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Colloidal Instability Fosters Agglomeration of Subvisible Particles Created by Rupture of Gels of a Monoclonal Antibody Formed at Silicone Oil-Water Interfaces

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ABSTRACT

In this study, we investigated the effect of ionic strength (1.25-231 mM) on viscoelastic interfacial gels formed by a monoclonal antibody at silicone oil-water interfaces, and the formation of subvisible particles due to rupture of these gels. Rates of gel formation and their elastic moduli did not vary significantly with ionic strength. Likewise, during gel rupture no significant effects of ionic strength were observed on particle formation and aggregation as detected by microflow imaging, resonance mass measurement, and size exclusion chromatography. Subvisible particles formed by mechanical rupturing of the gels agglomerated over time, even during quiescent incubation, due to the colloidal instability of the particles.

Introduction

Prefilled syringes are increasingly used as delivery devices because of their associated improved patient compliance, dose accuracy, and ease of administration.[1] To aid in the syringe plunger movement prefilled syringes typically are lubricated with silicone oil.[2] Much research has focused on the mechanisms by which proteins may be destabilized, aggregate and form particles as a result of their adsorption to oil-water interfaces.[3-10] In contrast, less emphasis has been placed on the effect of protein adsorption on the colloidal stability of silicone oil droplets.[11]

According to regulatory expectations,[12] particles within protein formulations must be characterized and quantified. Furthermore, protein formulations must be developed to minimize particle formation. Therefore, for products in prefilled syringes it is important to understand not only the potential effects of silicone oil on protein aggregation, but also the ways in which proteins may influence the emulsion stability of silicone oil droplets.[11]

Previously, we established that proteins adsorbed at silicone oil-water interface may form viscoelastic gels.[9] Furthermore, mechanical perturbation of these gels (e.g., due to interactions with air bubbles[7]) may result in particle formation.[9]

Interface-induced aggregation of proteins may also be influenced by their conformational[6] and colloidal instability.[10,13] Thirumangalathu et al.[10] found that decreased colloidal stability of a monoclonal antibody (mAb) led to enhanced aggregation when the protein was agitated in the presence of a silicone oil emulsion. Basu et al.[3] showed that the presence of 150 mM NaCl reduced colloidal stability of an antibody, and accelerated its aggregation during agitation in the presence of silicone oil-coated glass beads. Similarly, Chou et al.[13] found that the presence of 150 mM NaCl decreased colloidal stability of albinterferon-α_{2b}, leading to increased aggregation upon agitation.

In this study, we investigated how particle formation and growth are affected by colloidal instabilities of both mAb and particles formed from silicone oil and mAb.[9] We modulated the colloidal stability by changing the ionic strength of the mAb

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solution, and we hypothesized that conditions resulting in lower colloidal stability in the bulk solution would also create stronger interfacial gels at the silicone oil-water interface. Furthermore, we hypothesized that colloidal instability of particles formed by mechanically perturbing the interfacial gels would dictate their subsequent growth and agglomeration kinetics.

Materials and Methods

Materials

Purified mAb was provided by MedImmune (Gaithersburg, MD) in a lyophilized formulation. The lyophilized material was reconstituted with water for injection to obtain 50 mg/mL mAb in 10 mM histidine, 6% (wt/vol) trehalose, 2% (wt/vol) arginine, and 0.025% (wt/vol) polysorbate 80 at pH 6.0.

United States Pharmacopeia grade reagents such as L-histidine, NaCl, and silicone oil (50 cst) were purchased from Fisher Scientific (Fair Lawn, NJ). Pierce Slide-A-Lyzer cassettes (Thermo Scientific, Rockford, IL) with 10 kDa molecular weight cutoff were used for dialyzing mAb solutions. Silicone oil (1000 cst) used to study rupture of interfacial gels was of medical grade and purchased from Dow Corning (Midland, MI). Unless otherwise indicated, deionized MilliQ® water was used to prepare all solutions. Lyophilization vials (2 mL) and caps were purchased from West Pharmaceutical (Lionville, PA). The rotating mixer was purchased from Appropriate Technical Resources (Laurel, MD).

Preparation of mAb Formulations

Following reconstitution of the lyophilized formulation with distilled water, the mAb solution was dialyzed into various formulations. The formulations tested for this particular study were 10 mM histidine (His) and 10 mM His with various NaCl concentrations: 50, 100, 150, and 230 mM. All formulations were at pH 6.0. The solution ionic strengths were, respectively, 1.25, 51.3, 101, 151, and 231 mM. Only 10 mM His and 10 mM His plus 230 mM NaCl formulations were tested in the post rupture quiescent incubation study described below. Each dialyzed mAb solution was diluted to a final mAb concentration of 1 mg/mL, unless otherwise indicated.

