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Evaluation of Glass Delamination Risk in Pharmaceutical 10 mL/10R Vials

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ABSTRACT

Glass delamination is characterized by the dissociation of glass flakes from the glass surface. Since glass delamination is time dependent, 5 vial types were investigated to assess delamination under accelerated stress conditions published as quick tests in literature and compared to stress testing recommended per United States Pharmacopoeia <1660>. A broad panel of analytical techniques was employed to test the solution for visible/subvisible particles and leachables and characterize topography and composition of the surface. The vial types showed significant differences in surface durability when applying the same stress conditions. An increase in glass leachables and change in topography were shown for uncoated vials. An indication for an elevated delamination risk was confirmed for Expansion 33 vials only by the compiled analytical data set including particle assessment and change in elemental composition of the near glass surface investigated by dynamic secondary ion mass spectrometry. The delamination test protocols differ in test solution, handling, and time. Before choosing the most appropriate protocol to predict delamination propensity and mimic real-time conditions, long-term storage data are needed. A combination of analytical techniques to study the risk for long-term corrosion of glass is highly recommended covering the 3 aspects: visible/subvisible particle assessment, solution analysis, and surface characterization.

Introduction

In recent years, glass particles, so-called lamellae, have been observed in a couple of parenteral products filled in glass containers. These visible glass particles resulted in product recalls due to the parenteral administration of the products to ensure patients' safety.[1-3] Therefore, biopharmaceutical industry and regulatory agencies nowadays pay considerable attention to particle formation, especially to glass delamination from glass containers for therapeutic parenteral products, including biologics.[4,5] An informal monograph in the United States Pharmacopoeia (USP <1660>[6]) has been released in 2012 to provide guidance for pharmaceutical industry related to evaluating glass delamination.

Glass delamination is described in literature.[7-11] It is characterized by the dissociation of glass in the form of sheets and flakes from the product contact surface. White and Zoitos have suggested different mechanisms to describe the corrosion of glass[12]: (1) congruent dissolution by simple dissociation or by chemical reaction with the solvent referred to as homogeneous dissolution in this article. This leads to enhanced concentration of the respective glass elements in the formulation. Concentrations of the ions are equivalent to the weight ratios of the elements in the bulk glass. (2) Incongruent dissolution with the formation of crystalline reaction products or the formation of noncrystalline layers and (3) ion exchange. Mechanism (2) results in ion concentrations in the solution, which are significantly different from the bulk concentrations (inhomogeneous dissolution).

The predominant mechanism depends on the solution in contact with the glass surface. When glass surfaces are exposed to water, the glass surface is hydrated and an alkali-depleted,

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silica-rich layer is formed.[13] This involves an ion exchange between hydrogen ions from the water and alkali ions in the glass. The presence of water promotes hydrolysis of the silicon-oxygen bond, forming a silica-gel layer. At higher pH values, the mechanism of glass dissolution is assumed to change from the leaching of alkali elements to the dissociation of the silicate network.[6]

Glass delamination in vials for parenteral products on the one side depends on the glass composition and the glass/vial manufacturing processes,[7,11] and on the other side, process conditions during drug product manufacturing[14-16] such as terminal sterilization[10] of glass vials and potentially depyrogenation after vial washing.[17] In addition, the dosage form and formulation (compound,[9] pH,[4,18] ionic strength,[17,18] and buffer components[19,20]) and storage conditions (temperature and time)[21-23] impact the risk and likelihood for glass delamination during a product's shelf life. For example, liquid (refrigerated and ambient) formulations are expected to interact stronger with the glass surface compared to dried (lyophilized) formulations and thus would be considered a higher risk for delamination. Frozen formulations in comparison to dried formulations would also be of higher concern for delamination given that freezing and thawing can pose stress on the vial.[15] Jiang et al.[15] have reported that freeze/thaw cycles of protein formulations may induce formation of glass lamellae by mechanical stress due to rapid movement of the frozen plug from the glass surface at temperatures below −50°.

Glass delamination is evident by visible glass flakes (particles) in solution and can show up as subvisible particles (SVPs).[4] Delamination is a matter of time, and glass particles may only occur after months or years of drug product storage. If delamination is observed in vials, it is assumed to occur mostly at areas where heat was applied during the vial forming process, that is, at the bottom and shoulder of a vial.[24] These areas have a lower hydrolytic/chemical resistance and form so-called reaction zones when in contact with the drug product. This is also why glass delamination is likely less of an issue for syringes, given that these areas are usually located outside of the product contact region of the glass barrell.[25] By applying extreme stress conditions to glass containers,[6,17,18,24,26] glass delamination can be accelerated. These accelerated test methods may provide a rough evaluation of the potential and the risk for delamination of the respective glass vial. These conditions, however, do not necessarily relate to actual long-term storage at intended conditions. For the time being, these tests are unable to predict likelihood of occurrence or even time point of glass delamination but are used to assess the relative risk of occurrence and to determine the risk for glass delamination of a given container. These tests are thus of value for the general assessment of the glass container to be chosen.

The present study assesses stress protocols to accelerate glass delamination in pharmaceutical glass vials. First, the interior surface of 5 different vial types was characterized after using established stress conditions to force glass delamination. Second, the 3 applied accelerated delamination tests were compared. A broad panel of conventional and advanced analytical techniques was employed for testing the (1) stressed solutions, for example, visible and SVPs by light obscuration and Micro-Flow Imaging (MFI), particle identification by scanning electron microscopy with energy dispersive X-ray spectrometry (SEM-EDS), pH shift, and leaching of elements from the glass by inductively coupled plasma spectrometry (ICP) and (2) characterizing the inner vial surface after particular stress treatment by colorimetric staining, SEM, and dynamic secondary ion mass spectrometry (D-SIMS). The results obtained were further related to results of a delamination study performed in alignment with USP chapter <1660>, section Screening Methods to Evaluate Inner Surface Durability.[6]

Materials and Methods

Glass Vials

Five vial types in a 10 mL/10R format were used in the present study. Table 1 summarizes the nominal glass composition, thermal expansion coefficient, the presence and type of coating surface modification, and the surface properties of these vials as provided by the vendors.[27] Uncoated Expansion 33 and Expansion 51 vials were purchased at Schott North America, Inc. (Elmsford, NY) and Schott AG (Müllheim, Germany), respectively. Three vial types of 51 expansion glasses with an additional interior surface modification, which are referred to as Siliconized, TopLyo™, and Type I plus® vials, were obtained from Adelphi Healthcare Packaging (Haywards Heath, UK). The vials are referred to as "surface-modified" vials rather than "coated" vials in the following as the terminology "coated" suggests only a physical application of material that can be easily removed or extracted. All vials comply with hydrolytic class I.

Preparation of Vials for Stress Test

Washing of Vials

Vials were washed with water for injection (WFI) (~75°C) in a Belimed LA280 cleaning and disinfectant instrument (Belimed Sauter AG, Sulgen, Switzerland) and allowed to dry under laminar air flow for several hours (<24 h).

Table 1
Summary of Chemical Composition and Physical Data of Investigated Glass Vials as Provided by the Vendors[27]

Trade Names	Expansion 33	Expansion 51	Siliconized	TopLyo	Type I plus
Chemical glass composition	(wt.%)	(wt.%)	(wt.%)	(wt.%)	(wt.%)
SiO_2	81	75	75	75	75
B_2O_3	13	10.5	10.5	10.5	10.5
Al_2O_3	2	5	5	5	5
Na_2O	3.5	7	7	7	7
CaO	–	1.5	1.5	1.5	1.5
K_2O	0.5	–	–	–	–
Thermal expansion coefficient	$3.3 \times 10^{-6}\ K^{-1}$	$4.9 \times 10^{-6}\ K^{-1}$	$4.9 \times 10^{-6}\ K^{-1}$	$4.9 \times 10^{-6}\ K^{-1}$	$4.9 \times 10^{-6}\ K^{-1}$
Layer	No	No	$SiOCH_3$	$Si_aO_bC_cH_d$	SiO_2
Layer thickness	–	–	~10-60 nm[a]	~40 nm	100-200 nm
Coating method	–	–	Baked-on	PICVD	PICVD
Surface property	Hydrophilic	Hydrophilic	Hydrophobic	Hydrophobic	Hydrophilic

PICVD, plasma-impulse chemical vapor deposition.
[a] Theoretical calculation based on the amount of sprayed-on silicon emulsion assuming homogeneous silicon layer.

Depyrogenation of Vials

Depyrogenation of the vials was performed in a Heraeus® WU 6100 heating and convection oven (Thermo Electron LED GmbH, Langenselbold, Germany). After loading the vials, the oven was heated to 300°C with a ramp of 6°/min, followed by a holding step at 300°C for 10 min. Immediately thereafter, the vials were allowed to cool down to room temperature for at least 30 min (standard depyrogenation). Vials used for testing according to USP were filled with 200 μL of WFI and subjected to the same temperature cycle as described previously simulating residual moisture after vial washing step and worst case depyrogenation conditions.

Protocols for Delamination Stress Testing

Three established test protocols[17,24] (Nuova Ompi glass division—Stevanato Group. private communication) were evaluated and compared as described in the following section. The matrix solutions were freshly prepared and filtered using a 0.22-μm Millipore Stericup® Filter Unit (Merck, Darmstadt, Germany). The solutions were analyzed for SVPs using the light obscuration method as well as for pH. Twenty vials of each vial type were treated according to the test protocols. The protocols differ in test solutions and stress conditions used. Every protocol suggests its own test methods for analysis. To guarantee a similar evaluation of results and to be able to understand the advantage of each method when investigating glass delamination, all samples regardless of the test protocol applied, were analyzed using the full panel of test methods. The process descriptions in the following section follow the guidance given in the respective reference.

