Preservation by Foam Formulation

An Alternative to Freeze-Drying

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AUTHOR

Preservation by foam formation is thus permitting the delivery of products that currently cannot be delivered by conventional methods.

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Market projections indicate that the delivery of proteins and vaccines by inhalation and oral formulation will become increasingly important during the next 5–10 years. Significant research is being conducted to develop noninjection delivery mechanisms for biopharmaceuticals and vaccines, including pulmonary (inhalation), nasal, transdermal, and oral alternatives. To be effective, these delivery mechanisms will require better stabilization of biological drugs than is currently available so that they can maintain their potency and effectiveness at ambient temperatures for long periods of time.

Biologicals must be in a dry state to ensure long-term stabilization at ambient temperatures. Stabilization of biologicals in a liquid state is possible only for limited periods of time because of degradation resulting from molecular movement. To achieve long-term stabilization of sensitive biologicals, molecular mobility must be arrested to stop the degradation process during storage. This task can be achieved by vitrification, which is the transformation from a liquid into a highly immobile, noncrystalline, amorphous solid state, known as the glass state (1–3). In other words, to ensure long-term stabilization, specimens should be stored at a temperature below the liquid-to-glass transition temperature (T_g). For example, cryopreserved specimens typically are stored at −196 °C, which is lower than the −145 °C T_g value for pure water (4). The presence of water in a sample has a strong plasticizing effect, which decreases T_g and increases molecular mobility. Therefore, to preserve a specimen at ambient temperature, a strongly dehydrating drying method must be used. However, this method may also damage the sample.

Freeze-drying, or lyophilization, is the most common method used for the preservation of labile biological and pharmaceutical products in the dry state. This method involves freezing the materials as a first step. Then, during a primary stage, the pure ice crystals are sublimed under vacuum conditions from the partially frozen state at moderately low temperatures (generally between −50 and −20 °C). Afterward, secondary drying under vacuum is applied to remove water from the remaining material. At the end, the material looks like a dry cake.

The lyophilization process has a number of drawbacks. The major three drawbacks are as follows:

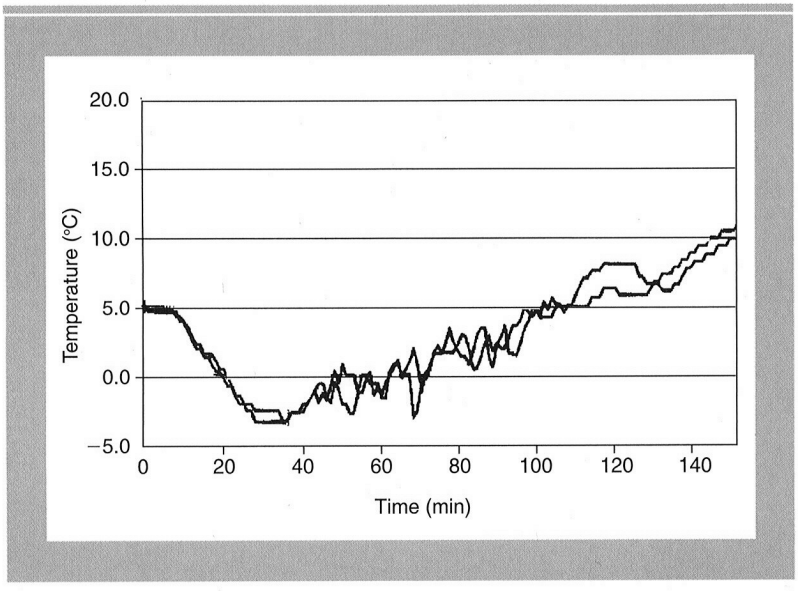

Figure 1: Typical dependencies of temperature inside the specimen over time during PFF primary drying. The initial volume of the specimen was 2 mL, and the vial volume was 20 mL.

- Freezing and subsequent equilibration at moderately low temperatures damage labile biologicals.
- The process takes several days.
- Even if successful, the lyophilized materials often still must be stored at refrigerated temperatures to ensure stability.

In addition, the freeze-drying process is not suitable for bulk aseptic production.

By comparison, preservation by foam formation (PFF) does not have the drawbacks of lyophilization. During PFF, the biological solutions or suspensions are first transformed into mechanically stable, dry foams by boiling them under vacuum at ambient temperatures above the freezing point (primary drying). Second, the samples are subjected to stability drying at elevated temperatures to increase the glass-transition temperature. Survival or activity yield after rehydration of preserved samples is achieved by proper selection of protectors (i.e., sugars) that are dissolved in the suspension before PFF and by proper selection of the vacuum and temperature protocol during PFF.

