Evaluation of the Effect of Syringe Surfaces on Protein Formulations

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Received 1 September 2010; revised 25 January 2011; accepted 26 January 2011

Published online 11 February 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22515

ABSTRACT: Packaging of drugs in prefillable syringes offers considerable advantages over conventional vials. Almost all major biotech molecules are available on the market today in prefilled syringes, and are safe and efficacious. Newer high-concentration liquid formulations, especially fusion proteins, however, can suffer from instability in prefilled syringes due to syringe components like silicone oil. To assess the effect of siliconized and modified syringe surfaces on protein formulations, the stability of the recombinant protective antigen (rPA) for anthrax, abatacept, a fusion protein formulation with known silicone oil sensitivity, and an antistaphylococcal enterotoxin B (anti-SEB) monoclonal antibody (mAb) was assessed in siliconized, uncoated, and BD-42-coated (a proprietary coating developed by BD Technologies) prefilled syringes under different conditions. Both the soluble protein content and the number of subvisible particles were followed over time. When filled in siliconized syringes, all three protein solutions showed increased number of subvisible particles relative to uncoated or BD-42-coated syringes; the abatacept formulation with known silicone sensitivity also developed visible particles. Although rPA and anti-SEB mAb formulations mainly showed individual droplets, presumably of silicone, the abatacept formulation also showed droplets entangled in a fibrous structure. Uncoated glass and BD-42-coated syringes considerably reduced the formation of both visible and subvisible particles after immediate contact and after agitation. The anti-SEB mAb also adhered as a thin layer to the siliconized surface after agitation, irrespective of storage temperature. The development of visible particles could not be correlated with the loss of soluble protein fraction at protein concentrations above 4 mg/mL. It appears that protein formulations interact differently with different surfaces. The BD-42 coating appears to be a promising solution for packaging silicone-sensitive proteins in prefillable syringes and needs to be investigated further. It is demonstrated that BD-42 provides an inert surface with adequate lubrication while limiting the formation of visible and subvisible particles. It is hypothesized that these particles are formed due to the release of silicone droplets in the solution and result in the formation of silicone-induced visible aggregates. J Pharm Sci 100:2563–2573, 2011

Keywords: biotechnology; protein aggregation; protein formulation; physical stability; materials science

INTRODUCTION

Prefillable syringes are the packaging of choice for biotech molecules and small molecule drugs because they allow rapid and accurate dosing, reduced dosing errors, reduced risk of microbial contamination, and dramatically reduced overfill. The biotech industry, in particular, has embraced the prefillable syringe with almost all major biotech molecules being available on the market today in prefilled syringes. Prefillable syringes have been perfectly acceptable as a primary container for the vast majority of therapeutic proteins; all of the proteins that are currently on the market in prefilled syringes are stable in this format. Recently, however, subvisible particles in the prefilled syringes resulting from the sloughing of silicone

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Journal of Pharmaceutical Sciences, Vol. 100, 2563–2573 (2011)

oil into the bulk over time or under agitation have come under scrutiny from the scientific community and the regulatory agencies. Visible and subvisible particles have become a cause for concern due to their immunogenic potential. It has been suggested by Carpenter et al.[1] that aggregates exhibiting native-like conformation are potentially immunogenic. An immune response against a protein therapeutic can result in dose neutralization leading to subpar therapy. In more severe cases, these immune responses can result in allergic reactions and at its worst, in the case of injected proteins that are also naturally present in the body, can trigger an autoimmune disorder.[2,3] In a response formulated by Singh et al.,[4] industrial scientists contend that the link between aggregates and clinical immunogenicity has not been unequivocally established; furthermore, they emphasize that such subvisible particles are and have been present in products that have been on the market for years and that these remain safe and efficacious.

Formulators in various laboratories have worked on assessing the effect of syringe components on their protein formulations. Through these studies, it has emerged that sensitivities of proteins to surfaces are varied and very subjective. Several formulators have evaluated syringe components such as silicone oil,[5] tungsten,[6] and leachables from rubber stoppers[7] as potential sources of incompatibilities. Silicone oil-induced protein instability has probably received the maximum scrutiny in the recent literature as a relatively large silicone oil-coated surface is exposed to the formulation.[5,8,9] Jones et al.[5] suggest that only at solution concentrations greater than 5 mg/mL, silicone oil can induce aggregation in proteins, significantly above the 0.3–0.7 mg/syringe that are realistically present in a prefillable syringe. Thirumangalathu et al.[8] suggest that although silicone oil adsorbs an IgG1, it induces aggregation only in the presence of another stressor like agitation. Ludwig et al.[9] corroborate the adsorption of proteins on silicone oil droplets in an emulsion system and suggest that the effect of silicone oil in the bulk of the formulation is minimal. These reports that focus solely on the effect of silicone do not suggest a direct role of silicone at concentrations typically found in prefillable syringes in inducing aggregation. At this time there does not seem to exist much literature on the effect of glass-adsorbed silicone oil interface on the stability of protein formulations.

In order to reduce the number of injections, for patient convenience, formulators have tried to increase the concentration of therapeutic proteins in solution; at times, preparing formulations with protein concentrations close to its solubility limit. In such formulations, the influence of syringe components on the protein solubility may be amplified and may result in instabilities. As we show in this article, these pipeline drugs may not be put into prefillable syringes as the silicone lubricant can be stripped off causing higher levels of subvisible particles. Silicone-induced visible particle formation has also been suggested in some fusion proteins that are inherently unstable.[10] For these formulations, there exists a need for a syringe lubricant that sheds fewer particles and provides an inert surface for the protein formulation. In response to this need, BD (Franklin Lakes, NJ) has developed a novel syringe coating, BD-42, which is designed to reduce the formation of particles, act as a lubricant, and provide an inert surface for the protein formulation. The experiments reported here were undertaken to compare the effect of the syringe surfaces (silicone oil, glass, and BD-42 coating) on protein formulations at low concentration [recombinant protective antigen (rPA) at 150 μg/mL], at medium concentration [antistaphylococcal enterotoxin B (anti-SEB) monoclonal antibody (mAb) at 4.9 mg/mL], and high concentration (abatacept at 25 mg/mL). The three proteins studied are very different in size and structure. They were chosen to represent the various types of proteins that comprise protein therapeutics. The rPA is a 82.5 kDa nonglycosylated protein, whereas abatacept is a 92 kDa homodimeric fusion protein with substantial heterogeneity associated with N- and C-oligosaccharides, and N- and C-terminals; the anti-SEB mAb is a 150 kDa mononclonal antibody. Abatacept also represents a relatively small class of fusion proteins that have silicone sensitivity. The results provide an understanding of protein aggregation and subvisible particle formation in protein formulations filled in prefillable syringes and the contributions of factors such as protein concentration, temperature, agitation, and duration of storage.