Test Protocol 1

Depyrogenized vials were rinsed 3 times with distilled water to remove any potential debris or dust and dried with a nitrogen gas stream (Nuova Ompi glass division—Stevanato Group. private communication). The vials were completely filled with 0.9% NaCl solution (Baxter AG, Volketswil, Switzerland) with the addition of 1 M NaOH (Sigma-Aldrich, Steinheim, Germany) to receive pH 8. The fill volumes for the vials were empirically determined as Expansion 51 = 12.6 mL, Expansion 33 = 12.2 mL, Siliconized = 12.4 mL, TopLyo™ = 12.6 mL, and Type I plus = 12.4 mL. The vials were wrapped with a piece of aluminum foil and were exposed to the autoclave's atmosphere 121°C for 60 min using a Zirbus HST 3 × 3 × 6 autoclave (Zirbus Technology GmbH, Bad Grund/Harz, Germany).

Test Protocol 2

Depyrogenized vials were rinsed twice with hot R1 water (B. Braun Medical AG, Sempach, Switzerland) (50°C-60°C) to remove any potential debris or dust.[24] In the next step, the vials were filled again with hot R1 water to the brim and allowed to stand for 5-10 min to cool down. Immediately before testing, the vials were emptied, rinsed 3 times with R1 water (25°C), allowed to stand at room temperature for 5-10 min, and finally dried with nitrogen gas. The vials were autoclaved bottom-up on an appropriate stainless steel net tray to allow permanent exchange with the autoclave's atmosphere at 121°C for 240 min using a Zirbus HST 3 × 3 × 6 autoclave (Zirbus Technology GmbH). Subsequently, the vials were allowed to cool down and then filled with 5 mL WFI. Each vial was capped with a piece of aluminum foil, and the vials were again exposed to the autoclave's atmosphere at 121°C for 120 min.

Test Protocol 3

Depyrogenized vials were filled with 200 μL WFI and placed in a Heraeus® WU 6100 heating and convection oven (Thermo Electron LED GmbH, Langenselbold, Germany) for 90 min that was preheated to 250°C.[17] After unloading, vials were allowed to cool down for approximately 30 min. The vials were then filled with 1 mL of a 20 mM aqueous glycine solution (Sigma-Aldrich) adjusted to pH 10 using 1 M NaOH (Sigma-Aldrich), wrapped with a piece of aluminum foil, and incubated in the Heraeus® heating and drying oven at 50°C for 24 h.

United States Pharmacopoeia <1660>

An aggressive delamination screening was additionally performed for a subset of vials, that is, for uncoated Expansion 33 and Expansion 51 vials in alignment with USP chapter <1660> Evaluation of the inner surface durability of glass containers,[6] section Aggressive Screening Conditions after worst case depyrogenation. Ten vials were exposed to each of the following screening conditions, according to the protocol as described in USP <1660>:

- USP solution 1: 0.9% KCl solution (Merck) with the addition of 1 M KOH to receive pH 8 (Merck); autoclaved at 121°C for 1 h, 2 cycles.
- USP solution: 2: 3% aqueous citric acid solution (Merck) adjusted to receive pH 8 using KOH (Merck); stored at 80°C for 24 h.
- USP solution 3: 20 mM aqueous glycine solution (Merck) adjusted to receive pH 10 using 1 M KOH (Merck); stored at 50°C for 24 h.

Analytical Methods

A panel of analytical methods was used for analysis of the vial surface or the stress solution. Three vials were pooled for light obscuration and MFI analysis and 10 vials for ICP analysis. SEM-EDS and pH measurements were analyzed from separate vials. Glass strips were generated for surface analysis by manually cutting the vials in several glass strips. Glass strips were blown off with nitrogen gas stream to remove any potential debris or dust.

Analysis of Isolated Particles and Particles in Solution

Visual Inspection. Visible particles were analyzed by visual inspection using a V90-T Seidenader inspection machine (Seidenader Maschinenbau GmbH, Markt Schwaben, Germany). The Seidenader instrument is equipped with a magnifying glass and light passes through the bottom, side, and top of the sample. Samples are set into rotation, and particles were detected by the reflected light.

Light Obscuration. SVPs were quantified by light obscuration using a HIAC Royco counting System 9703 (SKAN, Basel, Switzerland) equipped with an HRLD400HC sensor. Analyses were made with a flushing volume of 0.2 mL followed by a small volume method consisting of 4 runs of 0.4 mL each at a flow rate of 10 mL/min. The first run was rejected and the average and the standard deviation of the last 3 runs were reported as cumulative particle counts per mL for sizes ≥2, ≥5, ≥10, and ≥25 μm and corrected by the particle count of the matrix solution (blind value).

Micro-Flow Imaging. MFI was performed to assess the particle size and morphology using the MFI Flow Microscope model with a 100 μm flow cell DPA4200 and the MVSS V2.0 software (Brightwell Technologies Inc., Ottawa, Ontario, Canada). Analyses were made with 1 mL of the sample.

Scanning Electron Microscopy With Energy Dispersive X-ray Spectrometry. To isolate particles, solutions were filtered through a polycarbonate filter with a pore size of 0.8 μm (Merck Millipore,

Tullagreen Carrigtwohill, Ireland) and placed on a conductive adhesive tape for analysis. SEM pictures were acquired with a FEI Quanta FEG 250 (CSV B838) instrument (FEI, Eindhoven, the Netherlands) using an accelerating voltage of 5 keV for locating the relevant particle on the filter. The elemental composition of the located particles was further analyzed by EDS. For this analysis, the accelerating voltage was set to 10 or 15 keV.

Analysis of Solution

Inductively Coupled Plasma Spectrometry. Glass leachables/elements were measured in test solution by using ICP mass spectrometry (ICP-MS) on an Agilent 7500ce ICP-MS instrument (Agilent Technologies GmbH, Waldbronn, Germany) and ICP optical emission spectrometry (ICP-OES) on an Agilent 725 ICP-OES instrument (Agilent Technologies GmbH). ICP-MS was performed to investigate the boron (referred to as "B") and aluminum concentrations (referred to as "Al") in solution, whereas ICP-OES was used to determine the silicon concentration in solution (referred to as "Si"). The performance of the ICP-MS/-OES analysis was according to the European Pharmacopoeia (Ph.Eur.) 2.2.57[28] and Ph.Eur. 2.2.58,[28] respectively. Samples (pure water) treated according to the test protocol 2 were acidified with 60% HNO_3 (Merck) to obtain a final concentration of 2% HNO_3. Samples treated according to test protocols 1 and 3 were acidified with 2% HNO_3 and appropriately diluted (at least 1:10) before analysis. To note, for the present stress protocols, sodium concentrations were not determined by ICP analysis as sodium hydroxide was used for pH adjustment of the test solutions 1 and 3.

pH Measurement. pH value of the stress solution was measured because glass leachables are known to cause pH shifts in test solutions without buffering capacity.[6] The pH measurements were performed at room temperature using a 781 pH/Ion Meter (Metrohm Schweiz AG, Zofingen, Switzerland).

Characterization of Glass Surface

Colorimetric Staining. Colorimetric staining was performed to identify potential inhomogeneity of the glass surface (for area of analysis, see Fig. 1a). Vials ($N = 5$) were rinsed twice with distilled water, filled to the rim with a 0.05% methylene blue solution (Sigma-Aldrich), and equilibrated for 20 min. After rinsing them twice with distilled water, they were dried in a Heraeus® UT 6060 heating and drying oven (Thermo Fisher Scientific, Langenselbold, Germany) at 120°C for 10 min.

Scanning Electron Microscopy. The morphology of the vial surface was analyzed by SEM using a Σigma VP (Zeiss, Oberkochen, Germany) system under high vacuum at an accelerating voltage of 3 keV. The glass strips were coated with a thin film of gold using a Cressington 108 Auto sputter (Elektronen-Optik-Service GmbH, Dortmund, Germany) to increase the conductivity of the sample (120 s, 30 mA, 0.1 bar Ar). A line averaging of 17 scans per frame was applied to reduce the noise in the images, resulting in a full acquisition time of 44.6 s per image. An area of 92 μm × 68 μm was visualized by a matrix of images of ~23 μm × 17 μm at the bottom-near area on a single glass strip as indicated in Figure 1a.

Dynamic Secondary Ion Mass Spectrometry. SIMS sputter depth profiles were obtained on an IONTOF Time-of-Flight-SIMS 4 instrument in combination with the Surface Lab software package 6 (ION-TOF GmbH, Münster, Germany). The samples were prepared in the following way: the vials were cut into strips of ~5 mm × 30 mm in size, and the inner surface was coated with a thin film (~80 nm) of gold for charge compensation. The sputter depth profiling was performed using a dual beam technique: Cs^+ ions at 2 keV were used to sputter the sample, whereas Bi^{3+} ions (25 keV) were used for analysis. The size of the sputtered crater was 150 μm × 150 μm; the analysis was done inside those craters on an area of 64 μm × 64 μm. After depth profiling, the sputter rate was estimated by measuring the crater depth with a Wyko NT3300 white-light interferometer system (Veeco Instruments GmbH, Mannheim, Germany). The depth profiles were measured at different distances to the bottom of the vials (see Fig. 1b).

Methods Related to USP <1660>

Samples stressed according to USP <1660> were analyzed by ICP-MS/-OES as described previously.

