Inhibiting sterilization-induced oxidation of large molecule therapeutics packaged in plastic parenteral vials

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Abstract

For many years, glass has been the default material for parenteral packaging, but the development of advanced plastics such as cyclic olefin polymers (COP) and the rapidly increasing importance of biologic drugs have complicated this situation. In particular, many biologics must be stored at non-neutral pH, where glass is susceptible to hydrolysis, metal extraction, and delamination. Plastic containers are not susceptible to these problems, but suffer from higher gas permeability and a propensity for sterilization-induced radical generation, heightening the risk of oxidative damage to sensitive drugs. This study evaluates the properties of a hybrid material, SioPlas™, in which an ultrathin multilayer ceramic coating is applied to the interior of COP containers via plasma-enhanced chemical vapor deposition. We find that the coating decreases oxygen permeation through the vial walls 33-fold compared to uncoated COP, which should allow for improved control of oxygen levels in sensitive formulations. We also measured degradation of two biologic APIs that are known to be sensitive to oxidation, teriparatide and erythropoietin, in gamma and electron beam sterilized SioPlas™, glass, and uncoated COP vials. In both cases, APIs stored in SioPlas™ vials did not show elevated susceptibility to oxidation compared to either glass or unsterilized controls. Taken together, these results suggest that hybrid materials such as SioPlas™ are attractive choices for storing high-value biologic drugs.

Lay Abstract

The primary function of parenteral drug containers is safeguarding their contents from damage, either chemical or physical. Glass has been the container material of choice for many years, but concerns over breakage and vulnerability to chemical attack at non-neutral pH have spurred the rise of advanced plastics as alternatives. Plastics solve many problems associated with glass, but introduce several of their own, including increased gas permeation and generation of oxidizing radicals during sterilization. In this article, we evaluate SioPlas™, a hybrid material consisting of plastic with a thin multilayer ceramic coating applied to the interior, for its ability to overcome these two problems. We find that SioPlas™ is much less permeable to oxygen than uncoated plastic, and that two biologic drugs stored in gamma and electron beam sterilized SioPlas™ vials do not display elevated levels of oxidation compared to either glass or unsterilized vials. This suggests that hybrid materials such as SioPlas™ can exhibit the best qualities of both glass and plastic, making them attractive materials for storing high-value parenteral drugs.

Keywords

Primary containers, oxidation, permeation, sterilization, teriparatide, erythropoietin

Introduction

The most important function of primary packaging for parenteral drugs is to protect the active pharmaceutical ingredient (API) from degradation that could alter its efficacy or increase patient side effects. API degradation can take many forms, including physical, thermal and chemical induced damage. Biologic APIs (proteins, peptides and nucleic acids, among others) are typically more prone to degradation than small molecules and their degradation pathways are more difficult to predict (1, 2). This complicates proper primary container selection as biologics increase in clinical use. In particular, the choice of material used for primary packaging can have serious implications for the fate of the packaged drug.

Oxidation is a particularly important type of chemical degradation for protein and peptide APIs, as it is intimately related to protein structure and therefore function (2, 3). One form of oxidative damage is formation of non-native disulfide bridges between cysteine residues, sometimes termed 'disulfide scrambling'. Many proteins rely on disulfide bridges for proper folding, but unwanted disulfide bridges lead to improper folding and/or aggregation. Insulin is a canonical example of this; in its native, bioactive form, insulin is composed of two peptide chains linked by two disulfide bonds. Oxidative stress, particularly in the presence of metal ions, causes formation of disulfide bridges between dimers, and this covalent crosslinking results in aggregation and loss of activity (3, 4). Individual amino acids can also be oxidized, with methionine (to methionine sulfoxide), tryptophan (to 5-hydroxy-tryptophan, kynurenine, and others), and histidine (to 2-oxo-histidine) the most reactive (1, 2). Of these, methionine (Met) oxidation is the most studied, with examples including monoclonal antibodies, interferons, and other protein and peptide hormones recently reviewed by Torosantucci et al (3). In many cases,

Met oxidation causes protein conformational changes that reduce bioactivity, but local effects such as blockage of a binding site can also affect activity. Oxidation can be induced by a variety of species, including reactive oxygen species (ROS), metal ions, free radicals, and even atmospheric oxygen, which can either oxidize solvent-exposed Met residues directly (auto-oxidation) or by activating excipients in the formulation (1, 2, 5).