MATERIALS

BD HYPAK™ syringes [1 mL, long, Sterile, Clean, Ready to Fill (SCF)] with tip caps henceforth referred to as SGS (siliconized glass syringes), nonsiliconized glass barrels (1 mL, long, SCF) without through-hole for the needle, henceforth identified as NSGB, and nonsiliconized glass syringes (1 mL, long, SCF) with needle and tip caps were provided by BD Medical Pharmaceutical Systems (Le Pont de Claix, France). The latter were coated with BD-42, a proprietary nonionic coating that is neither hydrophilic nor very hydrophobic (less hydrophobic than silicone as measured using water contact angle measurements) developed at BD.[11] The coated syringes were rinsed with a mild jet of (~15 psi) distilled water for injection prior to being used for stability studies. The coating was not affected in any way by rinsing (data not shown).

Recombinant protective antigen (rPA) was purchased from Vaxgen (South San Francisco, CA). For

the stability study, rPA was used at a concentration of 150 μg/mL in a 20 mM Tris buffer (pH 7.4) formulation containing 0.9% (w/v) sodium chloride and 0.01% (w/v) polysorbate 80. Syringes were filled with 1.2 mL of rPA formulation filtered through 0.2 μm, stoppered with nonsiliconized stoppers W4023 with Flurotec®coating (Daikyo, Tokyo, Japan) leaving a head space of about 3–5 mm and maintained either at 25°C or at 2–8°C (under refrigeration) for 3 months. The syringes were sealed on the needle end by tip caps. Initial samples (in sets of three) were drawn and analyzed after 6 h and subsequent samples after 7, 14, 17, 35, 63, and 91 days. After 14 days of incubation, six syringes were shaken horizontally with a displacement of 1 cm along the axis of the syringe, for 3 days at 6.66 Hz while being maintained at room temperature (23–25°C). Three samples were analyzed immediately after shaking on day 17 (labeled as D17SH) and another set of three on day 35 (D35SH) after storing at either 2–8°C or 25°C for an additional 18 days. At each time point of analysis, all the samples were checked for the presence of visible particles. For analysis at each time point, rPA formulations were flushed out of the syringes into 1.5 mL centrifuge tubes. Stopper removal and decanting was necessary for NSGBs.

Abatacept was purchased as a lyophilized powder from McKesson Specialty Care Solutions (La Vergne, TN) as Orencia®. Prior to filling the syringes, the powder in the vial was reconstituted, according to the manufacturer's instructions, with 10 mL of water drawn from a freshly opened bottle of Water for Injection, United States Pharmacopeia (USP), using a silicone-free syringe provided by the manufacturer. After complete dissolution (examined visually), the protein solution was filtered though a 0.2 μm polyvinylidene difluouride filter and used at a nominal concentration of 25 mg/mL. Each milliliter of the solution contained 25 mg abatacept, 50 mg maltose, 1.72 mg monobasic sodium phosphate, and 1.46 mg sodium chloride. 1 mL of the solution was filled in each syringe and stored at 25°C for 91 days. Sampling time points and treatment of samples was similar to that described under rPA.

Antistaphylococcal enterotoxin B mAb 6469 was purchased from MorphoSys (Martinsried/Planegg, Germany). Samples were received from MorphoSys in 1× Dulbecco's phosphate buffered saline buffer without $CaCl_2$ and $MgCl_2$ (Catalog #14190094, Invitrogen, Carlsbad, CA). The specific mAb 6469 is a 150 kDa human IgG1 (kappa) with a calculated pI of 8.59. Similar antibodies have been recently described by Larkin et al.[12] For the stability study, the buffer was exchanged to a 20 mM phosphate buffer at pH 7.4 using dialysis. After four buffer exchanges lasting for 3 h each, the final antibody concentration was determined to be 4.9 mg/mL. Samples were stored at 25°C and 2–8°C for 17 days. After storage for 14 days, samples were shaken as described above. Only shaken samples were analyzed on day 17. Sampling time points were 1 h and 7, 14, and 17 days. Samples were treated similar to the rPA samples.

ANALYTICAL METHODS

It is well established that no single analytical technique is suitable for analysis of all types of protein aggregates that may range in size from few nanometers to more than 100 μm. Therefore, each sample was analyzed using size-exclusion chromatography (SEC), which is suitable for analysis of monomers and soluble aggregates smaller than 220 nm, dynamic light scattering (DLS), which can yield qualitative information about the presence of monomers and aggregates smaller than 1 μm, and micro-flow imaging (MFI), which can count the concentration of particles (#/mL) and measure the size of the particles in the micron range.

Size-Exclusion Chromatography

Size-exclusion chromatography was used to determine the fraction of soluble protein in the formulation. An Agilent 1200 Series LC system with ChemStation software (Agilent Technologies, Santa Clara CA) was used. Samples were filtered through 0.2 μm filters before SEC analysis. A Tosoh TSK-Gel 3000SW column (7.5 mm ID × 60 cm, 10 μm; Tosoh Bioscience LLC) was used with a mobile phase comprising of 90 parts of a mixture of 100 mM sodium phosphate and 300 mM sodium chloride, and 10 parts of isopropyl alcohol. The flow rate was 0.65 mL/min and detection was set at 280 nm.