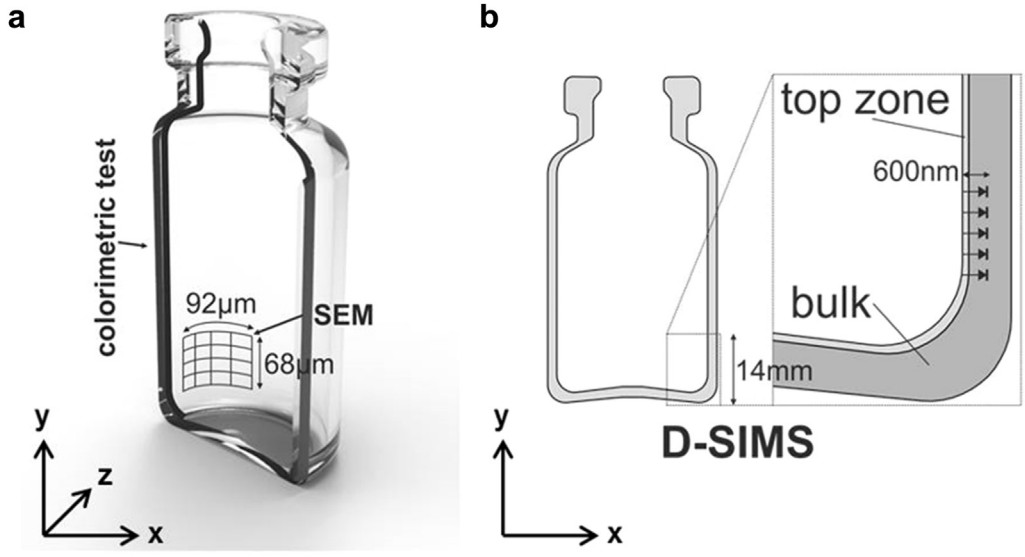

Figure 1. Area of analysis. (a) Along the vertical axis of the inner surface of the vial for the colorimetric test and at the bottom area for SEM and (b) into the depth of the glass vial at the bottom area for D-SIMS.

Results

Analysis of (Isolated) Particles in Solution

The 5 different types of glass vials were visually inspected after delamination stress testing using test protocols 1-3 referred to as 0.9% NaCl pH 8 (test protocol 1), WFI (test protocol 2), or 20 mM glycine pH 10 (test protocol 3). 20 containers were inspected for visible glass particles by Seidenader inspection machine for each protocol and vial type and classified as no (0), 1 to 5, 6 to 10, and more than 10 glass particles. The percentage of glass containers containing visible glass particles is presented in Figure 2. Figure 2 shows that glass particles were observed for all vial types when stressed with test protocol 2, as well as for Expansion 33 vials when stressed with test protocol 1. The observed particles were tiny and reflected light irregularly. The amount of visible particles observed was either one to 5 (light gray) or 6 to 10 (dark gray), but never in uncountable amounts. For further confirmation of the nature of the particles, they were isolated for selected samples and analyzed by their elemental composition using SEM-EDS. A representative SEM image with corresponding ED spectra is presented in the Supplementary Information (see Figure S-1). Considering detection and size range limits for visual inspection, glass particles were only confirmed for Expansion 33 and for Expansion 51 vials when stressed with test protocol 2. In particular, particles were between 5-10 μm, which is in a size range that does not readily allow visual detection and more commonly relates to the subvisible size range.

Subvisible particle analyses were further performed after delamination stress testing by 2 different methods. Cumulative particle counts per milliliter were determined by light obscuration (Figs. 3a-3c), and the particle morphology was characterized by MFI (Fig. 3d), with differentiation of glass particles, air bubbles, silicone oil droplets, and others (e.g., cellulose fibers). Figures 3a-3c show the cumulative subvisible particle counts of the samples for different size ranges subtracted by the number of SVPs of the extraction solution before filling the vials. An increase in SVP was observed for the lower size ranges, and a clear difference was observed between the stress protocols with highest counts for test solution 1 compared to protocols 2 and 3. For covalently surface-modified vials, particle counts were low or within the standard error of the method. The morphology of SVP was further characterized by MFI. A clear differentiation of the particle morphology was only possible for particles ≥10 μm based on the limitation of the MFI method. SVP ≥10 μm to max. 100 μm were identified as fibers or air bubbles. SVP in the size range 5-10 μm (Fig. 3d) were not clearly identified or differentiated as glass particles. However, this size range seems to be most relevant as already confirmed by SEM-EDS. Representative MFI pictures of this size range are shown in Figure 3d.

Analysis of Extraction Solution

Leaching of glass elements from the glass surface into the test solution after delamination stress testing was investigated by ICP-MS/-OES analysis and indirectly by pH measurement of the test solutions. Figures 4a-4c show the oxidized form of the extracted elements silicon (Si), boron (B), and aluminum (Al) for all vial types investigated. The concentration ratios of silicon oxide/boron oxide (SiO_2/B_2O_3) and Si/Al for the uncoated vials are depicted in Figures 4d and 4e. Expansion 33 vials revealed highest concentrations of SiO_2 and B_2O_3 for test protocol 3 followed by test protocol 1 (alkaline solutions). Lowest values were found for test protocol 2 (aqueous solution). The elemental concentration of Al_2O_3 was higher for test protocol 1 compared to test protocols 2 and 3. For Expansion 51 vials, highest concentrations of SiO_2 and B_2O_3 were quantified for test protocol 2, lower and nearly the same level for test protocols 1 and 3. Overall, concentrations of SiO_2 and B_2O_3 were significantly higher for stressed Expansion 33 vials compared to Expansion 51 vials. Considering surface-modified vials, Siliconized vials showed unexpected high concentrations of glass leachables which are at the same level as obtained for uncoated Expansion 51 vials especially when stressed with test protocol 2. TopLyo™ vials were the most resistant vials when stressed with any of the stress test solutions as leaching of glass elements was minimal and just in the range of limit of quantification (LoQ) of the analytical methods. Thus, the modified surface layer of the TopLyo™ vials seemed to be unchanged. The concentration of SiO_2 was high for Type I plus® vials but not for to B_2O_3 and to Al_2O_3 concentrations

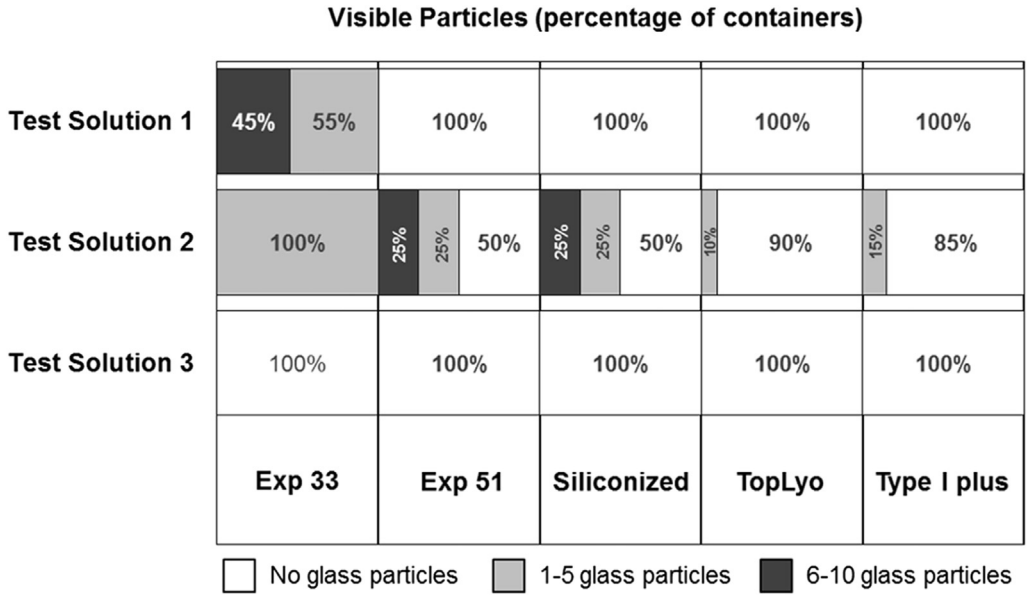

Figure 2. Visible particles after delamination stress testing with test protocols 1-3. 20 vials were inspected for visible glass particles by Seidenader inspection machine and classified as no glass particles; 1-5 glass particles; and 6-10 glass particles.

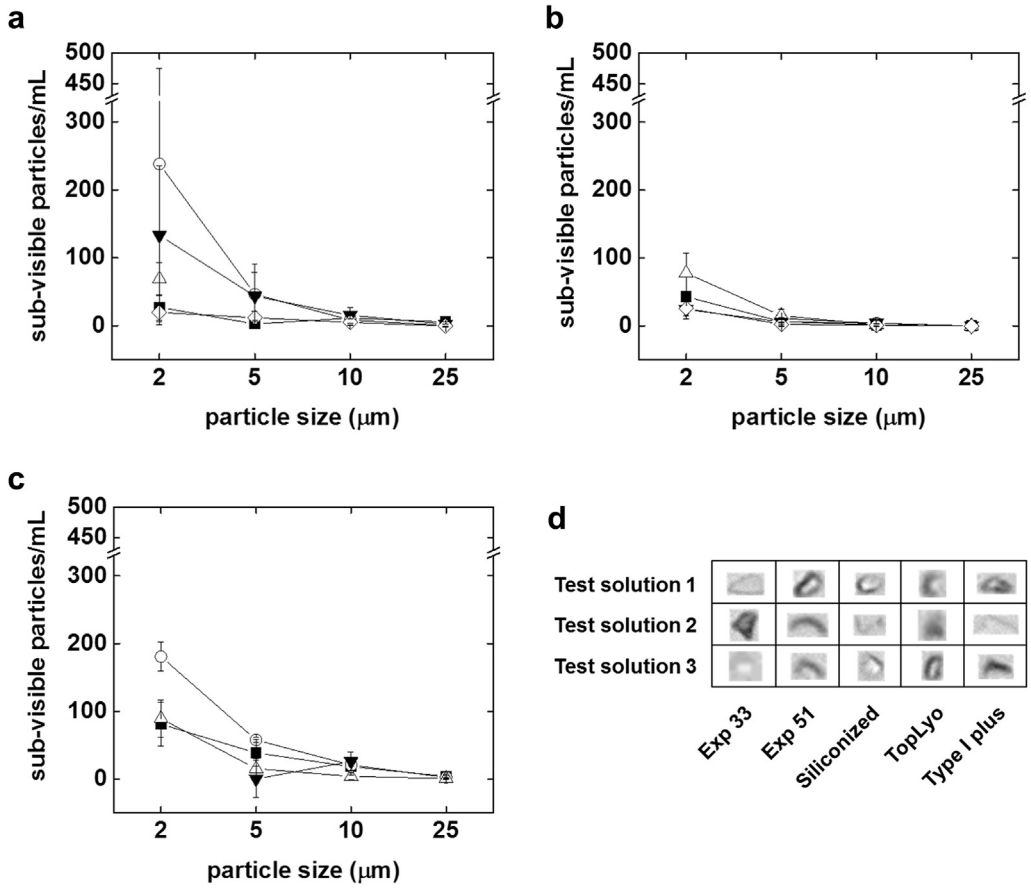

Figure 3. Subvisible particles after accelerated delamination. Cumulative subvisible particle count for (a) test solution 1, (b) test solution 2, and (c) test solution 3 analyzed by light obscuration presented as mean value with standard deviation: ■Exp. 33, ○Exp. 51, △Siliconized, ▼TopLyo, and ◇Type I plus. (d) Representative pictures of particle morphology ≥5 μm as analyzed by Micro-Flow Imaging.

being below their limit of quantification (LoQ of aluminum and boron <0.1 mg/L) especially when stressed with test solutions 1 and 3.

