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RESEARCH

Mechanistic Studies of Glass Vial Breakage for Frozen Formulations. II. Vial Breakage Caused by Amorphous Protein Formulations

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ABSTRACT: In an accompanying article we have described parameters that influence vial breakage in freeze-thaw operations when using crystalizable mannitol formulations, and further provided a practical approach to minimize the breakage in manufacturing settings. Using two diagnostic tools—thermal mechanical analysis (TMA) and strain gage, we investigated the mechanism of mannitol vial breakage and concluded that the breakage is related to sudden volume expansions in the frozen plug due to crystallization events. Glass vial breakage has also been observed with a number of frozen protein formulations consisting of only amorphous ingredients. Therefore, in this study, we applied the methodologies and learnings from the prior investigation to further explore the mechanism of vial breakage during freeze-thaw of amorphous protein products. It was found that temperature is a critical factor, as breakage typically occurred when the products were frozen to –70 °C, while freezing only to –30 °C resulted in negligible breakage. When freezing to –70 °C, increased protein concentration and higher fill volume induced more vial breakage, and the breakage occurred mostly during freezing. In contrast to the previous findings for crystallizable formulations, an intermediate staging step at –30 °C did not reduce breakage for amorphous protein formulations, and even slightly increased the breakage rate. The TMA profiles revealed substantially higher thermal contraction of frozen protein formulations when freezing below –30 °C, as compared to glass. Such thermal contraction of frozen protein formulations caused inward deformation of glass and subsequent rapid movement of glass when the frozen plug separates from the vial. Increasing protein concentration caused more significant inward glass deformation, and therefore a higher level of potential energy was released during the separation between the glass and frozen formulation, causing higher breakage rates. The thermal expansion during thawing generated moderate positive strain on glass and explained the thaw breakage occasionally observed. The mechanism of vial breakage during freeze-thaw of amorphous protein formulations is different compared to crystallizable formulations, and accordingly requires different approaches to reduce vial breakage in manufacturing. Storing and shipping at no lower than –30 °C effectively prevents breakage of amorphous protein solutions. If lower temperature such as –70 °C is unavoidable, the risk of breakage can be reduced by lowering fill volume.

KEYWORDS: Glass vial breakage, Mannitol, Crystalizable excipient, Amorphous, Frozen protein formulation, Freeze-thaw.

INTRODUCTION

In biopharmaceutical industry, simple and stable formulations are often used for toxicology evaluations and early clinical studies in phase I–II before the completion of commercial formulation development. Frozen formulations are suitable for this purpose because of increased product stability of protein molecules in the frozen state.

Vial breakage can occur during freeze-thaw, shipping, and handling for frozen products in glass vials. Both crystalline and/or amorphous excipients can be utilized as stabilizers, cryoprotectants, or bulking agents in protein formulations. Previously, we reported our studies in identifying the root causes and prevention

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strategies to reduce the glass vial breakage for crystalizable excipient (1). This paper will focus on the mechanistic studies of vial breakage during freeze-thaw for protein formulations containing only amorphous ingredients, which has not been reported in the literature before and is not well understood.

A series of experiments was performed to identify key factors influencing vial integrity and to find preventive approaches to minimize the breakage. Effort was made to diagnose the root cause of breakage through freeze-thaw experiments and mechanistic studies employing thermal mechanical analysis (TMA) and strain gage techniques. A novel mini-bag method for TMA enabled determination of thermal contraction/expansion for liquid protein formulations during the entire freeze-thaw process. A bi-axial strain gage mounted to the vial directly measured the deformation of the glass during freeze-thaw of protein products. Studies were performed to determine the strain on glass wall with varying protein concentrations, fill configurations, and freeze-thaw conditions. The vial breakage mechanism of amorphous protein formulations was found to be distinct from mannitol formulations. An understanding of the mechanism allows preventive approaches to be defined to reduce glass vial breakage for amorphous frozen protein formulations.

MATERIAL AND METHODS

Materials

Four Amgen protein products, including three antibodies (A, B, C) of ~150 kDa molecular weight and a smaller recombinant protein (D) of ~18 kDa molecular weight, were used in this study. A broad concentration range of these products was achieved by ultrafiltration or dilution of the bulk drug substance with formulation buffer containing only amorphous excipients. Packaging materials including the Type I tubing glass vials (5 cc, 10 cc and 20 cc), stopper, and seals were the same as reported in the mannitol study (1). Plastic vial trays were obtained from Amgen clinical manufacturing. The same equipment and settings of TMA and strain gage were employed in this study compared to the previous mannitol investigation (1).