Dynamic Light Scattering

Dynamic light scattering analysis was done using the Wyatt DynaPro DLS Plate Reader (Wyatt Technology Corporation, Santa Barbara, CA) to detect the presence of large molecular weight aggregates in protein solutions that may not be detected by MFI or SEC. An average of three measurements was reported for each sample. Individual protein solutions were used as they are or diluted as described: rPA 150 μg/mL (as it is); Orencia and anti-SEB mAb solutions were diluted to 5 mg/mL and 1.16 mg/mL, respectively, with 0.2 μm filtered Water for Injection, USP.

Micro-Flow Imaging

Subvisible particle analysis was carried out using MFI system (DPA-4100) from BrightWell Technologies (Ottawa, ON, Canada). The protein solution was introduced into the sampling port using a pipette tip-like sample holder. Approximately 0.3 mL of the solution was predispensed to generate a background free of Schlieren effects and then 0.3 mL of the solution

was actually analyzed for particle concentration (#/mL). The particle sizes were calculated as the effective circular diameter and used for the particle concentration analysis (number-based).

Break-loose and gliding forces were measured using an Instron 5500R Universal Testing Instrument (model 1122, Instron, Norwood, MA). Each syringe was filled with 1 mL of water for injection and the forces were measured as the stopper and plunger rod were displaced through a distance of 25 mm at a crosshead speed of 380 mm/min.

RESULTS

The observations made during this study were very formulation specific. The development of subvisible particles was particularly easy to detect in protein solutions with low concentration (i.e., the rPA solutions). Development of visible particles due to silicone oil sensitivity was more apparent in abatacept formulations than any other. In anti-SEB mAb formulation, the silicone oil sensitivity was manifested only under stressful conditions.

Development of Subvisible Particles in rPA Solutions Following Filling in Prefillable Syringes

It is clear from Figure 1, after comparing bars at $t = 0$ (formulation prior to filling) and $t = 6$ h (postfilling) that rPA solutions filled in NSGB do not show any increase in the number of subvisible particles. This suggests that the contribution of the glass barrel to the subvisible particle count in the rPA solution is insignificant. In contrast, the subvisible particle count in the rPA solutions filled in SGS syringes increased dramatically from approximately 583 to 12,950 ± 6539/mL. This is suggestive of silicone oil sloughing into the bulk of the solution. When this information is taken together with the near complete recovery of soluble protein (Fig. 2) at $t = 6$ h, it can be surmised that a significant number of the subvisible particles are comprised of silicone oil. The rPA solution filled in BD-42-coated syringes showed a modest increase in the number of subvisible particles from approximately 459 to 4121 ± 905/mL, suggesting that the BD-42 coating contributes significantly fewer ($p < 0.1$, $n = 3$) particles to the bulk of the protein solution when compared with sprayed silicone lubricant.

With increasing storage times, the amount of soluble protein decreases (~67% of the initial after 3 months at 25°C) independently of the syringe system suggesting that although silicone oil may contribute to the immediate increase in subvisible particles, it does not have any notable impact on the long-term storage stability of rPA.

Development of Visible Particles in Abatacept Solution Filled in Siliconized Syringes

Abatacept is a fusion protein with identified silicone sensitivity. Therefore, it was studied to assess the behavior of silicone-sensitive proteins when filled in prefillable syringes. When the abatacept solution was filled in SGS syringes, all the 21 syringes developed

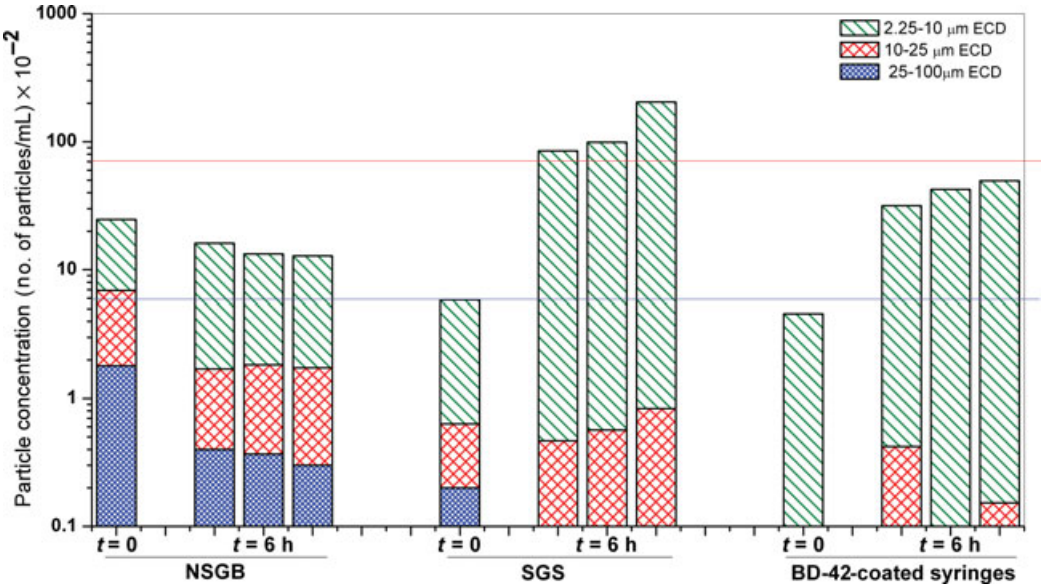

Figure 1. rPA solution prior to filling ($t = 0$) did not show any increase in particle concentration after filling in NSGBs after 6 h ($t = 6$ h) of storage at 2–8°C. A notable increase was observed in solutions filled and stored in SGS. In solutions filled and stored in BD-42-coated syringes the increase in subvisible particle concentration was moderate. Size is represented as effective circular diameter (ECD). Horizontal lines that represent 600 and 6000 particles/mL, respectively, are provided as references.

