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Freezing and Thawing Rate Controlled Validation 16-L Bag Study

Introduction:

It is well understood that freeze/thaw processes affect the product quality of biopharmaceuticals.[1-3] It has been reported that there is no consistent method of controlled freezing and thawing rates for biological formulations.[4] Traditionally, ultra-low temperature storage chambers that were not designed for freezing have been used to provide an energy state for the environment surrounding the product with very little excess capacity to change the state of the product.

This study shows a consistent method is available for controlled rate freezing and thawing. It also includes the effect of load and container's position on freeze rates. The freeze/thaw controlled rate chamber used in this study was a Model 4002 manufactured by Farrar Scientific. It permits rapid, uniform bulk freezing and/or thawing of products with temperature ranges from +40ºC to -80ºC. It has a minimum of 1.7 kw of net cooling and heating capacity over the entire temperature range. It is also equipped with programmable profiles, which allow flexibility to meet unique product conditioning.

Experimental Procedure:

The effect of the program temperature profile on the rate of product freezing and thawing were explored. Various temperature profiles were tested in order to determine the best for uniformly controlling the freezing rate to the 1st phase transition (0ºC), and the cooling rate from the 1st phase transition to -35ºC. The target product cooling rates from -5ºC to -35ºC were 0.13ºC/min to 0.94ºC/min. The target thaw rate was as fast as possible without overshooting a particular temperature.

Tap water was used to simulate a water-based biological formulation. Bags (16L fill capacity) were used in this study. Each bag was placed on a stainless steel shelf. For the 1 bag test, the bag was arranged in order maximize air flow to the entire outer surface as uniformly as possible (see attached picture Figure 1). The bag contained 16L of water. The bag had a thermocouple placed in the middle port with the tip located half way between the bag mid-point (hole) and the edge (Figure 2). The end of the thermocouple is also equal distance from the upper to the lower bag surface (Z direction). This is the expected last point to freeze.

Figure 1. Standard 1 Bag Position in Chamber.

Figure 2. Thermocouple placement in Bag.

In order to meet the freezing requirements, the controlled rate chamber was set up with the following program (Test 1).

Step 1. Timed Event to -20ºC in 10 seconds
Step 2. Wait for process
Step 3. Soak for 6 hours
Step 4. Timed Event to -40 in 10 seconds
Step 5. Wait for Process
Step 6. Soak for 12 hours
Step 7. Timed Event to -80ºC in 10 seconds
Step 8. Wait for process
Step 9. Soak for 6 hours
Step 10. Wait for event

Controlled Freeze Rate Results and Discussion:

Controlled Freeze Test 1 results are shown in Figure 3. Notice that it took 472 minutes to break from the phase change and 732 minutes to reach -35ºC. The cooling rates from -5ºC to -35ºC are shown in Table 1. The cooling rates are controlled at 0.08ºC/min to 0.30ºC/min. The cooling rate is at its slowest rate from -30ºC to -35ºC because the difference in air temperature (-40ºC) and product temperature is so small. Therefore, Test 2 was conducted where the 2nd plateau temperature was changed from -40ºC to -50ºC (Figure 4). The cooling rates are shown in Table 2. The cooling rates are within the target (0.13°C/min - 0.94°C/min) throughout the entire temperature range from -5°C to -35°C.

Figure 3.

