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Evaluation of aggregate and silicone-oil counts in pre-filled siliconized syringes: An orthogonal study characterising the entire subvisible size range

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ABSTRACT

Characterisation of particulates in therapeutic monoclonal antibody (mAb) formulations is routinely extended to the sub-visible size-range (0.1–10 μm). Additionally, with the increased use of pre-filled syringes (PFS), particle differentiation is required between proteinaceous and non-proteinaceous particles such as silicone-oil droplets. Here, three orthogonal techniques: Raster Image Correlation Spectroscopy (RICS), Resonance Mass Measurements (RMM) and Micro-Flow Imaging (MFI), were evaluated with respect to their sub-visible particle measurement and characterisation capabilities. Particle formation in mAb PFS solutions was evaluated with increasing polysorbate-20 (PS-20) concentrations. All three techniques provided complementary but distinct information on protein aggregate and silicone-oil droplet presence. PS-20 limited the generation of mAb aggregates during agitation, while increasing the number of silicone-oil droplets (PS-20 concentration dependant). MFI and RMM revealed PS-20 lead to the formation of larger micron-sized droplets, with RICS revealing an increase in smaller sub-micron droplets. Subtle differences in data sets complicate the apparent correlation between silicone-oil sloughing and mAb aggregates' generation. RICS (though the use of a specific dye) demonstrates an improved selectivity for mAb aggregates, a broader measurement size-range and smaller sample volume requirement. Thus, RICS is proposed to add value to the currently available particle measurement techniques and enable informed decisions during mAb formulation development.

1. Introduction

There is an estimated production of 3.5 billion pre-filled syringe (PFS) units per year for therapeutic biopharmaceutical drug (*e.g.* monoclonal antibody (mAb)) administration, with a potential to grow to 6.7 billion units by 2020 (TMR, 2013), (Rapra, 2015). The increase in PFS use is driven by factors such as the ease of use, advantages in safety, reductions in drug overfill and patient self-administration; all of which reduce the incidence of hospitalisation and associated costs (Condino et al., 2005).

One of the challenges for the formulation scientist is to ensure the stability of the formulated mAb throughout the products lifetime, in the preferred presentation. Protein aggregation has been found to arise during and after fill-finish steps; which may develop from mechanical and/or agitation stress or from interaction with primary packaging components (Baldwin, 1988). Silicone-oil is a widely-utilised lubricant in PFS, facilitating ease of plunger movement in syringes and injection with hypodermic needles (Thirumangalathu et al., 2009); however, exposure to sloughed silicone-oil droplets has been suggested to adversely impact formulation stability (Gerhardt et al., 2014; Shi and Ladizhansky, 2012). Initial indication of adverse effects from silicone-oil was found in the 1980s following correlation of insulin particle formation with elevated blood glucose levels, in diabetics administered with the product (Baldwin, 1988). Later studies on agitation stress have shown the loss of soluble protein in PFS to be a

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particular problem during transportation (Gerhardt et al., 2014). Furthermore, agitation at higher speeds was correlated with an increase in monomer loss in reported shaking studies (Thirumangalathu et al., 2009). Subsequently, a number of silicone-oil related mechanisms underlying particulate formation have been proposed, exemplified by dispersed droplets acting as nucleation sites for protein aggregation (Majumdar et al., 2011); adsorption-destabilization of protein onto the silicone-oil/water interface (Thirumangalathu et al., 2009); and silicone-oil droplet surface charge neutralisation by adsorbed proteins resulting in agglomeration (Basu et al., 2013; Ludwig et al., 2010).

The size range of protein and silicone-oil particulates is generally wide (Table 1 presents the various size ranges and common terminologies used) (Ludwig et al., 2011; Philo, 2006; Philo and Arakawa, 2009; Weinbuch et al., 2013b). The United States Pharmacopeia (USP) chapter 'Particulate Matter in Injections' ⟨788⟩ defines concentration limits for particles in parental solutions that are ≥10 and 25 μm (Pharmacopeia, 2012b). USP chapter 'Subvisible Particulate Matter in Therapeutic Protein Injections' ⟨787⟩ makes the recommendation to monitor particles <10 μm, with a supporting chapter ⟨1787⟩ giving guidance on the expanded techniques that can be used and size ranges (Pharmacopeia, 2012a). Based on the USP recommendations, the commercially available Micro-Flow Imaging (MFI) system, detecting particles from approximately 1 μm to 400 μm (Zolls et al., 2012), (Sharma et al., 2010b), is commonly used in the industry to assess sub-visible particulates alongside more established USP methods such as light obscuration (Pharmacopeia, 2012a,b). The potential immunogenic risk of smaller sub-visible aggregates (0.1–10 μm) has been discussed by Carpenter et al. (Carpenter et al., 2009) and Singh et al. (Singh, 2013; Singh et al., 2010) and regulatory submissions therefore may include quantitative characterisation of micron-sized aggregates (1–10 μm) and qualitative characterisation of sub-micron aggregates (0.1–1 μm) in the early stages of development (Pharmacopeia, 2011; Weinbuch et al., 2013b). With the current particle detection technologies, an 'analytical gap' around 1 μm still remains; consequently there is a drive for the development of new particle metrology tools (Gross et al., 2016). Furthermore, there is a high interest in developing technologies which are also capable of particle differentiation *i.e.* between protein and foreign matter, such as silicone-oil. In response to this predicament, in the last decade several new analytical technologies have been introduced in order to detect and characterise aggregates; offering the capability to extend the detectable size range of particles from 30 nm to 10 μm, through combining orthogonal technologies (Ríos Quiroz et al., 2015). For example, the recently developed Resonance Mass Measurement (RMM) system (Archimedes) has been utilised alongside MFI, as a particle metrology tool to bridge the analytical size 'gap' for particulates in the 0.5–5 μm size range, and similar to MFI, discriminate between silicone-oil droplets and protein aggregates. However, the focus of the study was on large sub-micron and micron-sized particles through the utilisation of the RMM 'micro sensor', with a lower detection limit of 0.5 μm (Pharmacopeia, 2011; Weinbuch et al., 2013b).