 DOI 10.1002/jps

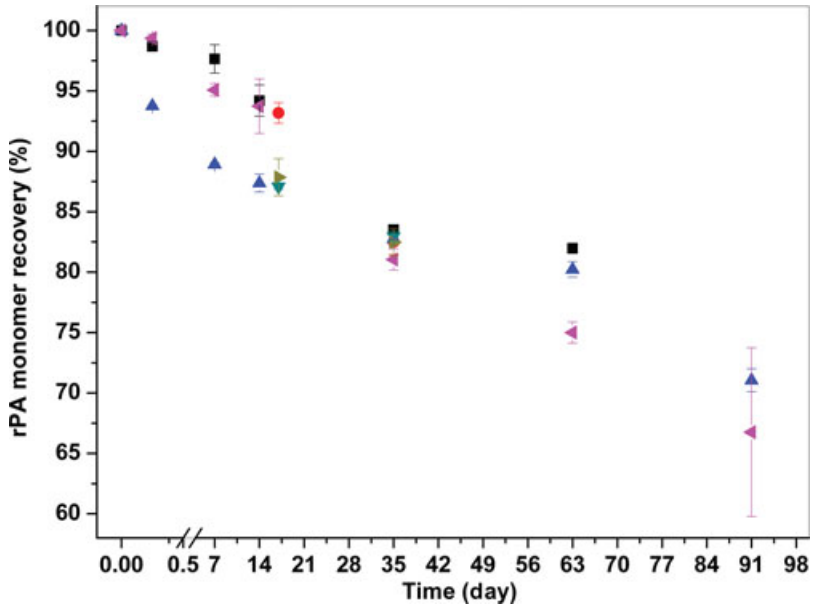

Figure 2. Soluble protein recovery as measured using SEC showed that about 67% of the protein was recovered after 3 months when stored at 25°C. The recovery was independent of the syringe system. SGS nonshaken (■); SGS shaken (●); NGSB nonshaken (▲); NGSB shaken (▼); BD-42-coated nonshaken (◄); and BD-42-coated shaken (►).

visible particles (Fig. 3a) within 20 min of filling. Also, the subvisible particles comprised presumably of silicone oil droplets (Fig. 3b) and silicone droplets with adsorbed particles (Figs. 3c and 3d). This striking observation is a reflection of the silicone-sensitivity of abatacept. The development of visible particles did not correlate with a measurable loss of soluble protein as measured using SEC (data not shown). Abatacept solutions stored in NSGB and BD-42-coated syringes, however, did not show immediate development of visible particles. Abatacept is provided as a lyophilized powder and is prone to developing visible particles when stored in solution state at 25°C regardless of the container. The rate of development of visible particles, however, is the slowest in glass containers. Therefore, BD-42-coated syringes were compared with NSGBs. Interestingly, the rate of development of visual particles in the BD-42-coated syringes was similar to that in NSGB (Fig. 3e). Both these observations highlight the ability of the BD-42 coating to reduce the formation of particles relative to silicone that can induce the formation of visible particles.

Trends in Subvisible Particle Counts in Abatacept Formulations

Figures 4a and 4b show the counts and size distribution of subvisible particles in abatacept solutions filled in SGS and BD-42-coated syringes. It can be seen that the particle concentrations show large variations due to syringe-to-syringe variability. We hypothesize that these variabilities are at least in part related to how the syringe was made, right from syringe forming, to cleaning and application of the lubricant. Therefore, particle concentration from each syringe was presented as one single bar to capture the inherent variability in the system and not as an average with error bars. Although the variability is high, it is real. The variability due to the MFI measurement was less than 5%.

Subvisible particle content analysis of initial abatacept solutions showed the presence of hardly any particle greater than 25 μm (blue bars). Upon contact with all syringe surfaces for 7 days, the particle concentration increased; the extent of increase, however, was greater in the silicone-coated SGS syringes (29,248 ± 24,153/mL) than in the BD-42-coated syringes (7446 ± 6865/mL). Over the long term, this trend persisted; that is, in abatacept solutions the total particle concentration (#/mL) in the size range of 2.2–100 μm was significantly lower when stored in BD-42-coated syringes than when stored in SGS syringes ($p < 0.01$, $n = 20$). Most striking are the decreased concentrations of 25–100 μm sized particles in the BD-42-coated syringes over the entire storage period. In addition, shaking did not significantly affect the particle concentration and size distribution in abatacept solutions stored in BD-42-coated syringes, whereas in siliconized syringes, shaking resulted in an increased count in the 10–25 μm and 25–100 μm size ranges. Further analysis with DLS (Figs. 5a and 5b) corroborated these findings as seen by the presence of larger species (box with dashed border) in solutions stored in siliconized syringes and the absence of larger species in solutions stored in BD-42-coated

