



Stability of Buffer Stored in Celsius-Paks

During and After Shipment on Dry Ice

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Sartorius Stedim Biotech is the world leader in controlled Freeze-Thaw technology

Executive Summary

Dry ice is the most common material used for maintaining frozen temperature of biopharmaceuticals during shipment. However, in some cases, carbon dioxide (CO₂) from sublimating dry ice can alter product stability or shelf life. Selecting a high gas barrier container that prevents CO₂ from contacting the product eliminates this problem. The gas barrier properties of the Celsius-Pak demonstrated a very limited transfer of CO₂ into the content of the Celsius-Pak. Following a three day storage in dry ice in Celsius-Paks, pH shifts of 0.16, 0.08 and 0.02 pH units were observed for 50 mM TrisHCl, 25 mM MOPS and 25 mM citrate buffer, respectively. Interestingly, allowing the frozen Celsius-Paks to degas in a conventional freezer provided a simple means of further limiting the pH variation.

Introduction

The pH shift of a low-buffered solution stored in screw-capped containers and shipped in dry ice is well known¹ and could result in loss of product stability². This pH variation is attributed to the formation of carbonic acid resulting from the dissolution of CO₂ entering through the bottle walls during shipment in solid carbon dioxide, or to a leakage through the cap, due to differential thermal expansion of sealing materials. Because of the high partial pressure of CO₂ resulting from the sublimation of dry ice, similar gas transfers should apply to material stored in the Celsius-Pak.

Key contributors to the pH variation were identified to be the buffer formulation (including buffer composition, concentration and pH), the gas barrier property of the S71 film (which the Celsius-Paks are made from), the gas barrier property of the optional over pouch, the time between shipment and thawing and the volume of solution in the Celsius-Pak. The objective of the following study was to analyze the impact of the above-interrelated variables on the pH stability of frozen buffer solutions stored in Celsius-Paks during and after storage in dry ice. The stability of three different buffered solutions stored in Celsius-Paks following exposure to dry ice, were tested at defined intervals by measuring the pH. In addition, the protective effect of very high gas barrier over pouches was tested. Finally, storage of buffer in screw-capped containers was undertaken for comparison.

Materials and Methods

A 50mM TrisHCl buffer (Tris), a 25 mM Citrate (Citrate) and a 25 mM MOPS buffer (MOPS) were prepared and the pH adjusted.

Filling of Celsius-Paks and bottles

Thirteen 100 mL Celsius-Paks per buffer were filled with 100 mL of each buffer using a sterile syringe. One sample per buffer was placed in a high gas barrier (multi layer aluminum foil) over pouch (OVP300, Sartorius Stedim Biotech) and sealed using an impulse sealer. One sample per buffer was placed in a food-grade, 3-ply polyallomer-polyethylene (PA-PE) plastic bag and sealed using a domestic hot blade welder. All the Celsius-Paks were placed in a -80°C freezer overnight, at which time they were completely frozen. The remaining buffer solutions were stored in the fridge at +4°C in a glass bottle.

Reproducing shipment in dry ice

Samples of each buffer were kept as controls at -80°C for varying periods. For each buffer, six other frozen unwrapped Celsius-Paks and two over wrapped Celsius-Paks (one aluminum foil and one PA-PE over pouches) were placed in two insulated shipping boxes (14in wide x 21in long x 14.5in tall) containing dry ice for 3 days. Following this storage, the day 0 samples were thawed, while the rest were transferred to the -80°C freezer and held there until being tested at defined intervals. For comparison, on day 0, a polypropylene (PP) 15mL centrifuge tubes were filled with 10 mL of each buffer and placed in dry ice for 24 hours.

Measurement schedule

As a measure of the amount of CO₂ that entered the buffers, the pH was measured after buffer preparation and also after 0, 1, 2, 3 and 10 days of frozen storage following the simulated shipment in dry ice. The results were compared with the samples stored at +4°C and at -80°C. Prior to pH measurement, frozen Paks were thawed at +45°C in a water bath and then left on the bench until the temperature dropped to 23°C. From each Celsius-Pak, three triplicate 5–8 mL samples were removed and placed in 15 mL centrifuge tubes. In addition, following the three-day storage in dry ice, one Celsius-Pak per buffer was vented with a syringe before being thawed. On the day of each measurement, the pH meter (Accumet Model 20) was calibrated using new reference solutions at pH 4, 7 and 10. The pH of each sample was measured when stable.