Table 1
Common terminology used for various protein aggregate size ranges (Carpenter et al., 2009; Narhi et al., 2012; Ríos Quiroz et al., 2015; Zolls et al., 2012).

Common terms	Size in Diameter
Nano-metre aggregate, oligomer	<100 nm
Sub-micron aggregates	0.1–1 μm
Smaller sub-visible aggregates	0.1–10 μm
Sub-visible particles, micron aggregates	1–100 μm
Visible particles	>100 μm
Analytical size gap	0.5–5 μm

Raster Image Correlation spectroscopy (RICS) is an image analysis tool, originally developed by Digman et al. (Digman et al., 2005). We recently reported a comparison of particle size distributions in the gap region with the novel application of RICS, by extrinsic aggregate labelling, against Dynamic Light Scattering (DLS) and MFI, in simple mAb formulations (*e.g.* in the absence of silicone-oil and surfactant). RICS was demonstrated to measure a broad particle size range (*i.e.* 10 nm–~100 μm) for stressed mAb samples (*i.e.* thermal and freeze-thaw stress) (Hamrang et al., 2015); thereby providing scope for the application of RICS in more complex formulations.

This manuscript reports the quantitative evaluation of protein and silicone-oil particulates formed in PFS solutions, both within and outside the analytical size gap range. We compare the complementary of RICS, detecting particles from 30 nm–10 μm, against RMM and MFI which are capable of particle sizing over the sub-micron (~0.1–~5 μm, through the use of the nano and micro sensor) and micron (>1 μm) sizes ranges, respectively. The PFS solutions, in the presence and absence of polysorbate-20 (PS-20), were subjected to agitation stress *via* end-over-end rotation, used to model stress during transportation (Gerhardt et al., 2014; V, 2011). There are numerous studies assessing the mechanisms of mAb aggregation (Li et al., 2011; Morris et al., 2009) and the effects of silicone-oil (Basu et al., 2013; Gerhardt et al., 2014; Jones et al., 2005; Weinbuch et al., 2013b) or polysorbate surfactants (Agarkhed et al., 2013; Khan et al., 2015) in influencing the aggregation process; such studies include novel methods to reduce *in situ* mAb aggregation in PFS (Depaz et al., 2014). However, the focus has been the larger sub-visible size range of particulates *i.e.* >0.5 μm (Felsovalyi et al. 2012; Krayukhina et al., 2015; Teska et al. 2016); due to the current lack of available technologies that are sensitive to the detection of smaller particles, whilst capable of differentiating between proteinaceous and foreign particulates (*e.g.* silicone-oil). Herein, the ability of RICS to characterise aggregates in solutions containing silicone-oil droplets *via* extrinsic fluorescent dyes is also evaluated: the selectivity of RICS (through the use of a specific dye) is compared with the efficiency of RMM and MFI (based on particle buoyancy and optical parameters for RMM and MFI, respectively) in particle differentiation. The assessment of size and concentration of particulates generated in siliconized PFS containing formulated mAb is reported utilising all three techniques.

2. Materials and methods

2.1. Materials

A bi-specific monoclonal antibody, herein termed 'COE-08′, was kindly provided by Medimmune (Cambridge, UK). 1 mL, long, sterile, ready to fill BD Hypak™ glass siliconized syringes were purchased from Becton Dickinson and Company (New Jersey, US).

All buffer components including sucrose, L-histidine and PS-20 were of analytical grade or higher, purchased from Sigma Aldrich (Dorset, UK) and used without further purification.

SYPRO® Red and SYPRO® Orange dyes were obtained from Thermo Scientific (Leicestershire, UK) at a concentration of 5000 × (in DMSO). All buffers and solutions were prepared with Millipore de-ionised water (18 MΩ.cm) and pre-filtered prior to stress experiments.

2.2. Methods

2.2.1. Sample preparation

All solutions were prepared in a pH 6 buffer composed of 25 mM histidine and 235 mM sucrose. COE-08 solutions were prepared at a final concentration of 10 mg/mL in the presence of 0,