A Case Study of Nondelamination Glass Dissolution Resulting in Visible Particles: Implications for Neutral pH Formulations

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ABSTRACT: Visible particles were unexpectedly observed in a neutral-pH placebo formulation stored in glass vials but were not observed in the same formulation composition that contained protein. The particles were identified as silica gel (SiO_2) and polysorbate 20, suggesting dissolution of the glass vial. Time course studies were performed to assess the effect of variables such as pH, excipients, storage temperature, and duration on particle formation. Data suggest that glass dissolution occurred during the storage in the liquid state, as shown by increased Si levels in solution. Upon freezing, the samples underwent freeze concentration and likely became supersaturated, which resulted in the appearance of visible silica particles upon thawing. The glass degradation described here is unique and differs from the more commonly reported delamination, defined by the presence of reflective, shard-like glass flakes in solution that are often termed lamellae. This case study underscores the importance of an early assessment (during formulation development) of potential incompatibility of the formulation with the primary container. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1104–1114, 2014
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INTRODUCTION

The presence of visible and subvisible protein-related particles provides a significant product quality challenge and is fairly common in drug product protein formulations.[1,2] Here, we report that visible particles were observed in placebo samples formulated at neutral pH in glass vials but no particles were observed in the equivalent protein-containing formulations also stored in glass vials. The composition of the isotonic placebo formulation was 10 mM potassium phosphate buffer at pH 7.0 with an amino acid excipient and the surfactant polysorbate 20. The presentation of both the placebo and protein-containing formulations was a 3.5 mL fill volume in 10 cc Type IA borosilicate glass vials.

It is highly unusual to observe particles in placebo formulations and not in the protein-containing formulation of equivalent composition; in fact, it is typically the opposite. As the particles were observed only in placebo formulations, protein was ruled out as a causative factor. To determine the cause of particulation in placebo samples, several potential contributing factors were assessed: buffer composition, raw material components (including both primary container and excipients), drug product physical properties [such as glass transition temperature (T_g')], filling procedure, as well as storage temperature and duration. The most notable difference between placebo and vials containing formulated protein was their storage temperature. Protein-containing vials were frozen immediately after filling. On the basis of the varying need for placebo controls during our studies, placebo vials were stored for varying durations at 2°C–8°C before freezing at −30°C. This analysis suggested that the formation of particles in placebo samples was inherent to the placebo composition in its primary container and storage condition, which made the system amenable to further investigations by varying those two critical parameters.

Glass primary containers are commonly used because of their presumed lack of reactivity and the extremely low solubility of silica (SiO_2), which at 2°C–8°C has been reported in the 10–130 ppm range.[3] However, it is well documented that under certain conditions, glass can be both chemically and physically reactive.[3–5] The interactions that occur when glass is in contact with aqueous solutions are well characterized and are the subject of a large body of literature dating back several decades and spanning several industries.[3–16] Reactions between glass and aqueous solution can be broadly categorized into glass dissolution/leaching and surface erosion (more commonly referred to as delamination), which is characterized by glass flaking off the interior surface of the vial. A hallmark of glass delamination is visible glass flakes in solution, but as pointed out by Iacocca et al.,[5] such visible glass flakes in solution are a lagging indicator of glass delamination. Before glass flakes in solution are large enough to be seen, subvisible glass flakes are often present. Moreover, Iacocca et al.[5] reported that dissolution of the silica network may be a preliminary indication of the probability of glass delamination. Glass dissolution occurs when the silicate network is disrupted with water, forming silicic acid (H_4SiO_4) and releasing sodium, boron, and aluminum into solution.[15]

$$(SiO_2)_n + 2H_2O \leftrightarrow (SiO_2)_{n-1} + H_4SiO_4(\text{silicic acid})$$

Abbreviations used: EDS, energy-dispersive spectroscopy; FTIR, Fourier transform infrared microscopy; ICP–MS, inductively coupled plasma–mass spectrometry; LOQ, limit of quantitation; MFI, microflow imaging; SEM, scanning electron microscopy.

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The SiO_2 concentration in solution is an accepted index of glass dissolution.[17] The rate at which glass dissolution occurs depends not only on the type and treatment of the glass containers but also on the solution conditions such as pH, buffer components, temperature, and the degree of supersaturation.[18] For example, citrate is reported to form soluble complexes with silica in neutral solutions.[15] Higher temperature storage of the glass vial may also increase the dissolution rate of glass. We explore the use of temperature as an accelerated condition to increase glass dissolution.

