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Research paper

# Rational design of lyophilized high concentration protein formulations-mitigating the challenge of slow reconstitution with multidisciplinary strategies

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ABSTRACT

An increasing number of protein therapies require chronic administration at high doses (>200 mg) by subcutaneous (sc) injection. Due to the injection volume limitation (<1.5 mL) associated with sc administration, high protein concentration formulations at or exceeding 100 mg/mL are required to achieve the dose. Development of a high concentration protein formulation can be challenging due to increased aggregation at higher concentration and/or chemical instability, which necessitates the development of lyophilized formulation for high protein concentration drug products. Unique challenges, such as long reconstitution time for a lyophilized high protein concentration drug product, can limit practical usage and commercial marketability of the product. In this paper, a systematic approach is presented to develop a lyophilized high concentration protein formulation. The focus is on achieving reasonable reconstitution times with multidisciplinary strategies. Many strategies have been shown to provide nominal improvement in reconstitution times, such as adding wetting agents in the diluents, incorporating high annealing steps in the lyophilization cycle and reconstituting under vacuum. The reconstitution strategy of reduced diluent volume, however, has enabled significant decrease in reconstitution time (4–7-fold) of lyophilized high protein concentration formulations.



## 1. Introduction

Development of high concentration protein formulations has its unique challenges in manufacturing, stability, analytical characterization, and drug delivery. At high concentrations, protein solutions are prone to instability. The principal stability challenge for formulations at these high concentrations is aggregation, which requires bimolecular collisions and, thereby, is highly concentration dependent [1]. In fact, aggregation is generally the primary degradation pathway in high concentration protein formulation [1,2]. Chemical degradation can also be an issue, as the formulation pH of maximum solubility may not be optimal for chemical stability. One approach to minimize aggregation and chemical degradation in aqueous liquid formulation is to lyophilize and store the product in a dried state. A lyophilized formulation with appropriate excipients can greatly enhance conformational stability of a protein by restricting mobility, and other hydrolytic reactions can also be minimized with the removal of water [3].

An additional challenge associated with lyophilized formulations at high protein concentrations is the long reconstitution time. The reconstitution time ranges from about 20 min [4] to hours [2,5,6]. In some cases, reconstitution properties can put limitations on the practical usage of the lyophilized drug product.

In this paper, we present the formulation development work for a lyophilized high concentration Fc-fusion protein. The Fc-fusion protein is composed of two identical human Fc-fusion polypeptide chains, with molecular weight around 57 kD. The targeted protein concentration was 100 mg/mL for both clinical and commercial formulations. In general, Fc-fusion proteins are less stable than antibodies, specifically more chemically labile within the active peptide moiety and especially if aglycosylated, i.e., expressed in *Escherichia coli* [7]. Fast et al. suggested that because Fc-fusion proteins do not benefit from the mutual stabilization from

*Abbreviations:* sc, subcutaneous; mAb, monoclonal antibody; UF/DF, Ultrafiltration/Diafiltration; SEC, size-exclusion chromatography; BET, Brunauer–Emmett–Teller; XRPD, X-ray powder diffraction; SEM, Scanning Electron Microscopy.

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inter-domain interactions in intact antibodies, they may have reduced stability compared to their monoclonal antibody counterparts [8]. The Fc-fusion protein in this study is no exception, and it has multiple degradation pathways, such as covalent aggregation and aspartic acid isomerization, which requires a lyophilized formulation for stability to achieve a 2-year shelf life.

This paper demonstrates that challenges in developing high concentration lyophilized formulations can be met by applying systematic approaches on (1) selection of the appropriate amount of excipient for maximum stability and (2) utilizing multidisciplinary strategies to reduce reconstitution times. The focus of the study is on mitigating slow reconstitution of high protein concentration lyophilized cakes with multidisciplinary approaches.

## 2. Materials and methods

### 2.1. Materials

The Fc- fusion protein was produced by microbial fermentation in *E. coli* and was purified by standard processes at Amgen (Thousand Oaks, CA, USA). The Fc-fusion protein samples were statically thawed at 4 °C for use in the formulation studies. The formulations with different protein concentrations were prepared either with Ultrafiltration/Diafiltration (UF/DF) or diluted from the bulk material. The UF/DF was performed using a Millipore Lab scale TFF system with a 100 mL vessel. Samples were lyophilized in a lab-scale lyophilizer (Virtis, SP scientific, Warminster, PA, USA).