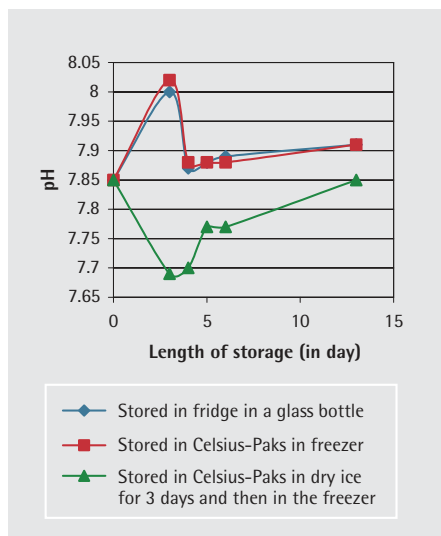


Figure 1: pH kinetics of 50 mM TrisHCl buffer stored in different conditions.

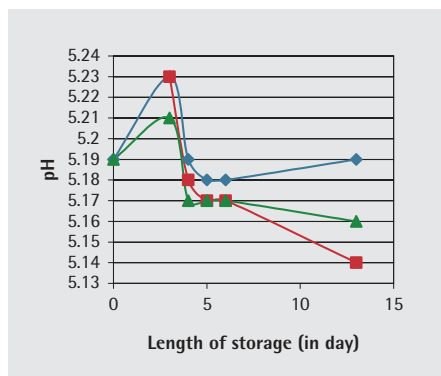


Figure 2: pH kinetics of 25 mM citrate buffer stored in different conditions.

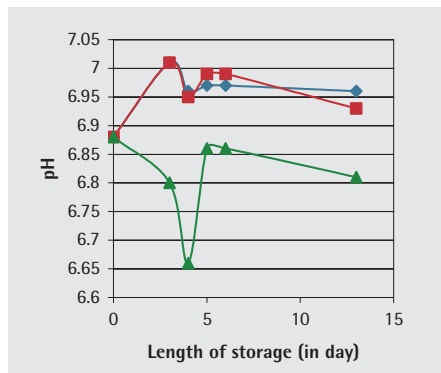


Figure 3: pH kinetics of 25 mM MOPS buffer stored in different conditions.

Results and Discussion

The TrisHCl buffer started at pH 7.85 and MOPS buffer at pH 6.88. After 3 days in a glass bottle at +4°C or in the freezer at -80°C, the pH value was 8.01 for the Tris buffer and 7.01 for the MOPS buffer. These shifts are independent of the materials of construction of the container and could be due to a variation in the standardization of the pH meter. Small pH shifts (0.16 pH compared to the initial value and 0.32 pH unit compared to the liquid stored Tris buffer and 0.08 pH compared to the initial value and 0.21 pH unit compared to the liquid stored MOPS buffer) were observed for the buffers stored in Celsius-Paks and exposed to dry ice. These drops were likely due to the carbon dioxide transfer through the Celsius-Pak film and dissolution in the thawed buffer solution. Short-term storage of frozen Celsius-Paks exposed to dry ice, in a freezer, had a positive impact on the pH as shown on figures 1 to 3. Two days in a -80°C freezer after three days in the dry ice allowed enough time to return to a pH value within the 1% of the original value or 3% of the value of the liquid stored buffer. The gas diffusion process is reversible and carbon dioxide diffused out of the frozen Celsius-Pak when exposed to the low CO₂ content of ambient air inside the storage freezer. Carbon dioxide solubilization in the frozen mass is negligible and cannot adversely impact product stability. Dry ice had little effect on the pH of the citrate buffer.

Figures 4 to 6 illustrate the limited pH drops observed for buffers stored in Celsius-Paks and exposed to dry ice. The barrier properties of the two over pouches are also illustrated in the above figures. Under the test conditions no significant difference in the barrier property is observed between the two types of materials (PA-PE and aluminum foil).

The above figures also show that venting a frozen Celsius-Pak following storage in dry ice provided a convenient means of limiting the pH shifts. The results obtained with this technique are comparable to the one obtained with over wrapped Celsius-Paks.

In addition, the gas barrier property of Celsius-Pak and PP centrifuge tube were compared. Although putting them in dry ice was outside the working range, no damage was observed after storage in dry ice.

