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READY-TO-USE PREFILLABLE SYRINGES: STERILISATION EFFECTS ON BIOPHARMACEUTICALS

In this paper, William Dierick, Director, Technology Development, and Koji Nakamura, PhD, Senior Manager, Business Development, both of Terumo Pharmaceutical Solutions, report studies comparing the effects of different sterilisation on the extractables and leachables content of biotherapeutic products in prefilled syringes, make links with free radical production and oxidisation, and PLAJEX™ with i-coating™ plunger stoppers creates a primary drug container that mitigates possible interactions from tungsten, silicone oil and their aggregation.

Pharmaceutical industry growth is buoyed this year by the expectation that 12 new drugs are forecast to reach blockbuster sales by 2020. Within this market, the biotech sector is a large contributor as indicated by the number of biopharmaceuticals within the top-10 drugs by worldwide sales in 2016. R&D in this space is forecast to continue at a high pace with four biotech products in the top-10 of the biggest launches in 2016.[1]

> "New developments have been made in recent years to establish a low-leachable PFS system with a focus on application with therapeutic proteins."

Therapeutic proteins are typically administered by injection and by using prefilled syringes (PFS). However, proteins may be sensitive to heat, oxidation and have the propensity to aggregate.[2-4] Protein aggregation and the elicitation of anti-drug antibodies (ADAs) may have detrimental effects on drug efficacy, pharmacokinetics and safety. There is evidence that protein aggregation may enhance immunogenicity and this can be an important factor in causing adverse events.[5-9] Immunogenicity has been reported with contributing factors related to excipients / formulation and interactions with contact materials of the primary drug container.[10-12]

Protein-aggregation factors derived from storage in PFS may include silicone oil, usually applied to improve the smooth gliding of the rubber plunger along the barrel, and tungsten oxide, a contaminant derived from the syringe glass manufacturing process.[13-16]

In 2014, these concerns led the US FDA to issue a Guidance for Industry: "Immunogenicity Assessment for Therapeutic Protein Products". It states that "interactions between therapeutic protein products and the container closure may negatively affect product quality and immunogenicity. These interactions are more likely with prefilled syringes of therapeutic protein products". In this Guidance, FDA recommends that sponsors should conduct a comprehensive extractables and leachables laboratory assessment using multiple analytical techniques to assess the attributes of the container-closure system that could interact with and degrade protein therapeutic products.[17]

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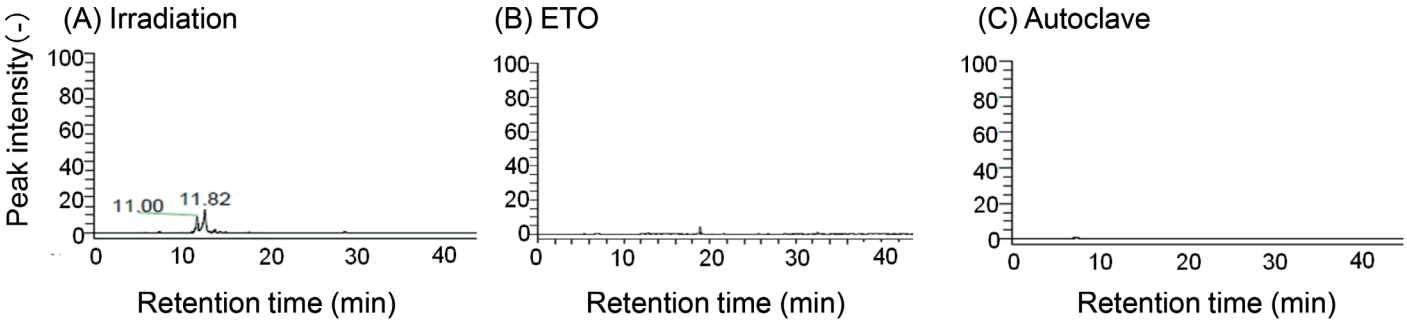

Figure 1: Comparison of extractables for different methods of sterilisation.