The protein formulations contained 10 mM glutamic acid as a buffer. The stabilizer and caking agent were sucrose and mannitol, respectively, at levels of either 2% sucrose (w/v) and 4% mannitol (w/v) or 4.5% sucrose (w/v), 2.5% mannitol (w/v). All formulations contained 0.01% polysorbate 20 as a surfactant. Sterile water for irrigation (WFI) (Baxter, Deerfield, IL, USA) was used as the diluent to reconstitute the lyophilized protein cakes. In one study where various diluents were evaluated, the diluents were prepared from the following chemicals with WFI to the targeted concentrations: Benzyl alcohol: (Sigma–Aldrich, St. Louis, MO, USA); Ethanol (200 Proof USP/NF): (Sigma–Aldrich, St. Louis, MO, USA); Pluronic® F 68 NF Prill Poloxamer 188: (BASF, Florham Park, NJ, USA); Glycerol: (Alfa Aesar, Ward Hill, MA, USA); polysorbate 20 and polysorbate 80 (Amgen internal inventory, Amgen Inc., Thousand Oaks, CA, USA). All chemicals and solvents used were reagent grade or better.

### 2.2. Size-exclusion chromatography (SEC)

Size-exclusion chromatography (SEC) was performed using an Agilent 1100 HPLC system equipped with the Chromeleon® software (Agilent, Palo Alto, CA, USA). SEC was conducted isocratically using a TSK-GEL G3000$W_{XL}$ column (Tosoh Biosciences, 5 μm, 78 mm × 300 mm). The mobile phase consisted of 50 mM sodium phosphate, pH 6.5, 300 mM sodium chloride, and 10% IPA. The protein was monitored and quantified with UV detection at 280 nm. The variability of the SEC assay was determined by several injections of a standard sample. The error bars or precision are then generated based on a standard deviation of a given integrated area (e.g. main peak).

### 2.3. Freeze–thaw studies

Two freeze thaw procedures were followed. In all procedures, the material was frozen to a target temperature of −30 °C. For procedure A, samples were frozen at a −30 °C freezer (Thermo Scientific Inc., Waltham, MA, USA) and thawed in the 2–8 °C cold room. For procedure B, a controlled rate freezer (CRF) (SPX Thermal Product Solutions, Rochester, NY, USA) was employed that has similar temperature targets to procedure A in freeze and thaw. However, the CRF was used to slow the rate of temperature change over time to mimic larger manufacturing scale conditions. After thawing, some samples were aliquoted for analysis, whereas the rest of samples were returned to the −30 °C freezer for the beginning of the next freeze thaw cycle. Three cycles were performed in this study.

### 2.4. X-ray powder diffraction

The X-ray powder diffraction (XRPD) analysis was performed using a $\theta/\theta$ diffractometer (X'pert MPD, Philips Analytical, Natick, MA) with Cu Kα radiation. All samples were analyzed with Bragg–Brentano geometry from 3° to 40° $2\theta$ at a step size of 0.01° $2\theta$.

### 2.5. Reconstitution

Samples were transferred from the incubators and refrigerators to the laboratory benchtop to equilibrate to the room temperature before the reconstitution. Reconstitution was done with 3 mL BD Luer-Lok™ syringe and BD PrecisonGlide™ 21 gauge needles (BD, Franklin Lakes, NJ, USA). Typically, the diluent was sterile water for injection (Baxter, Deerfield, IL, USA), except in the diluent agents study where various diluent agents were evaluated, as specified. Vacuum in the vial, if any, was released by inserting the needle through the septum. The predefined reconstitution procedures were followed in wetting the cake with 1.2 mL of diluent and initiating the timing of reconstitution times as soon as diluent was in the vial. The reconstitution time was recorded, and the reconstituted solution was inspected visually when the solids were totally dissolved.

### 2.6. BET

BET surface area measurement was performed on a Micromeritics Tristar II Surface Area and Porosity Analyzer 3020 (Micromeritics, Norcross, GA, USA) using Krypton. Adsorption volumes were determined at multiple points from 5% to 25% $p/p_0$ pressure range at 77.5 K. Samples were degassed using nitrogen prior to surface area analysis with a Micromeritics SmartPrep Programmable Degas System. The results were averaged from two analyses.

### 2.7. Microscopy

Photo images were taken using a Nikon Eclipse E600 Polarized Light Microscope (Nikon Instruments, Inc. Melville, New York, USA), equipped with a Nikon Digital Camera DXM1200, operated by ACT-1 Camera Control software for DXM1200, Version 2.11. Samples were dispersed in silicone oil on a microscope slide and examined under transmitted polarized light.

## 3. Results

### 3.1. Stability strategies

Lyophilized high concentration protein formulations usually include the protein, a disaccharide stabilizer, such as sucrose, buffer, and sometimes a small molecular weight polyol. Excipients such as sugars and polyols are used to mitigate protein aggregation by stabilizing the native conformation of the protein [9,10]. The mechanism by which sugars stabilize protein is by replacing water and hydrogen-bond to water-binding site on the dried protein [11]; therefore, the formulation has a certain sugar to protein ratio to provide optimal stability [12–14]. Shire et al. described a lyophilized mAb1, which showed increased stability with increasing