Compared to other chemical degradation pathways such as deamidation, formulation options for controlling oxidation are limited due to the diversity of oxidation pathways (1). Antioxidants such as ascorbate are effective at scavenging radicals and ROS, but often function in a limited pH range, can increase the reactivity of metal ions (6), and can generate unwelcome chemical changes (e.g. reduction in pH due to metabisulfite oxidation). Decreasing the storage temperature can actually increase oxidation rate due to increased solubility of oxygen in water. Lyophilization can be effective in preventing oxidation of some APIs (7, 8), but not all, and is not suitable for many APIs and applications (e.g. prefilled syringes). Two approaches which have been shown to be broadly effective are reducing exposure of the formulation to light and minimizing oxygen inside the storage container. The former is straightforward, but keeping oxygen out of a drug container over extended storage periods imposes strict requirements on the materials used for packaging.

Borosilicate glass (USP Type 1) has many favorable properties that have made it the ‘gold standard’ for primary parenteral packaging across much of the world, but also comes with a number of drawbacks (9, 10). Glass is essentially impermeable to both gases and liquids, which allows for good environmental control including oxygen level. Borosilicate glass is minimally reactive and somewhat pH-tolerant, and can be treated (e.g. by siliconization) to

create a hydrophobic surface in order to decrease interactions with sensitive APIs. It has a low coefficient of thermal expansion and is mechanically strong, but is brittle due to its amorphous structure. Mechanical shock can cause container fracture or spallation of small particles into the formulation. Particles can also be generated by a physio-chemical process known as delamination, in which alkali ions in the glass exchange with protons in the formulation, leading to an increase in solution pH and a decrease in mechanical strength of the inside surface of the glass, followed by shedding of particles (11, 12). Delamination, as well as hydrolysis and leaching, are more likely at pH values away from neutrality, which can be required to stabilize sensitive biologic APIs. Metal ion contaminants from glass manufacture can also leach into the formulation, contributing to API degradation by oxidation and other pathways.

Advanced plastics such as cyclic olefin polymers (COP) and copolymers (COC) solve many of the problems of glass, but come with their own set of drawbacks (9, 13). COP and COC are optically clear, with good strength and resistance to fracture and spallation. They have low surface energy, which minimizes API adsorption without siliconization, and can be produced with excellent dimensional tolerances. Importantly, they are non-reactive over a large range of pH values and do not delaminate. COP and COC exhibit low leaching of ions, but organic molecules such as plasticizers and antioxidants can be extracted. While COC and COP provide a good barrier against moisture, they are permeable to gases. This makes them unsuited to applications requiring low oxygen levels unless accompanied by an airtight secondary packaging system and secondary oxygen scavenger (14, 15). Another concern is radical generation during sterilization; Nakamura et al found that gamma irradiation at doses typical for sterilization (25 and 50 kGy) produced significant oxidation of COP in syringe barrels, and that erythropoietin

(EPO) stored in gamma-sterilized COP syringes oxidized significantly faster than did EPO in steam-sterilized syringes (15). They ascribe this to free radicals generated by the irradiation process, which presumably diffuse to the inner surface of the barrel and react with the dissolved API.