With emphasis on the interrelated areas (drug product formulation – container closure system – manufacturing) for therapeutic proteins, the method of sterilisation for prefillable syringes may also become a key factor to mitigate interactions with therapeutic proteins. For sterile ready-to-fill syringes, typical methods of sterilisation include Ethylene Oxide (EtO) gas sterilisation, irradiation (gamma irradiation or e-beam) and steam sterilisation, depending also on the applied materials used in the PFS system.

EXTRACTABLES / RESIDUES FROM DIFFERENT STERILISATION METHODS

New developments have been made in recent years to establish a low-leachable PFS system with a focus on application with therapeutic proteins; particularly PLAJEX™ a cyclo-olefin polymer (COP) PFS with i-coating™ plunger stoppers to eliminate the use of silicone oil as a lubricant of the syringe. These sterile ready-to-use syringes are steam sterilised within the tub/nest presentation.[18-21]

Terumo evaluated the material of PLAJEX™ prefillable syringes and compared the extractables (and residues) upon using different sterilisation methods. Sterilisation of the materials was conducted respectively with steam sterilisation (121°C for 30 min), EtO (EtO concentration 20%), and irradiation (25 kGy).

Extractables were prepared by extractions with water for injection (WFI) at conditions of 121°C for 60 min. The levels of organic compounds in the extractable were determined by liquid chromatography-mass spectrometry (LC-MS) according to methods reported previously.[20]

Figure 1 shows the graphics of relative abundance obtained by LC-MS. Peaks were detected for irradiated samples whereas no peaks were detected for steam sterilisation and EtO sterilisation. In addition, residual EtO was monitored over time by gas chromatography using headspace sampling. Figure 2 shows the

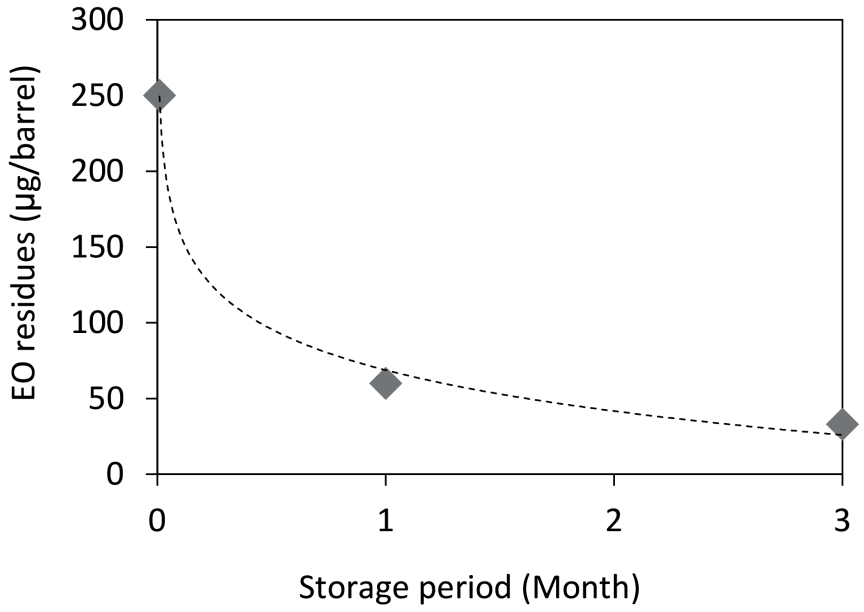

Figure 2: Residual EtO profile on polymer PFS.

