A POLYMER-BASED PREFILLABLE SYRINGE SYSTEM TO MINIMISE RISK OF PROTEIN OXIDATION

Here, William Dierick, Director, Technology Development, Terumo Europe; and Koji Nakamura, PhD, Global D&D, Terumo Corporation, explore oxygen and free-radical mediated mechanisms of protein drug degradation within prefilled syringes, present research data, and suggest that using a deoxygenated packaging system to reduce or remove dissolved oxygen is an effective means of minimising oxygen-mediated degradation, and that steam sterilisation, as against e-beam, could be a means to reduce free radical-mediated degradation.

INTRODUCTION

In the global market, prefilled syringes are preferred as a parenteral drug container in providing various advantages such as ease of use, less overfill compared to vials, and minimising errors in clinical use.[1] The applications and interest for prefilled syringes as a primary drug container are increasing due to the heightened importance of biopharmaceuticals in parenteral drug development.[2] In 2014, the US FDA issued a Guidance for immunogenicity assessment for therapeutic protein drugs.[3] Section 8 of this Guidance addresses the considerations with regard to container closure interactions, indicating that these are more likely with prefilled syringes of therapeutic protein products. The FDA cites that the following are container closure considerations pertinent to immunogenicity: protein aggregation and denaturation related to silicone oil, glass and air interfaces, as well as glass delamination and leached materials from the container closure system.

Taking this into consideration, Terumo has developed a silicone oil-free (SOF) prefillable syringe system comprised of a cyclo olefin polymer (COP)-based syringe barrel and i-coating™ stopper. The Terumo i-coating™ technology is a proprietary coating technology applied to the plunger stoppers to reduce the risk of protein aggregation induced by silicone oil.[4,5] In addition, the i-coating™ technology provides consistent performance of the break-loose and glide forces when the product is stored for an extended time, therefore providing a repeatable and predictable performance not found in siliconised glass prefillable systems.[4-6]

Protein degradation due to oxidation is well known.[1] Dissolved oxygen occurs naturally in the drug product during the formulation and filling processes. Nitrogen blanketing and other techniques are often used to reduce the effect of dissolved oxygen in the drug product. Based on recent research, this article will show how using a system approach to designing a deoxygenating package, can minimise protein degradation due to the presence of oxygen.[6-8] In addition, we will show how free radicals created during sterilisation can further contribute to oxidation and degradation of protein drug products stored in a prefilled syringe system.[8,9]

DISSOLVED OXYGEN

Generally, the polymer-based prefilled syringe has a relatively higher gas permeability than glass prefilled syringe. Over time, this may lead to increasing levels of dissolved oxygen in the drug product even with nitrogen blanketing or the anti-oxidising agents in the drug formulation. Glass prefillable syringes are often considered more appropriate than polymer-based prefillable syringes in applications with oxygen sensitive drug products.[7,8,10]

Dissolved oxygen is one of the factors causing protein degradation through the oxidation pathway.[6-8] To prevent oxida-