Methods

All glass vials were washed and depyrogenated prior to the freeze-thaw experiments. Amorphous protein formulations were filtered through 0.22-μm PVDF filters and were filled into vials at 18–80% fill ratios, that is, fill volume to nominal vial capacity. Freeze-thaw conditions included direct freezing to either –30 °C or –70 °C and thawing at room temperature (RT). In freeze-thaw experiments with an intermediate staging step, the vials were first frozen at –30 °C before being further cooled to –70 °C, followed by storage and thawing at room temperature. Thirty to 100 vials were filled for each freeze-thaw experiment. The vials were placed on plastic trays to mimic manufacturing conditions. Vial integrity was visually checked after the freezing step as well as after thawing.

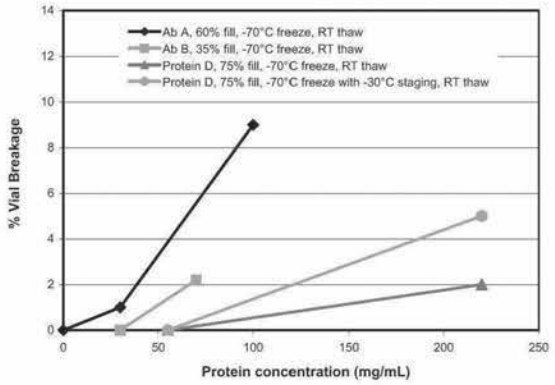

Figure 1

Impact of protein concentrations on vial breakage. With different proteins, fill levels, and thermal histories, vial breakage always increases as protein concentration increases.

The methods used in statistical analysis for vial breakage data and the techniques of TMA and strain gage are described in the accompanying paper (1).

RESULTS AND DISCUSSION

Identification of Key Parameters in Freeze-thaw Studies

Effect of Protein Concentration

In laboratory-scale freeze-thaw studies of amorphous protein formulations, higher vial breakage rate was found to be correlated with increasing protein concentrations, as shown in Figure 1. This occurred for both the antibodies and the smaller recombinant protein, although at any given concentration, more breakage was observed with the antibodies. In the manufacturing setting, concentration dependence was also evident. For example, product B at 70 mg/mL gave rise to

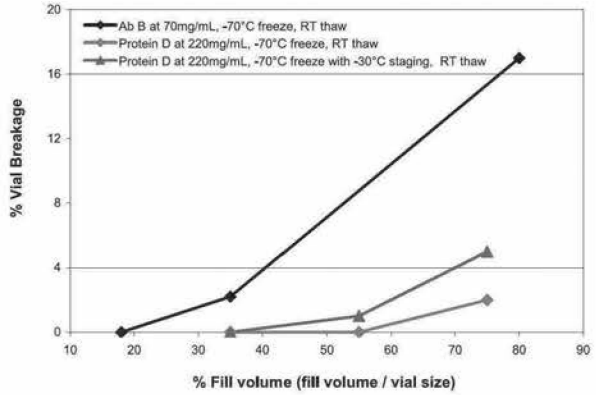

Figure 2

Impact of percent fill volume on vial breakage. With different proteins and thermal histories, vial breakage always increases as percent fill volume increases.

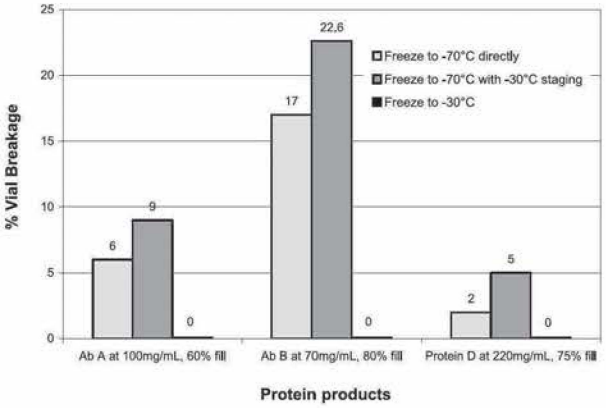

Figure 3

Impact of freeze-thaw condition on percent vial breakage.

a high breakage up to 50%, as opposed to the same molecule at 30 mg/mL in an identical formulation, which caused negligible breakage under same handling conditions.

Development of high concentration protein products has been a recent trend for therapeutic proteins requiring high dose and frequent or chronic administration, such as monoclonal antibodies (2). The increased vial breakage associated with higher protein concentration can be a concern and challenge for formulation development and fill-finish process.

Effect of Fill Volume

A strong correlation between vial breakage and fill volume percentage was observed with protein formulations (Figure 2). For example, the 70 mg/mL formulation of antibody B caused 0%, 2.2%, and 17% vial breakage at 18%, 35%, and 80% fill ratio, respectively. The vials were more susceptible to breakage with antibody B compared to recombinant protein D, even though the latter was formulated at a higher concentration, which could be associated with either the protein molecules or different formulation excipients. For instance, the glass transition temperature (T_g') of a frozen liquid formulation is affected by the protein and the excipient compositions. Considering that product B induced more breakage than other products, it was investigated in more detail as a model formulation in the subsequent TMA and strain studies.