An ideal primary packaging material would combine the barrier properties of glass with the mechanical properties of plastic, producing a container that was resistant to both chemical and mechanical stress. Ultra-thin film ceramic coatings have been applied to optical and electronic components using physical and chemical deposition techniques for over 40 years, and this technique has more recently been applied to pharmaceutical packaging (16, 17). In particular, plasma-enhanced chemical vapor deposition (PECVD) is capable of growing thin layers (tens to hundreds of nm or more) of silicon dioxide, silicon nitride, and other related materials on a variety of dielectric substrates, including plastics and glass. The thin layer is mechanically compliant, but provides an effective chemical and permeation barrier if deposited evenly (16, 18, 19). Schott Type 1a glass a well-known example of this technology: a silicon dioxide layer is deposited on the inside of borosilicate glass vials in order to reduce API adsorption and decrease leaching of metal ions into the formulation (20). SiO_2 Medical Products recently introduced SiOPlas™ as a material for primary parenteral packaging: PECVD is used to apply a multilayer coating to the inside of COP containers. The coating architecture is illustrated in Figure 1A: a thin organosilicate adhesion layer allows secure binding of a layer of pure silicon dioxide, which forms a barrier against gases, liquids, and diffusing ions, as in Type 1a glass. A thicker organosilicate top layer is then applied to provide pH and chemical resistance. By tailoring the layer properties, it should be possible to obtain high barrier quality

and chemical resistance while still retaining the desirable mechanical properties of the bulk COP. In this manuscript, we describe our measurements of COP, glass, and PECVD-coated SiOPlas™ materials as oxygen permeation barriers and their propensity for sterilization-induced oxidation of two biologic APIs: teriparatide and erythropoietin.

Materials and Methods

Materials

All chemicals were ACS analytical grade or better. Chromatography solvents were HPLC-grade, 0.2 µm sterile filtered. Ultra-pure water was obtained using a Milli-Q Reference system (Millipore, Milford, MA). 22 x 40 mm (6 mL) USP Type 1 borosilicate glass vials were purchased from International Vial Packaging (Lawrence, KS), along with 20 mm bromobutyl rubber stoppers and West flip-off seals. Cyclic olefin polymer (COP, Zeonex 690R) and PECVD-coated COP vials (SiOPlas™) were provided by SiO_2 Medical Products (Auburn, AL). Teriparatide (Human parathyroid hormone aa 1-34, > 95% pure by HPLC) was purchased from Toronto Research Chemicals (Toronto, Canada). Recombinant human erythropoietin was a kind gift from Amega Biotech (Buenos Aires, Argentina). Glu-C protease, MS grade (specific activity $\geq$ 500 U/mg) was purchased from Thermo Fisher Scientific.

Permeation testing

Oxygen permeation through the vial wall materials was measured using non-contact fluorescence quenching (optode) sensors (MOCON, Minneapolis, MN). A sensor was affixed to the interior of a vial, which was then sealed under low-oxygen conditions (< 0.2% oxygen) with

a glass slide and epoxy. Containers were stored at 25 °C and the oxygen levels were monitored using a MOCON Optech-O2 Platinum reader. The reader was calibrated at the start of the measurement using a calibration card supplied by the manufacturer that provides reference signals for air and “zero” oxygen. The same calibration setting was maintained for the duration of the measurement, and reader stability was periodically checked versus a sensor sealed inside a glass reference vial.

Sterilization

Vials were sterilized in cases of 96 using standard pharmaceutical procedures. Gamma sterilization was carried out by Steris AST (Spartanburg, SC) using a ^{60}Co source. The delivered dose was 31.1–36.7 kGy across the case. Electron beam sterilization was carried out by Steris AST (Denver, CO), with a delivered dose of 26.1–28.1 kGy.