Freeze-drying damages cells and other labile biologicals. Strong freeze-drying–induced injury occurs during both freezing (i.e., the formation of ice crystals) and during the subsequent equilibration of the frozen specimens at moderately low temperatures during ice sublimation. Well-known factors that cause cell damage during freezing include freeze-induced dehydration, mechanical damage of cells during ice crystallization and recrystallization, phase transformation in cell membranes, and increasing electrolyte concentration. However, the main physicochemical process that destroys frozen biologicals is most likely the abnormally large pH change in the liquid that remains unfrozen between ice crystals. This abnormal pH change, which can be as large as 5 units (i.e., pH >12), is associated with crystallization hydrolysis (5).

Crystallization hydrolysis occurs because ice crystals capture positive and negative ions differently. This creates a huge ($\sim 10^7$ V/m) electric field inside the ice crystals. Neutralization of this field occurs by electrolysis inside the ice crystals (with a rate proportional to the constant of water molecule dissociation in the ice), thereby resulting in a change of the pH of the liquid that remains unfrozen between the ice crystals.

The effect of freeze-induced damage could be decreased if biologicals were frozen in a concentrated solution of protectors (i.e., sugars) and then dried. However, conventional freeze-drying cannot be performed in this manner because the "cake" formed after such conditions of drying would collapse at ambient temperatures, thereby causing structural damage so that normal reconstitution would be impossible.

Freeze-drying legacy

For more than 50 years, freeze-drying has been a dominant method for the preservation of labile biologicals. The popularity of freeze-drying is most likely a result of the conventional belief that drying at lower temperatures should cause less damage and because there has been no known alternative.

The conventional belief about lower-temperature drying being less damaging is simply not accurate for the case in which ice crystals form in the specimen. However, the fact that there was no known alternative to freeze-drying is accurate. No scalable industrial technology has been developed that can preserve labile biologicals at ambient temperatures with high survival yield.

Preservation of biological materials at ambient temperatures

At the beginning of the past century, many scientists performed studies that compared the stabilizing effects of evaporation from the liquid state with that of freeze-drying. They reported, and it has been recently confirmed, that the stability of biologicals dried by evaporative drying of small drops is comparable with, and in many cases even better than, the stability of freeze-dried samples. For example, we were able to preserve labile enzymes (luciferase and isocitric dehydrogenase) by evaporative drying for more than a year at 50 °C without significant loss of activity during drying and subsequent storage at 50 °C (6, 7). Unfortunately, because dehydrated solutions containing protectors (i.e., sugars) become very viscous, too much time is required to evaporate water even from small drops of solution. Until now, for industrial applications, people have continued to use freeze-drying methods because evaporative drying is a diffusion-limited process and is not scalable.

History of foam formation preservation development

Approximately half a century ago, Annear (8) demonstrated that very viscous solutions and biological liquids (syrups) containing sugars or amino acids can be dried by foaming during vacuum application. Annear used this process to preserve several bacteria in the dry state. Unfortunately, he was able to execute this foaming process only in very small volumes. Because

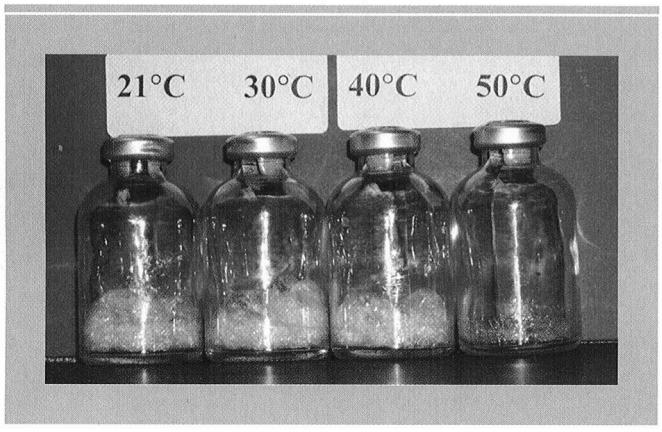

Figure 2: Appearance of the foam after 12 months of storage at 21, 30, 40, and 50 °C.

of that, he did not believe that this process could be used for industrial applications. Other scientists also avoided the foaming that usually occurred during freeze-drying at high shelf temperatures or during simple drying under vacuum because foaming was conventionally considered to be a damaging phenomenon for biological macromolecules. SpeedVac technology was invented and used extensively to avoid foaming during drying under vacuum.

In 1996, the idea that Annear's process could be used to scale up the drying process from the liquid state (desiccation) had crystallized. We were the first to report, against conventional belief, that biological macromolecules could be effectively stabilized by foam drying (9). At the same time, Roser and Gribbon (10) independently introduced an approach for stably incorporating biologicals in a dry foamed matrix .

The two methods differ in regard to how the syrup state is achieved. Our method initially vaporizes water by boiling to ensure scalability of the process. According to Dr. Roser (private communication), his method uses evaporative drying to obtain the syrup before foaming to avoid uncontrollable eruptions and spitting of the liquid to the wall of the vial. We believe that Dr. Roser's evaporative step limits scalability of the preservation process because of a strong increase of the evaporation time with increasing volume of the liquid to be dried, whereas the boiling method is more adaptable to the processing of large volumes.

