RESEARCH

Distribution of Silicone Oil in Prefilled Glass Syringes Probed with Optical and Spectroscopic Methods

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ABSTRACT: Prefilled glass syringes (PFSs) have become the most commonly used device for the delivery of recombinant protein therapeutics in parenteral formulations. In particular, auto-injectors preloaded with PFSs greatly facilitate the convenient and efficient self-administration of protein therapeutics by patients. Silicone oil is used as a lubricant in PFSs to facilitate the smooth motion of the plunger during injection. However, there have been few sophisticated analytical techniques that can qualitatively and quantitatively characterize in-situ the morphology, thickness, and distribution of silicone oil in PFSs. In this paper, we demonstrate the application of three optical techniques including confocal Raman microscopy, Schlieren optics, and thin film interference reflectometry to visualize and characterize silicone oil distribution in PFS. The results showed that a container coating process could produce unevenly distributed silicone oil on the glass barrel of PFSs. An insufficiency of the amount of silicone oil on the glass barrel of a PFS can cause stalling when the device is preloaded into an auto-injector. These analytical techniques can be applied to monitor the silicone oil distribution in PFSs.

KEYWORDS: Silicone oil, Prefilled glass syringe, Raman microscope, Interference reflectometry, Zebra Schlieren, Protein formulation, Drug delivery device.

Introduction

Prefilled glass syringes (PFSs) have become the primary container for protein therapeutics in parenteral formulation in recent years (1, 2). They offer several advantages over conventional primary containers such as glass vials. For example, PFSs eliminate several steps that are needed to inject a drug from the vial into a patient. They contain the exact dose to be injected, unlike in vials, which often are overfilled by up to 25% to ensure that the desired dose will be available for withdrawal and delivery, which may in turn lead to dosing errors. The precisely filled dose in PFSs also reduces the waste of precious protein therapeutics. The majority of protein therapeutics on the market is now contained in PFSs. Moreover, PFSs have been incorporated into auto-injectors for self administration of the drug by patients and for use in clinical centers for convenience and needle safety (3).

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In contrast to the many benefits PFSs present to patients and physicians, the change of primary container from glass vials to PFSs poses great challenges for formulation scientists and device and packaging engineers in the biopharmaceutical industry. The degree of complexity of a PFS and the auto-injector pre-loaded with a PFS is significantly increased compared to that of simple glass vials. Protein therapeutics in particular pose potential interactions between the protein and the additional materials that comprise the PFS. We have observed visible particulates that have formed due to the interaction of protein products with foreign matters left in PFSs during the PFS manufacturing process (4). For instance, metals used to make the syringe needle may generate iron rust, which could be hazardous to protein products. Residual tungsten left in PFS interacts with the protein to form visible particles and generate defects. Silicone oil is an essential material used as a lubricant for proper movement of the plunger in PFS. In contrast, vials are essentially free of silicone oil except for an extremely small quantity around the vial stopper for assembly purposes. Silicone oil has been observed to induce turbidity and particulate formation (5, 6) in insulin and other proteins (7).

Moreover, the performance of an auto-injector depends on the reduction of friction in the PFS using silicone oil.

It is, therefore, important to characterize the distribution of silicone oil in PFSs and to understand the complex interaction between protein therapeutics and silicone oil used in PFSs. In the present study, we investigate silicone oil distribution and its correlation with the function of PFSs and auto-injectors by using a number of optical techniques including optical microscopy, confocal Raman microscopy (8), Schlieren optics (9), and interference reflectometry (10). The results demonstrate that silicone oil could be unevenly distributed on the glass barrel of the PFS, or in extreme cases it could be completely absent in certain areas. The deficiency of and uneven distribution of silicone oil can result in the stalling of a PFS in an auto-injector. On the other hand, external force placed on excessive amounts of silicone oil localized in specific areas on the PFS can generate silicone oil microdroplets and contribute to particulate formation associated with proteins. These findings should be helpful to PFS manufacturers for improving the silicone oil coating process in PFSs and improving the overall quality of PFSs used in auto-injectors.

Experimental Design

Materials

PFSs with 1.0-mL barrels were obtained from a vendor without further treatment. (BD, Franklin Lakes, NJ) Syringes from three lots were selected as samples. Two lots were coated with silicone oil. However, one lot coated with silicone oil showed poor performance when the syringes were loaded onto the auto-injector for auto-injection testing. The other lot exhibited good performance. The third lot of syringes was not coated with silicone oil and was used to provide control samples. None of the syringes in these three lots were filled with product unless it was otherwise noted that a product-filled syringe had stalled during auto-injection testing. The product in the syringe was not emptied when it was examined using the Schlieren optical method.