Effect of Freeze-thaw Conditions

In contrast to the mannitol paper (1), where staging was found to minimize vial breakage, introduction of such a stage did not reduce breakage for amorphous protein formulations (Figure 3). Freezing to –70 °C (with and without an intermediate –30 °C staging step) led to significant vial breakage. No freezing or thawing breakage was found when the products were frozen to –30 °C only. The –30 °C staging did not help to reduce the breakage if the protein product was further cooled to –70 °C. In fact, such an intermediate holding condition even caused slightly higher breakage. No protein vials broke during –30 °C staging and breakage occurred after further freezing to –70 °C, typically with a crack appearing on the sidewall (Figure 4). This

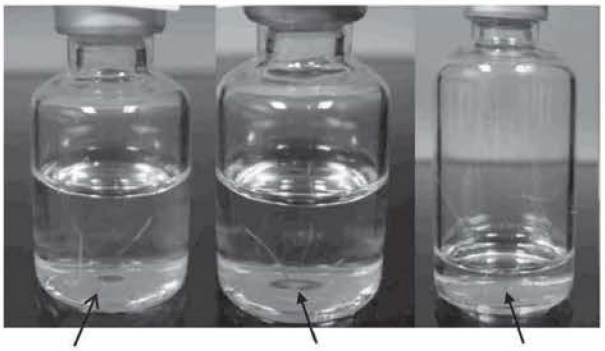

Figure 4

Typical vial breakage pattern during freeze-thaw of amorphous protein formulations (Product A–C from left to right, arrows pointing to the centers of the cracks).

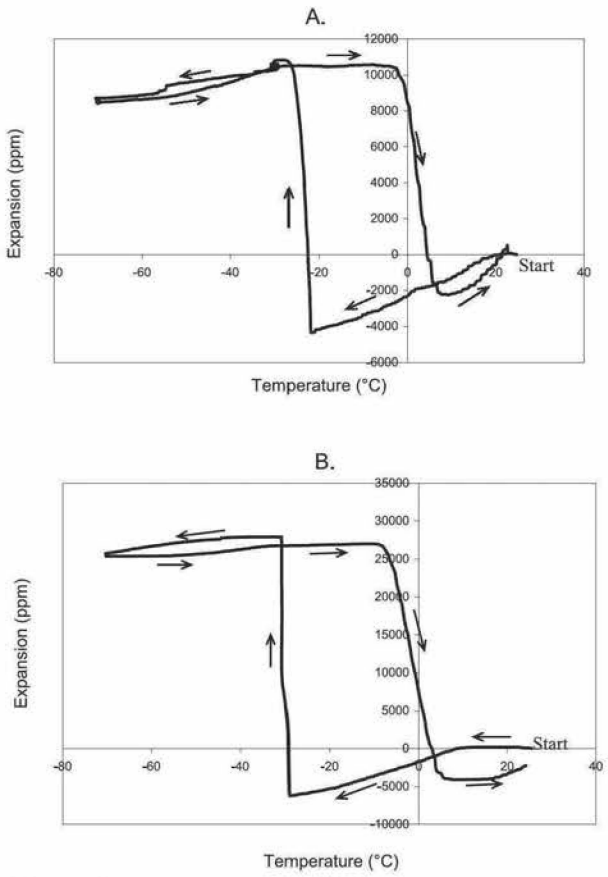

Figure 5

TMA profiles of antibody B products at 70 mg/mL (panel A) and 30 mg/mL (panel B).

breakage pattern is different from the breakage observed from mannitol formulations, where the vial bottom typically separates from the vial. Only few breakages of protein product vials occurred during thawing. The different vial breakage rates for mannitol solutions versus amorphous protein products in response to the –30 °C staging and the different vial break patterns further suggest distinctive mechanism of vial breakage involved for crystallizable and amorphous formulations. In practice, freezing to only –30 °C was found an optimal condition to avoid vial breakage for amorphous protein products.

