Journal of Pharmaceutical Sciences xxx (2016) 1-9

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

Synergistic Effect of Cavitation and Agitation on Protein Aggregation

Tetsuo Torisu [1,2], Takahiro Maruno [3], Yoshinori Hamaji [1], Tadayasu Ohkubo [2], Susumu Uchiyama [3,4,*]

[1] Biologics and New Modalities Development, Pharmaceutical Sciences, Takeda Pharmaceutical Company Limited, Hikari, Yamaguchi, Japan
[2] Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan
[3] Department of Biotechnology, Graduate School of Engineering, Osaka University, Suita, Osaka, Japan
[4] Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Okazaki, Aichi, Japan

ARTICLE INFO

Article history:
Received 27 July 2016
Revised 27 September 2016
Accepted 13 October 2016

Keywords:
monoclonal antibody
stability
protein aggregation
microparticles
biopharmaceutical characterization
particle size
adsorption
desorption

ABSTRACT

It was recently reported that dropping induces protein aggregation due to the occurrence of cavitation. Agitation also causes protein aggregation. In this study, vials filled with antibody solution were subjected to a cycle of dropping and shaking using the friability testing apparatus to examine the combined effect of cavitation and agitation on protein aggregation. A cycle of dropping and shaking generated a massive amount of subvisible particles. Comparison of aggregation rate at different fill volumes indicated that shaking plays an important role in protein aggregation due to combination stress. Furthermore, the impact of dropping on aggregate formation was apparent because aggregation rate under combination stress was much faster than that under shaking stress alone. Increase in aggregate concentration was observed after shaking of the antibody solution, which was freshly filled into vials that had been previously used in the dropping and shaking test. Polysorbate 80 was effective in inhibiting aggregate formation under combination stress. These results suggest the following particle formation pathway: cavitation caused by dropping promotes antibody unfolding, the unfolded antibodies adsorb on the inner surface of the vial, and subsequent shaking yields subvisible particles by desorbing the adsorbed antibodies.

© 2016 American Pharmacists Association®. Published by Elsevier Inc. All rights reserved.

Introduction

In recent years, the use of antibodies and antibody-based drugs has increased rapidly.[1] Protein aggregates are considered as impurities included in biopharmaceuticals and are one of the major concerns of pharmaceutical companies because protein aggregates can lower drug efficiency and potentially elicit adverse immune responses.[2,3] Therefore, regulatory agencies require characterization and establishment of control strategies for protein aggregation during the development of biopharmaceuticals.[4] Protein aggregates can be induced by various stresses including high temperature, low pH, and oxidation; the quality and quantity of the aggregates also depend on the type of stress.[5,6]

Dropping is also a type of stress that can induce protein aggregation. When containers, such as vials, containing protein solution are dropped on a solid surface, cavitation bubbles are generated and subsequently collapse.[7] Bubble dynamics model show that extremely high temperature and pressure are locally generated at the site of collapse.[8] Moreover, hydrogen and hydroxyl radical can be generated during cavitation because the high temperature decomposes water molecule.[9] Protein molecules can get degraded and aggregated due to the high temperature, high pressure, and the free radicals generated by dropping.[7] Agitation stress can also induce protein particle formation.[10] Aggregation induced by agitation is triggered by nucleation of protein molecules at the air–water interface,[11,12] and collisions among nuclei and other molecules lead to the formation of large particles.[13]

Cavitation and/or agitation can occur under several conditions during the manufacturing, shipping, and administration of biopharmaceuticals.[14-16] Although several studies have reported protein aggregation induced by different stresses,[7,10-12] the combined effect of cavitation and agitation on protein aggregation has not been investigated in previous studies.

Friability testing apparatus is a widely used pharmacopoeial instrument to test the hardness of tablets[17-19] in pharmaceutical industries. Although the friability testing apparatus is designed to test the ability to withstand against mechanical stresses which

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2016.10.015.

* *Correspondence to:* Susumu Uchiyama (Telephone: +81-6879-4216; Fax: +81-6879-7442).

E-mail address: suchi@bio.eng.osaka-u.ac.jp (S. Uchiyama).

http://dx.doi.org/10.1016/j.xphs.2016.10.015
0022-3549/© 2016 American Pharmacists Association®. Published by Elsevier Inc. All rights reserved.

tablets are subjected to during manufacturing and shipping,[20] it is considered that drug products of biopharmaceuticals are also subjected to the same kind of physical stresses as those generated by the friability testing apparatus during shipping. Here, to understand the combined effect of cavitation and agitation on protein aggregation during shipping, the friability testing apparatus was used to generate a cycle of dropping and shaking of vials. Aggregates induced by the friability testing apparatus were analyzed by size exclusion chromatography (SEC), dynamic light scattering (DLS), and flow imaging analysis (FI). Circular dichroism (CD) spectroscopy was used to evaluate secondary and tertiary structure changes, and liquid chromatography mass spectrometry (LC-MS) analysis was performed to examine chemical degradation due to the combination stress. The protective effect of nonionic surfactant against aggregation induced by dropping and shaking was also investigated.

The results obtained in this study revealed a synergistic effect of cavitation and agitation on antibody aggregation. The cyclic process of adsorption of unfolded antibodies to inner surface of the vial after dropping and subsequent desorption of the adsorbed antibodies by shaking contributes to the synergistic effect. Polysorbate 80 has a powerful protective effect on protein aggregation induced by dropping and shaking by lowering the surface tension of the solution, thus preventing proteins from adsorption to water-hydrophobic part interfaces.