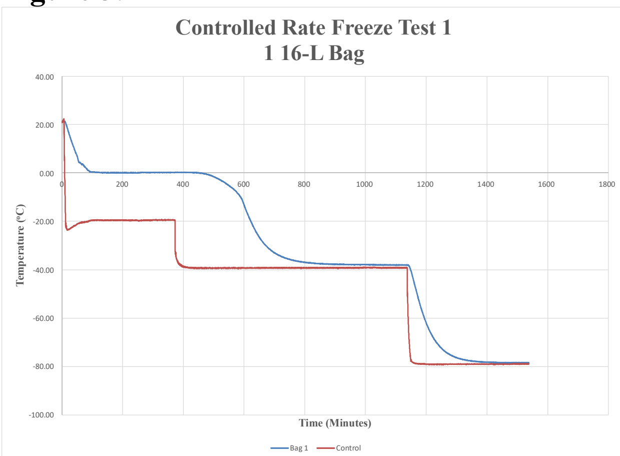
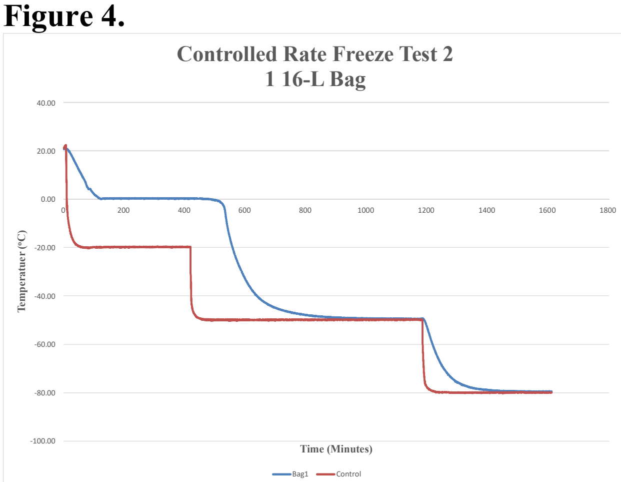


Table 1. Test 1 Cooling Rates.

Temp. Range (°C)	Cooling Rate (°C/min)
-5 to -10	0.12
-10 to -20	0.30
-20 to -30	0.20
-30 to -35	0.08

Figure 4.



Since the correct temperature profile was determined with 1 bag, the next test (Test 3) was conducted using the same temperature profile with 8 bags. For the 8 bag test, bags were arranged in order to maximize air flow to the surface of all bags as uniformly as possible (Figure

5). The bags were numbered 1-8 and were in consecutive order from top (Bag 1) to bottom (Bag 8). Figure 6 and Table 3 show the cooling rates of each bag. The 8 bags break from the phase change in the range of 429 to 555 minutes. The 1 bag (Test 2) broke from the phase change at 499 minutes, within the range of the 8 bags. This shows that the freezing rate is independent of product load. The cooling rate from -5ºC to -35ºC ranged from 0.13ºC/min to 0.73ºC/min. All bags had cooling rates within the target range. These results show that the cooling rate is not dependent on bag position in the chamber or load.

Table 2. Test 2 Cooling Rates.

Temp. Range (°C)	Cooling Rate (°C/min)
-5 to -10	0.88
-10 to -20	0.54
-20 to -30	0.33
-30 to -35	0.24

Figure 5. Standard 8 Bag Positions in Chamber.

Figure 6.