Mechanistic Studies

Thermal Mechanical Analysis (TMA)

The mini-bag method (1) was also used to determine the volume change of protein formulations during freeze-thaw. Figures 5A and 5B depict the thermal mechanical behavior of antibody product B at two concentrations, 70 mg/mL and 30 mg/mL, respectively. During freezing, the liquids became denser before transitioning into solid phase, where significant volume expansion arises due to lower density of ice as compared to water. Solidification occurred between –20 to –30 °C. After being completely frozen, the solid displayed thermal contraction from –30 to –70 °C with more apparent segment between –50 and –70 °C. Upon warming, the process was reversed; the thermal expansion took place from –70 to –30 °C followed by a plateau and then thawing. Between –70 and –30 °C, the coefficient of thermal expansion of protein formulations were estimated to be around 60 ppm/°C, more than one order of magnitude higher than that of glass (3.3 to 5.5 ppm/°C) (3). As discussed previously, vial breakage was not observed when freezing to –30 °C, most breakage occurred after further cooling to –70 °C. Therefore, breakage can be correlated to thermal contraction of protein formulation between –30 and –70 °C. Due to adhesion between the frozen plug and glass vial, such contraction would pull the wall of glass vials inward, storing significant potential energy in the body of the vial. This hypothesis was further confirmed in the later strain studies. The plateau region suggested that no significant thermal contraction or expansion took place in solid phase if the product is frozen to only –30 °C, which agrees well with the zero breakage in Figure 3. The volume expansion associated with liquid-to-ice phase transition was over two times higher in the 30 mg/mL formulation than in the 70 mg/mL formulation (34100 ppm vs. 15100 ppm, Figures 5A and 5B), but the vial breakage of the 70 mg/mL solution was significantly higher than that of the 30 mg/mL solution (Figure 1). Therefore, the freezing expansion has little influence on protein vial breakage because at this stage the product is semi-frozen and the expansion is accommodated by an upward movement of the remaining liquid, causing relative low strain on the glass vial.

Strain Gage Measurements

When external forces are applied to a stationary object, stress and strain are the result. Stress is defined as the object's internal resisting forces, and strain is defined as the displacement or deformation, calculated as the length of deformation divided by the original length (4). Bi-axial strain gages (1) were utilized to investigate the mechanism of breakage caused by amorphous protein formulations.

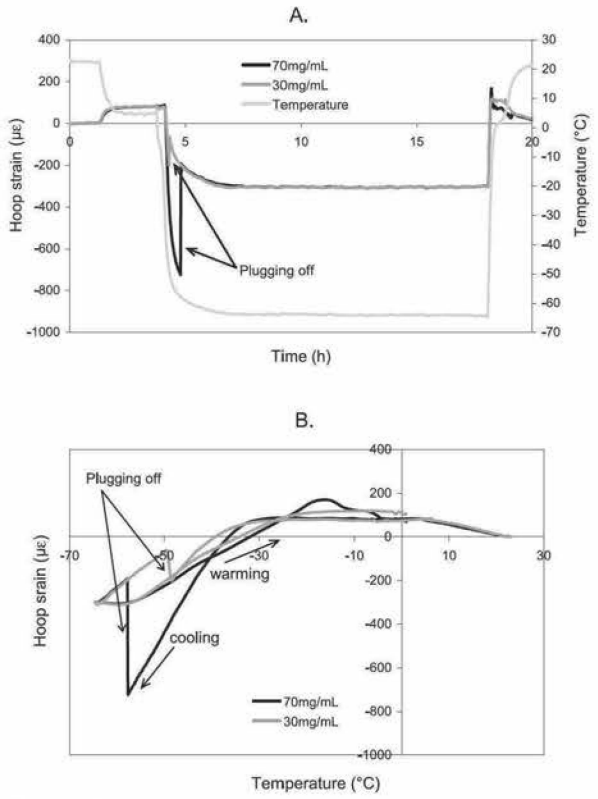

Figure 6

Strain profiles of 30 mg/mL and 70 mg/mL antibody B products during freeze-thaw cycle to –70 °C (3.5 mL in 10-cc vials, RT → 2–8 °C → –70 °C → 25 °C): (A) strain in time course; (B) strain as a function of product temperature.