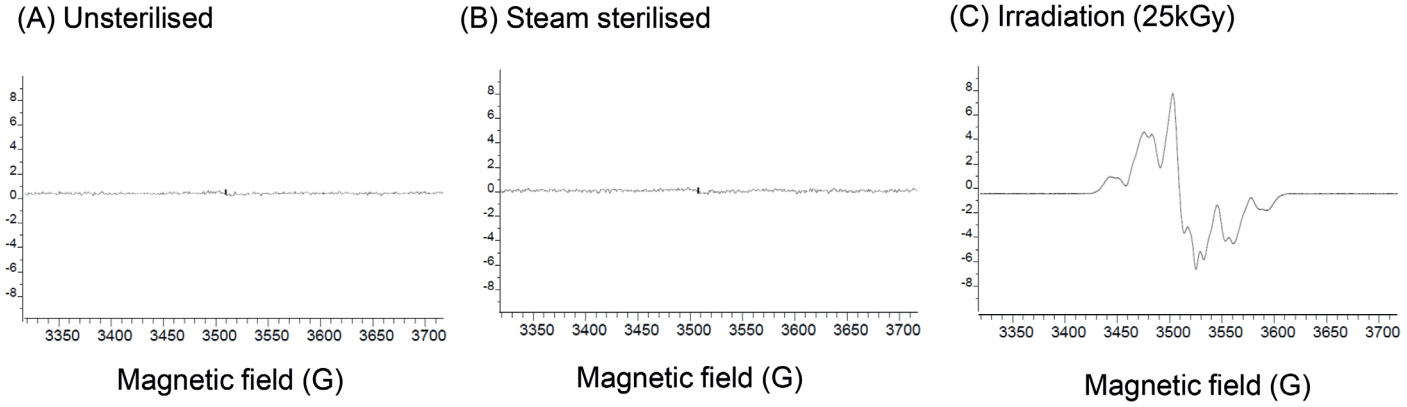

Figure 3: Typical ESR spectra of (A) unsterilised syringe, (B) steam sterilised syringe and (C) syringe irradiated at 25 kGy.

results of residual EtO over time of storage. The potential effect of residual EtO on biopharmaceuticals is not well known and will be subject to our additional research and experiments.

IRRADIATION: RADICAL GENERATION & INFLUENCE ON THERAPEUTIC PROTEINS

Sterilisation by irradiation is a technique commonly applied for polymer-based ready-to-fill syringes. Earlier publications indicated the occurrence of radicals from irradiated polymer-based PFS, affecting the level of protein oxidation as stored in these syringes.[23-26]

The generation of radicals after sterilisation of COP prefillable syringes, by irradiation (25kGy) or by steam sterilisation, was analysed using electron spin resonance (ESR) spectroscopy and the results are shown in Figure 3. There are no significant ESR spectrum changes in autoclaved syringes compared with control (non-sterilised syringe), whereas changes were observed after the syringe was sterilised by irradiation. Quantitative values of the generated radical amounts were calculated based on these spectra as shown in Figure 4.

> "We deduce that irradiation of prefillable syringes results in radical generation, resulting not only in syringe polymer oxidation, but also in an extended persistence of radicals."

Additionally, a protein oxidation study was conducted where erythropoietin (EPO) was dissolved in an aqueous solution containing 2 mM Na_2HPO_4 and 0.06 mg/mL polysorbate 80 to obtain a final concentration of EPO with 24,000 IU/mL. Analysis of oxidised methionine in the EPO solution was conducted in using HPLC. The results, shown in Figure 5, demonstrated that oxidation rate increased over time for the protein stored in prefillable syringes sterilised by irradiation. The oxidation rate of proteins stored in steam-sterilised prefillable syringes remains similar to that of non-sterilised syringes. Therefore, protein oxidation may be attributed to

