Particle Shedding from Peristaltic Pump Tubing in Biopharmaceutical Drug Product Manufacturing

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ABSTRACT: In a typical manufacturing setup for biopharmaceutical drug products, the fill and dosing pump is placed after the final sterile filtration unit in order to ensure adequate dispensing accuracy and avoid backpressure peaks. Given the sensitivity of protein molecules, peristaltic pumps are often preferred over piston pumps. However, particles may be shed from the silicone tubing employed. In this study, particle shedding and a potential turbidity increase during peristaltic pumping of water and buffer were investigated using three types of commercially available silicone tubing. In the recirculates, mainly particles of around 200 nm next to a very small fraction of particles in the lower micrometer range were found. Using 3D laser scanning microscopy, surface roughness of the inner tubing surface was found to be a determining factor for particle shedding from silicone tubing. As the propensity toward particle shedding varied between tubing types and also cannot be concluded from manufacturer's specifications, individual testing with the presented methods is recommended during tubing qualification. Choosing low abrasive tubing can help to further minimize the very low particle counts to be expected in pharmaceutical drug products. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1440–1450, 2015

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INTRODUCTION

The design of a suitable drug product (DP) manufacturing process is essential for biopharmaceutical DP quality. Because of the sensitivity of protein molecules, decisions should be taken with care.[1,2] For fill and finish processes, several types of pumps are generally employed, including rotary piston pumps, rolling diaphragm pumps, peristaltic pumps, and time-pressure fillers. Given the fact that pumping to dispense into units occurs after the sterile filter in order to avoid backpressure peaks and to ensure adequate dispensing accuracy, generated particles may directly end up in the final DP units.[1,3,4] The European and United States Pharmacopeia require parenteral DPs to be essentially/practically free from visible particles. Additionally, subvisible particle counts have to be below harmonized thresholds [not more than (NMT) 6000 particles ≥10 μm and NMT 600 particles ≥25 μm per container].[5] Therefore, DPs are carefully monitored for visible as well as subvisible particles, also in frame of continuous discussions about immunogenicity caused by protein particles.[1,6–9] Non-proteinaceous particles might pose a risk to patients because of capillary occlusion, although controlled studies on this subject are not available.[10]

To date, several studies indicate that the use of rotary piston pumps might increase the risk of particle formation in protein-based DP solution.[1,3,6] Upon filling, the protein possibly acts as lubricant and is exposed to significant forces.[1] In consequence, the formation of protein particles is frequently encountered. Moreover, stainless steel particles can be shed from the pump head.[6] In contrast, stress on the proteins exercised upon filling with peristaltic pumps has been shown to be minimal[2,11,12] and the use of peristaltic pumps has not led to a significant increase in particle counts and turbidity.[3] Another main benefit of filling with a peristaltic pump is the fact that the DP solution only comes into contact with the inner surface of the disposable tubing. This greatly diminishes the risk for cross-contaminations[13] and the need for cleaning validation of these components. Downsides of peristaltic pumps include the lower dosing accuracy in comparison with piston pumps.

For fill and finish operations with a peristaltic pump, platinum-cured silicone tubing is the material of choice as it releases less leachables than peroxide-cured silicone or tubing made from other plasticizer containing materials.[14] Concerns about extractables and leachables from these materials are generally low, given that additives for silicone tubing usually only involve fillers like fumed silica but not plasticizers. Moreover, silicone tubing exhibits excellent long-term durability, can be sterilized, is highly flexible, and therefore, is well-suited for usage in peristaltic pumps.[15] Nevertheless, potential drawbacks might result from enhanced particle spallation from the tubing material due to the mechanical stress exerted within the peristaltic pump. Presently, particle shedding and tubing wear have mainly been investigated in the context of medical use,[16–23] as silicone tubing is employed, for example, during hemodialysis and in cardiopulmonary bypass operations.[24] Silicone tubing was shown to be more prone for particle shedding as compared with tubing made from polyvinyl chloride and polytetrafluoroethylene composites.[25] Despite these findings, silicone

Abbreviations used: DP, drug product; NMT, not more than; WfI, water for injection; CIP/SIP/DIP, cleaning-in-place/sterilization-in-place/drying-in-place; NTU, nephelometric turbidity unit; DLS, dynamic light scattering; PdI, polydispersity index; LO, light obscuration; MFI, micro-flow imaging; NTA, nanoparticle tracking analysis; FNU, Formazine nephelometric units; RT, room temperature; SEM, scanning electron microscopy.

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tubing remains the standard material for operation in peristaltic pumps because of the above-mentioned advantages.

A study that characterizes particle shedding from different kinds of silicone tubing used during biopharmaceutical DP manufacturing is still lacking. We close this gap by presenting a comprehensive data set on the amount of shed particles, particle characteristics, and tubing wear of three different commercially available silicone tubing types. Influences of tubing pre-treatment, pumping time, recirculation medium (WfI and a surfactant-containing placebo solution), and possible batch-to-batch variations were considered. Surface characterization with 3D laser scanning microscopy of all tested tubing types was employed to identify the root cause for the observed differences in particle shedding. As experiments were performed under recirculating conditions, particle counts of this study were extrapolated to production conditions during fill and finish of a biopharmaceutical DP. Studying the influence of shed particles on the stability of a biopharmaceutical DP or interactions with protein was beyond the scope of this study, but was addressed in separate investigations which are to be published soon.