Formulation and container filling

Teriparatide was dissolved at 0.1 mg/mL in acetate buffer (0.1 mg/mL sodium acetate, 0.41 mg/mL acetic acid, adjusted to pH 4) as for the commercial formulation (Forteo – Lilly) with 0.05% sodium azide added to prevent microbial growth. Erythropoietin was dissolved at 24,000 IU/mL in 2 mM sodium phosphate buffer (1.2 mM dibasic, 0.8 mM monobasic sodium phosphate) with 0.06 mg/mL polysorbate 80 (15) and 0.05% sodium azide. 0.5 mL was loaded into glass, COP, and coated COP vials that had been sterilized by gamma or electron beam treatment, as well as unsterilized controls. The vials were sealed by manual crimping and stored

in low light conditions. At the indicated time points, the contents of 3 vials for each material/sterilization combination were removed and pooled for analysis.

Teriparatide Oxidation Measurement

Teriparatide oxidation was analyzed by reversed phase HPLC (Shimadzu) as follows: 200 uL of the formulated peptide solution was injected onto an XBridge C18 2.5 µm 4.6 x 50 mm column (Waters Corp., Milford MA) and analyzed by gradient elution (mobile phase A: 0.1% trifluoroacetic acid (TFA) in water, mobile phase B: 0.1% TFA in acetonitrile): 0–5 min, isocratic 25% B; 5–25 min, 25–45% B; 25–30 min, isocratic 90% B; 30-35 min, isocratic 25% B. The column temperature was 50 °C and the flow rate was a constant 1 mL/min. Species abundance was quantified by 220 nm absorbance peak area; equivalent results were observed using 280 nm absorbance peak areas. Oxidation standards were prepared by incubating peptide samples with hydrogen peroxide (0.01% v/v, from 30% stock) for 30 minutes at room temperature. HPLC chromatograms for the control and H_2O_2-oxidized sample are shown in Figure 1A. Three oxidized species were observed, as expected for oxidation of methionine at position 8 and/or 18 (21, 22). Total oxidation level was computed as the sum of oxidized species peak areas divided by the sum of oxidized and unoxidized peak areas; we estimate the uncertainty of quantification to be ≈ 3%.

Erythropoietin Oxidation Measurement

Erythropoietin oxidation was analyzed using a modified version of the glycopeptide mapping method described by Ohta et al (23) and Nakamura et al (15). 200 µL of protein solution was

mixed with 300 µL of 100 mM ammonium acetate solution (pH 8.0) and concentrated by diafiltration (Amicon Ultra 0.5, 10k MWCO, 14,000 x *g*, 20 minutes) to a volume of ≈ 25 uL. 2 µg of Glu-C protease in 30 µL ammonium acetate buffer (pH 8.0) was added and the mixture was incubated overnight at 37 °C. Digestion reactions were analyzed by RP-HPLC (Agilent 1200 series) using a Kinetex 2.6 µm EVO C18 2.1 x 50 mm column (Phenomenex, Torrance, CA) at a flow rate of 0.2 mL/min at 40 °C with the following gradient program (mobile phase A: 0.1% TFA in water, mobile phase B: acetonitrile): 0–5 min, isocratic 9% B; 5–23 min, 9–35% B; 23–28 min, isocratic 90% B, 28–35 min, isocratic 9% B. The injection volume was 50 µL and absorbance was monitored at 220 and 280 nm. Species abundance was calculated using A_{280}, and oxidation fraction was computed from the areas of the oxidized and native peptide peaks (Figure 3A). Oxidation standards were prepared by incubating EPO samples with hydrogen peroxide (0.03% v/v from 30% stock) for 30 minutes at 37 °C. We estimate the uncertainty in quantification of oxidized fraction to be ≈ 2% based on repeat LC analyses of samples from individual vials.