We have reported (1) that vial breakage occurred due to elongation strain induced by partial crystallization of mannitol during freezing and "secondary" crystallization of the remaining fraction during thawing. In comparison with mannitol, the amorphous protein formulations did not have any crystalline excipient and displayed completely different strain profiles. For example, 3.5 mL of antibody B formulations at two concentrations, 30 mg/mL and 70 mg/mL, were filled into 10-cc vials with a strain gage attached and cooled to 2–8 °C from room temperature, then frozen at –70 °C followed by thawing at room temperature. Figure 6A displays the strain and temperature in a time course, and Figure 6B shows the strain as a function of product temperature at different concentrations. As the vials were transferred to –70 °C and product temperature started to decrease, an increase in compressive strain was found. This correlates with the TMA data that showed thermal contraction of frozen liquids below –30 °C. The increase of negative strain was due to the compression force imposed by contraction of frozen protein solution. Soon after the products reached –50 to –60 °C, the negative strain suddenly bounced back with strain change amplitude at 510 με and 130 με, for 70 mg/mL and 30 mg/mL, respectively. Such phenomena indicate an abrupt separation ("plugging off") between the solidified plug and the inner surface of the vial, so the contraction force was suddenly relieved. The glass wall of the vial could be pictured as a very stiff spring held in compression by frozen plug. When the plug breaks loose, the release of stored energy causes the vial wall to rebound and move rapidly, even beyond its at-rest dimensions. During such rapid movement the integrity of glass can be easily disrupted, particularly at low temperature due to increased stiffness. The plugging off was a result of drastic difference between the coefficient of thermal expansion of the frozen protein formulation and the glass. Figures 6A and 6B demonstrate the comparison between the high and low concentrations. The 70 mg/mL vial underwent much higher negative strain before the frozen plug separated from glass than did the 30 mg/mL vial. As a result, much higher contraction potential energy was added to the glass wall of the 70 mg/mL vial. Such strain difference explains the concentration dependence of vial breakage described earlier. The higher compression strain associated with the higher concentration product could be related to higher viscosity, which held the frozen formulation as one unit (observed as a single, big, white plug) possessing stronger cohesive force. Frozen product at low concentration typically had fragments in the plug due to lower viscosity, and consequently, strain induced by small fragments was less significant. During thawing, the formulation expands upon warming and at approximately –20 °C, positive strain was found with the 70 mg/mL formulation, which may explain the occasional thaw breakage. Recent literature suggested that after the "plugging off" event, frost might fill the gap between plug and vial, resulting in high expansion strain upon thawing (5).

Figure 7 shows minimal strain of the above two concentrations of antibody B in vials freezing to only –30 °C. The TMA data in Figure 5 revealed negligible thermal expansion and/or contraction of the frozen product under this temperature condition. This is further confirmed by Figure 8, where the strain profile of the 10-cc vial filled with 3.5 mL of 70 mg/mL antibody B was found to be comparable to that of an empty 10-cc vial. Both TMA and strain gage findings confirmed that freezing amorphous protein formula-

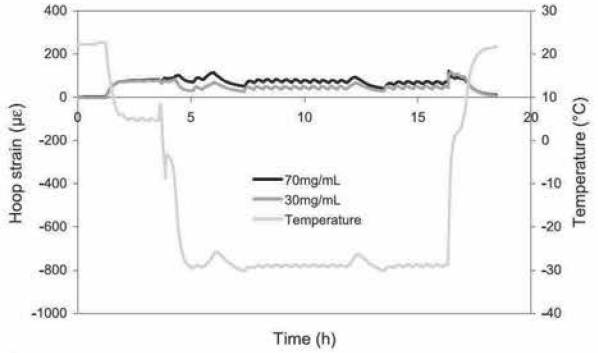

Figure 7

Strain profiles of 30 mg/mL and 70 mg/mL antibody B products during freeze-thaw cycle to –30 °C only (3.5 mL in 10-cc vials, RT → –30 °C → 25 °C).

tions only to –30 °C avoided excessive stress on glass vial.

The strain gage results also elucidated the vial cracking pattern on the side wall of frozen protein formulations. When product temperature decreased to below –30 °C, compression strain was more significant in the hoop direction than the axial direction (Figure 9A and 9B). When the negative strain was suddenly released during "plugging off", the abrupt strain change on the hoop direction (~560 με) was substantially higher than that on the axial direction (~360 με), suggesting more extensive circumferential inward deformation on the side wall before plugging off. This provides a good explanation for the vial breakage pattern with the cracking source on the vial side wall (Figure 4).

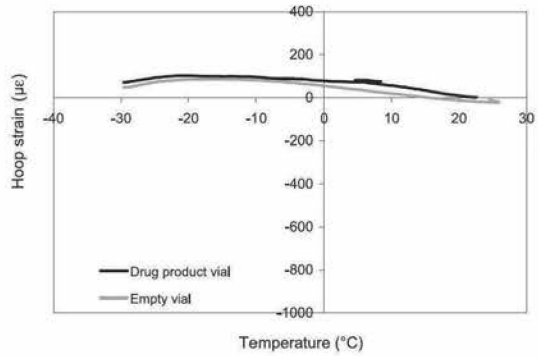

Figure 8

Comparison of strain profiles of product filled vial (70 mg/mL antibody B product, 3.5 mL in 10-cc vials) and an empty vial (10 cc) during freezing to –30 °C from room temperature.

