Influence of Formulation pH and Suspension State on Freezing-Induced Agglomeration of Aluminum Adjuvants

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Received 18 March 2011; revised 13 October 2011; accepted 21 October 2011

Published online 23 November 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22815

ABSTRACT: Freezing and thawing of vaccines containing aluminum adjuvants can lead to formation of aggregates and loss in vaccine potency. We sought to understand whether and to what extent the freeze–thaw damage to aluminum adjuvants would differ based on suspension state (flocculation and settlement) at the time of freezing. As flocculation and settlement characteristics of aluminum adjuvants are driven largely by the electrostatic charges on the adjuvant particles, which, in turn, are strongly influenced by the pH of the suspension, we conducted freeze–thaw studies on both Adjuphos and Alhydrogel™ samples at three pH levels (4, 6.5, and 7.2) in buffer solutions with 9% sucrose. Significantly less aggregation occurred in the buffered sucrose solutions at the pH furthest from the aluminum adjuvant point of zero charge during slow freezing at −20°C. The freezing-induced aggregation for the samples with 9% sucrose at each pH was minimal during fast freezing at −70°C and −115°C. Suspensions that were flocculated and settled to a greater extent experienced the most freeze–thaw aggregation, whereas suspensions that were frozen before significant flocculation and settlement occurred showed little or no aggregation. Because pH of formulation can affect flocculation and settling time, it indirectly affects the extent of freeze–thaw aggregation. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:1050–1062, 2012

Keywords: formulation; vaccine adjuvants; light scattering (static); microscopy; particle size; pH; physical stability; precipitation; stabilization

INTRODUCTION

Freezing of aluminum-adjuvant-containing vaccines may occur accidentally during storage and shipment.[1] In addition, the intentional freezing or freeze-drying of the vaccines could be desired for achieving appropriate stability for some antigens. It has been shown that the freezing of suspensions with aluminum adjuvants leads to irreversible aggregation/agglomeration of the adjuvant particles. Even though vaccine in an aggregated state may still be immunogenic,[2] the big drawback of freezing stress is manifested by low reproducibility of vaccine dose delivered after drawing into a syringe.[3] One of the possible mechanisms of aluminum adjuvant aggregation during freezing could be attributed to particles being forced too close together (into the primary energy minimum) by ice formation.[3] It was demonstrated that the aggregation of aluminum adjuvant during freezing can be avoided by either introduction of a steric barrier with surfactants or addition of glass-forming cryoprotectors (carbohydrates, polyols, and etc.) at a concentration above 10%.[1–5]

Most of the published findings on freeze–thawing of aluminum adjuvant suspensions focused on aluminum-hydroxycarbonate- or aluminum-hydroxide-containing suspensions at one pH without a clearly specified settlement state for the suspension.[3,4] We hypothesize that the state of a suspension (dispersed, flocculated, settled) that is influenced by both the magnitude of the zeta potential of aluminum adjuvant (or vaccine) particles and the formulation pH will influence aggregation damage from freeze–thaw. To further our knowledge in this area, we studied the effect of freeze–thawing of two aluminum adjuvants (Alhydrogel™ and Adjuphos™) at three pH levels (4, 6.5, and 7.2). The freezing was conducted at either −20°C, −70°C or the samples were blast frozen at −115°C. The state of the suspensions was controlled by allowing

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Journal of Pharmaceutical Sciences, Vol. 101, 1050–1062 (2012)

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flocculation and settlement to occur during controlled time intervals.Adjuphos™ exhibits a point of zero charge (PZC) of pH 5.2, whereas Alhydrogel™ exhibits a PZC of pH 8.4.

MATERIALS AND METHODS

Materials

All reagents used were of analytical grade and were purchased from Fisher Scientific (Pittsburg, PA, USA) and Sigma-Aldrich (Saint Louis, MO, USA). Alhydrogel™ (aluminum hydroxide adjuvant) and Adjuphos™ (aluminum phosphate adjuvant) were made by Brenntag Biosector (Frederikssund, Denmark).