Methods

We employed three optical techniques to visualize the silicone oil and measure in-situ the thickness of the silicone oil droplets. A confocal Raman microscope and Schlieren optics were used to observe the silicone oil droplets on the glass barrel. The Raman spectrum of silicone oil was used to confirm its identity. A visible reflectometry based on the light interference of transparent thin film (10) was applied to determine the thickness of silicone oil and its distribution along the longitudinal direction of the syringe barrel. The silicone oil coated on prefilled syringes could be treated as transparent thin film.

Optical Microscope: A Carl-Zeiss stereomicroscope (Stemi 2000C, Carl-Zeiss, Thornwood, NY) was employed to take micrographs of the empty glass syringes.

Confocal Raman Microscope: The optical microscope on the confocal Raman microscope (Senterra®, Bruker Optics, Billerica, MA) was employed to examine the silicone oil distribution, and the Raman module was used to confirm the identity of the silicone oil. Three objective lenses attached to the nosepiece with 20×, 50× and 100× magnification, corresponding to a spatial size of 5, 2, and 1 μm, respectively. Lower magnification objective lenses have a larger field of view and working distance, which enables ease of observation and sample handling. The total magnification is determined by the product of the objective lens and binocular eyepieces (10×). The syringe was placed on a XY-stage, which is precisely controlled by micrometers on the microscope. The light beam projected onto the sample syringe was vertically focused with a 4× objective lens.

The Raman spectrometer module accommodates two lasers for exciting Raman scattering: a red 785-nm diode laser and a green 532-nm green solid laser to facilitate the measurement of samples with different scattering natures. Variable laser power can be selected depending upon the sample signal. The maximum laser power is 100 mW for the 785-nm laser and 20 mW for the 532 nm laser. The spectral coverage is from 70 cm^{-1} to 3700 cm^{-1} with a spectral resolution of 4 cm^{-1}. Two special functions were implemented on the Raman microscope: the FlexFocus™ for confocal Raman detection and SureCal® for continuous automated frequency calibration. Both play an important role in the microscope's performance. The confocal function provides a significant improvement in both the contrast and the spatial resolution in the vertical (z) axis. The properly adjusted confocal optical configuration offers the capability of "optical sectioning" and depth profiling of heterogeneous and thin

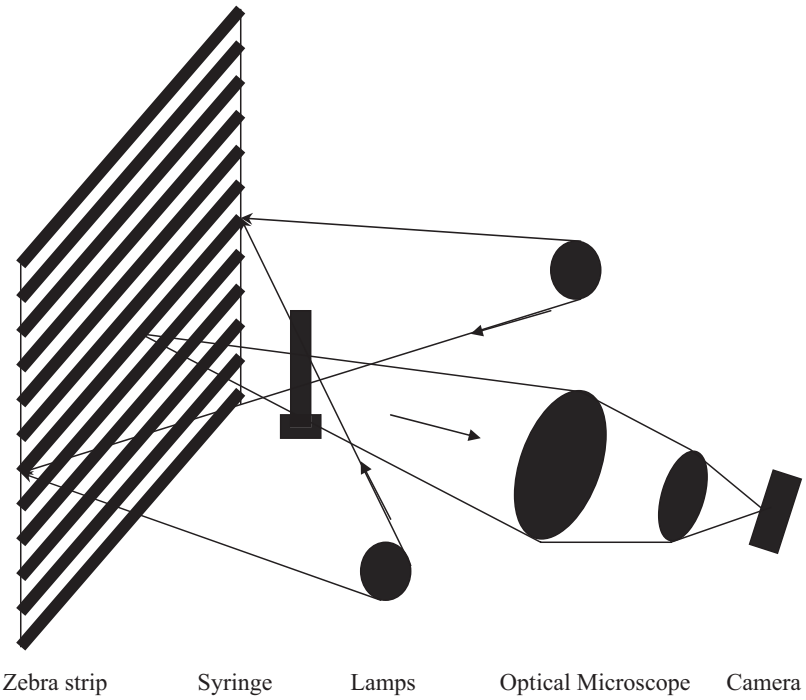

Figure 1

Schematic of a Zebra Schlieren optic set up for visualization of silicone oil in PFS. The light emitted from lamps is projected toward the Zebra strip passing through the syringe standing on the holder. The reflected light from the syringe is collected by the microscopic lens and a picture is then taken by a digital camera.

specimens, which is crucial for the successful observation of silicone oil micro-droplets on the glass barrel of PFSs.