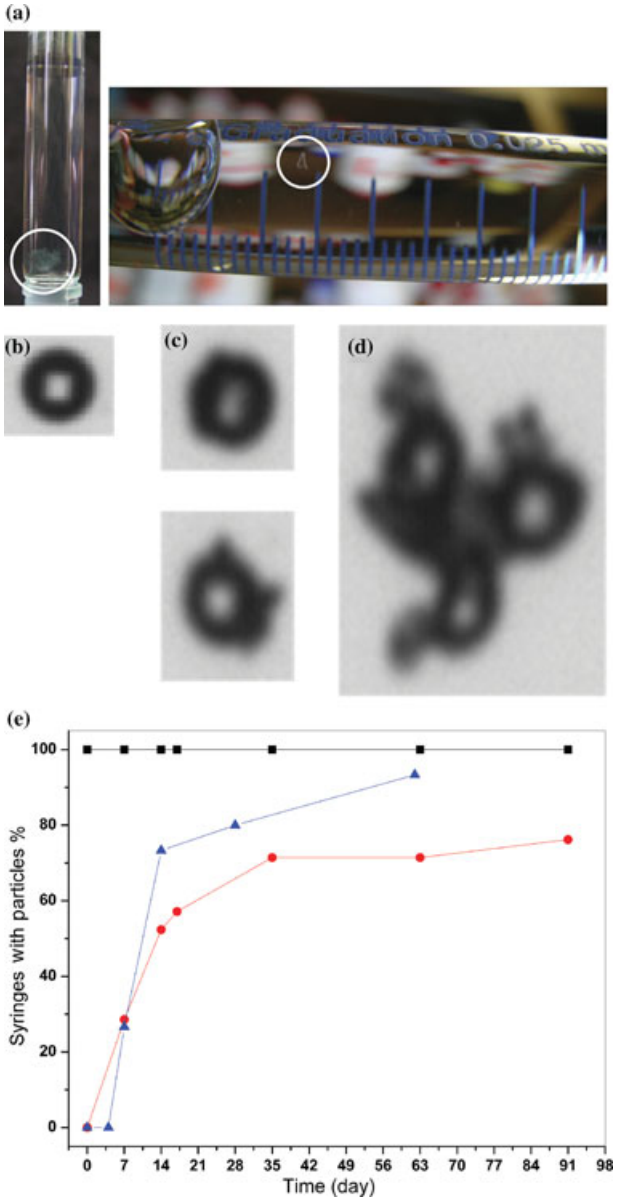

Figure 3. (a) Visible particles (encircled) seen in SGS syringes filled with the abatacept solutions. All the syringes showed visible particles at 20 min after filling. Subvisible particles observed in the abatacept solution ranged from (b) silicone droplets to (c) silicone droplets with adsorbed particles and to (d) agglomorates of silicone droplets with adsorbed particles. (e) Development of visible particles in the abatacept solution stored in various prefilled syringes. Standard SGS syringes immediately developed particles (■). The rate of development of visible particles in BD-42-coated syringes (●) is similar to that in NGSB (▲), much slower than that in SGS.

syringes. These data reflect the ability of BD-42 to reduce the formation of particles.

Manifestation of Silicone Sensitivity of an anti-SEB mAb Under Stressful Conditions

To gain a further understanding of the interaction of proteins with different syringe surfaces, the stability of anti-SEB mAb was also studied in prefilled syringes. This mAb is known to undergo reversible aggregation process and is projected to have a pI of 8.6. The antibody solutions filled in all three syringe systems were found to be fairly stable through the first 14-day storage period. Upon shaking, however, solutions stored in SGS formed a white layer on the interior surface of the syringe (Fig. 6). No such layer was formed in solutions stored in NSGB and BD-42-coated syringes. These data suggest that the siliconized surface of the SGS can potentiate the destabilizing influence of a stress treatment such as shaking on protein formulations. It may also be a function of being formulated at pH 7.4 close to the calculated p*I* of 8.6 of the mAb because such instability was not observed when the mAb was formulated at pH 4 (data not shown). Thus, several stress factors may combine to result in such instability. Unlike the abatacept formulations, the formation of a white film correlated with the loss of two aggregate species that accounted for about 2% of the total soluble protein (Fig. 7). Interestingly in this case, the subvisible particle concentration was also significantly increased in solutions that showed the visible film (Fig. 8). Even more striking is the dramatically increased concentration of particles greater than 25 μm in size (blue bars; 46,360 ± 43,288/mL in SGS relative to 3557 ± 5463/mL in BD-42-coated syringes). For the formulation at pH 7.4, these effects were dependent on shaking and independent of the storage temperature.

Lubrication of Prefillable Syringes

BD-42 was developed as an alternative to the silicone lubricant; without lubricant properties of its own it cannot function as a viable alternative. Therefore, lubricant properties of SGS and BD-42 were compared. Figure 9 shows that the break-loose force for a SGS is typically between 5 and 7 N and the gliding force is between 7 and 10 N when filled with water. The forces can be higher depending on the viscosity of the filled solution. It can be seen that the break-loose force for BD-42-coated syringes is between 7 and 9 N and the gliding force is between 9 and 11 N, well below the 15 N target, providing a viable alternative to the silicone oil lubricant.

DISCUSSION

The results suggest that silicone oil lubricant contributes to the subvisible particles. It is conceivable that silicone droplets released into the solution can provide a nucleation site for the aggregation of the proteins. Although silicone oil is known to slough into even Water for Injection, it is suggested that the surface-active nature of proteins causes more silicone to be released into the bulk. The relatively high concentration of abatacept may emulsify more silicone

