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DEVELOPMENT OF PREFILLABLE SYRINGES TO MITIGATE THE RISK OF PARTICLE FORMATION IN BIOPHARMACEUTICALS

In this article, Hideaki Kiminami, Research Manager, Core Technology Group, Terumo Corporation, and Philippe Lauwers, Director Technology Development, Terumo Europe, discuss the problem of particle formation in biopharmaceuticals packaged in prefilled syringes.

INTRODUCTION

In recent years, the safety of drugs has become a topic of significant importance. To enable patients and healthcare professionals to use drugs comfortably and safely, it is necessary to assess their safety at each phase of the manufacturing process,[1] from active pharmaceutical ingredients to finished formulations, and to verify the safety and efficacy of the drug product as marketed within its primary container and secondary packaging.[2] A major concern is that therapeutic proteins may denature or aggregate by physical or chemical stimulation to form particles,[3] leading to the development of immunogenic responses and, consequently, adverse reactions in patients.[4] This article discusses the main factors responsible for particle formation in biopharmaceuticals and also describes a conceptual and technical approach for the reduction of particle formation in prefilled syringe (PFS) systems.[5,6]

> "A major concern is that therapeutic proteins may denature or aggregate by physical or chemical stimulation to form particles, leading to the development of immunogenic responses and, consequently, adverse reactions in patients."

> "Proteins used as active ingredients in biopharmaceuticals are generally chemically unstable, and therefore likely to undergo denaturation or aggregation due to stresses such as heat, vibration, and impurities introduced during the manufacturing process."

ISSUES AND MEASURES IN BIOPHARMACEUTICALS

A variety of drug products has been developed for many therapeutic applications, from small-molecule drugs produced by chemical synthesis, to biopharmaceuticals produced by biotechnological processes, such as genetic recombination and cell fusion.[7]

Proteins used as active ingredients in biopharmaceuticals are generally chemically

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unstable, and therefore likely to undergo denaturation or aggregation due to stresses such as heat, vibration, and impurities introduced during the manufacturing process.[8–16] Protein aggregation poses an important risk, including reduced drug efficacy and an increased risk of immunogenicity.[17–20] In response to this, the US FDA issued a guidance for industry on the risk management of biopharmaceuticals in August 2014.[4] Manufacturers are required to assess particles in biopharmaceuticals appropriately, and to reduce the risk of protein aggregation.

A variety of particle sizes may be present in biopharmaceuticals, from the nanometre scale up to the order of micrometres. In the US Pharmacopeia (USP), European Pharmacopeia (Ph Eur) and Japanese Pharmacopoeia (JP), the test for insoluble sub-visible particulate matter is listed as USP<788>, Ph Eur 2.29.19 or JP<6.07> respectively, and assesses the number of particles with a size ≥10 µm and ≥25 µm. The assessment of insoluble particles in biopharmaceuticals must be performed in compliance with USP<787>. In addition, recent studies have emerged to indicate that particles between 0.1 and 10 µm in size have an immunogenic potential. The relationship between sub-visible particles (SVPs) and immunogenicity has been determined from experiments in mice,[12,21] and fatal adverse events that may have been triggered by the presence of SVPs in biopharmaceuticals were reported in March 2016 by the FDA.[22] Thus, assessment of SVP-sized particles should also be performed.

USP<788>, Ph Eur 2.29.19, and JP<6.07> (Insoluble Particle Matter Test) include the use of the light obscuration (LO) particle count test for counting the number of particles. The LO method is a highly reliable analytical procedure that determines the attenuation of light energy (i.e. the blockage of transmitted light) by particles passing through channels and thus the size and number of particles based on the frequency of blockage. In addition to the LO method, there are various analytical procedures to measure particle size, with some of these analytical procedures shown in Table 1.

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Analytical Procedure	Abbreviation	Detectable Range (µm)
Dynamic Light Scattering	DLS	0.001 – 10
Asymmetrical Flow Field Flow Fractionation	AF4	0.001 – 100
Analytical Ultracentrifugation	AUC	0.001 – 0.1
Hollow Fiber Flow Field Flow Fractionation	HF5	0.001 – 100
Size Exclusion Chromatography Multi Angle Light Scattering	SECMALS	0.001 – 0.1
Nanoparticle Tracking Analysis	NTA	0.02 – 1
Resonant Mass Measurement	RMM	0.1 – 5
Flow Cytometry	FCM	0.2 – 200
Quantitative Laser Diffraction	qLD	0.15 – 10
Flow Imaging	FI	1 – 200
Light Obscuration	LO	1 – 200

Table 1: Analytical procedures by particle size.