Zebra Schlieren Visualization: A Zebra Schlieren optics technique was developed to visualize silicone oil on the glass syringe barrel. This technique was used to observe the optical inhomogeneity in transparent media that the human eye cannot directly see because of an extremely small difference of refractive index (9). It exploits the Schlieren phenomena in solid, liquids, and gases that have refractive-index gradients in one, two, and three dimensions due to inhomogeneity or resulting from temperature change, high-speed flow, or the mixing of dissimilar media. This is a qualitative visualization method to display the optical inhomogeneity of a material system. Schlieren optics has been widely employed in many sciences and engineering fields, including the detection of gas leaks, jet stream, and liquid flow (9). However, to the best of our knowledge, this is the first time that Schlieren optics has been applied to view silicone oil droplets on PFSs. The PFSs coated with silicone oil have a refraction-index gradient along the glass syringe barrel due to the presence of silicone oil droplets. Both silicone oil and the glass barrel are transparent to visible light; however, the density and refractive index of silicone oil and glass differ from each other in that the silicone oil used in PFSs as a lubricant has a refractive index of ~1.42 at 20 °C, while the refractive index of the glass barrel is close at ~1.5. The PFS coated with silicone oil constitutes a Schliere. The small difference between the refractive index of the silicone oil coated on the boronsilica glass barrel makes it difficult to see the silicone oil with the naked eye or under a low-resolution optical microscope.

The optical set-up to observe a Schliere is fairly simple, yet it can visualize the silicon oil distribution over the PFS in a practical and dramatic manner. Figure 1 shows a schematic of the optical configuration for the Schlieren visualization of silicone oil on the glass barrel. A zebra strip pattern was placed in front of a screen. This is the key component allowing the Schlieren optics to dramatically reveal micron-size

droplets of silicone oil on a glass barrel. A glass syringe was placed vertically on a syringe holder between the zebra strip and a high resolution digital optical microscope (model VHX-600, Keyence, Woodcliff Lake, NJ). Two projector fluorescence lamps were placed on the left and right sides of the digital microscope to illuminate the syringe. All other background light was minimized to reduce disruption. The images of the syringes on whose inner surfaces silicone oil had been distributed were taken with a digital camera attached to the optical microscope. All pictures were recorded on a computer without further processing.

Interference Reflection Spectroscopy: A rap.ID Layer Explorer (rap.ID Particle Systems GmbH, Berlin, Germany), based on the principle of light interference of thin film and reflection spectrometry, was employed for the measurement of silicone oil thickness. The Layer Explorer employs a beam of white light that is projected onto the glass syringe, and the reflected light from the thin oil film on the syringe surface is collected with an optical fiber probe (10). The interference pattern generated by the interaction of white light with the thin silicone oil film was calculated to determine thickness. The instrument has a precisely controlled and automated XY-stage. The syringe was laid horizontally on the syringe holder, which is fixed on the XY-stage. The software automatically divides the length of the syringe along the longitudinal direction to the number of measurement points and moves automatically along the syringe and collects the data on each point. It takes only a few seconds to measure one point. The size of the spot measured was ~100 μm. The detection limit of the thin film thickness was 50 nm. Below 50 nm the thickness was displayed as zero because the optical interference will not occur when the film thickness is much smaller than the wavelengths of visible light.

Results

Microscopic Image of Silicone Oil in PFS

Figure 2 shows the layout of a glass syringe on the confocal Raman microscope's XY-stage, this syringe having been selected from a "syringe lot" that showed low performance in the syringe injection test. The syringe was not filled with product solution. A total of 30 images were taken of one syringe and are numbered accordingly from the plunger side to the needle end along the longitudinal direction. We selected 15 out of

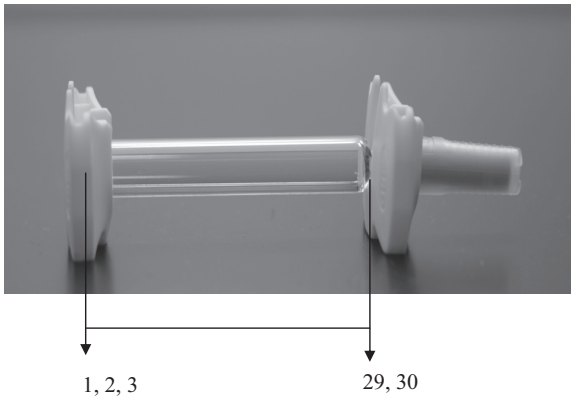

Figure 2

The layout of the syringe on the XY-stage of the Raman Microscope. Each number represents a 1.5-mm length of a segment of the syringe barrel of which a micrograph was taken. A total of 30 micrographs were taken sequentially with 15 of them exhibited in Figure 3.

the 30 images to show the silicone oil distribution on the barrel. Figure 3 shows the 15 individual images consecutively from the plunger side to the needle side of the glass barrel sections. Each picture corresponds to a 1.5-mm section of the syringe barrel. The silicone oil was seen as micro-droplets surrounded with a thin film layer, which under light illumination exhibited a typical thin film interference pattern. They resemble the Newton rings generated by a thin oil film on water under sunlight. These droplets and thin film layers were confirmed by Raman spectroscopy to be silicone oil. Figure 4 shows the Raman spectra of silicone oils on the syringe barrel and of a droplet of reference silicone oil on a glass slide. The Raman spectrum of the silicone oil droplets on the glass barrel was noisy, but the fingerprint pattern unambiguously confirmed that it was silicone oil.