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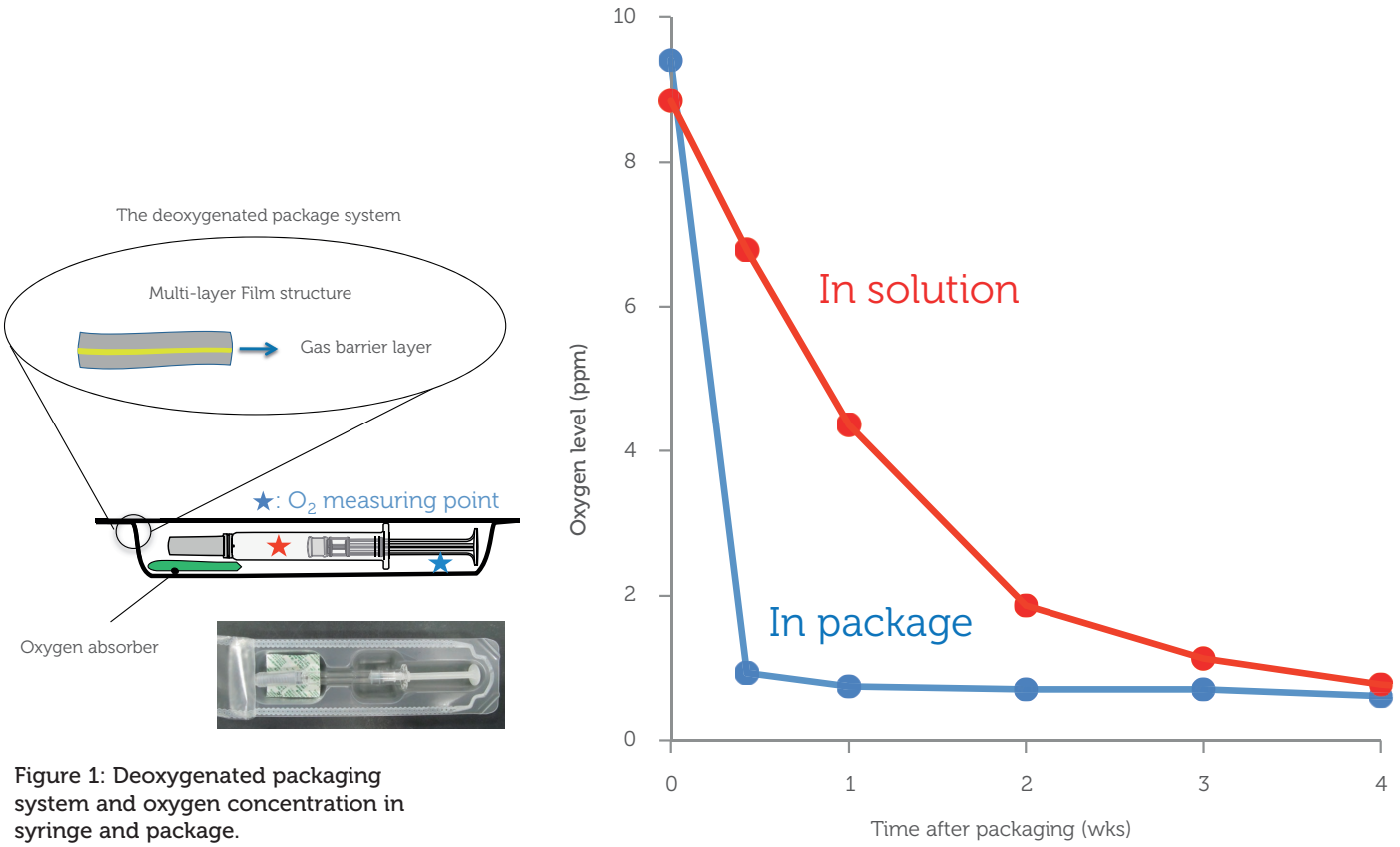

Figure 1: Deoxygenated packaging system and oxygen concentration in syringe and package.

tion of a drug product, measures should be taken, such as adding certain excipients into the drug formulation to control the oxygen level during manufacturing. An example of another method used is the Terumo prefilled syringe for multivitamins launched in 1999. This product is packaged into a deoxygenated packaging system composed of a low gas-permeable foil including an oxygen absorber as shown in Figure 1. Within this secondary packaging system, the oxygen level in the deoxygenated package decreased rapidly after sealing the package. Over time, the dissolved oxygen level in the solution within polymer-based prefilled syringe system also decreased.[6-8]

