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Assessment of the effects of sterilization methods on protein drug stability by elucidating decomposition mechanism and material analysis

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ABSTRACT

The effects of different sterilization methods on the stability of highly sensitive protein drugs were assessed by elucidating mechanism involved in the process of protein decomposition. Results demonstrated that the steam sterilized syringes produced less protein oxidation compared with sterilization by the electron beam method. Electron spin resonance analysis showed that while considerably high levels of radicals were observed in the electron beam-sterilized syringes, no radicals were detected with steam sterilization. To identify the factor involved in protein oxidation, stability of the chemical composition of the syringe material was investigated using various analytical methods. Results showed that the syringe material itself was oxidized and two forms of oxidation products were identified with electron beam sterilization. Protein oxidation was shown to increase over time, and this was thought to be as a result of persistent exposure to the oxidized syringe barrel surface, which induced further protein oxidation.

These results suggest that compared to electron beam sterilization, steam sterilization is a preferable method for the plastic prefilled syringe system, particularly for biopharmaceutical drug products that are highly sensitive to oxidization.

1. Introduction

Increase in the use of prefilled syringes (PFS) is motivated by the many advantages they afford over traditional ampoules and vials. These advantages include quick and accurate dosing, fewer dosing errors, reduction in the risk of biological contamination, greater convenience with ease of use, and reduction of drug wastage by elimination of overfilling. The demand for the use of PFS for a growing number of available biological drugs has considerably increased in recent years (Walsh, 2003).

Traditionally, glass has been widely used in the production of PFS on account of its availability and historical application. However, the fragile nature of glass presents inherent risks such as breakage and delamination, which may occur during the manufacturing processes and owing to storage under some specific conditions. Improving the breakage resistance of glass may be an option for mitigating potential risks, but the nature of the glass material presents limitations to eliminating the risks completely. In addition to the physicochemical weakness of glass, tungsten oxide in glass can leach from syringe barrels, causing protein aggregation at the distal tip (Krayukhina et al., 2014; Mensch and Davis, 2012; Seidl et al., 2003; Liu et al., 2010; Jiang et al., 1957).

It is well known that the use of silicone oil-coated syringes leads to undesirable and potentially dangerous protein aggregation with some silicone-sensitive protein-based drugs. Silicone oil-induced aggregation has currently become one of the most frequently discussed topics in the field of PFS, particularly for developers of highly sensitive biopharmaceuticals (Baldwin, 1988; Chantelau, 1989; Chantelau et al., 1986; Jones et al., 2005).

These circumstances have led to an increase in the demand for the development of novel PFS systems and various technologies have been proposed. For instance, silicone oil-free plastic PFS (P-PFS) is one of the options that is being suggested to be devoid of the risks associated conventional PFS (Yoshino et al., 2014).

Highly sensitive drugs are susceptible to degradation that can be induced by physical stress like agitation, heat, and surface adhesion. It is well known that formulation excipients such as

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buffers, salts, and adhesion inhibitors including surfactants, play important roles in preventing drug degradation. Interestingly, our previous studies showed that the sterilization method used for plastic PFS also plays a critical role in stability of the product (Nakamura et al., 2015).

There is currently no restriction on sterilization method of container intended for P-PFS. Therefore, it can be sterilized using methods including ethylene oxide gas sterilization, radiation sterilization (electron beam (EB) and gamma irradiation), and steam sterilization. Of these, radiation sterilization appears to be a common method because of its wide application for sterilization of disposable plastic syringes.

Results of our previous studies raised concerns regarding the use of the radiation sterilization method for P-PFS because it can cause generation of radicals on the syringe barrel (Nakamura et al., 2015). Furthermore, it is also known that the generated radicals can persist for long periods and react with drug product molecules, creating a chain reaction (Reich and Stivala, 1969). However, the impact of the P-PFS barrel on the stability of drugs packaged in P-PFS syringes has not been fully determined.