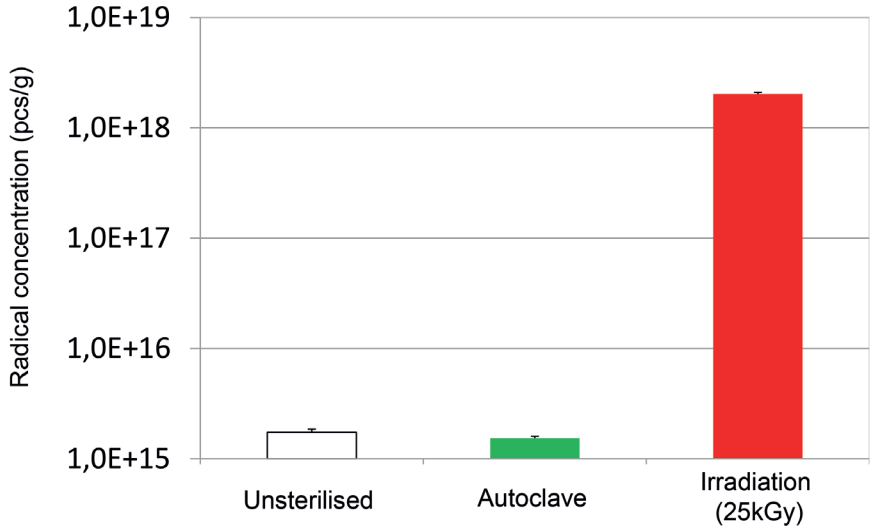

Figure 4: Residual radicals for different methods of sterilisation. Data presented as the mean ±SD (n=3).

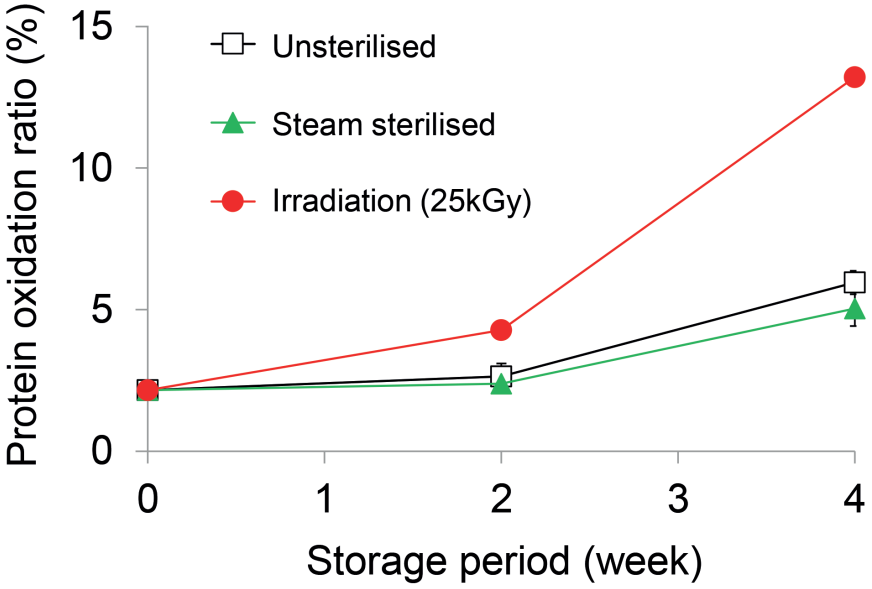

Figure 5: Difference in Oxy-Met production during storage of (square) EPO filled into unsterilised syringe, (triangle) steam-sterilised syringe, and (circle) irradiated syringe at 25 kGy, all at 25°C and 65% RH. Data presented as the mean ±SD (n=3).

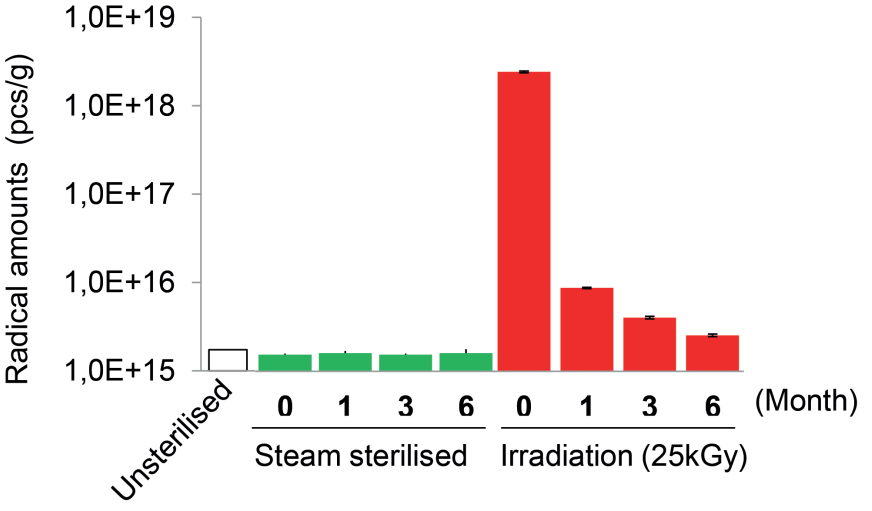

Figure 6: Transition of radical amount after storage of sterilised syringes by steam sterilisation and irradiation at 25 kGy (stored at 25°C and 65% RH).