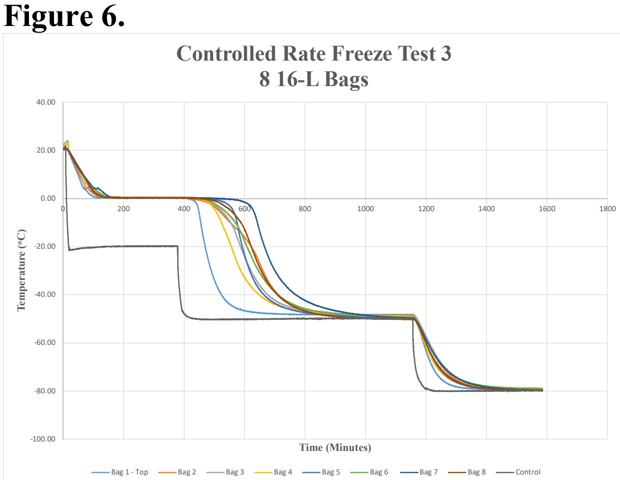


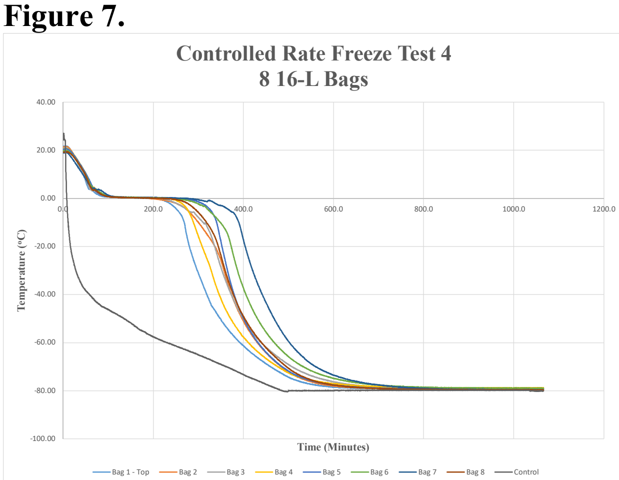
Table 3. Test 3 Bag Cooling Rates.

	Cooling Rate (°C/min)							
	Bag #							
Temp. Range (°C)	1	2	3	4	5	6	7	8
-5 to -10	0.73	0.13	0.16	0.24	0.38	0.14	0.38	0.15
-10 to -20	0.64	0.14	0.30	0.31	0.59	0.26	0.42	0.29
-20 to -30	0.50	0.26	0.29	0.32	0.40	0.26	0.29	0.30
-30 to -35	0.35	0.25	0.22	0.20	0.28	0.19	0.18	0.23

The top bag (Bag 1) was the fastest to break from the phase transition and the fastest cooling from -5°C to -35°C. This is to be expected since the air flow enters the chamber from the top left corner and flows down and out of the left duct toward the right side. What was unexpected was that the bottom bag was not the slowest to break from the phase transition. Bag 7 was the slowest to break from the phase transition. Even though the bottom bag has very little air flow underneath it, it apparently has a higher velocity air flow in contact with the top side of the bag compared to Bag 7. Bag 2 had the slowest cooling rate from -5°C to -35°C even though it was the second to break from the phase transition. It appeared that the other Bags that were still warmer kept Bag 2's cooling rate slower until they cooled to a lower temperature.

In order to determine if the cooling rates could be increased without exceeding the maximum (0.9°C/min) target, Test 4 was conducted where the temperature was decreased as quickly as possible (Time Event to -80°C in 10 sec). The cooling rate results are shown in Figure 7 and Table 4. All bags had cooling rates from -5°C to -35°C that were in the target range (0.13°C/min to 0.94°C/min). These results show again that the cooling rates were controlled to the desired target range independent of bag position in the chamber. In addition, the temperature at which each bag breaks from the phase transition ranges from 209 minutes (Bag 2) to 291 minutes (Bag 7). These results also show that freezing rates were controlled to a reasonable level.

Figure 7.



Another test was conducted (Test 5) in order to extend the time bags break from the phase transition. The temperature profile had a plateau at -15°C for 8 hrs before plunging to -80°C. Test 5 controlled cooling results are shown in Figure 8 and Table 5. The temperature at which each bag breaks from the phase transition ranges from 562 minutes (Bag 3) to 652 minutes (Bag 7). It took 353 more minutes for the 1st bag to break from the phase transition and 361 more minutes for the last bag to break from the phase transition compared to Test 4. The cooling rate for all bags from -5°C to -35°C were again within the target range.

Table 4. Test 4 Bag Cooling Rates.

	Cooling Rate (°C/min)							
	Bag #							
Temp. Range (°C)	1	2	3	4	5	6	7	8
-5 to -10	0.33	0.21	0.15	0.42	0.38	0.20	0.25	0.22
-10 to -20	0.87	0.27	0.39	0.51	0.85	0.41	0.69	0.38
-20 to -30	0.58	0.50	0.63	0.50	0.81	0.70	0.61	0.66
-30 to -35	0.46	0.64	0.52	0.61	0.75	0.60	0.50	0.59

Figure 8.