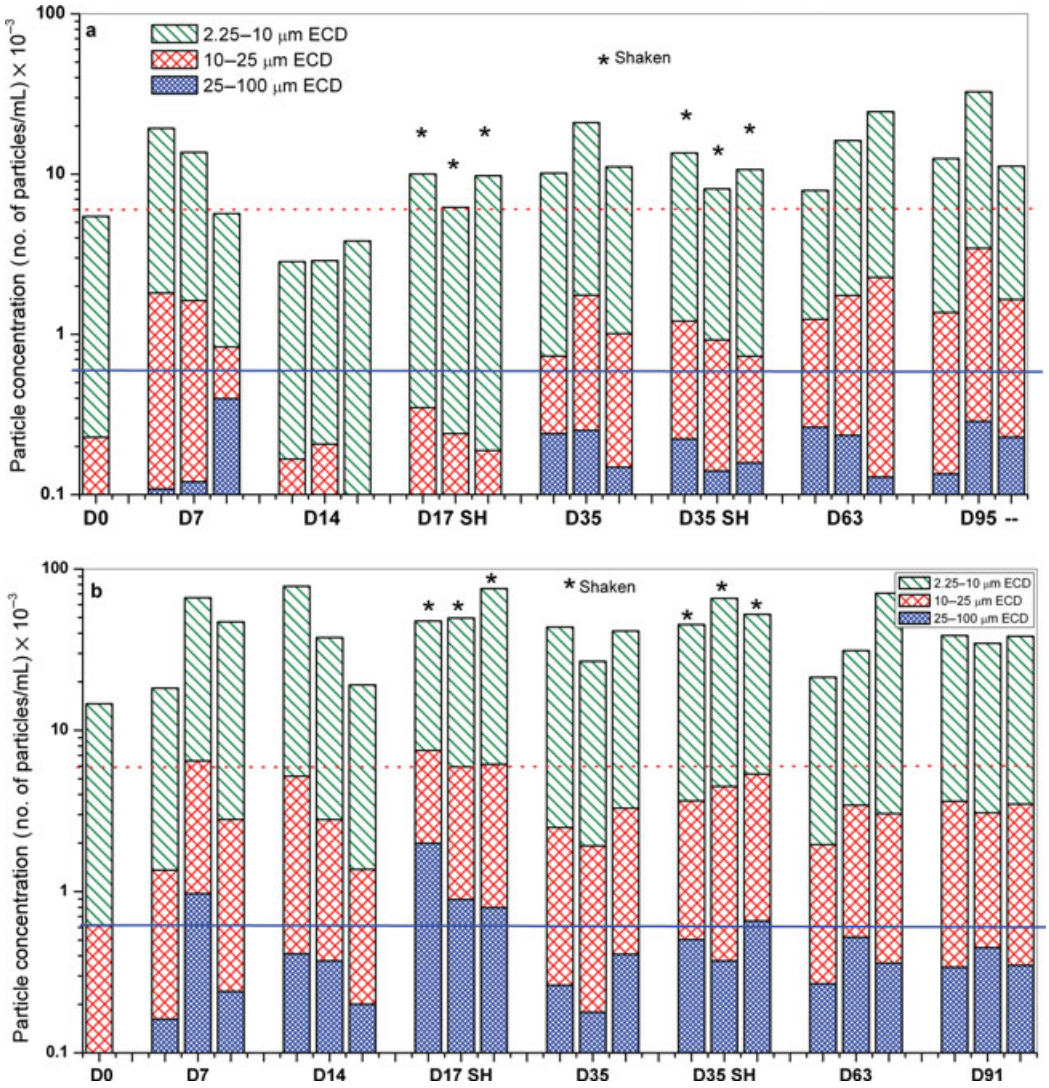

Figure 4. Subvisible particle concentration in abatacept solutions stored at 25°C in (a) BD-42-coated syringes and (b) SGS. Size is represented as effective circular diameter (ECD). Horizontal lines that represent 600 and 6000 particles/mL, respectively, are provided as references.

oil from the surface. Shaking resulted in an increase in particle concentration that can be attributed to both silicone particles released from the syringe surface and aggregation of protein due to the shear effect. This increase in subvisible particle content in itself does not appear a cause for concern as several protein-based therapeutics have been on the market in a prefilled syringe format and have been safe and efficacious.[4]

In some complex formulations, silicone oil can have a destabilizing effect. In this study, the destabilizing effect was manifested in different ways with each protein studied, sometimes as a conglomerate of subvisible droplets with fibrous strands as in the case of abatacept and at other times as visible particles in abatacept and anti-SEB mAb. The effect, however, may be equally complex and may depend on multiple factors in addition to silicone oil. In case of anti-SEB mAb, in which multiple formulations were studied, the effect was seen only at a pH close to its p*I* and after shaking. Therefore, it is suggested that silicone oil sensitivity may very well be as much dependent on formulation conditions and other stressors as the silicone oil.

Earlier studies have taken the approach of spiking protein formulations with silicone oil to study silicone sensitivity, often using quantities of silicone in large excess of what is encountered in commercial prefillable syringe systems.[5] Under such circumstances, the likelihood of inducing an artifactual silicone sensitivity, which may otherwise never be observed in a real system, is quite likely. Therefore, in these studies, prefillable syringe systems, specifically the siliconized syringes, were used in their unmodified form, which is with silicone oil at levels typical of real-world conditions.

