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A systematic evaluation of mechanisms, material effects, and protein-dependent differences on friction-related protein particle formation in formulation and filling steps

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ABSTRACT

Particle formation by physical degradation during the compounding step of biopharmaceuticals is a common concern and found in vessels with bottom mounted stirrers. It was potentially linked to sliding bearings, however, the exact mechanism was still unclear. In this study, custom designed small scale bearings in combination with an IgG1 antibody as model protein were used for investigations of the degradation mechanism inside a bearing. Thereby, abrasion of adsorbed proteins by contact sliding was identified as prevailing protein degradation mechanism and was quantified by an increase in turbidity and by monomer loss. As the protein degradation was highly dependent on combinations of the material of the bearing and the buffer solution, a test system was introduced which allowed to study these effects. Results from the test system using IgG1 and recombinant human growth hormone confirmed a protective effect of Polysorbate 80 by a reduction of protein adsorption, which was strongest in combination with a highly hydrophobic sliding material (PTFE). Finally, a comparison of degradation products from various stresses by ATR-FTIR revealed a high similarity between friction-related degradation products. Therefore, abrasion of adsorbed proteins is very likely the prevailing physical degradation mechanism in processing steps where contact sliding occurs.

1. Introduction

The manufacturing process of a typical protein pharmaceutical drug product includes so called formulation, fill and finish processing steps, where a purified and concentrated drug substance solution is processed into its final dosage form for the market. During the formulation step, also called compounding, the drug substance is diluted to a predetermined concentration and excipients are added to increase the stability of the molecule during processing, handling and long term storage (Rathore and Rajan, 2008). After homogenization by stirring in the compounding vessel the solution has the composition of the final drug product. Subsequent processing steps are sterile filtration, filling into the primary packaging and, if needed, lyophilization. In course of the processing steps the physical state of the protein can change. Diverse stresses have been described to affect the physical stability of proteins, including heat, freezing, light, dehydration, interfacial effects, shear, pressure and pH (Chang and Yeung, 2010). Exposure to such stress conditions can induce aggregation and particle formation which can change the biological activity of the drug and increase the potential for immunogenicity (Wang et al., 2010). Furthermore, particles created during compounding negatively influence the fouling behavior of the subsequent filtration step (Rajniak et al., 2008).

Here we want to focus on the stress that is caused by stirring in a compounding vessel with a bottom mounted magnetic type stirrer. Bottom mounted stirrers enable mixing of low volumes and are usually magnetically coupled impellers as this lowers the risk of contamination due to the absence of mechanical shaft seals that can fail (Chisti, 1992). Therefore, vessels with a bottom mounted stirrer are nowadays widely used for compounding. The impeller is kept in position by a sliding bearing, which consists of a fixed male part (stator) that is mounted to the vessel and a female part (rotor), that is embedded in the rotating mixing head (see Fig. 1). Typically, the bearing is manufactured of very hard ceramics (such as silicon carbide—SiC) which enable high wear and corrosion resistance (Somiya, 2013). The bearing is fully submerged inside the vessel.

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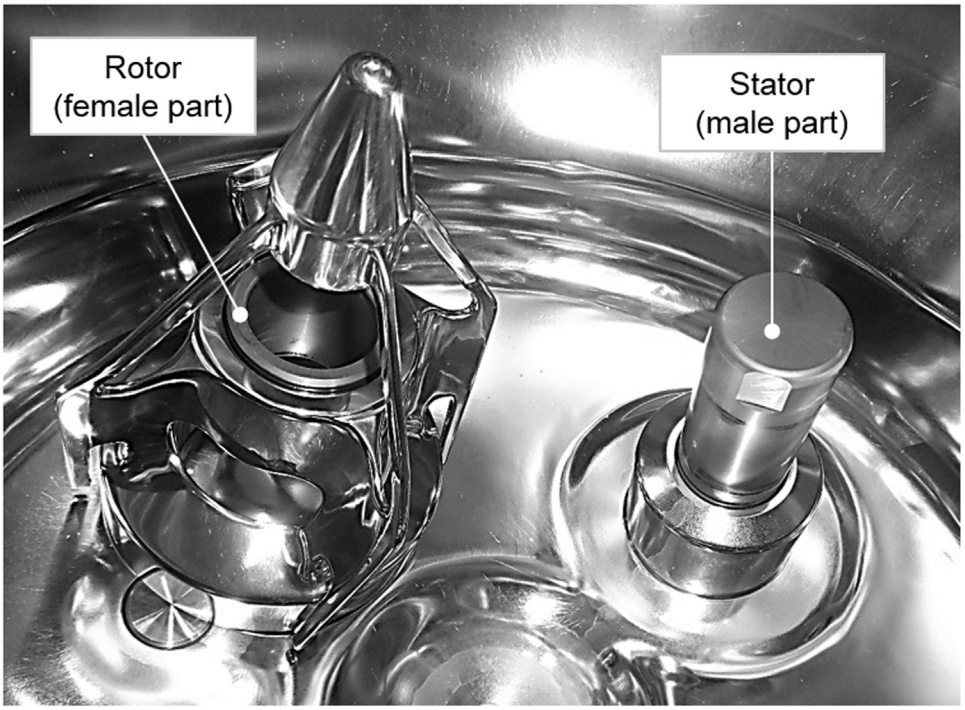