The first objective of this work was to identify and characterize the particles observed in the placebo formulation. Once the particles were determined to be silica gel (SiO_2) and polysorbate, we sought to understand the mechanism of particle formation in the placebo. A model for glass dissolution and subsequent silica polymerization is proposed to explain particle formation. Experiments were designed to test and validate the model. Studies were performed to assess different formulation parameters (storage temperature, excipient type, and pH) and identify those that are critical for particle formation. Finally, we strove to apply our understanding from this case study to provide a broader perspective on the compatibility of the primary container with excipients and implications for formulation development. A detailed and mechanistic understanding of glass degradation is necessary because glass is a preferred primary container for parenteral solutions.

EXPERIMENTAL

Materials

Sodium metasilicate nonahydrate (J.T. Baker, Phillipsburg, New Jersey) was prepared to a 1 g/L solution in water corresponding to 99 ppm Si. A 1000 ppm Si standard was also used (Ricca Chemical Company, Arlington, Texas). Polysorbate 20 and dibasic and monobasic potassium phosphate were used to prepare formulation buffers, termed "placebo." Placebo formulations were prepared in plastic containers. For studies, samples were stored in 10 cc Type IA glass vials. Vials were washed and depyrogenated before use using standard procedures on automated equipment. Polycarbonate (PC) containers (30 mL) were used for storage of controls (DS2127-0030; Nalgene, Rochester, NY).

Analytical Methods

Analysis of Isolated Particles and Inner Vial Surfaces

Particle Isolation on Gold Filters. The particles were isolated by filtration in a laminar flow hood. The filtration set was rinsed several times with Milli-Q water prior to filtration. The contents of the vial were filtered through a 0.8 μm gold-coated filter (rap.ID Inc., Monmouth Junction, NJ). The particles retained on the filter surface were further washed with Milli-Q water before examination by optical microscopy. No particulate manipulation was performed as the particles were retained on the gold-coated filter.

Optical Microscopy. The particles isolated on the gold filter were first examined with the Zeiss Stemi 2000 stereomicroscope (Carl Zeiss AG, Jana, Germany). A Keyence VHX-600 digital microscope (Keyence Corporation, Osaka, Japan) was used to examine particles and assess the inner surface of the vials for defects. The optical images of the sample were captured using the color digital camera attached to the microscopes and recorded by a computer.

F*ourier Transform Infrared Microscopy.* The filters were put directly onto a sample stage for analysis by Fourier transform infrared (FTIR) microscopy. FTIR analysis was performed via a Bruker Hyperion 2000 Infrared Microscope (Bruker Corporation, Billerica, MA) using a Bruker Vertex 70 bench as the IR source. FTIR spectra were collected in reflectance mode with 128 scans and 4 cm^{-1} resolution. Samples were compared with library reference spectra from the KnowItAll® database, version 6.0 Copyright 2001–2005 (Biorad Laboratories, Inc., Hercules, CA).

Scanning Electron Microscopy/Energy-Dispersive Spectroscopy. For energy-dispersive spectroscopy (EDS) analysis, the particles were transferred from the filters onto individual 13 mm double-sided carbon tabs on an aluminum mount for scanning electron microscopy (SEM)/EDS elemental analysis. For silicon mapping, the filter was directly put into the SEM sample chamber to retain particle morphology. For inner vial surface analysis, the vials were cut into strips by a diamond saw. The strips were then mounted on double-sided carbon tab. The SEM was used in variable pressure mode with an excitation voltage of 20 kV. Elemental analysis by EDS was performed using the attached INCAPenta FET×3 Energy EDS.

Analysis of Particles in Solution

Visual Assessment of Liquid Placebo in Glass Vials. Placebo formulations in glass vials were inspected for visible particles before and after a storage period of 1 week at −30°C. Any particles seen by the analyst were recorded as "visible." Figures display visual results as follows: if no visible particles are present, open symbols are shown, whereas filled symbols denote the presence of visible particles.

Particle Size and Quantitation by Microflow Imaging. Microflow imaging (MFI), with MFI Microscope model number DPA-4100-INS-D and MVAS software (Brightwell Technologies Inc., Ottawa, Ontario, Canada) was used to assess particle size and shape. Samples were degassed for 2 h. Approximately 1 mL of sample was used to purge the system, 200 μL was used to ensure system optimization, and a 1 mL sample volume was analyzed.

Analysis of the Solution Composition

Inductively Coupled Plasma–Mass Spectrometry. Inductively coupled plasma–mass spectrometry (ICP–MS) was performed on an Elan DRC II instrument (PerkinElmer Sciex, Waltham, MA)] that was optimized by following the standard procedures recommended by the vendor. Samples were quantitated for B, Na, and Al in standard mode and Si in dynamic reaction cell (DRC) mode using NH_3 collision gas. DRC mode is specifically for elements such as Si, which are difficult to analyze in standard mode because of polyatomic interferences. For standard