As these analytical procedures use different methods of detection and have varying levels of sensitivity, a wider detectable size range does not necessarily indicate a better analytical procedure. Additionally, the current research and development efforts of analytical instrument manufacturers have led to the emergence of instruments that provide highly accurate particle analysis over a wider detectable range. There is currently no single procedure that provides absolute quantification of the number of particles present in biopharmaceuticals, and therefore particle assessment using multiple types of analytical procedures is required.[23]

The recently increased interest in PFS is largely driven by their advantages compared with traditional ampoules and vials, such as allowing quick and accurate dosing; minimising dosing errors; reducing the risk of biological contamination; enhanced convenience and ease of use; preventing of overfill; and so on. With the increasing number of biological drugs becoming available, the demand for PFS has increased considerably in recent years.

It has been reported that silicone oil (SO) applied to the inner wall of PFS or tungsten oxide residues resulting from the glass forming process can cause the oxidation or aggregation of biopharmaceuticals.[14,16] Furthermore, it has been suggested that SO itself may adversely affect the human body.[24]

Terumo's core R&D group has analysed and considered containers that are more "biopharmaceutical friendly" to mitigate many of the shortcomings of PFS. The approach proposed in this article focuses on the following three aspects:

1. An SO-free (SOF) PFS system
2. A polymer-based primary container
3. Establishing measures against protein oxidation.[5,25-27]

To minimise the risk of immunogenicity, a major concern for therapeutic proteins, this study investigated whether the formation of aggregated particles, a major cause of immunogenic responses, could be reduced by the construction of the PFS system. Also tackled is how the application of SO lubrication and the method of sterilisation of ready-to-fill syringes may affect protein aggregation.

DEVELOPMENT OF THE PFS FOR BIOPHARMACEUTICALS TO REDUCE PARTICLE FORMATION

Effects of the Presence of Silicone Oil

Physical stimulation of therapeutic protein products in PFS has been reported to cause aggregation, leading to particle formation.[28] Prof John F Carpenter and Prof Theodore W Randolph, both from the University of Colorado (US), proposed a model to account for the particle formation in which, after the adsorption and gelation of proteins on the SO

