached.

Furthermore, protein formulations agitated in the presence of an air bubble in syringes that were siliconized with the baked silicone oil coating had particle concentrations that were only slightly higher than those observed in un-siliconized syringes or in syringes treated with the covalent method. The baked silicone oil coating had a thin layer of silicone oil that was
Figure 4. An example of the particles observed in 3M formulations after 1 day of end-over-end rotation in the presence of an air bubble in (a) covalently-coated syringes, (b) baked silicone oil-coated syringes, and (c) liquid silicone oil-coated syringes. The scale bar is 20 μm.
Figure 5. Force profiles (load vs. extension) for plunger depression in: (a) commercially-siliconized syringes, (b) liquid silicone oil-coated syringes, (c) baked silicone oil-coated syringes, (d) covalently-coated syringes, and (e) un-siliconized (bare glass) syringes. Each line represents the force trajectory for one syringe.

covalently attached to the glass wall. On top of the covalently attached layer was a thicker layer of silicone oil where cross-linking had occurred between the PDMS chains. These two features resulted in a silicone oil layer that was more adherent to the glass surface than the liquid silicone oil coating, where silicone oil was simply deposited on the syringe wall without further modification to attach the silicone oil to the surface. Because it was better adhered to the surface, capillary forces at the three-phase contact line in the syringe were not sufficient to remove the baked silicone oil coating along with the gelled protein, and thus, less particles were generated in syringes with this coating. This is consistent with the lower particle concentrations that were observed in syringes lubricated with baked-on silicone oil compared to those with sprayed-on silicone oil.

In addition, although the total numbers of particles varied between siliconization methods, the shape of the particle size distributions was not discernably different (data not shown) among the different methods.

Figure 4 shows examples of the particles observed by flow microscopy in protein formulations agitated in syringes lubricated with each of the three types of coatings. In syringes siliconized using the liquid silicone oil method, there were particles composed of spherically-shaped silicone oil droplets, irregularly-shaped protein aggregates, and agglomerates of protein and silicone oil. These images were similar to those observed for protein formulations agitated in commercially-siliconized syringes in the presence of an air bubble. Similar images were also observed for protein formulations agitated in the baked silicone oil-coated syringes. The particles observed here do not contain just silicone oil or protein, but rather consist of agglomerates of protein and silicone oil. Thus, the use of shape analysis to attempt to differentiate silicone oil droplets from protein particles would be inappropriate, as noted previously. However, the spherically-shaped particles that are characteristic of silicone oil droplets were not observed in protein formulations agitated in syringes siliconized by the covalent method because the covalently-attached coating was bonded to the surface.

**Force Trajectories in Siliconized Syringes**

The purpose of the silicone oil coating in syringes is to provide lubrication to the syringe barrel during plunging of the syringe. The effectiveness of the coating to provide the necessary lubrication can be evaluated by measuring the forces in the syringe as solution is being expelled. In this study, the liquid silicone oil coating caused the greatest particle generation but provided the lowest glide forces. The glide forces observed with this coating were similar to the glide forces measured in syringes siliconized by the syringe manufacturer. The baked silicone oil coating significantly decreased the particle concentrations, but the glide forces were higher, in this case. It has been suggested that higher temperatures alter the bulk properties of silicone oil and affect its ability to lubricate and improve gliding. The covalently-attached silicone oil coating yielded low particle concentrations but had the highest glide forces of the three siliconization methods tested. Thus, it would likely


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