RESEARCH ARTICLES
Pharmaceutical Biotechnology

Polar Solvents Decrease the Viscosity of High Concentration IgG1 Solutions Through Hydrophobic Solvation and Interaction: Formulation and Biocompatibility Considerations

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Received 10 September 2012; revised 23 December 2012; accepted 3 January 2013

Published online 28 January 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23453

ABSTRACT: Low-volume protein dosage forms for subcutaneous injection pose unique challenges to the pharmaceutical scientist. Indeed, high protein concentrations are often required to achieve acceptable bioavailability and efficacy for many indications. Furthermore, high solution viscosities are often observed with formulations containing protein concentrations well above 150 mg/mL. In this work, we explored the use of polar solvents for reducing solution viscosity of high concentration protein formulations intended for subcutaneous injection. An immunoglobulin, IgG1, was used in this study. The thermodynamic preferential interaction parameter (Γ_{23}) measured by differential scanning calorimetry, as well as Fourier transform infrared, Raman, and second-derivative UV spectroscopy, were used to characterize the effects of polar solvents on protein structure and to reveal important mechanistic insight regarding the nature of the protein–solvent interaction. Finally, the hemolytic potential and postdose toxicity in rats were determined to further investigate the feasibility of using these cosolvents for subcutaneous pharmaceutical formulations. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1182–1193, 2013

Keywords: immunoglobulin; monoclonal antibody; spectroscopy; calorimetry; viscosity; preferential interaction; high concentration; protein; formulation

INTRODUCTION

The advent of monoclonal antibody therapies for use in diverse medical indications requiring novel routes of administration has generated unique challenges for the pharmaceutical scientist. Of these challenges, the development of a low-volume protein dosage form for high dose applications has proven difficult cross functionally and throughout industry.[1,2] High doses often entail ultrahigh protein concentrations (>200 mg/mL) resulting in both high viscosity and stability limitations.[1–6] Previously, it was shown that increased intermolecular association of monoclonal antibodies can lead to increased solution viscosity possibly through the formation of network-like structures.[4–6] A particularly attractive solution to the issue of high viscosity and self-association is through the use of excipients. Currently, the amino acid, arginine (Arg), is added to formulations for a myriad of reasons, one of which is to reduce solution viscosity.[7] Although Arg is effective at increasing solubility and reducing viscosity and self-association, there are limitations to its use, and the search for more effective excipients is ongoing.

A recent series of experiments supports the idea that protein–protein interactions are responsible for increased viscosity at high protein concentrations.[3–6,8,9] Therefore, one strategy to reduce viscosity would be to disrupt or replace viscosity-increasing

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

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Journal of Pharmaceutical Sciences, Vol. 102, 1182–1193 (2013)

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protein–protein interactions with protein excipient interactions. We hypothesized that polar solvents, including dimethyl sulfoxide (DMSO) and dimethylacetamide (DMA), would disrupt protein self-association via direct interaction with hydrophobic protein residues while interfering with intermolecular hydrogen bonding leading to reduced solution viscosity. Indeed, polar solvents have been shown to preferentially solvate hydrophobic surfaces and interact with protein residues through hydrogen bonding and dispersive-type interactions.[10–13]

Large-length-scale water density fluctuations near hydrophobic surfaces have been shown to control hydrophobic assembly in aqueous solution.[14] If protein self-association is at least partially mediated via dispersive-type interactions, then at high concentrations as the two surfaces (protein–protein) approach each other, formation of the soft liquid–vapor-like interface characteristic of the hydrophobic force of assembly could be induced. At the interface between water and hydrophobic protein surfaces, water molecules form fewer hydrogen bonds compared with bulk resulting in a greater distance between the protein surface and the first hydrophobic hydration shell. The interface near relatively large patches of hydrophobic protein surfaces is thus characteristically similar to the liquid–vapor interface (oil–water interface) and forms the mechanistic basis of hydrophobic assembly. In contrast, density fluctuations near a hydrophilic surface are bulk like without vapor phase characteristics and eliminate the driving force for assembly. The presence of polar solvents should diminish the probability for large-scale density fluctuations of water near the hydrophobic surface by nature of the solvents' dual hydrophobic and hydrophilic character and thus eliminate the hydrophobic driving forces responsible for protein self-association. In addition, DMSO interacts with many hydrophilic amino-acid residues as determined by contacts in the Protein Data Bank (PDB).[10,12,13] Therefore, disruption of hydrophilic protein–protein intermolecular interactions via DMSO is also likely to facilitate reduced viscosity.

Polar solvents have been used extensively for altering the solubility, hydrolytic stability, and activity of therapeutic products including small molecules, peptides, and large proteins.[15] The applications of polar solvents in the biotechnology industry are numerous often involving purification and crystallization of biopharmaceuticals, spray drying and microencapsulation, and formulation. Similarly, some polar solvents, including DMSO and DMA, are currently used in marketed products.[16–18] However, the only polar solvents previously used in marketed injectable products are DMSO and DMA. Therefore, we limited our studies to these two polar solvents for potential indications requiring subcutaneous injections and ultrahigh protein concentrations.

In this work, we evaluate the use of DMSO and DMA as excipients for viscosity reduction of ultrahigh protein concentration formulations. Furthermore, to characterize the effects of these polar solvents on protein structure and to determine the mechanisms leading to their observed effects, spectroscopic and calorimetric methods were employed. Finally, the hemolytic potential and postdose toxicity in rats were determined for different formulations containing variable amounts of cosolvent (DMSO or DMA). We describe the physicochemical basis of a protein–polar solvent interaction while addressing the safety and feasibility for use in routine industrial practice.

MATERIALS AND METHODS

IgG1, a full-length antibody composed of κ-light chains, was used in this study. The antibody was cloned, expressed in Chinese Hamster Ovary cell, and purified at Genentech (South San Francisco, California). All reagents were ACS grade. The buffer solutions used in this study were 30 mM histidine, pH 5.5 with 0%, 1%, 2%, 5%, 10%, 15%, or 20% (v/v) DMSO (Amresco, Solon, Ohio) or DMA (Alfa Aesar, Ward Hill, Massachusetts). All Fourier transform infrared (FTIR), UV/vis, and Raman spectroscopy and differential scanning calorimetry (DSC) measurements were performed using protein concentrations of 150 mg/mL.

Viscosity Measurements

The viscosity of all solutions was measured using an Anton Paar Physica MCR300 rheometer with a CP25-1 24.972 mm cone (Anton Paar, Ashland, Virgina). A Peltier plate device was used to control temperature and measurements were made at 25°C. Three separate 75-μL samples of each formulation were measured 20 times over a 100 s interval, with a shear rate of 1000/s. The viscosity values are reported as the average ± standard deviation.

Differential Scanning Calorimetry

Differential scanning calorimetry thermograms were measured using a Microcal VP-Capillary DSC with autosampler (MicroCal, Northampton, Massachusetts) and calculated using Microcal subroutines. The scanning rate for all experiments was 60°/h, and a filtering period of 16 s was used. The DSC thermograms were recorded up to the temperature of reversibility and rescanned two to three times. The full DSC trace was also obtained in a separate experiment from which the transition midpoints were obtained. A Levenberg–Marquardt nonlinear least-squares method was used to fit the reversible transition utilizing a two-state model.

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