Trypsin stability study supports gel ice shipping conditions

Abstract

In order to mitigate the risk of pH shift and corresponding color shift caused by shipping on dry ice, we investigated the feasibility of shipping our Gibco® trypsin products on gel ice. From a thorough root cause analysis, it has been determined that this shift is caused by sublimating dry ice entering into the container headspace and mixing with the product. This report describes functional and stability testing carried out after trypsin products were exposed to simulated gel ice shipping conditions and additional freeze-thaw cycles. Spectroscopic activity assays of trypsin and chymotrypsin, as well as bioassay and viability tests, demonstrate that trypsin products meet the same stability and performance specifications as products shipped on dry ice. By shipping on gel ice, the risk of pH shifts from sublimated CO_2 is eliminated.

Introduction

Gibco® trypsin products have shipped on dry ice for many years, and sublimated CO_2 has been determined to periodically enter the bottle headspace and cause a shift in pH and corresponding color change in trypsin products with phenol red indicator. We have taken many steps to mitigate the risk of a pH shift in our trypsin products, including higher-torque bottle sealing and shipping in a heat-sealed plastic bag. Despite a significant reduction in the observation of the color change, a small number of occurrences persist. We have, therefore, explored an alternative shipping solution using a gel ice refrigerant, which does not sublimate. Over the last several years, we have demonstrated that many products are stable at temperatures above their storage temperature, especially over the short term experienced during transit [1-6]. By conducting extensive stability and performance testing on these products, we have shown that short-term exposure to elevated temperatures during shipping does not affect product quality or functionality.

This paper describes functional and stability tests carried out after trypsin products were exposed to simulated gel ice shipping conditions and additional freeze-thaw cycles. These experiments demonstrate that by shipping trypsin products on gel ice, not only can we supply researchers with the same high-quality products, but we can also mitigate potential shifts in pH due to CO_2 dissolving in the product solution. This change brings the additional benefit of waste reduction. By removing the dry ice, we do not have to ship trypsin products in a heat-sealed plastic bag, which enters our customers' waste streams. The gel ice packs are reusable and may act as an effective heat sink in the bottom of benchtop ice buckets.

Materials and methods

Products tested

We offer several Gibco® trypsin products formulated in different sizes and concentrations, as well as with and without EDTA and phenol red (Table 1). For this study, two trypsin products were chosen to represent the product line (Table 1, items 1 and 3). These products were selected based on formulation and potential sensitivity to thermal changes. To allow for "worst case" thermal and enzymatic concentration effects, we chose

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to evaluate the smallest product volume, 100 mL, at two different concentrations, 0.5% and 0.05%. Both test products contain EDTA; one is formulated with phenol red and one without. Neither EDTA nor phenol red is known to have stability concerns under frozen or refrigerated shipping conditions.

Sample acquisition

Several 100 mL vials of each manufacturing lot were selected from inventory, and half were stored at –20°C (control samples) while half were exposed to simulated gel ice shipping conditions (test samples). During the course of the study, test samples and matched controls were evaluated side by side so that the impact of the simulated gel ice shipping conditions could be determined.

During the simulated shipping test, the product remained in the original packaging. After completion of the shipping test, the test and control bottles were opened and aliquoted for use during the stability study.

Simulated shipping conditions

Gibco® trypsin products may be shipped and stored several times during transport from the manufacturing site to distribution centers and finally to customer sites. To simulate gel ice shipments under this transport model we created an 8-day, 4-part model (Figure 1) that includes exposure to gel ice shipping temperatures (estimated at 4°C) [7] and recommended storage temperatures (estimated at –20°C). We also introduced a freeze-thaw cycle (4 hours at 9°C) between each segment to capture a worst-case scenario in which the product is allowed to completely thaw before being stored at –20°C. Shipping conditions were simulated in a Thermotron® S-16 programmable environmental test chamber. Temperatures were recorded on Sensitech TempTale®4 temperature monitors with thermocouples placed directly next to the product containers.

Table 1. Trypsin products covered in this study; items 1 and 3 were tested.

Item	Lots tested	Product	Volume	Cat. No.
1	1417191	Trypsin-EDTA (0.05%), phenol red	100 mL	25300054
2	NA	Trypsin-EDTA (0.05%), phenol red	500 mL	25300062
3	1394360 and 1369111	Trypsin-EDTA (0.5%), no phenol red	100 mL	15400054
4	NA	Trypsin (0.25%), phenol red	100 mL	15050065
5	NA	Trypsin (0.25%), phenol red (EU only)	100 mL	25050014
6	NA	Trypsin (0.25%), phenol red	500 mL	15050057
7	NA	Trypsin-EDTA (0.25%), phenol red	100 mL	25200056
8	NA	Trypsin-EDTA (0.25%), phenol red	500 mL	25200072
9	NA	Trypsin (2.5%), no phenol red	100 mL	15090046

Bioassay and viability test

Functional performance testing was conducted using the *in vitro* bioassay. This assay is performed as part of quality control release specification criteria for all trypsin products and employs A549 adenocarcinoma human alveolar basal epithelial cells to measure cell removal time, morphology, and viability.