The most striking observation was that the distribution of silicone oil on the barrel syringe was unevenly spread from one end (plunger side) to the other (needle side) throughout the syringe barrel. For the syringe examined, the first few segments of the syringe barrel on the plunger side contained much more silicone oil than did segments in other areas. It can be seen that there are many small silicone oil droplets that are surrounded with the thin layer. However, towards the needle end, there were fewer oil droplets with no thin oil film surrounding them. The last few segments at

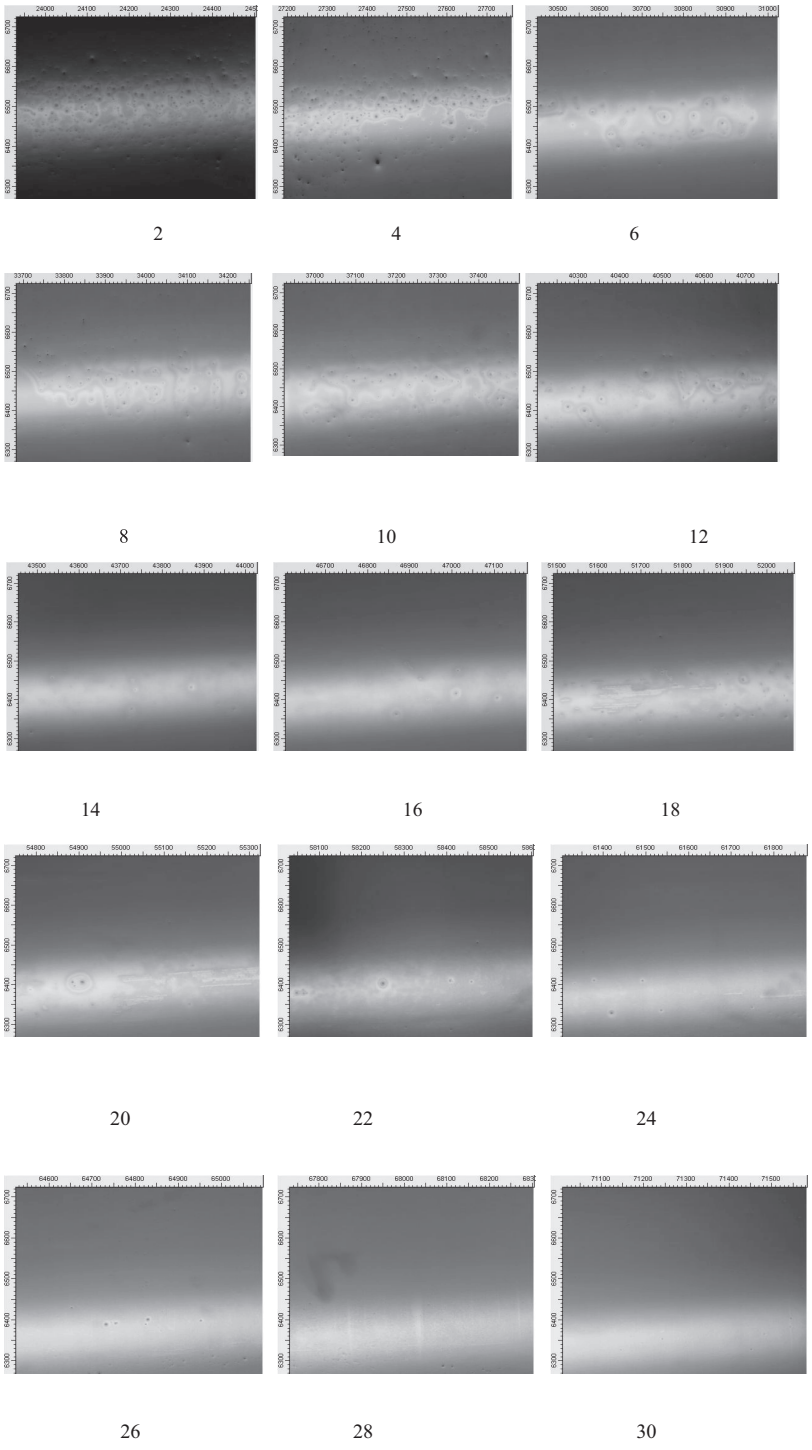

Figure 3

Micrographs of a glass syringe barrel obtained using the confocal Raman microscope. Each image from left to right represents a 1.5-mm section of the syringe barrel. An uneven distribution of silicone oil from the plunger side to the needle end can be seen.

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