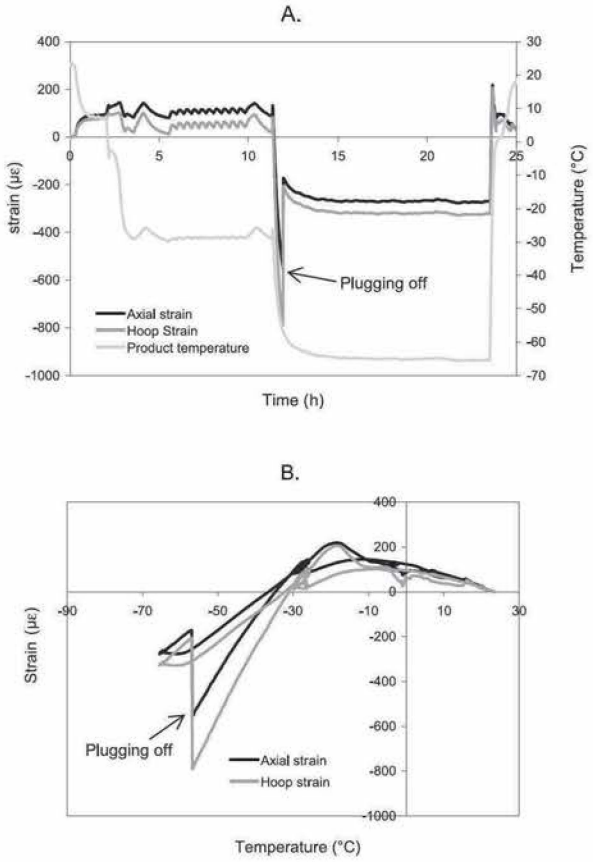

Figure 9

Axial versus hoop strain profiles of 70 mg/mL antibody B product during freeze-thaw (5.8 mL in 10-cc vials, RT → 2–8 °C → –30 °C staging → –70 °C → 25 °C): (A) strain in time course; (B) strain as a function of product temperature.

Previously in Figure 3, it was found that freezing to –70 °C with an intermediate –30 °C staging step gave rise to slightly more vial breakage than without the staging step. The strain gage data provided more evidence of this. Figure 10 represents the strain profiles with and without staging using a 10-cc vial filled with 5.8 mL antibody B at 70 mg/mL. This vial survived the direct freeze-thaw but broke during –30 to –70 °C cooling after the –30 °C staging step. Holding the vial at –30 °C prior to freezing to –70 °C produced a higher magnitude of compressive strain before the frozen plug separates from vial. Consequently, higher potential energy was then released and caused vial crack. The –30 °C staging step may enhance development of contact and adhesion between frozen formulation and glass. Therefore, staging of product at an intermediate temperature while freezing to –70 °C should be avoided.

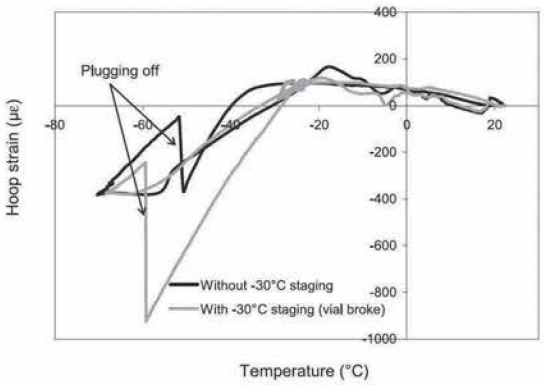

Figure 10

Difference in strain profiles of 70 mg/mL antibody B product during freeze-thaw with and without –30 °C staging (5.8 mL in 10-cc vial, RT → –70 °C with or without –30 °C staging → 25 °C).

It was also investigated whether the level of compression force and potential energy is site-specific on the side wall. In Figure 11, strain was determined at three positions relative to the fill line of the product solution, at a height corresponding to the top, middle, or bottom of the solution. This was achieved by fixing the strain gage location at a 3-mm height from the vial bottom and changing the formulation fill volume. Similar negative strain minima (~–950 με) and comparable strain changes were found at the various relative positions. Such information suggests that the amplitude of strain is relatively uniform across the glass surface contacting the plug.

Strain change during plugging off is an abrupt, transient event. In our setting of strain measurements, the strain data is collected at 1-min intervals. Therefore, the magnitude of the compressive strain minima could be underestimated. In the future, data acquisition at a higher frequency, for example every second, will allow us better accuracy in observing the event and capturing the details.

Preventive Approaches To Minimize Vial Breakage

Freezing to only –30 °C was found to reduce vial breakage. Nevertheless, this condition may not be suitable for product needs, or may be difficult to implement. For example, the vials for reference standard in stability testing are typically stored at –70 °C. Some clinical sites receiving the products only have –70 °C freezers. Additionally, shipping frozen products usually utilizes dry ice at approximately –70 °C. Under these constraints where –70 °C storage is inevitable, it was necessary to identify preventive approaches to minimize vial breakage.