Results

Oxygen permeation

We first measured how effective the PECVD coating was at reducing oxygen permeation compared to uncoated COP plastic. In order to isolate permeation through the vial walls vs oxygen ingress through the rubber stopper, vials were sealed with glass plates in an inert atmosphere, as described above, and oxygen levels were monitored for 15 days. Figure 1B shows the results of these tests: the oxygen partial pressure increased much faster in the uncoated COP vial than in the PECVD-coated vial. If the permeability of the material remains

constant vs. time, the oxygen partial pressure inside the vial should recover to ambient levels following first order kinetics:

$$p(t) = p_{amb}\left(1 - \left[1 - \frac{p(0)}{p_{amb}}\right] e^{-\frac{t}{T_P}}\right) \quad (1)$$

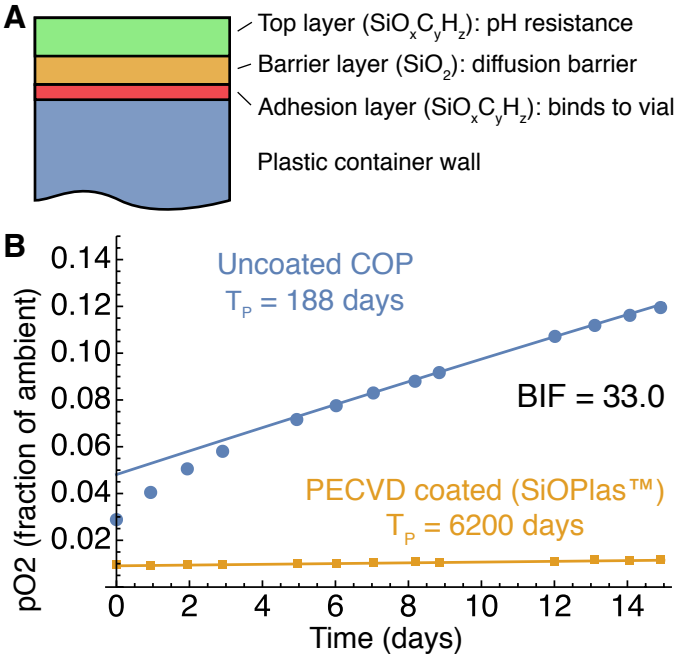
with p_{amb} the ambient (external) partial pressure of oxygen and $p(0)$ the partial pressure of oxygen inside the vial at $t = 0$. The characteristic permeation time T_p is related to the permeability coefficient P of the material (in moles O_2 / atm / day) and the container volume V by the ideal gas law and Eq. 1:

$$\frac{d}{dt}p(t) = \frac{RT}{V}P\big(p_{amb} - p(t)\big);\ T_P = \frac{V}{RTP} \quad (2)$$

where *T* is the temperature in Kelvin and *R* the ideal gas constant. The solid lines in Figure 1 show fits to Eq. 1 for the two materials, along with the fitted values for permeation versus time. The ratio of characteristic times

$$BIF \equiv \frac{T_{P,coated}}{T_{P,uncoated}} = \frac{P_{uncoated}}{P_{coated}} \quad (3)$$