The purpose of this study was to comparatively investigate the impact of both steam sterilization and radiation irradiation sterilization methods on the stability of drugs packaged in syringes. Regarding with radiation sterilization, there are two methods which have been already established, but in this study EB sterilization was employed because of EB sterilization being preferred as container sterilization method. In addition, we sought to identify the factors affecting drug stability and mechanism mediating drug degradation

2. Material and methods

2.1. Materials

PLAJEX™ [1 mL-long staked needle (27 G)] silicone-oil-free syringe (SOF) system, newly developed P-PFS composed of cyclic olefin polymer (COP) with a butyl rubber plunger stopper coated by means of i-coating™ technology (a proprietary SOF coating technology) (Yoshino et al., 2014), were provided by Terumo (Tokyo, Japan). erythropoietin (EPO) and endoproteinase Glu-C (Glu-C) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All buffer salts (sodium phosphate monobasic, sodium phosphate dibasic, and sodium chloride) were purchased from Kanto Chemical Co. (Tokyo, Japan). All other chemicals used in this study were analytical grade.

2.2. Sterilization of polymer-based syringes

PLAJEX™ syringes were sterilized by two different methods; namely steam and EB sterilization. The irradiation intensity for EB sterilization was 25 kGy. This intensity is commonly employed in sterilization of medical products. The steam sterilization was operated under the condition to meet the overkill.

2.3. Protein oxidation study

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. Following dissolution, syringes sterilized by either steam or EB at 25 kGy were filled with 1.0 mL of EPO solution, and the plunger stoppers were assembled to achieve the pre-determined head space of 0.2 mL. The syringes were then stored at 25 °C and 65% room humidity (RH) for 2 and 4 weeks, after which the content was evaluated using HPLC (Shimadzu, Kyoto, Japan) as described below.

2.4. Analysis of oxidized methionine in EPO preparation

Analysis of oxidized methionine in the EPO solution was conducted using HPLC following the modified method described by Ohta et al. (2001). Briefly, a mixture of 100 μL of the EPO solution and 400 μL of 100 mM ammonium acetate solution at pH 8.0 was placed in a Amicon Ultra-0.5 10 K (Millipore Ireland Ltd., Ireland) filter and centrifuged at 14,000 × g for 15 min. The unfiltered sample trapped on the filter was carefully collected and ammonium acetate solution (100 mM, pH 8.0) was added to obtain a final volume of 50 μL. To separate the oxidized methionine (Met-Oxy) and intact methionine (Met) fragments, 1 μg/mL Glu-C in 100 mM ammonium acetate at pH 5.6 was added to the samples. The samples were then incubated at 37 °C for 24 h and diluted with 100 mM ammonium acetate solution (pH 8.0); Met-Oxy and Met were analyzed using HPLC (Shimadzu, Kyoto, Japan).

HPLC conditions were as follows:

- Mobile phase A: 0.05% trifluoro acetic aqueous solution.
- Mobile phase B: acetonitrile containing 0.05% trifluoro acetatic acid.
- Column: Inertsil ODS-3 (5 μm, 250mm × 2.1mm i.d.).
- Column temperature: 40° C.
- Injection volume: 30 μL.
- Total flow rate: 0.25 mL/min.
- Wavelength: 280 nm.
- Flow mode: linear gradient mode.

Table 1 shows the time program for mobile phases A and B.

In Fig. 1, HPLC chromatogram obtained following the above method is shown, and according to the following equation the percent of Met-Oxy was calculated.

$$\%\ \text{of Met-Oxy} = \frac{\text{Met-Oxy peak area}}{\text{Met peak area} + \text{Met-Oxy peak area}} \times 100$$

2.5. Electron spin resonance

Residual radicals on the syringes were measured with electron spin resonance (ESR) in accordance with previously reported methods (Jiang et al., 1957; Swartz, 1972). Briefly, the barrels of the syringes sterilized by steam or EB were cut in 3 × 3 mm squares. Radical levels in these specimens were determined using ESR (ESP350E, Bruker, MA, USA) under the following analytical conditions; measurement temperature, room temperature; central magnetic field, around 3337 G; scanning range of the central magnetic field, 400 G; modulation, 100 kHz (2 G); microwave, 9.46 GHz (0.1 and 1.6 mW); scanning time, 83.89 × 4 times, and time constant, 163.84 ms.

Table 1
Time program for mobile phases A and B.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	99	1
5	99	1
135	65	35
140	20	80
150	20	80
155	99	1
190	99	1