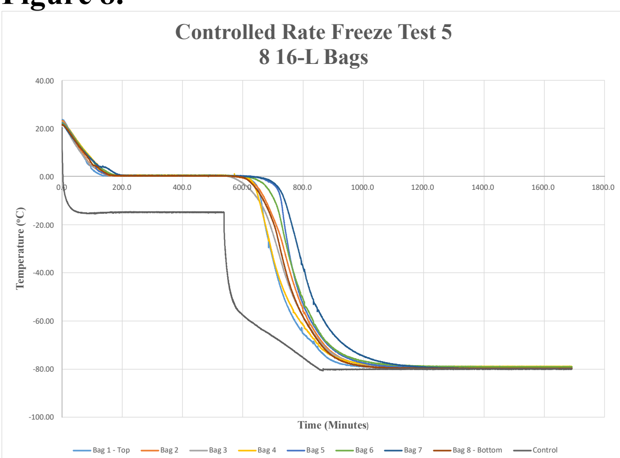


Table 5. Test 5 Bag Cooling Rates.

	Cooling Rate (°C/min)							
	Bag #							
Temp. Range (°C)	1	2	3	4	5	6	7	8
-5 to -10	0.47	0.22	0.15	0.41	0.38	0.21	0.26	0.19
-10 to -20	0.80	0.29	0.27	0.50	0.92	0.43	0.43	0.29
-20 to -30	0.56	0.39	0.41	0.52	0.75	0.55	0.48	0.49
-30 to -35	0.52	0.48	0.43	0.54	0.65	0.50	0.48	0.52

Since the correct temperature profile was determined with 8 bags, the next test (Test 6) was conducted using the same temperature profile with 4 bags. For the 4 bag test, bags were arranged in order to maximize air flow to the surface of all bags as uniformly as possible (Figure 9). The bags were numbered 1-4 and were in consecutive order from top (Bag 1) to bottom (Bag 4). The temperature profile was programmed to match the actual temperature profile of Test 5. It is shown below.

Step1. Timed Event to -15ºC in 10 seconds
Step 2. Wait for process
Step 3. Soak for 8 hours
Step 4. Timed Event to -56ºC in 10 seconds
Step 5. Wait for Process
Step 6. Timed Event to -80ºC in 4 hours and 26 minutes
Step 7. Wait for process
Step 8. Soak for 11 hours
Step 9. Wait for event

Figure 9. Standard 4 Bag Positions in Chamber.

The cooling rates of each bag are shown in Figure 10 and Table 6. The temperature at which each bag breaks from the phase transition ranges from 575 minutes (Bag 2) to 618 minutes (Bag 1). This is within the range that the 8 bags broke from the phase transition in Test 5. This shows again that the freezing rate is independent of product load. In addition, all bags had cooling rates from -5ºC to -35ºC within the target range. These results show that the cooling rate was not dependent on bag position in the chamber or load.

Figure 10.