Glass manufactures and USP <1660> suggest a comparison of SiO_2/B_2O_3 and Si/Al ratio for better assessment of vial types with respect to delamination propensity. The SiO_2/B_2O_3 ratio of intact vials is 6.2 for Expansion 33 and 7.1 for Expansion 51 vials, whereas the Si/Al ratio is 36.6 and 13.6, respectively (for initial glass composition, see Table 1). A decrease in SiO_2/B_2O_3 or Si/Al ratio is considered to indicate dissolution of the glass network.[29] Thus, these ratios may provide information about either inhomogeneous dissolution (ratio: ~2:3) indicating a severe change of the glass surface and potential glass delamination or homogeneous dissolution being in the theoretical range of intact bulk glass. The ratios were not determined for surface-modified vials to avoid a misleading interpretation due to an increased SiO_2 concentration originating from a dissolved surface modifications, that is, this approach cannot be applied for coated or surface-modified vials. Thus, the concentration ratio values were only determined for Expansion 33 and Expansion 51 vials (Figs. 4d and 4e). For Expansion 33 vials, when stressed with test protocols 2 and 3, the SiO_2/B_2O_3 concentration ratio value were 1.7 and 2.6, respectively, indicating an inhomogeneous dissolution and preferential dissolution of boron. In contrast, vials stressed with test protocol 1 showed only a tendency towards inhomogeneous dissolution of the glass (SiO_2/B_2O_3 ratio of 4.1). The determined Si/Al ratio for Expansion 33 vials when stressed with test protocols 1 and 2 showed also a tendency towards inhomogeneous dissolution of the glass, while Si/Al ratios were not further assessed for these vials when stressed with test protocol 3 due to the low Al concentration <0.1 mg/L. On the one hand, the SiO_2/B_2O_3 concentration ratio value obtained for Expansion 51 vials when stressed with test protocols 1 and 2 indicate a slight decrease; however, this may be still be considered as homogeneous dissolution of the inner surface (SiO_2/B_2O_3 ratio of 6.1 and 6.0). On the other hand, the lower ratio obtained after treatment with test protocol 3 indicated a tendency to inhomogeneous dissolution (SiO_2/B_2O_3 ratio of 3.7). Si/Al ratios determined for Expansion 51 vials when stressed with test protocols 2 and 3 confirmed a homogeneous dissolution.

Ion leaching with exchange of H^+ ions and glass network dissolution (dissolved Si anions), impact the pH value of the test solution. Thus, pH measurements were performed for the test solutions. Figure 5 shows the pH after delamination stress testing for all different vial types. For test solutions 1 and 2, a pH shift was detected as the extraction solutions have no buffering capacity. Vials stressed with test solution 1 showed an increase in pH between 0.2 and 1.8 pH units. For vials stressed with test solution 2, the pH value was found to increase between 0.1 and 2.8 units. For all vial types stressed with test solution 3, only a slight decrease in pH value was observed due to the buffering capacity of the extraction solution.

Characterization of the Glass Surface

Structural and chemical analysis of the inner surface of the glass vials was performed using the colorimetric test, SEM, and D-SIMS

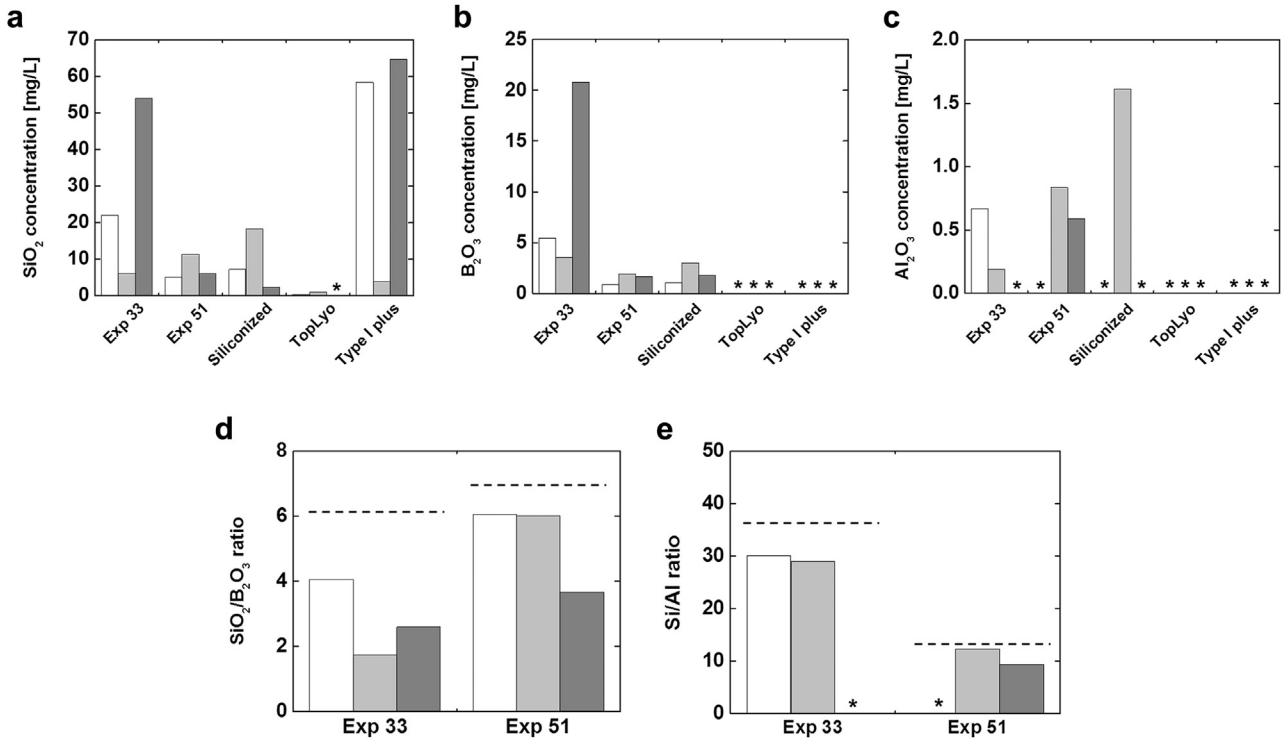

Figure 4. Extractable elements analyzed by ICP after delamination stress testing for test solutions 1-3. (a) SiO_2, (b) B_2O_3, and (c) Al_2O_3, (d) SiO_2/B_2O_3 ratio, and (e) Si/Al ratio. White = test solution 1, light gray = test solution 2, and dark gray = test solution 3. Dashed line indicates nominal glass bulk ratio. *LoQ.

after delamination stress testing: The colorimetric staining was performed to visualize differences and changes in the surface roughness and the morphology (see Fig. 6a). The blue colorations indicated an alteration of the glass surface which is a result of corrosion of the interior vial surface. Figure 6a shows that the vial types were stained differently when exposed to different extraction media indicating stronger coloration with methylene blue for more corroded areas. Uncoated vials (1, 2) showed a diffuse coloration already after washing and depyrogenation as a reference. Even more colored areas were observed for Expansion 33 and Expansion 51 vials after delamination stress test using test solution 1 (6 and 7), test solution 2 (11 and 12), or test solution 3 (16 and 17) in form of diffuse blue colored areas or distinct blue ring at the bottom or at the shoulder of the vial. The strongest coloration was observed for Expansion 33 (6, 11, and 16) vials at the bottom area after treatment with any of the 3 solutions as well as for Expansion 51 vials at the vial shoulder after treatment with test solution 2 (12). In contrast to the uncoated vial types, coloration was only observed after treatment with test protocols 2 (13) and 3 (18) for Siliconized vials, and test protocol 1 (10) for Type I plus® vials. TopLyo™ (4, 9, 14, and 19) vials did not show any coloration at all.

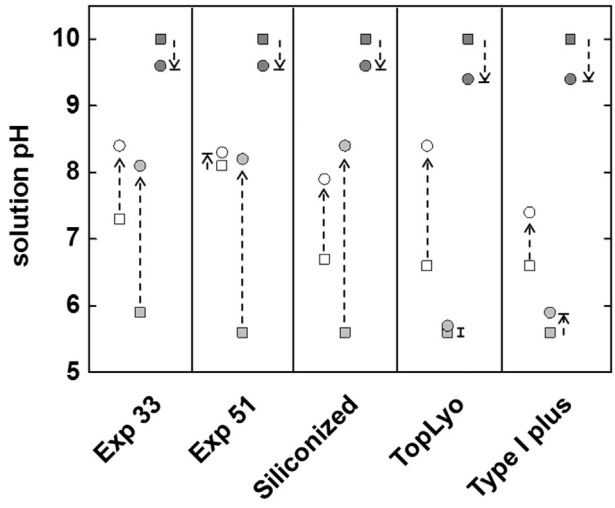

Figure 5. pH shift after delamination stress testing for the 5 different vial types. □Before stress test and ○after stress test. White = test solution 1, light gray = test solution 2, and dark gray = test solution 3. The direction of the arrows indicates pH value increase/decrease.

