Effects of Syringe Material and Silicone Oil Lubrication on the Stability of Pharmaceutical Proteins

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Received 16 July 2014; revised 28 August 2014; accepted 2 September 2014

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24184

ABSTRACT: Currently, polymer-based prefillable syringes are being promoted to the pharmaceutical market because they provide an increased break resistance relative to traditionally used glass syringes. Despite this significant advantage, the possibility that barrel material can affect the oligomeric state of the protein drug exists. The present study was designed to compare the effect of different syringe materials and silicone oil lubrication on the protein aggregation. The stability of a recombinant fusion protein, abatacept (Orencia), and a fully human recombinant immunoglobulin G1, adalimumab (Humira), was assessed in silicone oil-free (SOF) and silicone oil-lubricated 1-mL glass syringes and polymer-based syringes in accelerated stress study. Samples were subjected to agitation stress, and soluble aggregate levels were evaluated by size-exclusion chromatography and verified with analytical ultracentrifugation. In accordance with current regulatory expectations, the amounts of subvisible particles resulting from agitation stress were estimated using resonant mass measurement and dynamic flow-imaging analyses. The amount of aggregated protein and particle counts were similar between unlubricated polymer-based and glass syringes. The most significant protein loss was observed for lubricated glass syringes. These results suggest that newly developed SOF polymer-based syringes are capable of providing biopharmaceuticals with enhanced physical stability upon shipping and handling.

Keywords: protein aggregation; prefilled syringe; physical stability; silicone oil; biopharmaceuticals characterization; subvisible particles; HPLC (high-performance/pressure liquid chromatography); analytical ultracentrifugation; UV/Vis spectroscopy; imaging methods

INTRODUCTION

Pharmaceutical companies are increasingly using prefillable syringes as an alternative to traditional vial packaging for the delivery of injectable drug products. Significant advantages of prefillable syringe systems over vial packaging include a greater patient safety by reducing the risk of needle exposure to pathogens when drawing solution from the vial, improved dosing accuracy, and convenience for patient in terms of product storage, administration, and disposal.[1] The majority of prefillable syringe systems available on the market are made from glass. Glass syringes have a long history of use, and have proven their performance and reliability among healthcare providers. Disadvantages associated with application of glass-made syringe system include breakage, potential surface reactivity, possibility of delaminated glass contamination, and requirement of silicone oil lubrication. To overcome these problems, in recent years, polymer syringes mainly manufactured from cyclic olefin polymer (COP) have been successfully developed and are being promoted to the pharmaceutical market. Polymer-based syringes have significant advantages of increased break resistance, decreased surface reactivity, and compatibility with the broad range of pH. In addition, by using a novel proprietary coating technique for plunger stopper, polymer-based silicone oil-free (SOF) prefillable syringe system has been developed.[2] The potential disadvantages of polymer-based syringes compared with glass syringes include higher susceptibility to scratches, fair chemical resistance against strong acids and alkalis, higher gas permeability, and relatively short usage history. It should be mentioned though that polymeric materials used to manufacture syringes utilized in the present study have been extensively tested and have been demonstrated to be safe and biocompatible materials for medical packaging application.[3]

For the therapeutic proteins, it is critically important that the protein oligomeric state remains unchanged, as aggregates and particles that can be composed of a protein alone or protein adsorbed onto foreign material are suspected to invoke immunogenicity.[4–9] During shipping and handling, liquid pharmaceutical formulations are exposed to agitation that induces aggregation and particle formation through protein interaction with the interphases, such as air–solution and vial/syringe surface–solution interphases.[10,11] The factors which affect the adsorption of protein to interfaces include protein properties, interface properties, and solution conditions. In the present study, to elucidate independent and combined effects of syringe barrel material, silicone oil lubrication, and different formulation conditions on aggregation and/or particulate formation, we placed two different pharmaceutical proteins, abatacept and adalimumab, formulated at two different pH, into a 1.0-mL SOF and silicone oil-lubricated glass and polymer-based syringes and subjected them to agitation stress to

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This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com/.

Journal of Pharmaceutical Sciences

reproduce conditions similar to those experienced by the drug products during shipping. The resulting changes in the soluble aggregates level were evaluated by size-exclusion chromatography (SEC). Because of the potential inaccuracies in amounts of aggregates determined by SEC related to nonspecific column adsorption,[12] SEC results were verified with analytical ultracentrifugation sedimentation velocity (AUC-SV), which has been demonstrated as the most appropriate orthogonal method to SEC.[13,14] In accordance with the current regulatory expectations for the analysis of subvisible particles,[15] resonant mass measurements (RMM) and dynamic flow-imaging analyses were also performed to analyze particles in the range of a few hundred nanometers to approximately hundred micrometers. The evaluated aggregates and particles amounts serve as a guideline to understanding the effects of different syringe materials in the presence or absence of silicone oil lubrication on the stability of the pharmaceutical proteins.