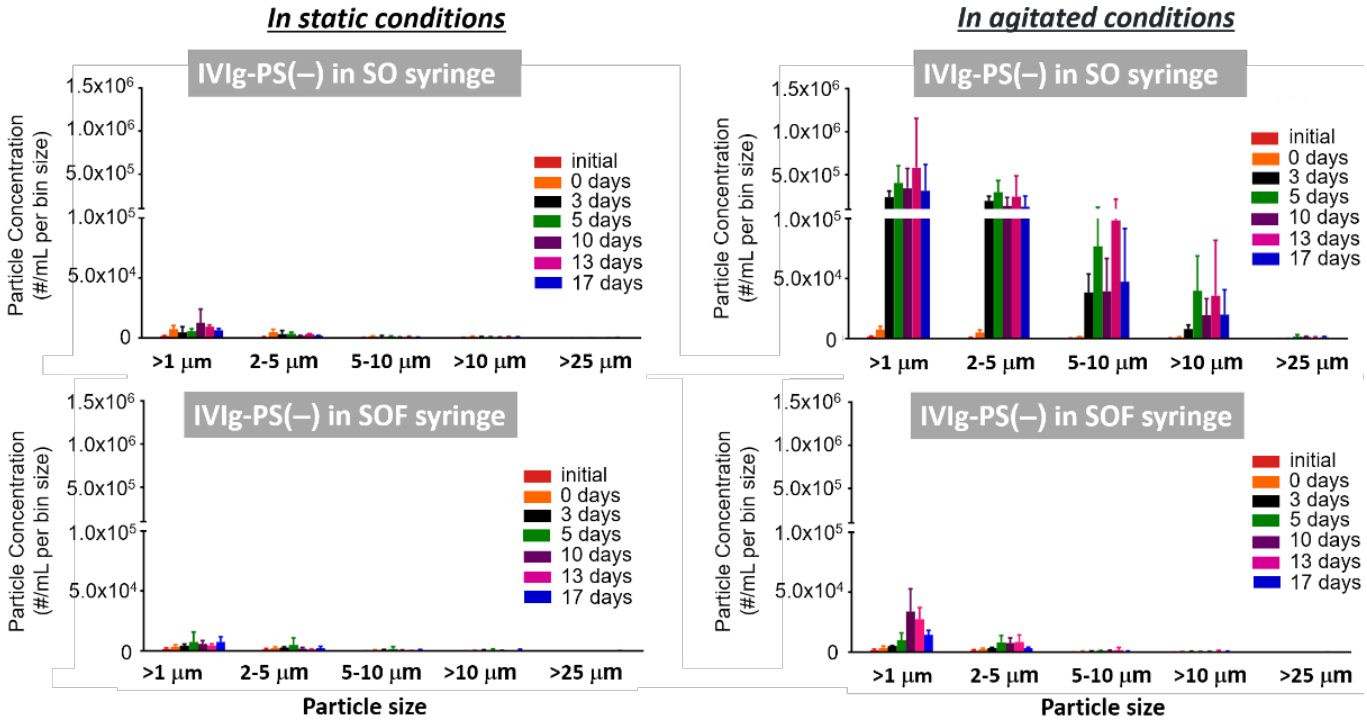

Figure 1: Results of particle assessment of IVIg products with and without SO in static and agitated conditions.

layer of the inner surface of a PFS, the layer of air remaining in the PFS is moved by physical stimulation, such as agitation, to remove the SO protein.[16] Terumo performed a particle assessment using the flow imaging (FI) method to determine the effect of SO on the aggregation of biopharmaceuticals under agitation, simulating physical stress during transportation, or during manipulation and administration procedures. The systems compared were PLAJEX™, a cyclo-olefin polymer (COP) ready-to-fill SOF system, and a siliconised PFS (Figure 1). This assessment used intravenous immunoglobulin (IVIg) as a model protein.

Under the conditions of static storage, the number of particles was only slightly increased in the SO PFS compared with that in the PLAJEX SOF PFS. However, with agitation, simulated transportation, and use, the number of particles was markedly increased in the SO PFS, while this increase was clearly minimised in the SOF PFS. These results indicate that the use of the SOF PFS system for biopharmaceuticals mitigates particle formation caused by physical stimulation in biopharmaceuticals.

Effects of Drug Composition

Proteins applied in biopharmaceuticals are composed of approximately 40–1000 amino acids (with the mean number of amino acids estimated to be approximately 300) which have a molecular weight of approximately 100 Da.[29] These amino acids contain both hydrophobic and hydrophilic groups, which makes many protein drug products poorly soluble in water. Many biopharmaceuticals therefore have polysorbate (PS), added as a surfactant to the drug formulation. Although the addition of the surfactant has been shown to reduce protein aggregation, recent investigations have suggested that additives may cause protein aggregation and SO particle formation, depending on the conditions of use.[30]

Therefore, Terumo performed a particle assessment of IVIg products containing PS by using the FI method for SO and SOF PFS under conditions that simulated actual drug formulation (Figure 2). In the PLAJEX SOF PFS, no increase in the number of particles was observed, despite the addition of PS. In the SO PFS, in contrast, the addition of PS caused a marked increase in the number of particles. As the particles observed in this assessment were either protein aggregates or SO, a particle image analysis was performed based on the FI analysis (Figure 3).