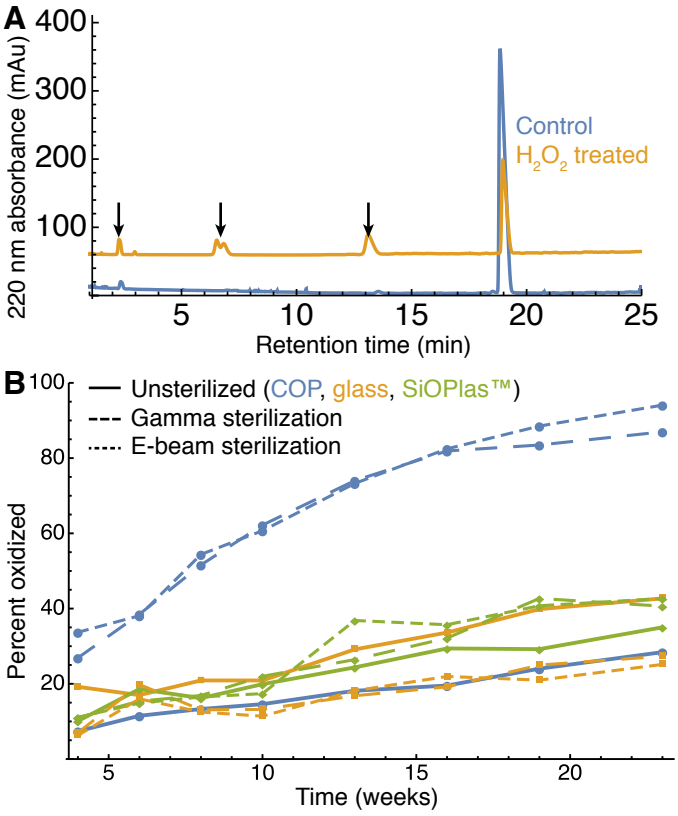
is referred to as the barrier improvement factor (BIF) and reflects the relative rates of permeation: the PECVD coating slows oxygen ingress through the vial walls by a factor of 33 compared to the uncoated vials. Note that while Eq. 1 describes both sets of data well beyond t = 4 days, the earlier time points for the uncoated COP vials show a faster rise (leading to a larger fitted value for $p(0)$). We believe this is due to the oxygen initially dissolved in the COP vial walls prior to filling with nitrogen, and these data are consequently excluded from the fit. Corroborating this explanation, measurements in which COP containers were soaked in a nitrogen atmosphere for several days before measurement do not show this effect.



Sterilization-induced oxidation: teriparatide

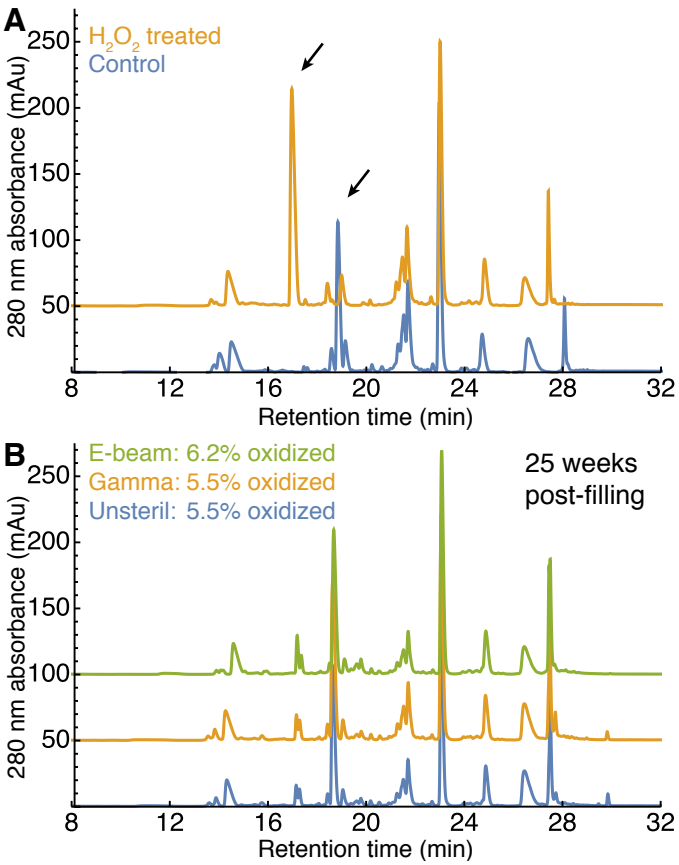
In order to assess the effect of container material on API oxidation due to sterilization-induced radicals, we selected two biologic APIs with known sensitivity to oxidation: teriparatide and erythropoietin. Teriparatide is a peptide comprising the N-terminal 34 amino acids of human parathyroid hormone (PTH) with bioactivity equivalent to that of the full hormone (24). PTH is a critical regulator of serum calcium levels, and recombinant teriparatide (sold by Eli Lilly and Co. as Forteo™) is used to treat bone density loss due to osteoporosis. Methionine residues at positions 8 and 18 are prone to oxidation, and their oxidation synergistically reduces bioactivity by disrupting helices that are essential for proper folding of the peptide (22, 24). As shown in Figure 2A, the single- and doubly-oxidized peptides are readily distinguished from the native form by RP-HPLC (22), making teriparatide an attractive system for studying oxidation.