Enzymatic assays

Spectroscopic activity assays were used to measure the activity of trypsin and chymotrypsin in test and control samples. Trypsin activity was determined by following hydrolysis

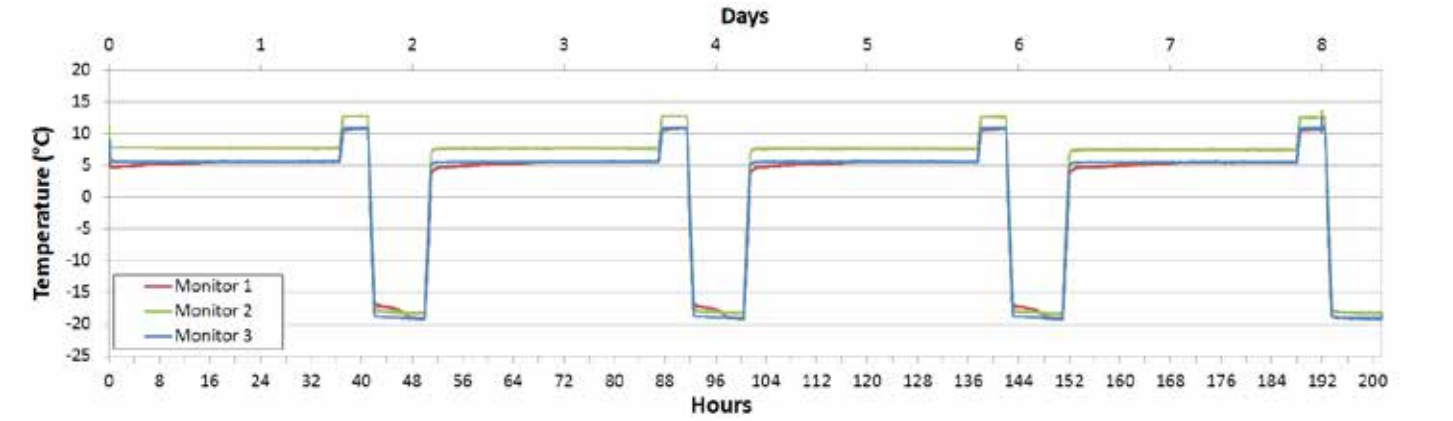


Figure 1. Simulated shipping conditions. Actual temperatures measured during the 8-day simulation used to model gel ice shipping conditions.

of the ester linkage in *N*-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) by the change in optical density (OD) at 253 nm [8,9]. Similarly, chymotrypsin activity was determined by measuring hydrolysis of the ester linkage in *N*-acetyl-L-tyrosine ethyl ester hydrochloride (ATEE) by the change in OD at 237 nm [10,11]. OD was recorded over 5 minutes to obtain a linear response, which was used to calculate activity.

Testing schedule

Products were tested according to the timeline in Table 2. At each time point the test sample that was exposed to simulated gel ice shipping conditions was compared to the corresponding control sample. At the time of this publication, stability has been assessed up to a 12-week period, and will continue to be monitored over a 56-week period. At 12 weeks, no differences were observed between gel ice–shipped products and controls.

Table 2. Testing schedule for trypsin products following simulated gel ice shipping conditions.

Weeks post-shipping	Enzymatic assay	Bioassay and viability test
1		✓
4	✓	✓
8	✓	✓
12	✓	✓
26	TBD	TBD
56	TBD	TBD

Results

Bioassay and viability test

The trypsin bioassay was conducted by measuring the time to release human epithelial cells from DMEM with 10% FBS in 75 cm^2 T-flasks grown at 34–38°C in 5% CO_2.

Figure 2 shows the bioassay results of the gel ice–shipped (stress test) samples compared with controls. In all cases the stress test and control samples showed similar release times, indicating that samples shipped on gel ice show no difference in performance.