Interfacial Shear Rheology Measurement

In order to study gelation of the mAb at the silicone oil-water interface as a function of NaCl concentration, a custom-built interfacial shear rheometer was used. Instrument calibration, data acquisition methods, and analysis of results were as previously described.[9,14-16]

The rheometer was calibrated using placebos for each formulation condition. For analysis of solutions containing mAb, 40 mL aliquots of mAb at 50 μg/mL were prepared in various NaCl buffers. Then, the rheology measurement was started immediately, and typically carried out over 20 h.

In order to quantify the effect of NaCl on elastic modulus of interfacial gels, we fit a single exponential equation to the curve of elastic modulus versus time in order to obtain a plateau value. The curve fitting was carried out using GraphPad Prism (GraphPad software, Inc., La Jolla, CA).

Periodic Mechanical Perturbation of mAb Gels at the Silicone Oil-Water Interface

The system used to study the effects of ionic strength and mechanical perturbation of interfacial gels of mAb has been described previously.[9] The experimental apparatus contained 1 mL of mAb solution at 1 mg/mL, with 200 μL of silicone oil (1000 cst) layered on top of the mAb solution. The concentration of silicone oil used here was higher than that encountered by therapeutic proteins formulated in prefilled syringes. However, this level of oil was required in our studies to form the water-silicone oil interface in the samples.[9] The interfacial gel layers that formed as a result of mAb adsorption at the oil-water interface were repeatedly ruptured by a stainless steel needle, at a controlled frequency of 0.25 or 0.5 Hz. Triplicate sample vials were prepared for each time point and each interface-rupturing condition. Samples were removed from the vials and analyzed on days 0, 1, 2, and 3 as described below.

Microflow Imaging for Particle Counting

Samples subjected to periodic rupturing of interfacial gels and unperturbed control samples were analyzed for subvisible particles of sizes $\geq$1 μm using microflow imaging (MFI, model # DPA 4100; Protein Simple, Santa Clara, CA). The instrument was configured in set-point 3 mode, and a 100 μm flow cell (Part number: 4002-002-001) was used. The total volume of sample dispensed into the flow cell was 0.5 mL, and 0.15 mL of the sample was allowed to flow through the cell prior to acquisition of data. Each of the triplicate samples from above was analyzed once.

Resonance Mass Measurement of Particles

Analysis of protein particles and silicone oil droplets was carried out using an Archimedes particle metrology system (Affinity Biosensors, Santa Barbara, CA). A "micro" format resonant mass sensor (channel cross section 8 $\times$ 8 μm^2, resonant frequency 400 kHz) measured particles in a size range 0.2-4 μm diameter. For protein particle measurements, a density of 1.4 g/mL was assumed,[17,18] and for silicone oil droplets a density value of 0.97 g/mL was used.[18] ParticleLab software version 1.8.510 was used to obtain particle concentration for different samples. The data were reported as total masses of silicone oil and protein particles.

Size-Exclusion Chromatography

Samples subjected to periodic rupturing of interfacial gels and unperturbed control samples were analyzed for loss of soluble mAb and the presence of soluble high molecular weight species using size exclusion chromatography (SEC). A Tosoh TSKgel G3000SW xl column was used, and mAb in the eluate was quantified using absorbance at 280 nm. Prior to loading the samples, the column was equilibrated with 0.1 M Na_2SO_4, 0.1 M Na_2HPO_4, pH 6.8, which was used as the mobile phase at a flow rate of 1 mL/min. Percent recoveries of soluble mAb and percent soluble aggregates were calculated by normalizing against total peak area of chromatograms for day 0 samples of each formulation condition.

Post Rupture Quiescent Study to Investigate the Effect of Formed Particles

Interfacial gels in mAb solutions formulated at 1 mg/mL mAb in the absence and presence of 230 mM NaCl were subjected to periodic rupturing at a frequency of 0.25 Hz over a period of 1 day, as described above. Following the periodic rupturing of the interfacial gel, the samples were removed from the gel rupture apparatus and incubated quiescently at room temperature for the next 13 days. Samples were analyzed for subvisible particles at various time points over the 14-day period using MFI, resonance mass measurement (RMM), and SEC, as described above. As controls, samples