Figure 2 shows the comparative study between polymer-based prefilled syringes

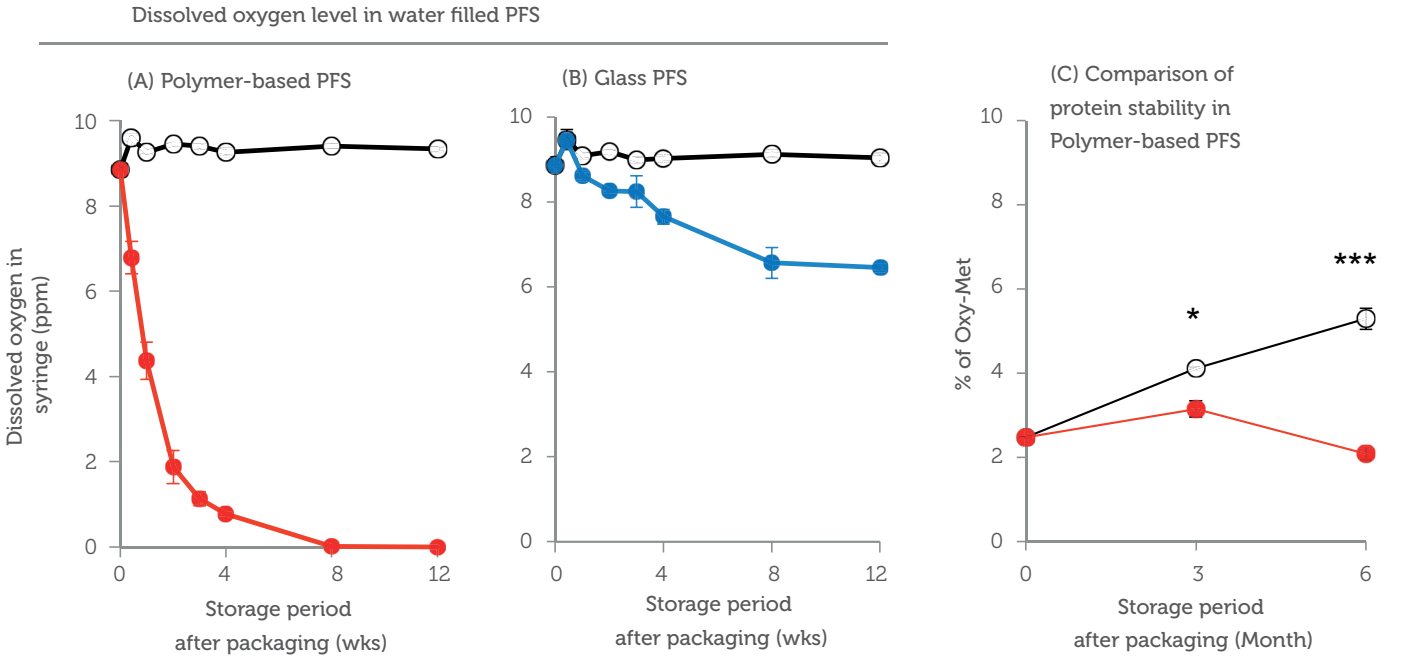

Figure 2: Reduction profile of dissolved oxygen in water filled (A) polymer-based PFS and (B) glass PFS, and (C) comparison of protein stability with and without deoxygenated packaging system. Open symbol: without deoxygenated packaging system, Closed symbol: with deoxygenated packaging system. (A), (B): Dissolved oxygen is measured by OXY-4 (PreSens). The value represents the mean +/- SD (n=3). (C) Data represents the mean +/- S.D. (n=3). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ against data from deoxygenated packaging system.