MATERIALS AND METHODS

Materials

Silicone oil-free 1-mL polymer-based syringes (PLAJEX™) with long staked needle (27G), henceforth referred to as "polymer-SOF," were provided by Terumo Company (Tokyo, Japan).[2] PLAJEX™ syringe is a recently developed prefillable syringe made from COP with a novel butyl rubber plunger stopper coated using proprietary coating technique. Silicone oil-lubricated PLAJEX™ syringes with an uncoated plunger stopper, henceforth referred to as "polymer-so+," were also provided by Terumo Company. SOF 1-mL borosilicate glass syringe barrels (conformed to the ISO 11040-4 standard), henceforth referred to as "glass-SOF," and silicone oil-lubricated 1-mL glass syringes barrels (conformed to the ISO 11040-4 standard), henceforth referred to as "glass-so+," were provided by TOP Company (Tokyo, Japan). Glass syringes were used with stainless steel needles and uncoated plunger stopper.

Abatacept (Orencia, molecular mass 92 kDa, pI = 4.5–5.5[16]), a recombinant fusion protein consisting of the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) linked to a modified Fc portion of human immunoglobulin G1 (IgG1), was purchased as a lyophilized powder for intravenous infusion from Ono Pharmaceutical Company, Ltd. (Osaka, Japan) at a stock concentration of 250 mg. Adalimumab (Humira, molecular mass 148 kDa, pI = 9.01 as calculated using the SEDNTERP software), a recombinant fully human IgG1 monoclonal antibody, was purchased from Eisai Company, Ltd. (Tokyo, Japan). Gibco phosphate-buffered saline (PBS), pH 7.4 (10×), was purchased from Life Technologies. Acetic acid was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Methods

Accelerated Stress

To elucidate the effect of formulation conditions on the levels of aggregation/particles formation, two different buffers with different pH were used in the present study. In our previous studies of adalimumab stability against aggregation using a number of formulations with different pH, we have detected that agitation for 96 h induced noticeable aggregation in the formulations with pH 4.0, 5.0, and 7.0 as indicated by an increase in turbidity. Because of concerns that pH 4.0, which is lower than that of stock formulation (pH 5.0) can result in the unfolding of the protein, pH 5.0 and pH 7.4 were selected. For abatacept, the same buffer conditions were chosen: pH 7.4 that is similar to that of stock formulation (pH 7.2–7.8) and pH 5.0 that is close to abatacept pI value.

A vial containing 250 mg of lyophilized abatacept was reconstituted with 10 mL of water for injections according to the manufacturer's instructions. First, sucrose was removed by extensive dialysis against Millipore water for 24 h at 4°C with two water changes. The obtained solution was further dialyzed into 1× PBS, pH 7.4, or 10 mM acetate buffer, pH 5.0 using slide-a-lyzer dialysis cassettes (MWCO 10,000 Da; Thermo Fisher Scientific Inc., Waltham, MA) with two buffer exchanges over 24 h at 4°C. The adalimumab stock solution was buffer-exchanged to 1× PBS, pH 7.4 trough gel filtration chromatography using the AKTAprime plus HPLC system with HiLoad™ 16/60 Superdex™ 200 prep grade column (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). The level of polysorbate 80 in the resulting solution was below quantification limit of 0.001% as was confirmed using modified Dragendorff reagent. A portion of the resulting solution was further dialyzed to 10 mM acetate buffer at pH 5.0 using dialysis cassettes. Protein concentration was determined using UV absorption and no significant protein loss was confirmed.

The buffer-exchanged protein solutions were diluted to 1 mg/mL by a respective buffer and filtered through a 0.22-μm polyvinylidene fluoride syringe filter (Millipore, Billerica, MA). One milliliter of each protein solution formulated at either 1× PBS or 10 mM acetate buffer was placed into 1-mL syringes, leaving a headspace of 5 mm. Four different types of syringes were used (three syringes of each type): (1) polymer-SOF; (2) glass-SOF; (3) polymer-so+; and (4) glass-so+. To reproduce the conditions experienced during shipping of therapeutic proteins, syringes were subjected to shaking at 500 rounds/min in a desktop orbital shaker Mix-VR (TAITEC Company Ltd., Saitama, Japan) at 4°C for 1 week. The samples before stress treatment were used as controls.

Absorbance and Transmission Measurements

Absorbance at 280 nm (A280) and transmission at 350 nm (T350) of the samples were acquired using DU-500 UV/Visible spectrophotometer (Beckman Coulter Inc., Brea, CA) and a 2-mL disposable cuvette with a cell length of 1 cm (Eppendorf, Hamburg, Germany). Samples recovered from syringes were centrifuged for 30 min at 15,600g, and percentage of insoluble large aggregates was determined from the difference of A280 before and after centrifugation, taken as a percentage of the A280 present before centrifugation.

High-Performance SEC

The samples were analyzed using the Alliance 1100 HPLC system (Waters, Milford, MA), with simultaneous UV absorbance detection at 215 and 280 nm with a TSK gel G3000SWXL column (Tosoh Bioscience, Tokyo, Japan) and 1× PBS pH 7.4 as the mobile phase. Twenty microliter aliquot was injected into the HPLC system. Flow rate was 0.5 mL/min, and elution time was set at 30 min. High protein mass recovery (>98%) was confirmed for all studied formulations, with the exception of glass-so+, for which the recovery was about 85%.

 DOI 10.1002/jps.24184