# Tungsten-Induced Protein Aggregation: Solution Behavior

YIJIA JIANG,[1] YASSER NASHED-SAMUEL,[1] CYNTHIA LI,[1] WEI LIU,[2] JOEY POLLASTRINI,[1] DAVID MALLARD,[3] ZAI-QING WEN,[1] KIYOSHI FUJIMORI,[1] MONICA PALLITTO,[1] LISA DONAHUE,[1] GRACE CHU,[4] GIANNI TORRACA,[1] AYLIN VANCE,[1] TONY MIRE-SLUIS,[5] ERWIN FREUND,[2] JANICE DAVIS,[6] LINDA NARHI[1]

[1]Formulation and Analytical Resources, Amgen, Inc., One Amgen Center Drive, Thousand Oaks, California 91320

[2]Drug Product and Device Development, Amgen, Inc., One Amgen Center Drive, Thousand Oaks, California 91320

[3]Ross University, 499 Thornall St., 10th Floor, Edison, New Jersey 08837

[4]AML Quality, Amgen Manufacturing, Limited (Puerto Rico), PO Box 4060, Juncos, Pureto Rico 00777

[5]Corporate Product Quality, Amgen, Inc., 4000 Nelson Road, Longmont, Colorado 80503

[6]WindRose Analytica, Inc., 5217 Verdugo Way, Suite A, Camarillo, California 93012

*Received 3 September 2008; revised 17 February 2009; accepted 27 February 2009*

*Published online 30 July 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21778*

**ABSTRACT:** Tungsten has been associated with protein aggregation in prefilled syringes (PFSs). This study probed the relationship between PFSs, tungsten, visible particles, and protein aggregates. Experiments were carried out spiking solutions of two different model proteins with tungsten species obtained from the extraction of tungsten pins typically used in syringe manufacturing processes. These results were compared to those obtained with various soluble tungsten species from commercial sources. Although visible protein particles and aggregates were induced by tungsten from both sources, the extract from tungsten pins was more effective at inducing the formation of the soluble protein aggregates than the tungsten from other sources. Furthermore, our studies showed that the effect of tungsten on protein aggregation is dependent on the pH of the buffer used, the tungsten species, and the tungsten concentration present. The lower pH and increased tungsten concentration induced more protein aggregation. The protein molecules in the tungsten-induced aggregates had mostly nativelike structure, and aggregation was at least partly reversible. The aggregation was dependent on tungsten and protein concentration, and the ratio of these two and appears to arise through electrostatic interaction between protein and tungsten molecules. The level of tungsten required from the various sources was different, but in all cases it was at least an order of magnitude greater than the typical soluble tungsten levels measured in commercial PFS.  J Pharm Sci 98:4695–4710, 2009

**Keywords:** protein aggregation; prefilled syringe; tungsten; precipitation

## INTRODUCTION

In recent years, the range of protein biopharmaceuticals in the marketplace has changed dramatically, with an increase in the number of approved products, in the modalities of the proteins (cytokines, growth factors, monoclonal antibodies, etc.) and in the types of delivery

Abbreviations: DLS, dynamic light scattering; SEC, size exclusion chromatography; ICP, inductively coupled plasma; UV, ultraviolet; CD, circular dichroism; FTIR, Fourier-transformed infrared; PFS, prefilled syringe.

*Correspondence to*: Linda Narhi (Telephone: 805-447-3104; Fax: 805-499-3654; E-mail: lnarhi@amgen.com) and Yijia Jiang (Telephone: 805-447-1116; Fax: 805-499-3654; E-mail: yjiang@amgen.com)

Journal of Pharmaceutical Sciences, Vol. 98, 4695–4710 (2009)

devices and/or primary containers used (vial, PFS, etc.).[1,2] With the change in complexity of protein products and delivery devices comes increased complexity in the interactions of the protein with components of the device and in protein self-association. These interactions may lead to protein unfolding or aggregation and have been the focus of much research effort and regulatory attention due to concerns about product quality and patient safety.

In our earlier investigation, an increase in protein aggregation and visible particle formation was observed in a protein formulation in prefilled syringes (PFSs) that had been manufactured using a tungsten pin to make the needle hole.[3] These particles were shown to consist of protein and tungsten.[3,4] Initial unpublished observation showed that increases in protein aggregation were also observed during stability studies of other proteins in PFS that were manufactured using tungsten pins.[5] In our previous study of a model protein in PFSs, we found that syringes with particles and higher amounts of protein oligomers generally contained a higher level of tungsten than those syringes without particles.[4]

Tungsten pins are commonly used in the syringe-forming process.[3] Material from these pins is believed to sublimate in the presence of hot glass and air, leading to deposits of tungsten species on the interior of the syringe.[6] Polytungstate is known to cause protein aggregation, and indeed this characteristic has been exploited to remove proteins from biological samples prior to testing.[7] Specific interactions between tungsten polyanions and human serum albumin have recently been demonstrated.[8] We therefore attempted to generate protein particles by spiking proteins in syringes with varying levels of tungsten species from $WO_3$, $H_2WO_4$, and $Na_2WO_4$ and from the extract prepared from tungsten pins that had been used in the syringe manufacturing process.

The reversibility of protein aggregation and particle formation and the conformation of the protein molecules both in solution and in the protein aggregate were assessed to better understand the mechanism of protein assembly in the presence of tungsten. The reversibility of particle formation was assessed by redissolving the particulates or diluting the aggregated protein samples and analyzing the resulting solutions by size exclusion chromatography (SEC) and/or dynamic light scattering (DLS). The conformation of the proteins in solution and in the solid state was analyzed by circular dichroism (CD), infrared (IR), or Raman spectroscopies. This article describes the effect of tungsten on aggregation of two model proteins in syringes and proposes a possible mechanism for this phenomenon.

## MATERIALS AND METHODS

### Materials

The two proteins studied were purified at Amgen (Thousand Oaks, CA) and are >98% pure by SEC. The two proteins were chosen as models to represent protein classes with high helical content and Fc fusion proteins, respectively. The alpha-helical protein contains four aspartic acid, nine glutamic acid, four lysine, five arginine, and five histidine for the charged amino acids. The numbers of each of the charged amino acids in the Fc fusion protein are: 20 aspartic acid, 25 glutamic acid, 26 lysine, 21 arginine, and 11 histidine and among them 12 aspartic acid, 16 glutamic acid, 20 lysine, 6 arginine, and 7 histidine are from the Fc part of the molecule. The alpha-helical protein was prepared at low protein concentration (<1 mg/mL) in a buffer at pH 4 (buffer A). The fusion protein was prepared at high concentration (>30 mg/mL) in a buffer at pH 6.3 (buffer B). The levels of tungsten in buffers A and B are below 0.5 ppb, the limit of quantitation of the inductively coupled plasma (ICP)–MS instrument.

### Preparation of Soluble Tungsten Species from Commercial $WO_3$, $H_2WO_4$, $Na_2WO_4 \cdot 2H_2O$, and from Used Tungsten Pins

Tungsten pins that had been used in syringe manufacturing processes were placed into glass vials and the corresponding buffers used for the alpha-helical and fusion proteins were added so that most of the heat-affected areas of the pins were covered. The capped vials were placed into a temperature-controlled sonicator at 45°C. They were sonicated for approximately 1.5 h, then incubated in an oven for 12 h at approximately 45°C followed by an additional approximately 1.5 h of sonication. The extracts were transferred to microfuge tubes and centrifuged to remove visible particulates. The supernatants were transferred to clean microfuge tubes and 50 μL of the solutions was removed for analysis of tungsten concentration by ICP–MS. The final measured