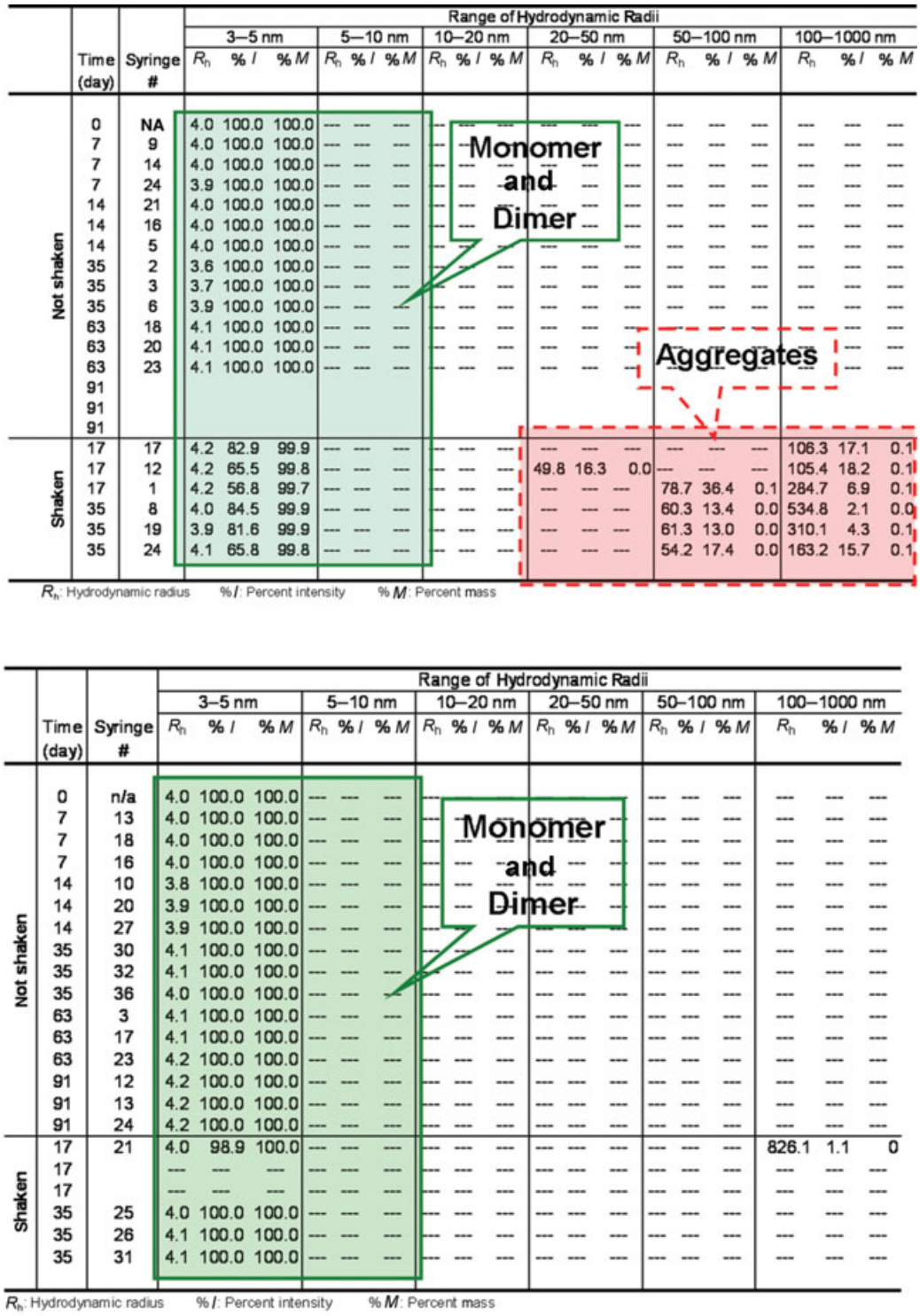

Figure 5. (a) DLS data showed that shaking the abatacept solution in SGS syringes resulted in the development of higher order aggregates (radius ≥ 10 nm), highlighted by the box with dashed border at the bottom right. Monomer and dimer content is highlighted by the box with solid border (radius ≤ 10 nm). (b) DLS data showed that shaking the abatacept solution in BD-42-coated syringes did not lead to the formation of higher order aggregates (radius ≥ 10 nm) as characterized by only the presence of monomer (box with solid border, radius ≤ 10 nm, includes dimers) and the lack of aggregates (box with dashed border seen in Fig. 5a).

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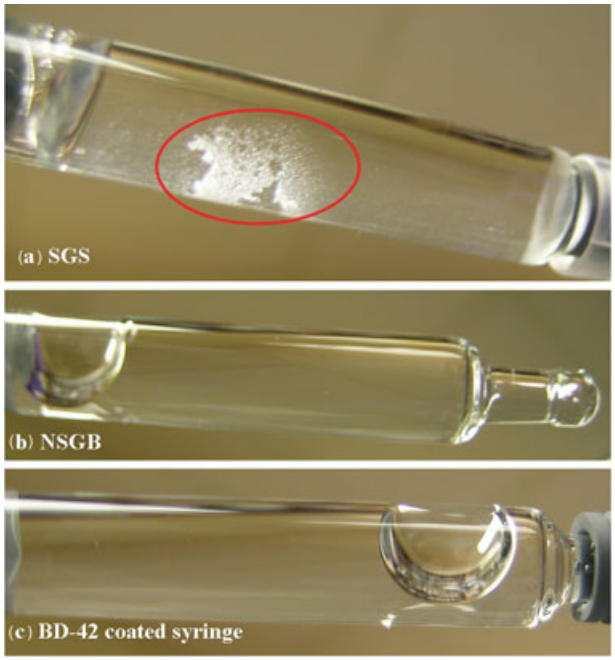

Figure 6. (a) After shaking, only SGS syringes show the development of a film on the syringe surface. (b) NGSB and (c) BD-42-coated syringes do not show any film formation.

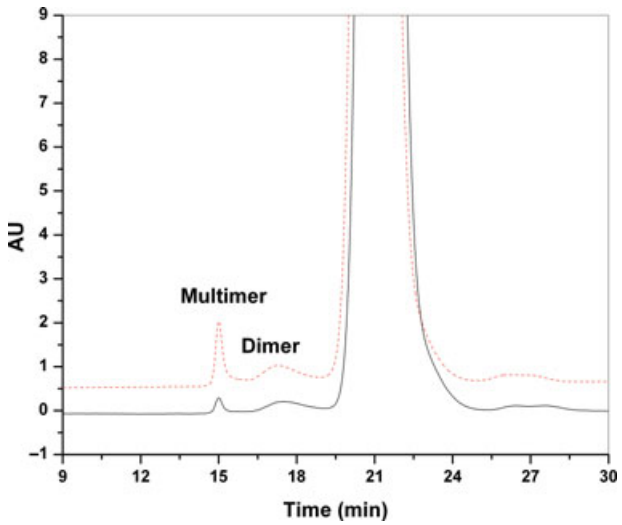

Figure 7. SEC profiles of anti-SEB mAb solutions show the loss of the multimer and the dimer in the initial sample (14 days —-) after shaking (17 days _____). Monomer content was unchanged. Chromatograms are offset by 0.5 AU and 0.2 min.

Traditionally, SEC has been a workhorse for formulation scientists and used as a stability indicating assay for quantitating aggregation.[13,14] As is apparent from the abatacept formulations, SEC does not always provide sufficient sensitivity for quantifying the protein loss due to particle formation because the protein aggregates and subvisible particles amount to an extremely small fraction of the total protein mass that cannot be detected using SEC. To gain perspective, 1000 spherical particles with a diameter of 4 μm will constitute a mass of only 0.033 μg (assuming density of 1 gm/cc). It is difficult to detect such a small change in mass using SEC in a protein formulation at a concentration of 25 mg/mL. Formulation scientists have, over the years, recognized the complex nature of proteins themselves and their interaction with containers and closures, and have expanded their arsenal of analytical techniques used to characterize the product. In this study, it was found that MFI, a technique relatively new to the pharmaceutical formulator, provided great value in imaging some of the aggregates formed and characterizing the behavior of silicone oil used in prefillable syringes. For instance, the rPA monomer content, as measured using SEC, was not influenced by whether the formulation was stored in SGS, NSGB, or BD-42-coated syringes. MFI, however, clearly showed the formation of additional particles in the formulation stored in SGS. In addition, the ability to clearly image particles above 20 μm was very valuable.[15] The results presented here clearly demonstrated that substantial information can be derived regarding the stability of protein formulations and their compatibility with storage containers by the use of techniques aimed at measuring particle size, concentration, and assessing their shapes. Characterization of protein aggregates in the size range of 0.1–10 μm still continues to be a challenge due to the lack of appropriate analytical techniques to quantitate and characterize them. It is hoped that future generations of the imaging instruments may help characterize even these smaller particles well. Next, analytical capabilities that allow the identification of the particles, for example, distinguishing proteinaceous from nonproteinaceous matter, will provide a significant boost to our ability to thoroughly characterize drug formulations and their interactions with containers and closures.