SEM analysis was used to visualize surface morphology of the inner glass surface, in particular so-called pits, bumps, and lens-shaped rings as shown in Figure 6b of each of the 5 vial types after delamination stress testing. The area of analysis was the lower part of the vial as depicted in Materials and Methods, which is the one being the most prone to glass corrosion when in contact with drug product. All vials (1-3 and 5), except for TopLyo™ vials (4), showed changes in glass surface morphology in form of bumps already after depyrogenation. In addition, lens-shaped rings were observed for Expansion 51 (2), Siliconized (3), and Type I plus® vials (5). After treatment with test solution 1 (6-8) and test solution 2 (11-13), the surface for Expansion 33, Expansion 51, and Siliconized vials changed to the presence of a lot of pits. After treatment with test solution 3, lens-shaped rings could be observed for Expansion 33 (16) and Siliconized (18) vials. In contrast, Expansion 51 (17) vials still showed pitting and in addition lens-shaped rings, however to a smaller extent after exposure to the test solution 3. TopLyo™ vials did not show any changes after treatment with all extraction protocols (9, 14, and 19), and the modified surface layer remained unchanged. Type I plus® vials showed a slight surface change with a reduction of bumps and an increase in lens-shaped rings for stressed vials (10, 15, and 20).

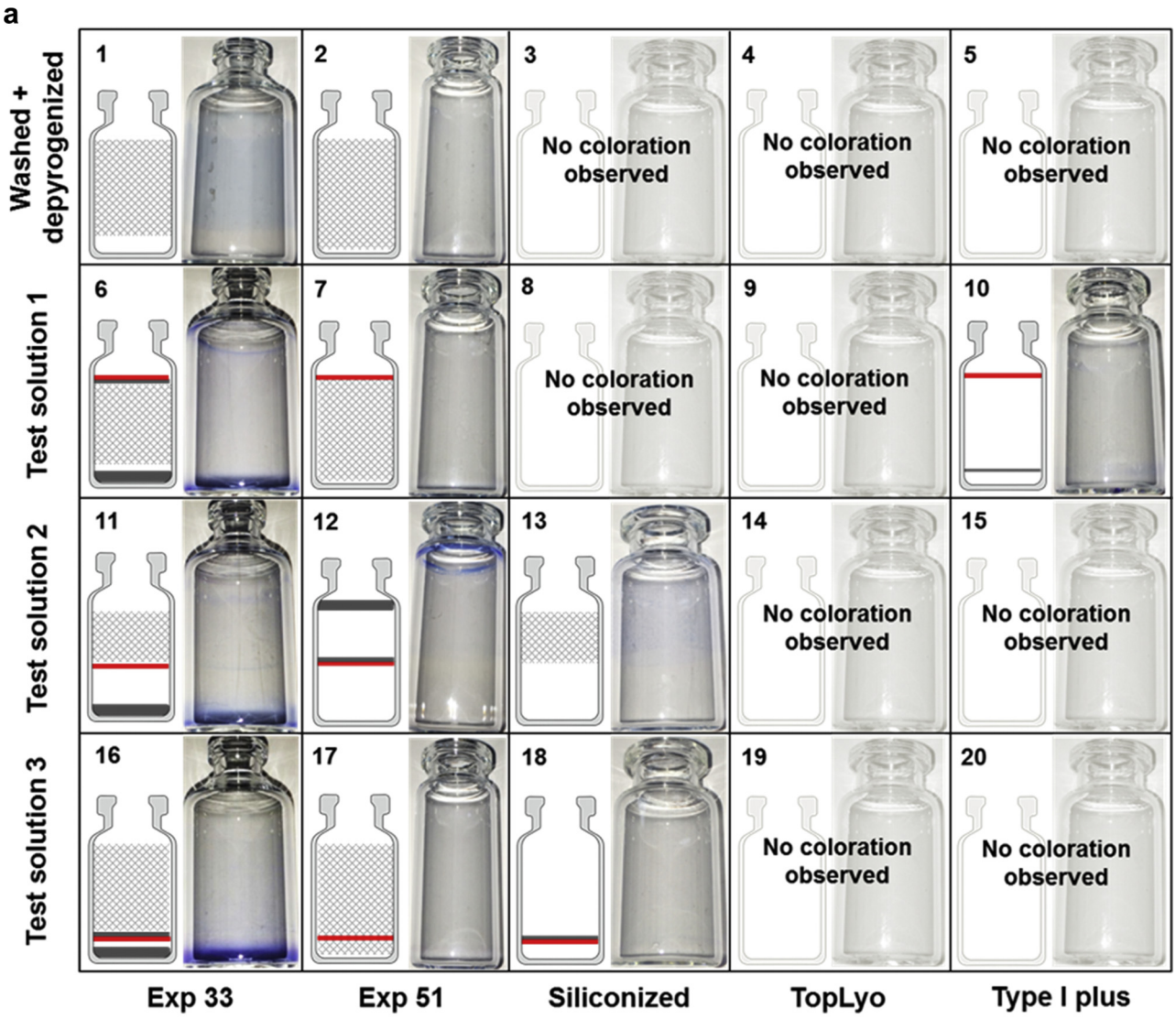

Figure 6. (a) Schematic and original pictures of colorimetric test showing blue coloration of altered glass surface. Diamond pattern = diffuse, pale colored surface, bar = colored surface in form of a ring, strength of the bar = intensity of coloration (pale/strong), red line = filling level. (b) Representative SEM images of glass surface morphology after delamination stress test using test solutions 1-3 for the 5 different vial types.

For Expansion 33 and Expansion 51 vials, D-SIMS analysis was performed with the objective to determine the depth-resolved chemical composition of the glass material in the surface near region (referred to as top zone). The analysis was performed for vials before and after exposure to test solutions 1-3. Depth profiles of the glass elements Si, O, B, Al, Na, Ca, and K were obtained at different measuring positions along the vertical axis of the vial, starting with a distance of ~1 mm from the vial bottom. Figure 7a shows depth profiles measured for Expansion 33 vials before (1) and after (2) exposure to test solution 2 as an example. The graphs display the elemental concentrations of B and Al at different positions of the side wall of the vial as a function of the sputter depth (nm). In the initial depyrogenized state, Expansion 33 vials show a clear position-depending enrichment of B in the bottom-near region for the first 200 nm of the glass material. In contrast, the Al concentration was nearly constant for the different measuring positions (Fig. 7a, 1). After the exposure to test protocol 2, a change in the surface near glass composition was found. The enrichment of B before stress testing changed to a depletion of B depending on the measuring position. In addition, an enrichment of Al at the same depth area was observed. The depletion of B and the enrichment of Al increased when approaching the vials bottom (Figs. 7a, 2).

For a quantitative comparison of the elemental changes in the surface near region of the vials, numerical values were obtained by investigating the elemental concentration over sputter depth representative for elemental enrichment or depletion for the individual measuring positions. In the first step, the difference between the depth-resolved elemental concentration and the nominal bulk material concentration (>200 nm) was determined as a function of sputter depth. In the second step, the area under the curve for the different elements was integrated, which is a measure of the enrichment or depletion of the elements in the top zone. Figure 7b summarizes the results of this analysis for Expansion 33 and 51 vials for the characteristic elements B (1), Si (2), and Al (3) depending on the distances to the vial bottom (mm) before and after exposure to test solutions 1-3. Expansion 33 vials showed a significant variation in elemental composition in the top zone of the glass vial for the first ~5 mm along the vertical axis of the vial starting from the bottom. For the initial depyrogenized state of the Expansion 33 vials, an enrichment of B (1), sodium, and potassium

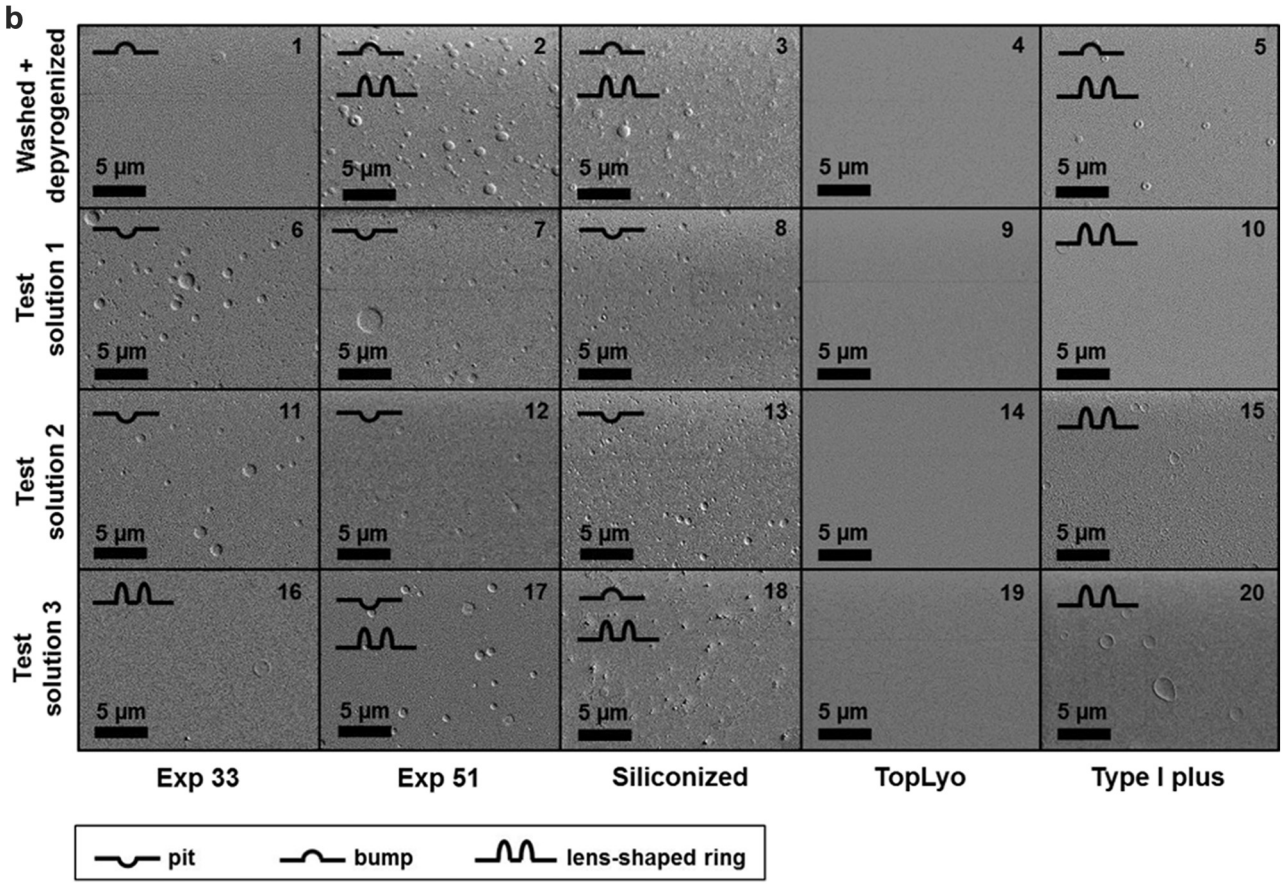

Figure 6. (*continued*).