Fig. 1. A typical bottom mounted-magnetic type stirrer. The mixing head with the embedded female part of the sliding bearing has been removed from the male part of the bearing for better visualization.

Thus, the liquid containing the active pharmaceutical ingredient is serving as lubricant for the sliding bearing.

There is a growing awareness throughout the pharmaceutical community that bottom-magnetic type stirrers have an impact on drug product quality. In a study performed by Ishikawa et al. a mixing system using a top entering type stirrer was compared to a bottom-magnetic type stirrer. Stirring of a monoclonal antibody solution resulted in particle formation and therefore filter fouling in a subsequent filtration step when a bottom-magnetic type stirrer was used. No formation of particles could be observed for the top entered stirrer (Ishikawa et al., 2010). An evaluation of the impact of the design of different types of bottom mounted stirrers on monoclonal antibody solutions was performed later (Gikanga et al., 2015). The authors could show that mixers which were designed to have only a small gap between rotor and stator or even touching parts induced particle formation upon rotation. Both studies draw the conclusion that shear stress inside the gap of the sliding bearing was the most likely mechanism for the observed physical protein degradation. Cavitation alone as well as cavitation in combination with shear was additionally considered by Gikanga et al. However, it has to be mentioned that the studies were designed to evaluate the best mixing system and not to identify the exact degradation mechanism.

From the physical protein degradation mechanisms that are currently under discussion in fill and finish steps, we identified 5 mechanisms that could be responsible for protein degradation inside the sliding bearing.

1) Heat induced protein denaturation: Contact sliding of the inner and outer part of the bearing could generate heat. Heat induced protein denaturation is a well-known phenomenon (Chang and Yeung, 2010) and could be responsible for the reported protein degradation by rotational movement of the sliding bearing.
2) Nano/micro particle heterogeneous nucleation: Micro/nano particles of stainless steel have been described to cause aggregation of IgG1 over 30 min of incubation (Bee et al., 2009a). Furthermore, they are under discussion to promote IgG1 particle formation by heterogeneous nucleation during stainless steel rotary piston pump filling operations (Tyagi et al., 2009). Contact sliding of the male and female part could introduce ceramic particles into the sample solution, which could serve, similar to steel particles, as heterogeneous nuclei for particle formation.
3) Cavitation and air/liquid interfacial effects: The phenomenon of rapidly forming vapor cavities (bubbles) inside of a liquid is called cavitation. Upon collapse of the bubbles, shock waves, extreme pressure, temperature or highly turbulent flow conditions could be responsible for protein aggregation, as well as a potential formation of hydrogen radicals (Mahler et al., 2009). Furthermore, cavitation creates a temporary air/liquid interface, which is destroyed again after the bubble is burst. It could be shown that especially the dilation and subsequent compression of air/liquid interfaces causes particle formation of a monoclonal antibody (Bee et al., 2012). Finally, it has to be mentioned, that additional air/liquid interfacial effects exist during stirring (e.g. vortex formation), which are not directly related to a sliding bearing. As these effects are also found in systems having top entering type stirrers, they were not considered as potential root cause of particle formation in our magnetic-bottom stirring studies.
4) Shear stress induced unfolding: Unfortunately, the term “shear” can be interpreted in different ways. Here we want to follow the definition of Thomas and Geer, who described shear as the effect of hydrodynamic forces in terms of velocity gradients on proteins in free liquid (Thomas and Geer, 2011). Shear related effects at interfaces, on the other hand, are considered below (see abrasion of adsorbed proteins). The effect of shear on proteins in solution is an interesting and debated topic with many conflicting studies. Whereas some authors claim to have observed shear induced unfolding already at moderate shear rates below $10^3\ s^{-1}$ (Ashton et al., 2009; Bekard et al., 2011, 2012; Tirrell and Middleman, 1975), contrary papers report