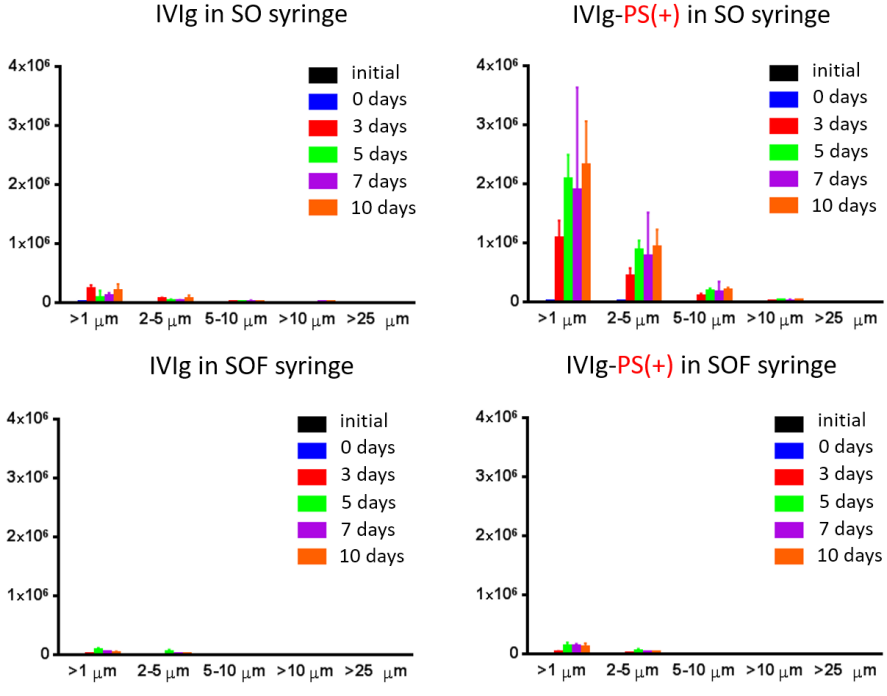

Figure 2: Results of particle assessment of IVIg products with and without PS80 in SO and SOF syringes.

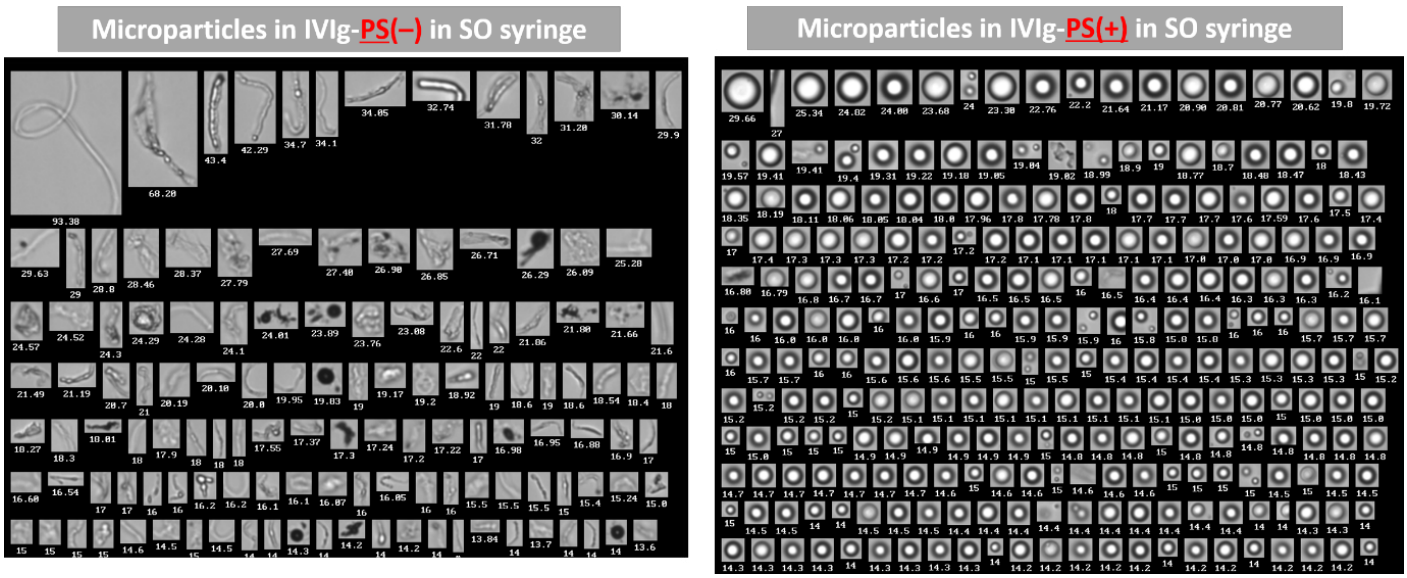

Figure 3: Results of the particle image analysis of IVIg products with and without PS in the SO syringe.

The analysis of images of particles in IVIg products showed that IVIg products filled in the SO PFS contained an abundance of long and thin filamentous particles, i.e. protein aggregates. In contrast, IVIg products containing PS were found to contain an abundance of spherical particles, i.e. SO. These results suggest that the clear increase in the number of particles in the SVP size range observed in the system with PS may be triggered primarily by an increase in SO particles rather than protein aggregates. In addition to the SO particles, as shown in the right panel of Figure 3, the IVIg product with PS was found to contain protein aggregates, shown in the left panel of Figure 3. These findings suggest that the PLAJEX SOF PFS may be effective in reducing particle formation when PS, an essential formulation component in biopharmaceuticals, is present.