(data not shown for the latter), as well as a depletion of Al (2) and Si (3) in comparison to the glass bulk composition was determined. After treatment with test solutions 1-3, a pronounced enrichment of Al (2) and Si (3) as well as a pronounced depletion of B (1) was observed. However, the depletion of B (1) after treatment with test solution 1 was found to a smaller extent than for test solutions 2 and 3. Expansion 51 vials showed a completely different behavior. In their initial depyrogenized state, a slight enrichment of B (4) was observed within the first 3 mm from the vial bottom. Furthermore, a slight and constant Al (5) enrichment over the complete area of analysis was obtained for Expansion 51 vials. Si (6) concentration was the same as for the bulk over the whole area of analysis. No significant changes in the chemical composition of the top zone was determined for Expansion 51 vials after any delamination stress testing not even depending on the measuring position.

United States Pharmacopoeia <1660>

USP chapter <1660>, section Screening Methods to Evaluate Inner Surface Durability provides guidance on how to assess the propensity of glass vials for glass delamination by the use of different extraction media (referred to as USP solution 1-3) and recommendation of a panel of analytical methods (SEM-EDS, particle size analyzer, conductivity/pH, ICP-MS or ICP-OES, differential interference contrast microscopy or electron microscopy, and SIMS). To investigate worst-case conditions, Expansion 33 and Expansion 51 vials were exposed to the proposed conditions according to USP <1660> after worst-case depyrogenation and analyzed for leaching of glass elements by ICP-MS/-OES as a selected method. Figure 8 shows the oxidized form of extractable elements (Si, B, and Al) as well as the concentration ratios of SiO_2/B_2O_3 and Si/Al for both vial types. For Expansion 33 vials, highest concentrations of dissolved glass elements (SiO_2, B_2O_3, and Al_2O_3) were obtained when stressed with USP solution 2 (3% citric acid pH 8) and 3 (20 mM glycine pH 10) as well as significantly lower concentrations when stressed with USP solution 1 (0.9% KCl pH 8). In contrast for Expansion 51 vials, significant leaching was only observed for USP solution 2. When comparing the elemental ratios SiO_2/B_2O_3 and Si/Al as presented in Figures 8d and 8e, the data indicate that either a homogeneous dissolution or a trend to inhomogeneous dissolution was observed after stress testing according to USP for both vial types. Results will be compared to the results from the other test protocols and discussed in the following section.

Discussion

Delamination Stress Testing in Glass Vials

The present study investigated 5 different vial types (see Table 1) for their propensity for glass delamination. The different vials were stressed by 3 different well-established stress protocols,[17,24] (Nuova Ompi glass division—Stevanato Group. private communication) and samples were subsequently analyzed by different analytical techniques. Our experiments showed that the solutions used for forcing delamination have a major impact on the occurrence of glass delamination. There are different mechanisms on how solutions interact with the glass surface. When glass

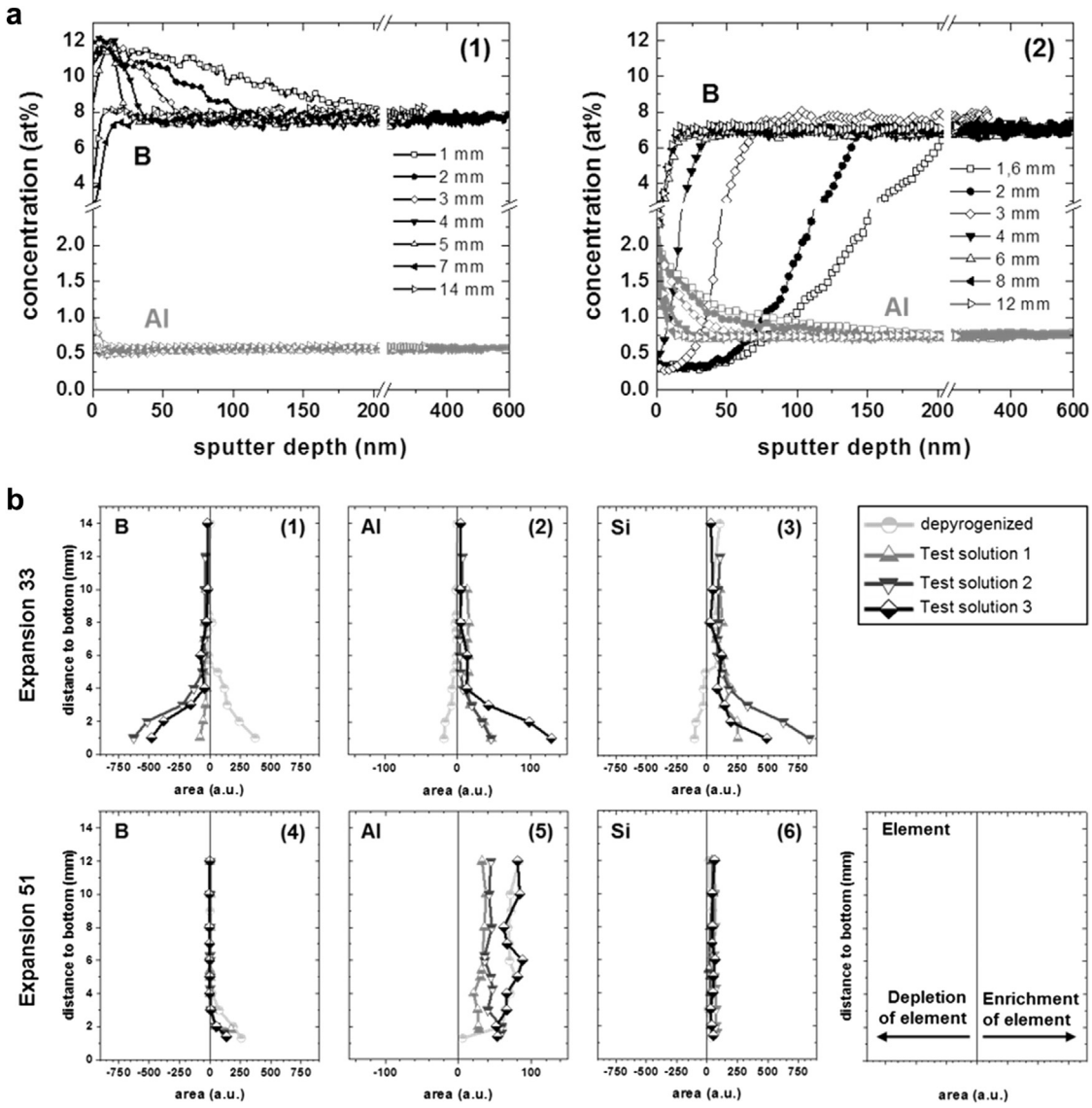

Figure 7. (a) Depth profiles for B and Al measured at different positions of the inner side wall of an Expansion 33 vial before (1) and after (2) delamination stress test with test solution 2. Displayed is the elemental concentration (%) for the elements boron (black) and aluminum (gray) as a function of sputter depth for different distances from the vial bottom (1-14 mm). (b) Enrichment or depletion of glass elements (B, Al, and Si) at the inner side wall in the bottom area of Expansion 33 and Expansion 51 vials before and after delamination stress testing (test solutions 1-3). The difference of elemental concentration to the glass bulk was integrated over the sputter depth. Numerical values (arbitrary units) are displayed depending on the distance to the vial bottom.

surfaces are exposed to water (test protocol 2), the glass surface is hydrated, and an alkali-depleted, silica-rich layer is formed.[13] This involves an ion exchange between the hydrogen ions in the solution and glass network modifiers (Na^+, K^+, and Ca^+). The presence of water (equilibrium of H^+ and OH^- ions) promotes hydrolysis of the Si—O bond forming a silica-gel layer which is referred to as glass network attack in the following.[6] At higher pH values such as for test solutions 1 and 3, the mechanism of glass degradation changes from leaching of alkali elements to the dissolution of the silicate network by hydroxyl ions. Both mechanisms can be confirmed by testing the glass elements in solution (Si, B, and Al) by ICP and of the glass by D-SIMS, as well as by a shift in pH of the test solution.