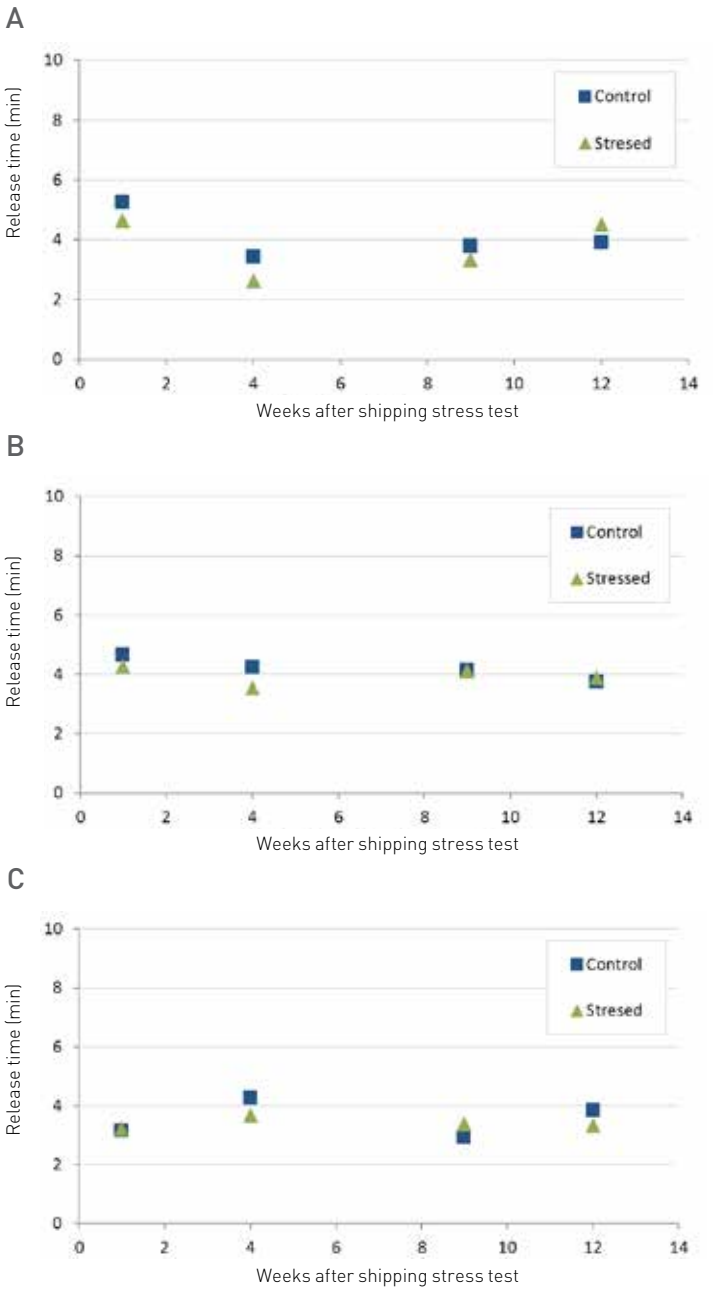
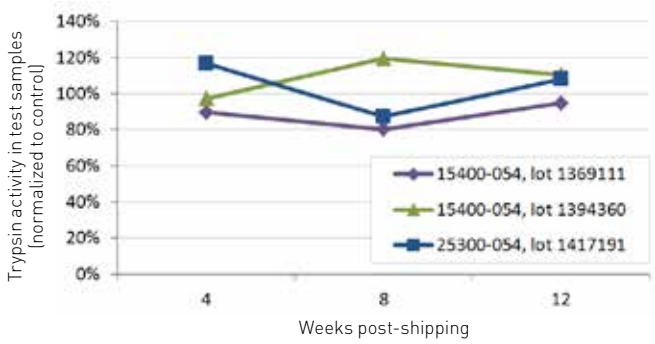


Figure 2. Bioassay test results. (A) 0.5% trypsin, no phenol red (Cat. No. 15400054, lot 1369111), **(B)** 0.5% trypsin, no phenol red (Cat. No. 15400054, lot 1394360), and **(C)** 0.05% trypsin, phenol red (Cat. No. 25300054, lot 1417191). No difference was observed between test samples that were put through a gel ice shipping simulation (green triangles) and controls (blue squares).

Enzymatic assays

Trypsin and chymotrypsin activity were assessed in spectroscopic activity assays. Figure 3 shows the activity of trypsin and chymotrypsin in the test samples, normalized to the controls. Twelve weeks after the ambient shipping simulation, the test and control samples show similar activity, further indicating that products shipped on gel ice maintain the same enzymatic activity as those shipped on dry ice.

A



B

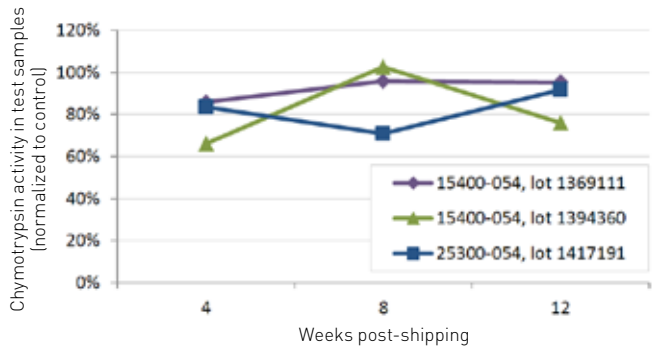


Figure 3. Enzymatic activity assays. (A) Trypsin and **(B)** chymotrypsin activity for each product tested. Activity of the test samples has been normalized to the controls. At 12 weeks, no significant difference was observed between test samples that were put through a gel ice shipping simulation and controls.

Conclusions

The data described in this paper demonstrate that gel ice shipping conditions have no effect on the quality and functionality of Gibco® trypsin products. Representative products were functionally tested, and they showed performance and enzymatic activity similar to those of controls. These results substantiate the change to gel ice shipping conditions and provide researchers with confidence that when shipped under these conditions, trypsin products will exhibit function and stability similar to unshipped controls.

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