Since "plugging-off" was found to be the root cause for vial breakage caused by amorphous protein formulations, our initial attempt was to reduce the fill volume in order to avoid the separation between glass and frozen formulation. However, the elimination of "plugging off" was found impractical at production scale where each vial is surrounded by other vials, which creates thermal mass and non-uniform cooling/heating. Figures 12A and 12B depict the strain profiles during freeze-thaw of 10-cc vials in duplicates with and without surrounding thermal mass. All four vials contained 2.0 mL antibody product C (100 mg/mL) and underwent the same freeze-thaw cycle. It was noted when the vials stood alone without surrounding vials (Figure 12A), the plugging off of frozen formulation did not occur. However, when thermal mass existed such as in production scale, the plugging off can still occur in some vials because of non-uniform heat transfer, as seen in Figure 12B. Therefore, a preventive approach was identified below based on projecting appropriate fill volumes to minimize risk of vial breakage. This is estimated from calculating the energy involved in separation between glass and frozen formulation at different fill volumes.

Because amplitude of strain is relatively uniform across the glass surface contacting the frozen plug, it

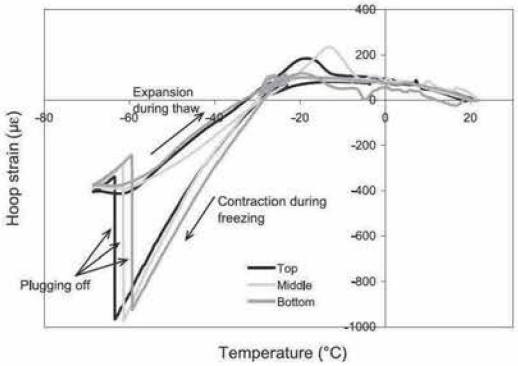

Figure 11

Comparison of strain profiles at different positions from the fill line of 10-cc vials filled with 70 mg/mL antibody B product during freeze-thaw (RT → 2–8 °C → –30 °C staging → –70 °C → 25 °C). Three relative positions were examined: close to the top of the solution, in the middle of the solution, and close to the bottom of the solution.

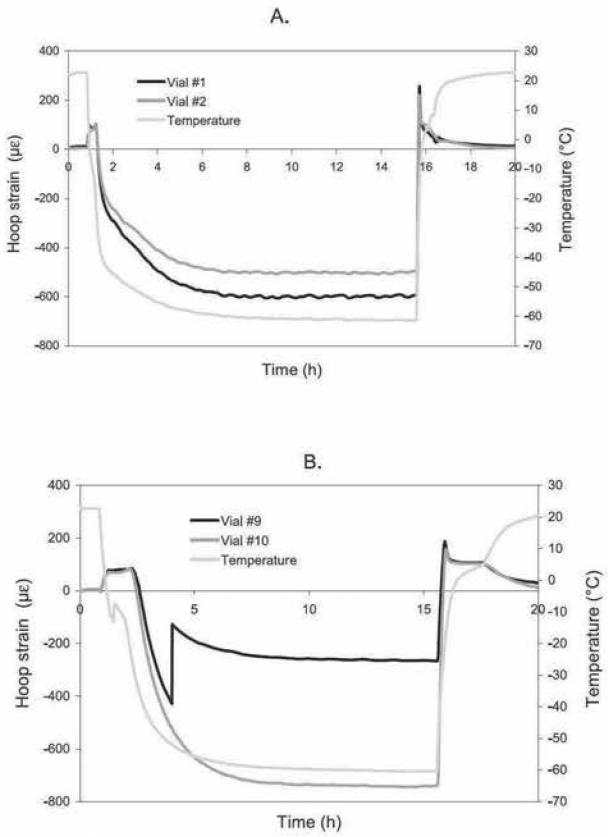

Figure 12

Strain profiles of 100 mg/mL antibody C product vials (3.6 mL in 20-cc vials) during freeze-thaw (RT → –70 °C → RT). (A) two stand-alone vials (#1 & #2); (B) two vials (#9 & #10) in thermal mass, i.e., surrounded by placebo vials.

can be considered that the contraction stress is identical throughout the contact surface between formulation and glass. In this case, the estimation of potential energy is simplified using Hook's law ($E = 1/2\ kX^2$). X is the displacement or strain in this case, and k is proportional to the surface area under strain. The surface under strain includes the part of vial wall in contact with the product and the vial bottom. As fill volume decreases, the contact surface area reduces and, consequently, results in a lower k, lower potential energy, and lower vial breakage. Using this principle, the appropriate fill volume can be estimated.