We have demonstrated that PFF has been successfully applied to dry various volumes of biological liquids in 1-, 3-, 5-, 10-, 20-, 50-, 100-, 200-, 500-, 4000-, and 100,000-mL vials or containers (11–13). These results suggest that the volume of liquid to be dried must not be more than 10–20% of the container volume because the sample expands during formation of the foam.

Because boiling is a very intensive process, the time required for the PFF primary drying step is much less than that required for freeze-drying (typically several days) (14). For example, the time required for primary PFF drying of 2-mL specimens is ~2.5 h (see Figure 1). As described in Reference 12, the PFF primary drying time of 400-mL samples is only two times as long or ~ 5 h.

Therefore, the PFF process is a scalable, turbulent process. In addition, PFF better lends itself to aseptic processing because the vapor pressure above the specimen during PFF is much higher than that during freeze-drying, so less surface area exposure of product and less exposure time is required.

PFF has the potential to make freeze-drying (lyophilization) obsolete for many applications for the following reasons:

- It does not require freezing of samples before drying, and thus is more efficient, gentle, and less damaging.
- It is less time-consuming and more energy efficient.
- It is a more scalable process compared with freeze-drying, which is limited by the maximum allowable cake height.

Activity of biologicals after PFF preservation

Our experiences with applications for PFF have shown that this process allows effective high ambient-temperature stabilization of biologicals (8, 11–13, 15–19) with minimum loss of activity during drying and subsequent storage. The following biologicals have been stabilized with the use of PFF technology.

Labile enzymes and pharmaceuticals. PFF has been used to stabilize amphotericin, urokinase, luciferase, β-galactosidase, lactate dehydrogenase, isocitric dehydrogenase, ice nucleating protein, Taq DNA polymerase, and others at temperatures of 37 °C or higher. There was no significant loss of activity during drying and subsequent storage at a temperature below T_g achieved during drying. For example, Figure 2 shows enzyme activity of lactate dehydrogenase specimens preserved by PFF after 12 months of storage at 21, 30, 40, and 50 °C. The enzyme activity in the specimens stored at 21, 30, and 40 °C did not change during 12 months of storage and was insignificantly different from the control samples. However, activity decreased to ~60% of the original value for specimens stored above the T_g value at 50 °C. The T_g values of these specimens were measured after drying as the onset temperature of specific heat change during glass-to-liquid transformation. For these specimens, T_g was ~41 °C. Figure 2 also shows that foams in the specimens stored at 50 °C collapsed after a year of storage above the T_g value.

Live viruses from various taxonomic groups. The foaming technology can be used to stabilize herpesviridae (bovine rhinotracheitis), paramyxoviridae (bovine RSV, bovine parainfluenza, canine parainfluenza, canine distemper), flaviviridae (bovine viral diarrhea), parvoviridae (canine parvovirus), and retroviruses (MLV) at room temperature or higher temperatures without significant loss of activity.

Gram-negative and Gram-positive bacteria. PFF can be used to stabilize Gram-negative bacteria such as *Escherichia coli* and *Bordetella bronchiseptica*, and Gram-positive bacteria such as *Lactobacillus acidophulus* and *Lactococcus lactis* subsp. *cremoris* at temperatures of 37 °C or higher with 40% or less loss of viability in both log or stationary phases.

PFF drying equipment

No industrial-scale equipment is available for the PFF process to prepare either bulk production powders or market-ready vials of material. Researchers have demonstrated that, after some modification of controls and process-programming software, conventional freeze-dryers can be used to execute an effective PFF process in glass vials. These modifications are required to

execute "two dimensional" drying protocols, during which both vacuum and temperature are controlled simultaneously. We have been collaborating with VirTis Company on the design and development of controls and software for equipment to be modified. New models of VirTis equipment is capable of running both the freeze-drying and the PFF processes in vials or lyophilization trays.

Pharmaceutical, microbiological, and other industries are suffering from an absence of effective drying equipment that could aseptically produce bulk products that are stable at ambient temperatures. Such equipment potentially could be developed using the PFF process. An aseptic bulk PFF dryer is described in Reference 13. However, the design and development of equipment for aseptic bulk drying is still in an embryonic stage.

Directions for future development

PFF technology is new and so far has been used only for a few applications. Few scientists are experienced in PFF technology. The process still needs improvements to better serve potential users. For example, better drying protocols should be developed to eliminate noncontrollable eruptions and spitting of the material during boiling in glass vials. More work should be conducted to directly compare the positive qualities and drawbacks of freeze-drying, PFF, and other drying processes that are known or will be invented. We believe that our experience in using the PFF process as an alternative to freeze-drying will stimulate development of new processes and equipment for the preservation of labile biologicals in the dry state.

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