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

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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 antibody formulations in the absence 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 interface[4–6] and that, as a result, proteins may experience conformational perturbations or form aggregates.[6–11] Also, silicone oil droplets may leach from the syringe wall into the protein formulation.[12–16] 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.[12]

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,[17] and the application of silicone oil to a surface makes the surface hydrophobic because of the sidechain methyl groups of PDMS.[18] Below 150°C, silicone oil is chemically inert and resistant to decomposition.[18] 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.[19]

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.[12] 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.[20,21] 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.[1,22] 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,[15,18,23] 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.[24] On top of this thin bonded layer is another thicker layer of silicone oil where cross-linking occurs between the PDMS chains.[23] 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

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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.[25] 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.[26–31]

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 albinterferon α_{2b} formulation, simultaneous exposure to both silicone oil microdroplets and agitation induced greater monomer loss than exposure to silicone oil microdroplets or agitation alone.[11] 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.[6,8] The excipient-free albinterferon α_{2b} 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.[11] 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.[10] 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.[10] 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.[32] In that study, protein aggregates, silicone oil droplets, and agglomerates of protein aggregates and silicone oil were all observed.[32]

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.[32] 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.[32] 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).[33] 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.[32] 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 reagent 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.[32]

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

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