The lowest fill volume at which unacceptable rate of breakage occurs is derived from freeze-thaw experiments at discrete fill volumes. The potential energy corresponding to that configuration was designated as $E_{breakage}$. For example, as observed in Figure 2, 54% fill ratio of antibody B (70 mg/mL) caused unacceptable rate of breakage at approximately 8%. So the potential energy associated with 54% fill was assigned as $E_{breakage}$. As calculated in Table I, lowering fill fractions reduced the potential energy to 80.5%, 66.7%, and 52.8% of $E_{breakage}$, allowing higher likelihood to retain vial integrity. Estimation of optimal fill volume for a particular product should be made according to a risk assessment, taking into account the practicality of various product dosing options.

In the long run, as alternative to glass, other containers possessing better elasticity, such as C-Z plastic resin vials, may better accommodate thermal contraction of frozen formulations (6). Additionally, certain surface modification of glass vials such as PICVD coating (7) may change interaction between the glass and protein formulation. Also, vials with greater strength may be found from different vendors. It could be worth investigating these container products.

SUMMARY AND CONCLUSIONS

Key parameters and mechanistic insights into vial breakage during freeze-thaw operations of amorphous protein formulations are described in this study.

TABLE I
Theoretical Assessment for Fill Volume To Reduce Vial Breakage Using 20-cc Vial as an Example (vial internal diameter = 26.35 mm).

Fill Configuration	Height of Solution (cm)	Bottom Surface Area (cm^2)	Area of Sidewall in Contact (cm^2)	Total Surface Area in Contact (cm^2)	Potential Energy
54% fill (10.8 mL)	1.981	5.450	16.388	21.838	$E_{breakage}$
40% fill (8.0 mL)	1.468	5.450	12.146	17.596	80.5% $E_{breakage}$
30% fill (6.0 mL)	1.101	5.450	9.110	14.560	66.7% $E_{breakage}$
20% fill (4.0 mL)	0.734	5.450	6.073	11.523	52.8% $E_{breakage}$

Higher rate of vial breakage is associated with higher protein concentration and increase in fill volume. Freeze-thaw temperature conditions have great impact on vial integrity. Vial breakage of protein products was found during –70 °C freeze-thaw but negligible during –30 °C freeze-thaw. TMA and strain gage results revealed that primary cause for vial breakage during freezing was the significant thermal contraction of protein formulation when cooled to below –30 °C, causing inward deformation of glass adhering to the frozen plug and subsequent rapid movement of glass when the sidewall separated from frozen plug ("plugging off"). Conversely, thermal expansion during thawing resulted in positive strains and explained the occasional breakage during thawing. Based on these observations, recommendations were developed to minimize vial breakage with frozen amorphous protein formulations. Where practicable, freezing to –30 °C only is recommended to avoid vial breakage during freeze-thaw. In the circumstances where –70 °C storage is unavoidable, there will be a risk of glass vial breakage, as the thermal contraction is an inherent property of the frozen formulations. It is recommended to reduce fill volume based on estimating the level of potential energy using breakage data from freeze-thaw experiments.

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References

1. Jiang, G.; Akers, M.; Jain, M.; Guo, J.; Distler, A.; Swift, R.; Wadhwa, M. S.; Jameel, F.; Patro, S.; Freund E. Mechanistic studies of glass vial breakage for frozen formulations. I. Vial breakage caused by crystalizable excipient mannitol. *PDA J. Pharm. Sci. Tech.* **2007,** 441–451.

2. Shire, S. J.; Shahrokh, Z.; Liu, J. Challenges in the development of high protein concentration formulations. *J. Pharm. Sci.* **2004,** *93,* 1390–1402.

3. Williams, N. A.; Guglielmo, J. Thermal mechanical analysis of frozen solutions of mannitol and some related stereoisomers: evidence of expansion during warming and correlation with vial breakage during lyophilization. *J. Parenter. Sci. Technol.* **1993,** *47,* 119–123.

4. Strain Gage Workshop Course Manual, W65, Vishay Measurements Group, Inc, Raleigh, NC (2004).

5. Craig, G. D.; Moreno, M.; Mishra, D.; Milton, N.; Roy, M.; Yu, L. Use of the Strain Gage To Study the Dynamics of Stress Generation in Vials during Freeze-Drying. Breckenridge Protein Stability Conference, Breckenridge, CO, July 28–31, 2004.

6. Bouma, M.; Nuijen, B.; Harms, R.; Rice, J. R.; Nowotnik, D. P.; Stewart, D. R.; Jansen, B. A.; van Zutphen, S.; Reedijk, J.; van Steenbergen, M. J.; Talsma, H.; Bult, A.; Beijnen J. H. Pharmaceutical development of a parenteral lyophilized formulation of the investigational polymer-conjugated platinum anticancer agent AP 5280. *Drug Dev. Ind. Pharm.* **2003,** *29,* 981–995.

7. Kuhr, M.; Bauer, S.; Rothhaar, U.; Wolff, D. Coatings on plastics with the PICVD technology. *Thin Solid Films* **2003,** *442,* 107–116.

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