Effects of the Sterilisation Method

Medical devices and prefillable ready-to-use primary drug containers are sterilised using various methods, those shown in Table 2 are commonly applied to PFS. Several of these sterilisation methods may result in some chemical or physical effects on prefillable syringes, for example radiation sterilisation causes the generation of radicals[25] and ethylene oxide (EtO) sterilisation leaves EtO residuals.[6]

Such effects and residuals may lead to the denaturation of biopharmaceuticals and radiation-sterilised PFS may lead to protein oxidation, as has been discussed in other publications.[5,25] Therefore, to determine the effects of various sterilisation methods on the denaturation and aggregation of biopharmaceuticals, Terumo assessed particle formation in erythropoietin (EPO) filled into PLAJEX SOF PFS, by examining aggregation using size exclusion chromatography with multi-angle light scattering (SEC-MALS), shown in Figure 4, and particle measurement using the FI method, shown in Figure 5. This assessment used non-sterilised PFS as a reference.

Method	Radiation Sterilisation		Ethylene Oxide Gas (EtO) Sterilisation	High-Pressure Steam Sterilisation
	Electron Beam Sterilisation	Gamma Sterilisation		
Instrument	Electron beam accelerator	Radiation source	Gas steriliser	Steam steriliser
Parameter	Dose	Dose	Time, temperature, pressure, etc.	Time, temperature, pressure, etc.
Permeability	Yes	Yes	No	No
Material	Radiation-resistant	Radiation-resistant	Gas permeability	Heat- and pressure-resistant
Treatment method	Continuous	Continuous	Batch treatment	Batch treatment
Duration of treatment	Several seconds to several minutes	Several hours to several days	Several hours	Several hours
After-treatment	Not required	Not required	Gas purging	Drying

Table 2. Sterilisation processes used for PFS.

The SEC-MALS profile of the EPO product in the steam-sterilised PFS was similar to that in the non-sterilised PFS, which indicated that no aggregation of EPO occurred in steam-sterilised PFS. In contrast, high molecular weight components tended to increase over time in the radiation-sterilised PFS, which suggest that the residual radicals induced the aggregation of EPO. Also, an increase over the components detected in the steam-sterilised PFS was seen at approximately 5.3 minutes in the EtO-sterilised PFS.

The FI measurement showed that particles in the EPO product considerably increased in the radiation-sterilised PFS at least four weeks after filling. In contrast, no remarkable increase in the number of particles was found in the steam-sterilised or EtO-sterilised PFS, with the number of particles similar to that in the non-sterilised PFS over time.

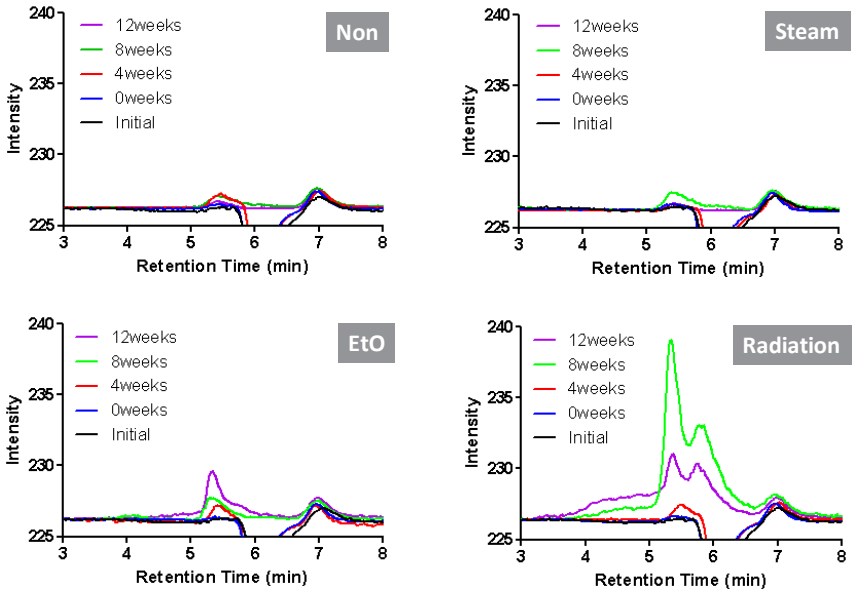

Figure 4: Results of the SEC-MALS measurements after storage of EPO products in sterilised and non-sterilised PFS.