In order to maximize the effect of any radicals generated in the vial walls during sterilization, we filled 6 mL parenteral vials with 0.5 mL of formulated API. This produces a larger surface area / volume ratio than a full container, and also approximates the ratio found in pre-filled syringes. The vials were filled and sealed in ambient conditions, and no oxygen scavenger was added to the solution. Under these conditions, teriparatide is expected to oxidize at a moderate rate (25); radical-induced oxidation would manifest as an increased oxidation level in the sterilized containers vs the unsterilized controls. The results of our measurements are shown in Figure 2B: we observe a dramatic increase in oxidation for the peptide stored in sterilized, uncoated COP vials compared to the unsterilized case. Neither the glass nor the CEPVD-coated materials displayed a significant increase in oxidation due to sterilization.



Sterilization-induced oxidation: erythropoietin

Erythropoietin (EPO) is a highly glycosylated (≈ 40% by mass) protein hormone (166 amino acids, molecular weight ≈ 34 kDa depending on glycosylation state (26)) that stimulates red blood cell production. Several versions of EPO (Epogen™, Aranesp™, EPREX™, etc) are used clinically to treat anemia arising from chronic kidney disease, cancer, and other causes. Erythropoietin contains one internal methionine residue at position 54, and oxidation of this residue is associated with decreased bioactivity (27). Although detection of oxidation is challenging in the full-length protein, digestion into smaller fragments by the sequence-specific protease Glu-C allows detection of oxidation in the fragment containing Met-54 (15, 23) by RP-HPLC. Figure 3A shows a clear shift in retention time for the peptide fragment containing Met-54 after treatment with hydrogen peroxide, while the other peaks are largely unchanged. As with teriparatide, we examined the effect of sterilization by storing EPO without stabilizers in vials under ambient conditions. Figure 3B shows results for the SioPlas™ vials: after nearly six months of storage, no significant increase in oxidation level is seen for either the gamma- or electron beam-sterilized vials compared to the control. A similar level of oxidation (≈ 6%) was observed in the glass and unsterilized COP vials, consistent with the results reported by Nakamura et al (15) for COP syringes stored under similar conditions. Gamma- and electron-beam sterilized uncoated COP vials showed elevated levels of oxidation (≈ 12% for both sterilization methods), though the values varied from vial to vial, perhaps reflecting differing radiation doses.



Discussion and Conclusions

In this study, we examined the effect of container material on two phenomena associated with oxidation of parenteral biologics: oxygen permeation and sterilization-induced radicals. In both cases, we found that SiOPlas™, cyclic olefin polymer with a multilayer PECVD coating, showed significant advantages as a vial material compared to uncoated COP. The coating reduced oxygen permeation rate through the container walls by a factor of 33, and two biologic APIs did not show elevated levels of oxidation after storage in sterilized SiOPlas™ vials compared to unsterilized controls. Both effects are likely due to the same cause, namely the diffusion barrier provided by the silicon dioxide layer. Gas transport through materials is often described by a solubility-diffusion mechanism, in which the permeation rate depends both on the solubility of

the gas in the material and how quickly molecules can move from one side to the other. The bulk material (COP) is the same for both the coated and uncoated vials, so the lower observed permeation rate can be ascribed to a low diffusion rate through the coating, as measured for other SiO_2 thin films (16). The thinness of the coating relative to the walls also means that radicals induced by radiolysis will primarily be created in the wall material, and are therefore prevented from contacting the API by the SiO_2 barrier layer. Multiple studies have shown that diffusion through thin films is dominated by the frequency of defects (16, 18, 19), suggesting that further improvements to permeation may be possible as manufacturing techniques improve.