Materials and Methods

Materials

Stock solution of humanized immunoglobulin G1 (IgG1) antibody at a concentration of 10 mg/mL in phosphate-buffered saline (PBS) was used in this study.[21] PBS (pH 7.4) and 10× concentrated PBS (pH7.4) were purchased from Invitrogen (Carlsbad, CA). Polysorbate 80 was purchased from J.T. Baker (Center Valley, PA) and NOF Corporation (Tokyo, Japan). Other reagents were purchased from Wako Chemicals (Osaka, Japan), unless otherwise specified. IgG1 solution was diluted to 0.9 mg/mL with 1× PBS. The diluted IgG1 solutions were filtered through 0.22-μm polyethersulfone filter and filled in 2.5-mL Crystal Zenith® vials purchased from Daikyo Seiko, Ltd. (Tokyo, Japan). The vials were sealed with fluoropolymer-coated rubber stopper and crimped with aluminum caps.

Combination Stress Conditions

FRV 2000 (Copely Scientific Ltd., Nottingham, UK) friability testing apparatus, compliant with United States Pharmacopoeia <1216>, European Pharmacopoeia <2.9.7>, and Japanese Pharmacopoeia <G6>, was used to study the combined effect of dropping and shaking on aggregation of IgG1 antibody. IgG1 solution was filled in the vials at fill volumes of 1.0, 1.5, and 2.0 mL. The vials were placed in the drums of the apparatus and a cycle of dropping and shaking was generated by rotating the drums at 50 rpm for 100 and 400 min. IgG1 diluted with 1× PBS containing 0.1% polysorbate 80 was also prepared and subjected to the dropping and shaking stress to evaluate inhibition effect of polysorbate 80 on the protein aggregation induced by the combination stress.

Shaking Stress Conditions

To evaluate the contribution of dropping stress to protein aggregation induced by the combination stress, IgG1 samples were prepared and subjected to only shaking stress using the TAITEC Mix-VR orbital shaker (Saitama, Japan). Vials filled with IgG1 solution were placed on the orbital shaker horizontally and shaken at 300 rpm for 100 and 400 min. IgG1 solution subjected to shaking stress was then compared with the samples subjected to the combination stress (prepared using the friability testing apparatus, as described earlier).

Investigation of the Synergistic Effect of Dropping and Shaking

IgG1 solution was dropped and shaken by the friability testing apparatus at 50 rpm for 10 min. The stressed IgG1 solution was removed, and the vial was refilled with fresh (unstressed) IgG1 solution. The vials were then shaken using the orbital shaker at 300 rpm for 30 and 100 min. The vials prepared as the control were not subjected to shaking. As shown in Figure 1a, 2 controls and 3 samples were prepared: control F1, dropped and shaken with 1.0 mL of IgG1 solution and then refilled with 1.0 mL of unstressed IgG1; control F2, dropped and shaken with 2.0 mL of IgG1 solution and then refilled with 1.0 mL of unstressed IgG1; sample F1A1, dropped and shaken with 1.0 mL of IgG1 solution and then refilled and shaken with 1.0 mL of unstressed IgG1; sample F1A2, dropped and shaken with 1.0 mL of IgG1 solution and then refilled and shaken with 2.0 mL of unstressed IgG1; and sample F2A1, dropped and shaken with 2.0 mL of IgG1 solution and then refilled and shaken with 1.0 mL of unstressed IgG1. Particle concentration was measured after the shaking and compared with that of the control. In addition, IgG1 solution was subjected to stress using the friability testing apparatus at 50 rpm for 10 min, transferred to a fresh vial, and then shaken using the orbital shaker for 30 and 100 min (sample F1A1_NV, Fig. 1b). Particle concentrations of IgG1 solution before and after the shaking were compared.

Flow Imaging Analysis

DPA-4200 (Brightwell Technologies Inc., Ottawa, Canada) equipped with silane-coated flow cell was used for quantification of particles in the micrometer range. The unstressed and stressed samples were diluted 100-fold with PBS. Samples were left to stand at room temperature for at least 2 min to remove air bubbles in the sample solutions. The system was flushed using 0.5 mL of the sample solution and then analysis was started. Analyzed volume was 0.25 mL ($n = 3$) and purge volume was 0.12 mL. Flow rate was set at 0.17 mL/min. Particles that are >1 μm were counted and edge particles eliminated by the MVSS software, version 2. The flow cell was flushed with water and PBS between measurements. For samples diluted with PBS with 0.1% polysorbate 80, sample dilution and flow cell flush were conducted using PBS with 0.1% polysorbate 80.

Size-Exclusion Chromatography

SEC was performed to quantify the amount of monomers and soluble aggregates in unstressed and stressed samples. Waters alliance 2695 system equipped with 2487 UV detector (Milford, MA) and Tosoh TSK gel G3000SWXL (Tokyo, Japan) were used for the analyses. For separation, 90% of 2× PBS and 10% of acetonitrile was used at a flow rate of 0.5 mL/min. The unstressed and stressed solutions were centrifuged at 13,200 × g for 10 min before the analysis; 20 μL of supernatant was injected into the system. UV absorbance detection was performed at 280 nm. Areas under the curves of monomers and high−molecular weight (HMW) species were calculated. In the analyses of samples containing polysorbate 80, peak area derived from polysorbate 80 was subtracted from peak area of HMW and monomer because the polysorbate 80 peaks overlapped with HMW peaks and partly with monomer peak (Supplementary Fig. S1). Samples were analyzed in duplicate, and the average monomer area and monomer area % were reported.

Download English Version:

https://daneshyari.com/en/article/8514433

Download Persian Version:

https://daneshyari.com/article/8514433

Daneshyari.com