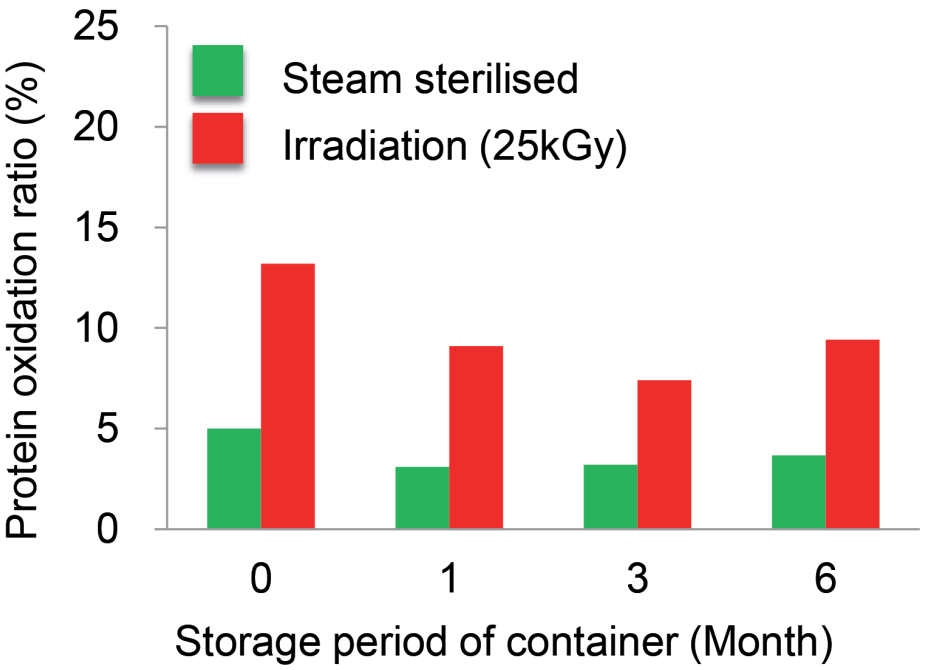

Figure 7: Comparison of protein oxidation after filling protein (EPO) into the sterilised syringe and stored at 25°C and 65% RH.