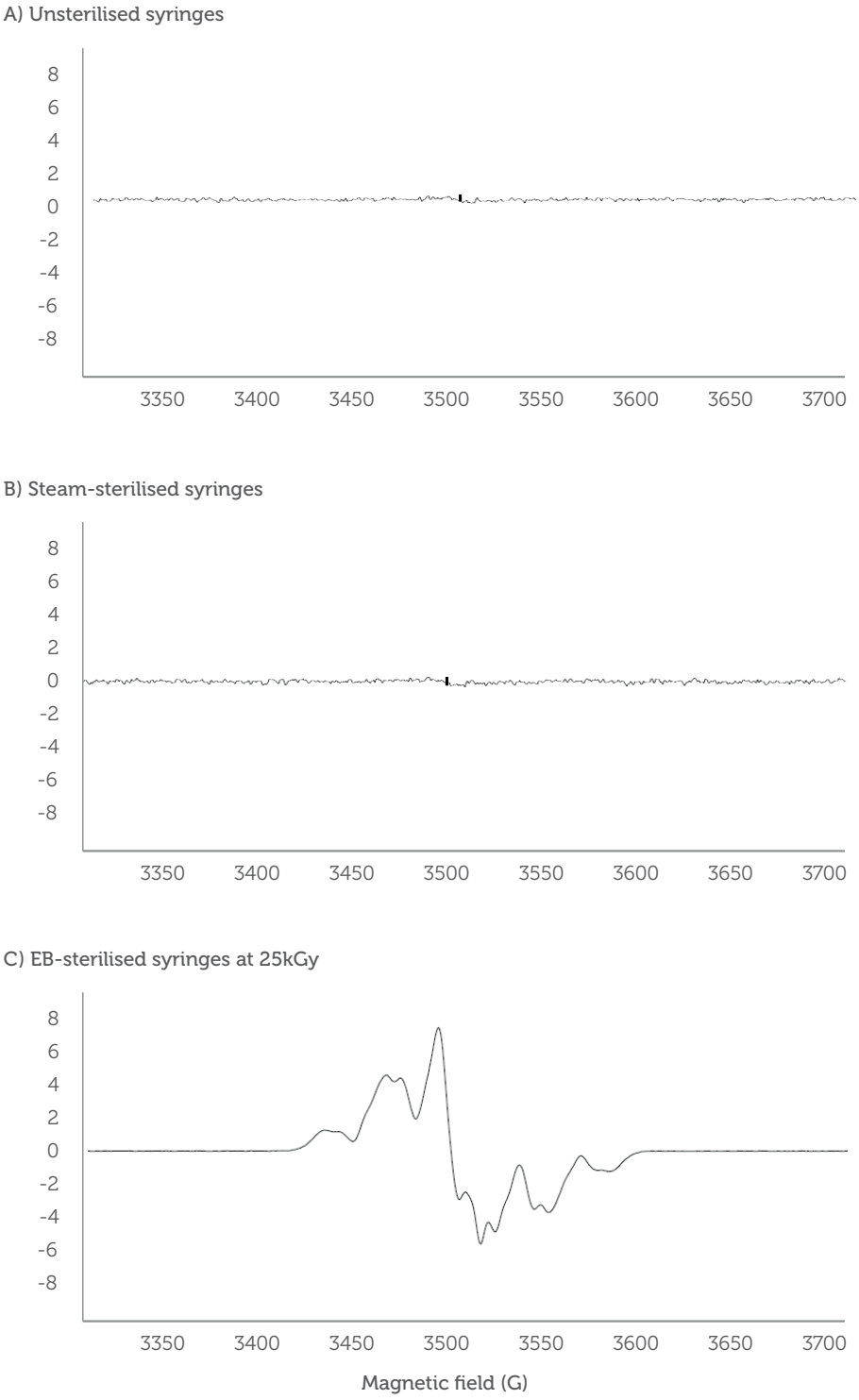

Figure 3: Typical ESR spectra of (A) unsterilised syringe; (B) steam-sterilised syringe; and (C) EB-sterilised syringe at 25 kGy.

and glass prefillable syringes packed with or without the use of a deoxygenated packaging system. With deoxygenated packaging systems, the dissolved oxygen level within the polymer-based prefilled syringes dramatically decreased compared to the non-deoxygenated (A). This decrease in dissolved oxygen within the glass prefilled syringes, irrespective of the deoxygenated packaging system, was not observed (B). To show the effectiveness of the proposed deoxygenated packaging system, a comparative study was also conducted to demonstrate the difference in protein oxidation between polymer-based prefilled syringes packed with or without a deoxygenated packaging system (C). As a result, the protein drug without the deoxygenated packaging system revealed a significantly higher Met-Oxy ratio than the samples kept within the deoxygenated packaging system at all time points, except time zero.

> "A deoxygenated packaging system for polymer-based prefilled syringes is very useful for protein drug products that are vulnerable to degradation by dissolved oxygen"

Through our research, this concept has been proven: a deoxygenated packaging system for polymer-based prefilled syringes is very useful for protein drug products that are vulnerable to degradation by dissolved oxygen.

The differences of oxygen elimination between glass- and polymer-based prefilled syringes are considered to be as a result of the difference in gas permeability characteristics. Gas permeability is extremely low for glass prefilled syringes compared to polymer-based prefilled syringes. Only polymer-based prefilled syringes can achieve oxygen control within a deoxygenated packaging system, while maintaining adequate container closure.[6-8]

RADICALS FROM STERILISATION BY IRRADIATION

Decomposition of protein drug products may result in unexpected side effects and/or reduced effectiveness, therefore controlling and preserving the product quality is of paramount importance.[3] Polymer-based prefillable syringe barrels can be sterilised by ethylene oxide gas, steam, and irradiation. It is generally understood that radicals are generated by irradiation, but so far there are few reports that show the impact of radical formation on protein stability. Sterile, single-use syringes (also called disposable syringes) are often sterilised by gamma or electron-beam (EB) sterilisation. There is minimal risk from the effect of radicals on drug stability because the drug solution taken into these syringes have a relatively short contact period within the syringe and are immediately administered to patients. However, in the case of prefilled syringe systems intended as the primary container for medicinal products, the storage period is substantially longer and generated radicals may have a greater impact on drug product quality.[8-9]

The amount of generated radicals can be quantitatively calculated based on the

results of electron spin resonance (ESR) according to the method as previously reported.[11-12] Typical spectra of ESR analysis on the polymer-based prefillable syringe after the EB sterilisation at 25 kGy or steam sterilisation are shown in Figure 3. The results reveal no significant differences between ESR spectra of unsterilised (A) and steam-sterilised syringes (B). However, considerable differences in ESR spectra are observed between the steam-sterilised (B) and EB-sterilised syringes (C).