Container	Celsius-Pak	Centrifuge tube
Storage conditions	3 days in dry ice	1 day in dry ice
pH shift for MOPS	0.08	1.14
pH shift for Tris	0.16	0.41
pH shift for Acetate	0.02	0.11

As shown in the table above, lower pH drops are observed for the buffers stored in Celsius-Paks and exposed to dry ice compared to centrifuge tubes, even with a three times higher contact time. The pH shifts are dependent on the composition of the buffer. As noted previously, a small variation was observed for the citrate buffer. The impact of dry ice was more pronounced for the TrisHCl and MOPS buffers. In all conditions, Celsius-Pak provided a significantly more efficient barrier to the intrusion of CO₂ than PP centrifuge tubes.

CO₂ permeability is a function of the surface area of the container. However, the impact of CO₂ permeation depends on the total volume of solution within the container. Therefore, the relevant parameter to extrapolate the pH shift results is the ratio between container surface area and contained volume. As the container surface-to-volume ratio of Celsius-Pak decreases with increasing container capacity, the results obtained with the 100 mL Celsius-Pak can be considered as a worst-case condition. This observation is valid for the range of recommended filling volumes of large Celsius-Paks. Therefore, the results obtained with the 100 mL Celsius-Paks can be safely extrapolated to larger Celsius-Pak sizes.

	When prepared
	In glass bottle, +4°C fridge
	In Celsius-Pak, -80°C freezer
	In Celsius-Pak, 3 days in dry ice
	In Celsius-Pak, 3 days in dry ice and vented
	In Celsius-Pak and PA-PE over pouch, 3 days in dry ice
	In Celsius-Pak and OVP300 over pouch, 3 days in dry ice

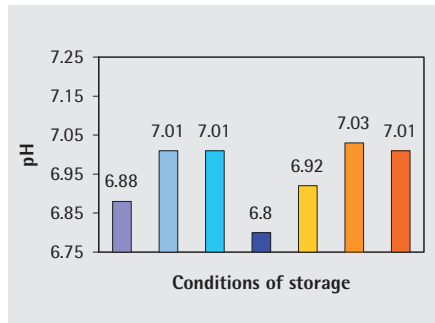


Figure 4: pH of 25mM MOPS buffer pH 6.88 stored 3 days in different conditions.

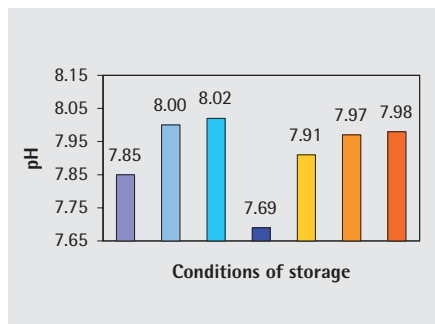


Figure 5: pH of 50mM TrisHCl buffer pH 7.85 stored 3 days in different conditions.

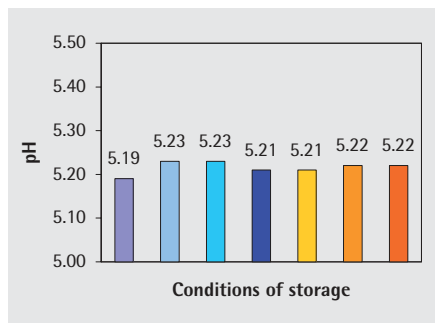


Figure 6: pH of 25mM Citrate buffer pH 5.19 stored 3 days in different conditions.

Conclusion

The drop in pH induced by transporting buffers stored in screw cap container in dry ice is well known and was reproduced in this study. The pH drop is dependent on the composition of the buffer. It is less pronounced for citrate buffer than for TrisHCl and MOPS buffers. The gas barrier properties of the Celsius-Pak ensure very limited pH shift following a three-day shipment in dry ice. pH variations of 0.16, 0.08 and 0.02 were observed for TrisHCl, MOPS and citrate buffer, respectively. Allowing frozen Celsius-Paks to degas in the storage freezer for two days can further reduce the pH shift. This duration was sufficient to return to pH value within the 1% of the original one.

Only in the most stringent situations when a pH variation lower than 0.1 unit is allowed, would over wrapping of Celsius-Paks be required. For these situations, the plastic PE-PA over pouch and the aluminum foil provides similar protections.

References

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