An increase in pH was evident for uncoated vial types (Expansion 33 and Expansion 51) for test solution 2 due to the decrease of hydronium ions from the solution. The covalently bound surface modification of TopLyo™ and Type I plus® vials was preventive against ion exchange within the surface layer of the glass resulting in no increase of solution pH, whereas Siliconized vials (baked-on coating) showed the same behavior as vials without any surface modification. Thus, the baked-on silicone layer is likely either not covering the complete surface or it is not a 100% diffusion barrier. The first was confirmed by ICP as discussed in the following section as well as by SVP analysis. A glass network dissolution caused by alkaline attack (test solutions 1 and 3) was only confirmed by pH measurement for samples stressed with test protocol 1 showing also an increased pH value. The increase in pH for the surface-modified Type I plus® and TopLyo™ vials was not a straight forward result due to their protective properties as shown for test protocol 2 as well as their intact barrier properties against glass delamination confirmed by colorimetric test and D-SIMS, which will be discussed in detail in the following section. However, for the Type I plus® vials, ICP data confirmed a partly dissolved surface modification caused by the alkaline attack. This was already described previously by Klause et al.[30] For TopLyo™ vials, the

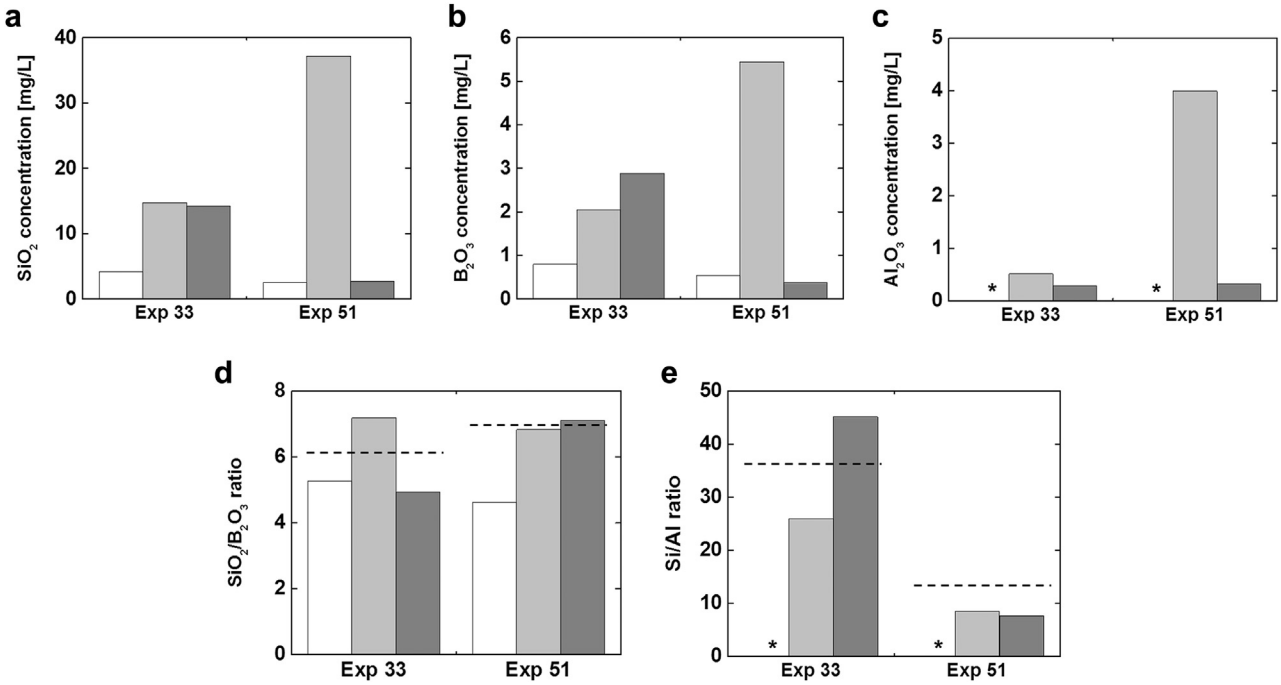

Figure 8. Extractable elements analyzed by ICP after delamination stress testing with USP solutions 1-3 for Expansion 33 and Expansion 51 vials. (a) SiO_2, (b) B_2O_3, and (c) Al_2O_3, (d) SiO_2/B_2O_3 ratio, and (e) Si/Al ratio. White = USP solution 1, light gray = USP solution 2, and dark gray = USP solution 3. Dashed line indicates nominal glass bulk ratio. *LoQ.

increase in pH was not expected. The surface modification of TopLyo™ vials seem not to be attacked, as confirmed by ICP data and morphology investigations. The more or less stable pH value for samples stressed with test protocol 3 (~ −0.4 units for all samples) was explained by the buffering capacity of the test solution, thus not suitable when assessing pH values only.

For uncoated vials (Expansion 33 and Expansion 51), ICP analysis revealed increased concentrations of Si, B, and Al in the leachate of stressed samples (presented as oxides for ICP). However, a change in glass composition in the surface near region at the vial bottom was only confirmed for Expansion 33 vials by D-SIMS analysis but not for Expansion 51 vials. In detail, for Expansion 33 vials, when comparing test solutions 1-3, the concentration of dissolved SiO_2 was high for both stress test 1 and 3; elemental ratios indicated clear or at least a tendency towards inhomogeneous dissolution of the glass for all 3 test protocols. In line, the corresponding D-SIMS results showed significantly smaller silicon enrichment in the bottom-near area when stressed with the test solutions 1 and 3 compared to test solution 2 (see Fig. 7b). The depletion of boron and the enrichment of aluminum, which are in line with the ICP results for the different protocols as well, finally confirm a change in the glass surface and the potential for glass delamination. Conversely, for Expansion 51 vials, test solution 2 is considered more aggressive compared with the alkaline solutions (test solutions 1 and 3) due to the increased concentrations of glass leachables Si, B, and Al as determined by ICP analysis. However, the ratios of glass leachables did not reveal a clear picture indicating homogenous dissolution of the glass surface. D-SIMS data finally confirmed that almost no change was observed upon stress testing in comparison to the initial state before testing at the bottom-near region. In summary, taking together particle, solution, and glass surface analysis, Expansion 33 vials exhibited indications for an elevated delamination risk upon stress testing, but not for Expansion 51 vials. The predictive power of the quick tests with regard to actual long-term storage data and the final occurrence of glass particles is the subject of ongoing research. Besides the uncoated vials, Siliconized vials showed the same trend of glass leachables as observed for Expansion 51 vials when stressed with any of the test solution. None of the test solutions (i.e., 1-3) resulted in an attack of the covalently bound $Si_aO_bC_cH_d$ layer of TopLyo™ vials, while the covalently bound SiO_2 modification of Type I plus® vials suffered by an alkaline attack (test solutions 1 and 3) as described previoulsy. The glass network of Type I plus® vials stayed stable. Detailed results for the surface-modified vials will be discussed further in the section Differences Between Vial Types.

All applied test protocols have an easy handling procedure; however, there are a few disadvantages for each of them. Instability of the pH value during pH adjustment of test solution 1 is the first one. Test protocol 2 was time consuming compared to the other 2, and test protocol 3 used a low filling volume (1 mL), which is challenging during visual inspection. Besides this challenge, the filling volume is crucial as it is reported that the most critical zone prone to delaminate is not only the bottom area of a vial but sometimes also the vial shoulder.[24] Our experiments showed that the critical delamination zone was within the first centimeter of the bottom area for the vials tested, starting upwards along the vertical axis. This was confirmed by D-SIMS analysis. All test protocols used filling volumes covering the bottom area, whereas the vial shoulder was covered by only one protocol (test protocol 1). Thus, the filling volumes used for delamination testing should be initially adapted to cover both potential delamination zones (bottom and shoulder) when forcing delamination independent on the protocol.

A delamination study in alignment with the USP chapter <1660>, section Screening Methods to Evaluate Inner Surface Durability[6] was performed for uncoated Expansion 33 and Expansion 51 vials using test solutions of USP test solutions 1-3 (0.9% KCl pH 8, 3% citric acid pH 8, and 20 mM glycine pH 10). Since the 20 mM glycine pH 10 solution was used in test protocol 3 and is part of the USP <1660> monograph (USP solution 3), the results obtained were set into relation. This stress testing established the same rank order for uncoated containers with respect to their chemical durability as the established test protocols 1-3. For both uncoated

vial types, the concentration of the glass leachables was higher after their treatment with test protocol 3 compared to the USP protocol. The analysis after using the established test protocol 3 indicates clear inhomogeneous dissolution of the glass surface for Expansion 33 vials but only a tendency for Expansion 51 vials in contrast to testing with USP solution 3 showing tendency for inhomogeneous dissolution for both vial types confirmed by the calculated concentration ratios of the glass leachables. The reason is that an additional depyrogenation step was performed for test protocol 3 compared to the preparation of the vials tested according to USP. The additional depyrogenation step weakens the surface which is thus more prone to delaminate.

The test solutions recommended by USP comprise buffered and unbuffered solutions, however only in the alkaline pH region. Thus, the underlying mechanism was an alkaline network attack as described previously. When comparing the test solutions in rank order, USP solution 2 was the most aggressive, followed by USP solutions 1 and 3. In case of USP solution 2, an ion exchange occurred in addition prior to the network attack weakening the glass surface, thus being the most aggressive one.

Differences Between Vial Types

Five different vial types investigated in this study differed in their chemical composition, surface properties, and the presence/absence of a surface modification respectively coating (see Table 1). Thereby, bulk glass composition has a direct influence on glass stability, but chemical durability is mainly driven by the surface near region.[24] The chemical durability is higher for Expansion 33 vials compared to Expansion 51 glass due to their higher B_2O_3 content in the bulk glass composition (see Table 1). However, due to the chemical glass composition of Expansion 33 vials, this vial type requires higher working temperatures for the vial forming process. High temperatures during the hot forming process of the vials (tubular glass) are reported to be the main root cause for changes in glass composition at the surface near region and potential subsequent delamination.[10,17,18,24] Fast physical and chemical mechanisms like evaporation, condensation, and diffusion of mainly sodium borates allow a new arrangement of local chemical bindings. Consequently, the glass composition of the top zone is altered and a glass phase separation into a silica- and borate-enriched phase is initiated.[31] Interestingly, in our study, uncoated Expansion 33 and Expansion 51 vials in the unstressed state showed a silica-depleted and borated-enriched area at the side wall near the vial bottom as shown by D-SIMS data. However, these vials were tested after washing and depyrogenation and not as provided by the vendor. The boron and silicon extent in the surface near region of the vials was changing in the first 5-8 mm along the vertical axis, starting at the vial bottom. Thus, topic of ongoing research is the influence of vial washing and depyrogenation process on surface properties of glass vials.[17] Rupertus et al.[24] have reported that glass delamination starts at the areas where heat was applied during the vial forming. It can be often observed near the bottom and sometimes at the shoulder of the vial. These surface areas are weakened and thus vulnerable for alkaline and hydrolytic attack like for test solutions 1-3. Indeed, the lower resistance against alkaline and hydrolytic attack of Expansion 33 vials compared to Expansion 51 vials was confirmed by the current study (ICP, colorimetric staining, D-SIMS). Although both uncoated vial types showed ion leaching (ICP), only Expansion 33 vials and not Expansion 51 vials did show the potential for glass delamination. This was indicated by the combination of analytical data including a change in chemical composition of the surface near region determined by D-SIMS upon stress testing. The suitability of the methods panel to assess glass delamination will be discussed in the following section.