The results obtained by calculating the generated radicals based on the ESR spectra are shown in Figure 4. These findings suggest that the EB sterilisation causes radical generation in polymer-based prefilled syringes.

The effects of such radicals from EB sterilisation on protein oxidation have been investigated in experiments with erythropoietin; tests conducted at conditions of 25 °C and 65% RH. Figure 5 shows the profile of observed Oxy-Met in erythropoietin solution after filled into EB-sterilised syringes and steam-sterilised syringes. The Oxy-Met level in the steam-sterilised syringes is similar to the non-sterilised syringes whereas the EB-sterilised syringes show an enhanced yielding of Oxy-Met compared with the steam-sterilised syringes. This difference in protein stability is attributed to the presence of radicals on the polymer-based syringes.

Furthermore, material analysis by Fourier transform-infrared spectroscopy (FTIR), and electron spectroscopy for chemical analysis (ESCA), was also conducted. Alongside the ESR analysis, FTIR spectra obtained in EB-sterilised syringes differ from that of steam-sterilised syringes. FTIR spectra obtained in steam-sterilised syringes resemble that of unsterilised syringes (Figure 6).

"In the case of prefilled syringe systems intended as the primary container for medicinal products, the storage period is substantially longer and generated radicals may have a greater impact on drug product quality"

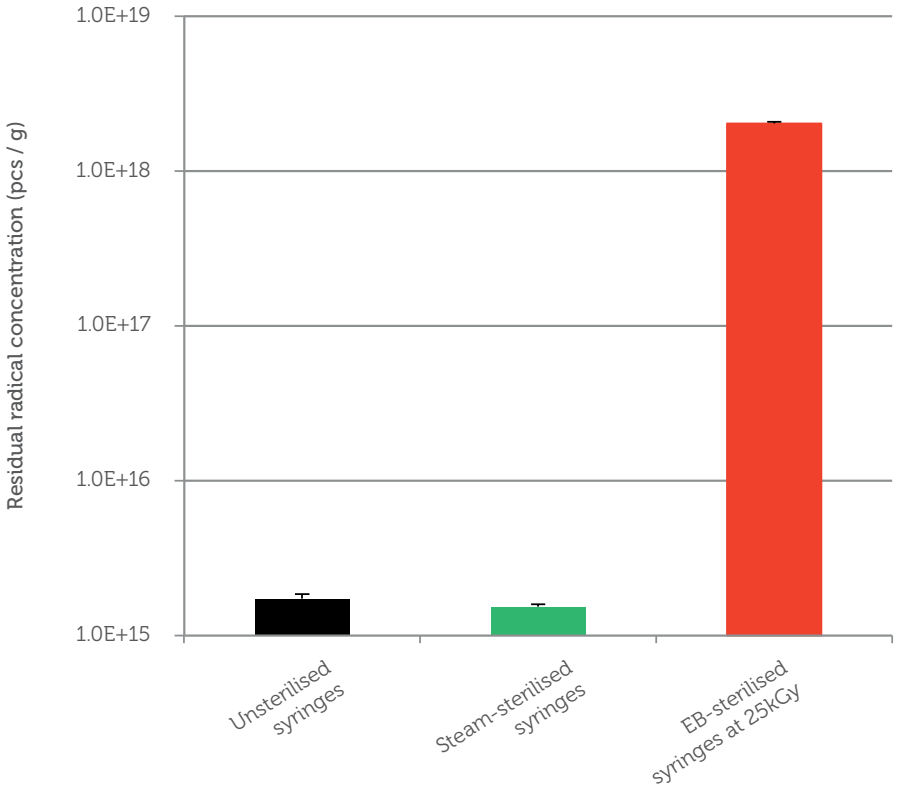

Figure 4: Residual radical amounts in unsterilised syringe, steam-sterilised syringe, and EB-sterilised syringe at 25 kGy. The data are presented as the mean +/- SD (n = 3).