MATERIALS AND METHODS

Materials

Water for injection (WfI) was produced via distillation (Muldestor Modell SE, Wagner + Munz, München, Germany) from deionized water. Placebo pH 6.0 containing 10 mM L-histidine monohydrochloride monohydrate and 10 mM L-histidine (Ajinomoto Europe S.A.S, Louvain-la-Neuve, Belgium), 200 mM sucrose (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), and 0.02% polysorbate 20 (Croda GmbH, Nettetal, Germany) was prepared with highly purified water (purification system arium pro with Sartopore 2 150 0.2 µm filter capsule from Sartorius AG, Göttingen, Germany). After filtration (0.2 µm, cellulose acetate; Sartorius) the buffer was stored at 2°C– 8°C until further use.

Three different types of commercially available silicone tubing (A, B, and C) were tested. Tubing sets employed in recirculation experiments consisted of two 30 cm long pieces for location within the pump head connected to 100 cm long tubing pieces via polypropylene Y-connectors (Kartell, purchased from VWR International GmbH, Darmstadt, Germany). The inner diameter of the investigated tubing was 6.0 mm for tubing A and B and 6.4 mm for tubing C. Sets were used either untreated, washed (washing cycles with highly purified water of 80°C, drying with filtered air at 110°C), and sterilized (30 min at 126°C and 2 bar) or cleaning-in-place/sterilization-in-place/drying-in-place (CIP/SIP/DIP) treated [washing cycles with highly purified water of 80°C, sterilization for 30 min at 131°C and 3 bar, drying with sterile nitrogen at room temperature (RT)]. This material processing was performed in analogy to production conditions and parameters. Unless otherwise stated, tubing was used as received from the manufacturer (= untreated).

Methods

Online-Turbidity Monitoring

Pumping cycles were performed under a laminar flow cabinet to avoid external particle contamination. Tubing sets were flushed with 4 L of highly purified water and 1 L of WfI prior to the recirculation experiment using a laboratory vacuum pump (LABOPORT, KNF Neuberger GmbH, Freiburg, Germany). Flush volumes were based on initial experiments (data not shown). The low-pressure flow-through cuvette set (HACH LANGE GmbH, Düsseldorf, Germany) as well as the outer surfaces of the ends of the tubing set were rinsed with highly purified water and WfI until the rinsing fluid was visually free from particles. Online-turbidity monitoring was performed with 250 mL of WfI or placebo in recirculation over 24 h with the setup shown in Figure 1. The operation parameters of the Flexicon PD12 peristaltic pump (Watson-Marlow Flexicon, Ringsted, Denmark) were based on previous experiments (data not shown) and set as follows: dispensing mode, 180 rpm, acceleration of 60, starting filling volume of 5.0 mL. As occlusion pressure is known to have an impact on particle shedding,[16,17,19] this parameter was kept at factory settings resulting in an occlusion pressure of 1.1 bar (tubing A) or 1.3 bar (tubing B and C) upon operation in air (accuracy class 2.5 manometer from WIKA Alexander Wiegand SE & Company KG, Klingenberg, Germany). In continuous mode, these settings resulted in a flow rate of 23 mL/s. Online-turbidity monitoring was carried out with a HACH 2100AN turbidimeter connected to a computer using HachLink 2000 V.2.9 alpha software. The operation mode of the turbidimeter included activated ratio function, auto range function and signal averaging ($n = 15$). Data points were collected every 60 s. Turbidimeter functionality was confirmed with turbidity standards (<0.15 NTU stray light standard, 0–2/0–20/0–200/0–2000 NTU Gelex Secondary Standards from HACH; maximum deviation of ±5 % in comparison with precedent measurement) prior to every experiment. Different pre-treatments of each tubing type were investigated with $n = 2$.

Dynamic Light Scattering

Particle characterization in the nanometer range was performed by dynamic light scattering (DLS) using the Zetasizer Nano-ZS (Malvern Instruments Ltd., Herrenberg, Germany). The undiluted, neither filtered nor centrifuged samples were measured in disposable Plastibrand semi-micro PMMA cuvettes (Brand GmbH, Wertheim, Germany) at a backward scatter of 173° after 20 s equilibration time at 25°C and with water as dispersant (viscosity of 0.8872 cP). Positioning, attenuation selection, and measurement duration as well as number of sub runs for the three performed measurements per sample were optimized automatically for each run by the Zetasizer Software 6.32. Z-average and polydispersity index (PdI) were calculated applying the "General purpose (normal resolution)" analysis model.

The ζ-potential of the particles was measured with the same instrument. An aliquot of 800 µL of the final recirculate was analyzed in disposable folded capillary cells (Malvern) with three measurements of 10–30 sub runs each at a voltage of 50 V. Zeta-potentials were derived from electrophoretic mobility data applying the monomodal analysis model and Smoluchowski theory.

Light Obscuration

The final recirculate was analyzed for particles in the micrometer range by light obscuration (LO) (in analogy to USP 788 and Ph Eur 2.9.19 requirements) with a SVSS-C instrument (PAMAS, Partikelmess- und Analysesysteme GmbH, Rutesheim,