Historically, silicone oil has been an established lubricant in medical devices including prefillable syringes due to its safety. Its properties enable the formation of a seal between the stopper and the barrel, and lubricate the gliding of the stopper on the syringe barrel for the proper functioning of prefilled syringes. It is only recently, with the development of fusion proteins and complex formulations that incompatibilities associated with silicone oil have been discovered. To mitigate these sensitivities, BD-42 was developed as an alternative lubricant coating. Initial tests on the novel BD-42 coating yielded results that suggest that BD-42 coating not only provides an inert surface that does not interact with the protein formulation but is also lubricious. The BD-42 surface is hypothesized to present an electrically neutral and much less hydrophobic surface than silicone oil to the protein solution, thus preventing any aggregation due to charge interaction with the protein molecules. Lack of visible

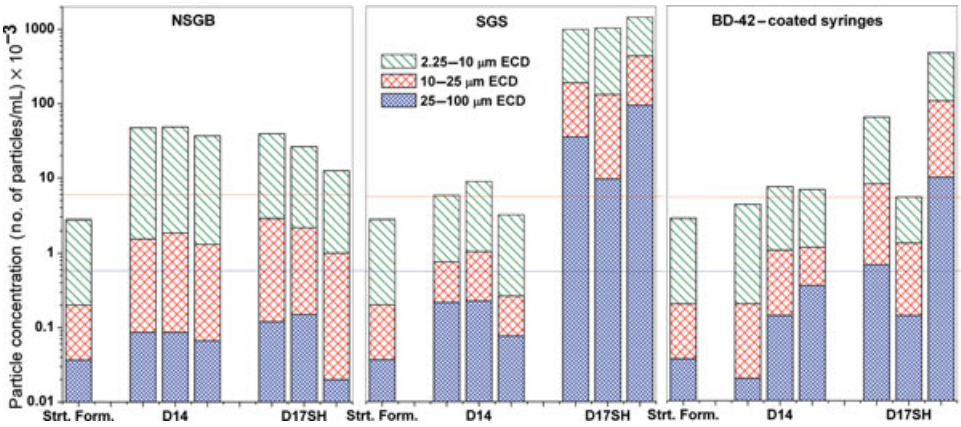

Figure 8. Subvisible particle concentration in anti-SEB mAb solutions (pH 7.4) stored in NGSB, SGS, and BD-42-coated syringes at 2–8°C. Samples were shaken for 3 days at 400 rpm horizontally along the axis of the syringes. Shaking tremendously increases the subvisible particle content for the SGS syringes. Increase in particle content was noticeably less in BD-42-coated syringes. Size is represented as effective circular diameter (ECD). Horizontal lines that represent 600 and 6000 particles/mL, respectively, are provided as references. Strt. Form., starting formulation.

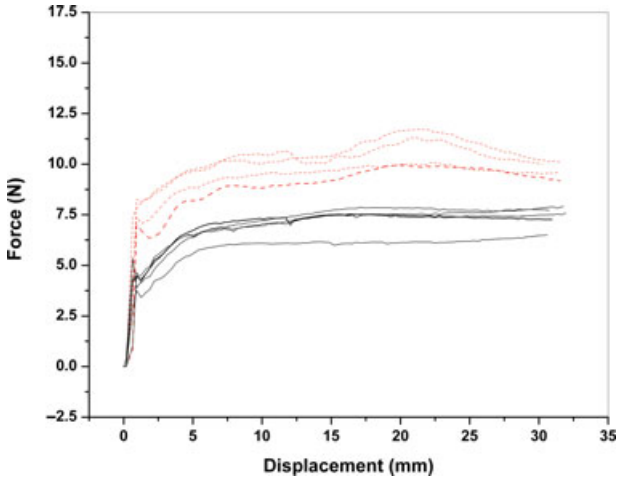

Figure 9. Glide forces in SGS and BD-42-coated syringes. The break-loose and glide forces generated with BD-42 (—-) are comparable to SGS (_____). Multiple lines of a kind represent replicates.

particle formation in the abatacept formulation indicates the effectiveness of this coating. Also, subvisible particle analysis of all the three protein formulations show that BD-42-coated syringes effectively limit the dramatic increase in particle content after shaking for 3 days.

CONCLUSION

The data presented here demonstrate that the presence of silicone oil in the container closure system can increase the subvisible particle content and at times destabilize the protein formulation. The particle content in rPA formulations was probably due to both silicone oil and protein aggregates. Sensitivity of protein to silicone oil may be a function of its structure and the formulation conditions. SEC analysis may not provide a complete picture of the instability in the protein solution, requiring that additional techniques be used to characterize the stability. Subvisible particle analysis using MFI can help present a more complete picture of the solution and in some cases also help to identify the origins of the particles. Thorough analysis of the data presented here conclusively establishes the effectiveness of BD-42 coating in limiting subvisible particle formation and mitigating the silicone-sensitivity of one of the most sensitive protein molecules.

ACKNOWLEDGMENTS

This work was funded in part by a grant from the US Department of Defense (Award No. DAMD 17-03-2-0037).

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