Figure 7 summarises the chemical identification result based on the analysis by FTIR and ESCA. This result suggests that polymer barrel material (Cyclic Olefin Polymer, COP) is also subject to oxidation since C=O and C-O bonds are identified only from EB-sterilised

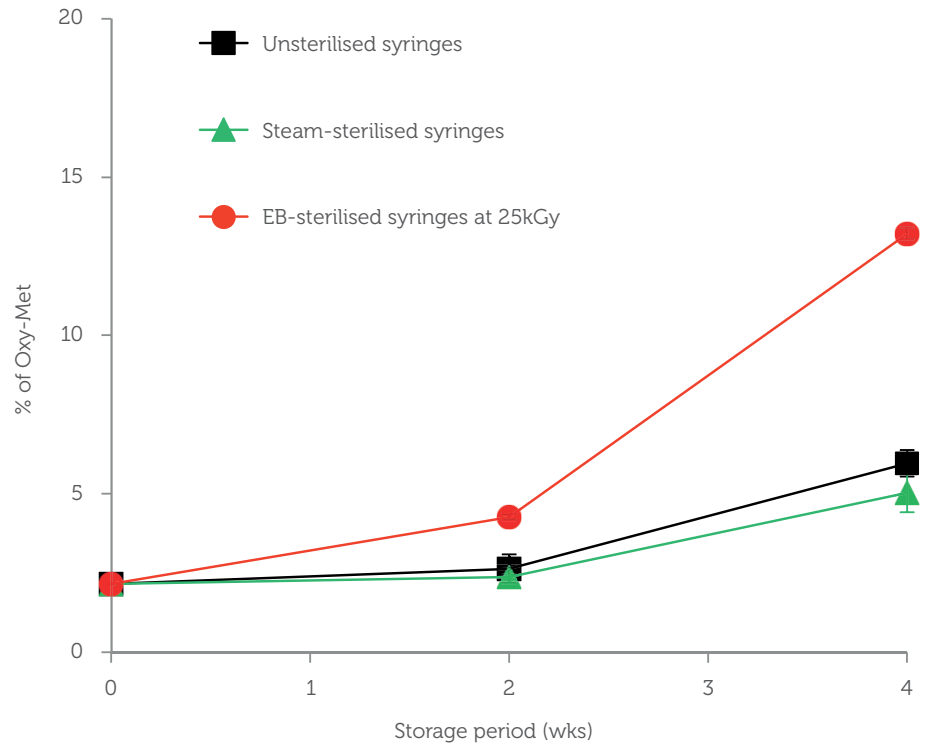

Figure 5: Difference in Oxy-Met production during the storage of erythropoietin filled into unsterilised syringe (square), steam-sterilised syringe (triangle), and EB-sterilised syringe at 25 kGy (circle) at 25°C and 65% RH. The data are represented as the mean +/- SD (n = 3).

Figure 6: IR spectra of (A) unsterilised syringe; (B) steam-sterilised syringe; and (C) EB-sterilised syringe at 25 kGy. Data are results of a single measurement.

syringes. These findings suggest that steam sterilisation is the preferred sterilisation method for polymer-based prefilled syringe systems, due to lower radical generation and therefore no enhancement of protein oxidation.

CONCLUSIONS

The root cause of protein oxidation have been identified as being dissolved oxygen within the drug product and the effect from radicals generated within polymer-based prefilled syringes by EB sterilisation.

Oxidation reaction due to dissolved oxygen can be minimised by controlling the oxygen content by reduction or complete removal of the dissolved oxygen using a deoxygenated packaging system. Radical generation within polymer-based prefillable syringes due to irradiation is not well known and is presented here for discussion. This presents a very important and critical aspect compared with the control of dissolved oxygen because it was thought that any sterilisation method for prefillable syringes was acceptable and would not result in reduction of product quality.

Through our experiments and results, the main oxidation pathway of a protein has been identified as dissolved oxygen and radical generation within polymer-based prefillable syringes. This report also demonstrates several solutions for controlling oxidation by means of applying a deoxygenated packaging system as well as utilising steam sterilisation as a method of sterilisation for polymer-based prefillable syringes.

(Note: research data in this article are on file at Terumo and are based on earlier publications from Terumo as referenced).

	Identified chemical bond	
	C=O bonding	C-O bonding
Unsterilised syringe	NONE	NONE
Steam-sterilised syringe	NONE	NONE
EB-sterilised syringe	IDENTIFIED (FTIR, ESCA)	IDENTIFIED (ESCA)

Figure 7: Summary of Chemical Identification Results Based on FTIR & ESCA analysis.

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