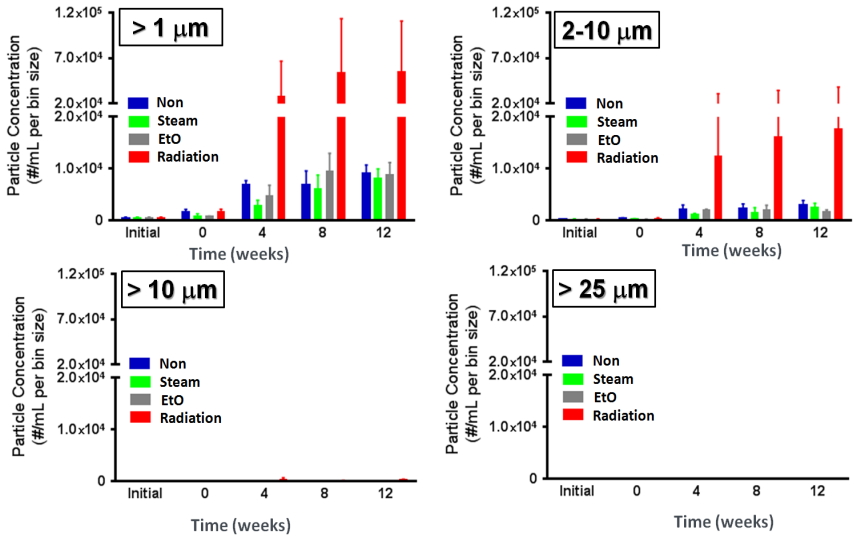

Figure 5: Results of the particle measurement by FI after storage of EPO products in sterilised PFS.

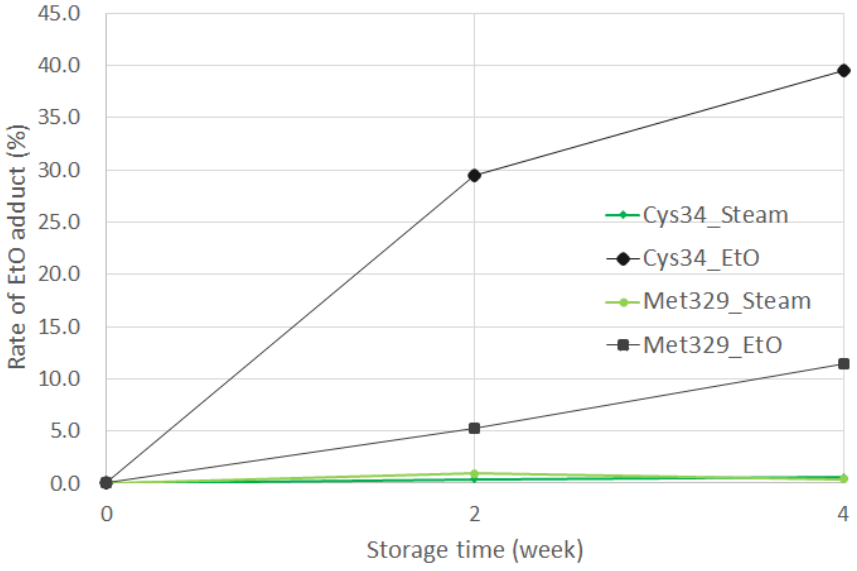

Figure 6: Rate of EtO adduct to HSA in the EtO-sterilised and steam-sterilised PFS.

Therefore, Terumo determined the effect of EtO molecules remaining in EtO-sterilised PFS on biopharmaceuticals (Figure 6).[6,31] After EtO sterilisation, an SOF PFS was left alone for four weeks, allowing for the period from sterilisation to filling and the period from filling to use, then filled with human serum albumin (HSA) solution and then stored at room temperature for four weeks. Terumo determined the rate of formation of EtO adducts with HSA. The results showed that approximately 39.5% and 11.5% of EtO molecules were added to Cys34 and Met329, respectively, in HSA. These results indicated that residual EtO molecules formed adducts with HSA, which resulted in structural changes to the drugs.

CONCLUSION

This article has discussed how, in comparison with an SO PFS, PLAJEX mitigates particle formation in biopharmaceuticals. As such, PLAJEX may be considered as a preferred primary container for biopharmaceuticals, owing to the SOF system and good response to steam sterilisation, which will help to minimise protein aggregation and the formation of particles in biopharmaceuticals, a problem that may be associated with a reduction in drug efficacy and the development of immunogenicity.

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ABOUT THE COMPANY

Terumo develops alliances with pharmaceutical companies on a global scale, using the company's technology to develop, manufacture and supply carefully crafted solutions to customers' injectable drug delivery challenges. Terumo prides itself on offering a full portfolio of products and services for the pharmaceutical industry, backed by unrivalled scientific expertise and know-how. By anticipating new trends and maintaining a constant dialogue, the company provides a first-class customer experience.

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