Surface-modified vials (Siliconized, TopLyo™, and Type I plus®) have the same glass composition as Expansion 51 vials but show different surface properties due to the respective surface modification. Unlike baked-on silicone (Siliconized vials), plasma impulse chemical vapor deposition places a covalently bound silicone layer onto the glass surface providing vials with an improved barrier against glass network attack and ion leaching thereby minimizing drug-container interactions within this study. The barrier properties of TopLyo™ vials with a covalently bound silicon oxygen carbon hydrogen layer ($Si_aO_bC_cH_d$) were confirmed after delamination stress testing (ICP, SEM, colorimetric test). Type I plus® vials also showed no delamination; however, unusually high SiO_2 concentrations were observed by ICP analysis after stress treatment under conditions of test protocols 1 and 3. For these protocols, the SiO_2 coating has likely to be dissolved because of the alkalinity of the test solutions. As a consequence, these vials may have partly lost the protective barrier of the surface modification. Nevertheless, the glass remained intact and was not affected by test solutions 1 and 3 as confirmed by the concentrations found for B_2O_2 and Al_2O_3 below their LoQ. Siliconized vials (baked-on coating) in the contrary were not stable under all test protocols. In 2 cases (test protocols 1 and 2), it seemed that silicone barrier properties were no longer present, suggesting that the silicone layer was either inhomogeneous or partly dissolved/detached.

In summary, the different vial types showed significant differences in their inner surface durability when applying the same stress conditions. The most vulnerable vial type with respect to delamination was Expansion 33 vials, followed by Expansion 51, Siliconized, and Type I plus®. The most resistant vial type was TopLyo™. To highlight, TopLyo™ vials showed better durability compared to Type I plus® vials with respect to maintaining its surface modification and with regards to delamination propensity, although Type I plus® vials are recommended especially for liquid products, whereas TopLyo™ vials are often recommended for freeze-dried products.

Proposed Strategy to Study Glass Delamination

A huge panel of analytical techniques is used in different studies when assessing glass delamination. Several analytical techniques are reported in the literature and can be clustered into investigation of (1) particles[3,4,32] in the visible and subvisible size range, which can be further identified and characterized by for example, SEM-EDS, (2) stress solution regarding its pH[4,18] and its chemical composition by ICP,[33-35] and (3) surface morphology of the glass vial by atomic force microscopy,[33] SEM,[36] differential interference contrast microscopy,[37] and colorimetric staining test,[26] as well as elemental composition by X-ray photoelectron spectroscopy[33] and SIMS.[38] In the following, the suitability of the methods used in the present article and a recommendation given on how to assess delamination with a minimum of methods.

Visual inspection is a simple test method to detect and count visible (glass) particles. However, it is not possible to provide detailed information about the particles' identity although the shape of the particles may already indicate their origin. Thus, an additional method like SEM-EDS is recommended.[3] SEM-EDS was able to identify the particle by its chemical composition. To highlight, within this study many particles identified as glass were in the subvisible size range between 5 and 10 μm. Thus, SVPs should be quantified by, for example, light obscuration providing the number of SVPs for sizes between ≥2 and 50 μm. Since the light obscuration technique does not provide information about the particle nature, an imaging technique, which was MFI in this study was used to elucidate particle morphology. This technique differentiates glass particles from air bubbles, silicone oil droplets, and

other particle morphologies and is thus suitable to confirm glass particles within a certain size range. However, this applies for particles >10 μm only.

USP chapter <1660>, section Predictive Screening Methods[6] suggests different methods for particle identification (SEM-EDS, particle size analyzer). USP specifically suggests using a particle size analyzer for investigating size of particles in the visible and subvisible size range. A particle size analyzer measures the intensity of light scattered by diffusing particles. However, particle size analyzers do only provide a distribution of particle size and do not account for different particle numbers and in particular their morphology. To account for this, it is suggested to use a combination of adequate and pharmacopendial techniques, namely light obscuration (e.g., Ph.Eur. monograph 2.9.19[28]) followed by MFI in combination with visual particle assessment (e.g., Ph.Eur monograph 2.9.20[28]), as shown in this study.

An indirect evaluation of glass vial delamination is achieved by pH measurement and ICP analysis within this study, as also proposed by Guadagnino and Zuccato.[18] The solution pH was investigated to provide information about the underlying corrosion mechanism, while the absolute concentrations of glass elements such as Si, B, and Al were determined by ICP providing information about the extent of dissolution of the glass matrix. The study showed that pH measurement was not suitable when using buffered stress solutions. ICP on the other hand was suitable for all test protocols. However, we suggest aligning on either using oxide or pure elemental concentrations for data reporting as well as calculation of boron ratios only but not aluminum ratios due to the low concentrations of the latter. In general, glass leachables are typically not a general concern and do not necessarily relate to delamination as highlighted in this study. Thus, further confirmation is needed to assess for glass delamination as described in the following section.

Besides methods for particle analysis and for investigations on glass leachables in solution, solid surface characterization methods were evaluated in the present study. The colorimetric test as proposed by Schmid and Zuccato[26] and SEM analysis as performed within the study are useful tools for obtaining information on the inner surface characteristics and morphology of the vials. The colorimetric test is a method which could be used immediately after glass vial production as a destructive test, in order to evaluate a properly controlled thermal process or an early alteration of the inner glass surface. Conventional SEM analysis allows the determination of morphological surface changes at the microscale and even nanoscale level. However, surface morphology alone may be misleading. “Pitting” for example is a known surface characteristic that can be easily detected by SEM analysis[14] yet it does not necessarily relate to delamination. Surface alterations in form of pits can already occur during glass manufacturing or vial depyrogenation or during subsequent storage with the formulation. It is therefore not justified to assume that pitting alone is an indication for glass delamination. Subject of ongoing research is the assessment to link changes in surface morphology, for example, depending on the drug product manufacturing process and glass delamination. The use of SEM to characterize the cross section of the inner surface of a vial would allow visualization of reaction zones or actual delaminated structures giving proof of delamination even if glass flakes cannot be observed as suggested but only recently disclosed in literature.[29] Alternatively, elemental concentrations of the inner surface can be determined at a nanoscale level by D-SIMS especially for the delamination zone at the bottom-near area of the vials. The present study showed that D-SIMS is a powerful method and finally confirmed the change in surface composition after stress testing for Expansion 33 but not for Expansion 51 vials. This is to highlight as pH and ICP methods suggested differently.

In summary, most current publications either discuss particle analysis[3] or surface characterization techniques[36,37] alone when assessing glass delamination. To avoid misinterpretation, we conclude that it is highly recommended to use analytical methods covering the characterization of glass surfaces, determination of glass leachables, and identification of visible and SVPs in solution using adequate methodology. Our study showed that a minimum of well-chosen analytical techniques such as D-SIMS, ICP, and particle assessment including SEM-EDS is sufficient when assessing glass delamination.

Conclusion

Five vial types were investigated for the purpose of assessing their delamination propensity. The 5 vial types were exposed to different established test protocols to force delamination as well as according to USP <1660>. Interestingly, most glass particles identified were in the subvisible size range. Hydrophilic or hydrophobic surface modification of the inner surface of glass vials increase their hydrolytic resistance and thus decrease their propensity for glass delamination. The study showed that covalently bound $Si_aO_bC_cH_d$ layer (TopLyo™) was most protective against delamination compared to Type I plus® (SiO_2-layer) vials and baked-on Siliconized vials, where the coating was partly dissolved. Uncoated vials, in the contrary, showed ion leaching (ICP), which together with results from other analytical techniques were an early indication for a change in chemical composition of the glass surface and increase the propensity to delaminate. However, inhomogeneous change in glass composition upon stress testing was only confirmed by D-SIMS analysis for the Expansion 33 vials and not for Expansion 51 vials. This makes it evident that the assessment of glass surfaces and leachables itself are not sufficient to study delamination. The applied test protocols differ in test solution, handling, time expenses, and recommended analytical techniques. Filling volumes should be adapted to cover both potential delamination zones (bottom and shoulder) when forcing delamination for any of the protocols. Test protocol 2 is considered to be the most aggressive one with respect to the underlying mechanisms (ion exchange and glass network attack) and the applied stress test conditions (autoclaving time and temperature). It is subject of ongoing research to confirm how these results of the different test protocols will translate into long-term storage data from parenteral drug products since time and storage conditions and composition contribute to the delamination potential.

The criticality and concern related to delamination of a given drug product relates to the findings of particulates in solution during long-term storage. Sole alternations in vial morphology or occurrence of glass leachables are mostly uncritical to overall drug product quality. However, previous studies focus on either SVP identification, solution analysis, or solid surface characterization separately. This can result in misleading interpretations when assessing the delamination propensity of each glass vial. Thus, a broad panel of conventional and advanced techniques was performed and compared in this study, covering (visible and subvisible) particle assessment, solution analysis, and glass surface characterization. It is highly recommended to perform a minimum of well-chosen analytical techniques from these 3 fields of analysis when assessing glass delamination.

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