Sample Preparation

The aluminum adjuvant suspensions were prepared at 450 μg/mL final concentration of aluminum, unless stated otherwise. Both the Adjuphos™ and Alhydrogel™ containing suspensions were prepared by the dilution of stock adjuvant suspensions at 10.1 or 10.2 mg/mL aluminum with appropriate buffer. Twenty millimolar acetate buffer at pH 4.0, 20 mM histidine buffer at pH 6.5, and 20 mM HEPES buffer at pH 7.2 with and without 9% (w/v) sucrose were utilized. All aqueous solutions of the buffers were passed through a 0.22 μm filter prior to formulation with the aluminum adjuvants. Samples were prepared in triplicates, where 3 mL of a suspension was aliquoted into a 10 cc glass vial with a screw cap. All samples, unless specified, were resuspended immediately before freezing (some settling could occur during the shelf freezing). A box with approximately 100 vials was placed on a shelf in either a −20°C freezer (Puffer Hubbard freezer with six shelves, Fisher Scientific, Ashville, NC, USA) or a −70°C freezer (Harris freezer with six shelves, Thermo Fisher Scientific, Ashville, NC, USA), or samples were blast frozen at −115°C (Kwik-Freeze freezing system, model KFB-N, Airco Carbon dioxide Division, Murray Hill, NJ, USA). During the blast freezing, shelves were cooled to −115°C with vaporized liquid nitrogen, vials were loaded on the cold shelf, and were subjected to blast cooling at −115°C for 15 min.

The effect of the suspension state on freezing-induced aggregation was evaluated by allowing suspensions to settle for either 5, 15, 30 min or 1, 2, 3 h prior to blast freezing at −115°C. A few suspensions were allowed to settle at 2°C–8°C for 1 h prior to freezing at −70°C. Another set of suspensions was allowed to completely settle at 2°C–8°C for 48 h prior to freezing at −20°C. All vials were thawed at ambient room temperature for 1 h. One set of samples was subjected to three freeze–thaw cycles. Control samples were stored at 2°C–8°C for the duration of the study. Samples were thoroughly resuspended prior to analysis.

Particle Size Measurements by Static Light Scattering

Size of the particles was measured by utilizing a static light scattering (SLS) instrument (Malvern Mastersizer 2000; Malvern Instruments Ltd., Worcestershire, UK). One to two milliliters of a sample was mixed with 20 mL of saline. The data were fit into a general-purpose distribution model for irregular-shaped particles with refractive index of 1.55 and absorption of 0.1 (water was selected as dispersant). Each sample was measured thrice. The average distribution of the particles for three samples, in the majority of cases, was reported in volume percentage. Standard deviation of mean particle diameter was ±0.5 μm for control samples (stored at 2°C–8°C) and ±5 μm for most of the freeze–thawed samples.

Particle Size and Number Measurements by Microscopy Flow Imaging

Micro-Flow Imaging™ (MFI) was performed by utilizing a Brightwell DPA 4100 particle analyzer (ProteinSimple, Ottawa, Ontario, Canada) at the low magnification set point (set point 1, SP1) with 5× magnification, 1760 × 1400 μm field of view, using MVSS version 2 software (MFI view system software). SP1 was used to count particles in the size range of 2 to 300 μm. One milliliter of aluminum adjuvant sample diluted 100× with saline was loaded on the instrument using a 1 mL barrier pipette tip (Neptune Scientific, San Diego, CA, USA). Each run was performed using sample dispensed as the run termination type, with a total available volume of 0.9 mL, a purge volume of 0.2 mL, and an approximate analyzed volume of 0.59 mL. Flow rate of 0.22 mL/min was utilized. Each sample out of the triplicate set of samples was measured once. Data are provided as particle counts in bins of 2–5, 5–10, 10–15, 15–20, 20–30, 30–40, 40–60, 60–100, and 100–300 μm. Error bars show standard deviation of number of particles per milliliter for each bin size. Please refer to the supplementary materials for representative MFI images.

The 100× dilution for the samples was selected on the basis of evaluation of 50×, 100×, and 200× dilutions of both the Adjuphos™ and Alhydrogel™ samples in saline. On the basis of the number of the particles in both control and frozen samples (in respect to the specified SP1 maximum concentration limit for the instrument of 175,000 particles/mL ≥2.5 μm),[6] reproducibility of measurements, and appearance of the images (to assure proper separation of the particles and no residual background), the 100× dilution in saline was performed for all samples prior to the MFI analysis. Please refer to supplementary materials for data on the dilution screening.

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