This study focused on electron beam and gamma irradiation sterilization methods, as ionizing radiation had been previously shown to cause oxidative damage to APIs stored in COP containers. Interestingly, we found that the two methods caused very similar rates of sterilization-induced oxidation for the applied standard sterilization conditions, as shown in Figure 2B. Our data does not permit conclusions regarding the generality of this finding, but it would be interesting to know whether this is true for broader classes of biologics.

While our measurements indicate that SiOPlas™ is a promising material for parenteral packaging, pharmaceutical application requires consideration of the entire API-formulation-packaging construct as a whole, which is more complex than the relatively simple systems studied here. In particular, we did not evaluate the effect of container closures on oxygen permeation rate or the effect of excipients on oxidation sensitivity. High-barrier containers

must be accompanied by similarly impermeable closures for low oxygen levels to be maintained, and as discussed above, excipients can have significant effects on oxidation sensitivity, both positive and negative. Whole-article tests with complete formulations will be required to evaluate the suitability of packaging systems for any particular API. Nonetheless, there are few APIs that would not benefit from greater isolation from their storage environment, and the combination of permeation barrier, chemical resistance, and mechanical durability offered by PECVD-coated plastics such as SiOPlas™ suggests that they may be superior materials for many parenteral applications.

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Conflict of Interest Declaration

S.J.M., A.P.B., and C.M.W. are employees of SiO_2 Medical Products. M.V.T. is a paid science advisor to SiO_2 Medical Products. SiO_2 Medical Products has provided unrestricted research support to the Institute for Molecular Engineering.

Figure Captions

Figure 1. SiOPlas™ multilayer coating reduces oxygen permeation. A) Schematic architecture of the multilayer coating. Layers of organosilicate and silicon dioxide are deposited on a COP surface by plasma-enhanced chemical vapor deposition to produce a diffusion barrier that is resistant to chemical attack. B) Oxygen permeation studies. The rate of oxygen ingress into oxygen-free sealed containers was measured for uncoated COP and SioPlas™ vials. The PECVD coating reduces oxygen permeation rate by a factor of 33. Fits to Eq. 1 are shown, with the fitted values of permeation time. As discussed in the text, the first four time points are not included in the fits to account for oxygen initially dissolved in the container walls.

Figure 2. Effect of sterilization-induced radicals on oxidation rate of teriparatide. A) HPLC traces of native (blue) and H_2O_2-oxidized (orange, vertically offset for clarity) teriparatide. Species (black arrows) with one ($T_r \approx 6.8$, 13.2 minutes) and both methionine residues oxidized are clearly distinguishable from the native peptide ($T_r \approx 19$ minutes). B) Effect of sterilization method and container type on oxidation. Sterilization by gamma (long dashes) and electron beam (short dashes) irradiation causes a large increase in oxidation rate for teriparatide stored in uncoated COP containers (blue traces). Glass (orange) and SioPlas™ (green) do not show a similar difference between sterilized and unsterilized vials. Error bars (estimated at ≈ 3%) are suppressed for visual clarity.

Figure 3. Sterilization effects on erythropoietin oxidation. A) HPLC traces of native (blue) and H_2O_2-oxidized (orange, offset for clarity) EPO, after protease digestion. Under the conditions

used here, Glu-C protease selectively cleaves the peptide backbone on the C-terminal side of glutamic acid residues, splitting EPO into 13 peptide fragments (see ref (23); only some absorb at 280 nm). The fragment containing Met-54 is marked with arrows; these peak locations were used to quantify oxidation fraction for the stored EPO samples. B) Oxidation due to radical species generated by sterilization would appear as an increase in the oxidized peak for the samples in sterilized vials compared to the unsterilized control. No significant increase in oxidation is observed for the samples stored in sterilized SiOPlas™ containers.

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