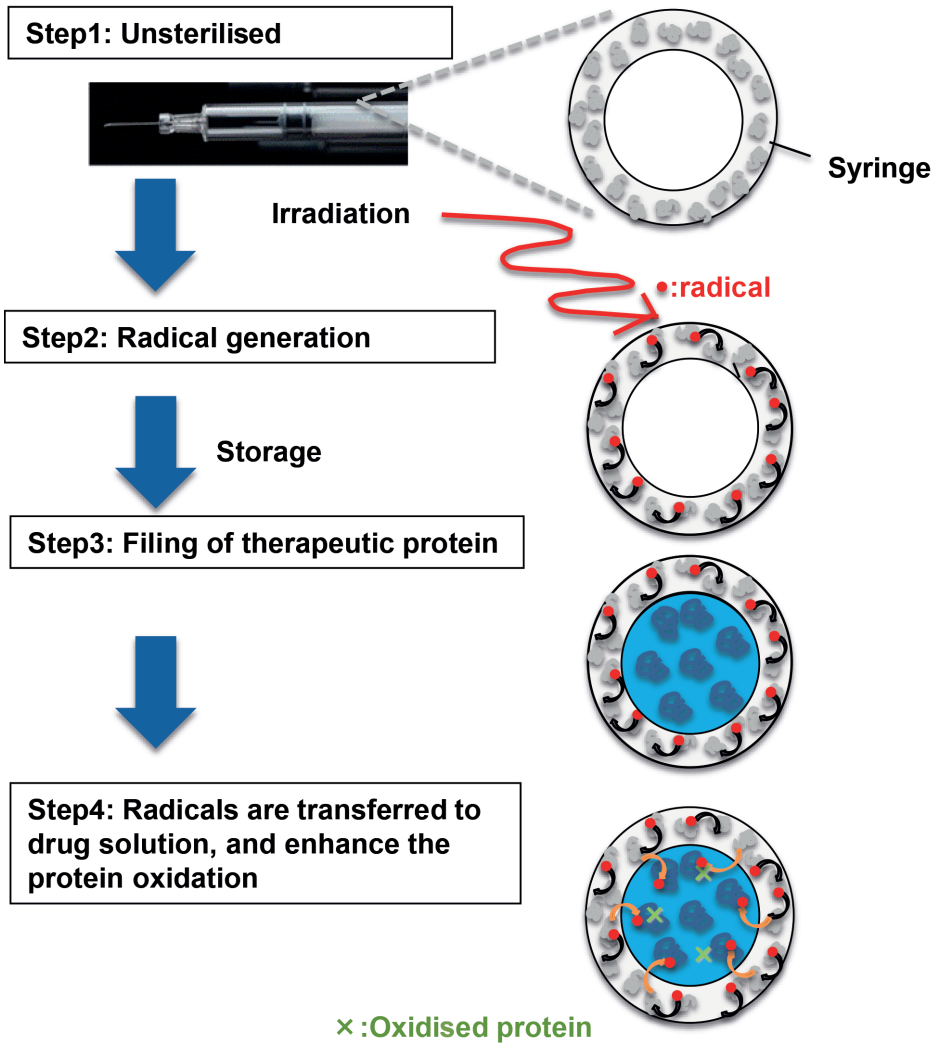

Figure 8. Hypothesis of the protein oxidation mechanism.

the generation of radicals present in the irradiated syringes.

It is generally believed that radicals persist for a very short period and more investigations were made therefore to verify the effects of time elapsing between irradiation and the time of filling of the tested protein solution (EPO solution). Samples were stored at 25°C prior to filling and filled syringes were also stored at 25°C for one month before oxidised methionine was quantified. Figure 6 shows the quantity of detected radicals over time and Figure 7 shows the protein oxidation ratio as measured for those time points. A decrease in oxidation ratio can be noted but the experiments suggest that the effect on the therapeutic proteins may persist over a longer period of storage time after sterilisation. Such effects can be mitigated by applying steam sterilisation for polymer ready-to-use syringes.

According to a report by Reigh *et al*,[27] radicals occur after polymer irradiation, and accelerate the continuous polymer oxidation. In this process, C=O or C-O bonds are produced (auto-oxidation). Development of this auto-oxidation cycle provides radicals to each molecule, resulting in a longer-term persistence of radicals. Our experiments reveal a similar reaction to the aforementioned auto-oxidation. We deduce that irradiation of prefillable syringes results in radical generation, resulting not only in syringe polymer oxidation, but also in an extended persistence of radicals (Figure 8). In our hypothesis, these residual radicals migrate into the biopharmaceutical solution, resulting in an increased oxidation of the protein drug product as was shown in Figure 6.

CONCLUSION

In consideration of the complexity and challenges associated with the development of therapeutic proteins, different regulatory guidance and directions have been developed over the years, emphasising the importance of assessing the possible interactions between biotherapeutics and the container closure system, as it may have detrimental effects on therapeutic protein quality and immunogencitiy.

PLAJEX™ with i-coating™ plunger stoppers creates a primary drug container that mitigates possible interactions from tungsten, silicone oil and aggregation thereof. Using steam sterilisation as the sterilisation method of ready-to-fill COP

syringes may assist in mitigating interactions with the therapeutic protein by reducing the risks of protein oxidation.

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