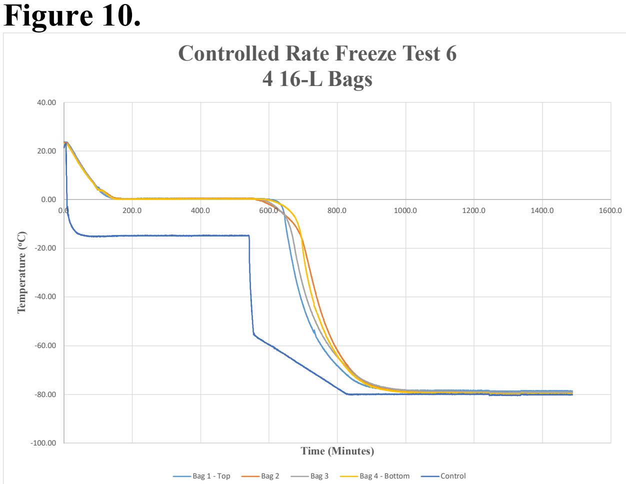


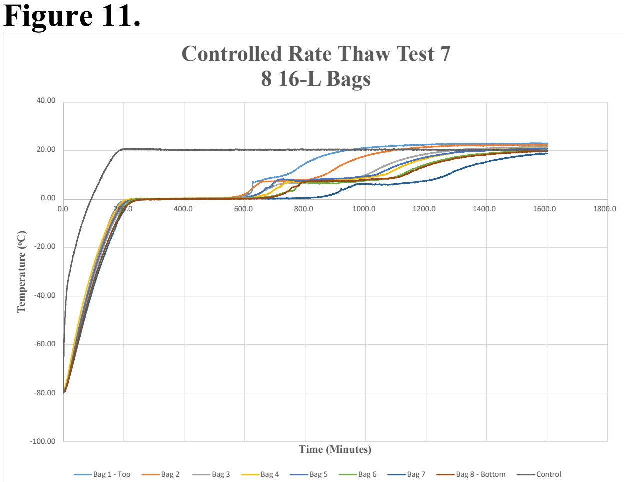
Table 6. Test 6 Bag Cooling Rates.

	Cooling Rate (°C/min)			
	Bag #			
Temp. Range (°C)	1	2	3	4
-5 to -10	0.94	0.14	0.22	0.23
-10 to -20	0.81	0.32	0.59	0.74
-20 to -30	0.71	0.58	0.70	0.88
-30 to -35	0.62	0.58	0.56	0.67

Controlled Thaw Rate Results and Discussion:

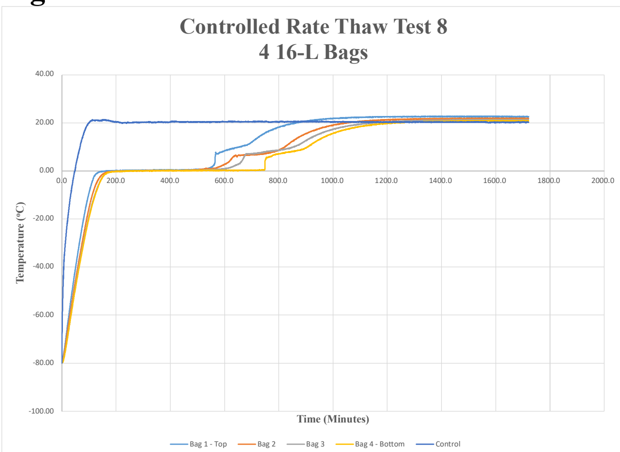
It has been reported that fast thaw rates are best for avoiding damage to biological formulations.[1] However, most biological formulations are temperature sensitive. Therefore, this study shows how the maximum temperature of the product could be controlled as well as controlled thaw rates. The thaw test temperature profile starts with product temperatures at -80ºC and ramps to +20ºC as fast as possible. For the purpose of this study, the time it took for each bag to reach to +2ºC is recorded as the thaw time. Test 7 thaw results are shown in Figure 11. The 8 bags reached +2ºC in the range of 599 minutes (Bag 2) to 890 minutes (Bag 7). As was the case with the slower freezing of Bag 7, it is in a position in the chamber that has the least amount of air flow. Seven out of the 8 bags show tight thaw rate control with only 124 minutes separating the 1st bag to thaw from the 7th.

Figure 11.



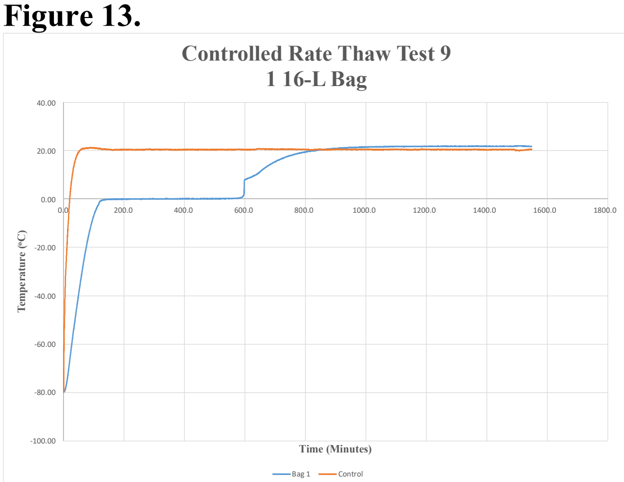
In order to test the effect of load, thaw Test 8 was conducted with the same conditions using 4 bags (Figure 12). The 4 bags reached +2ºC in the range of 557 minutes (Bag 1) to 749 minutes (Bag 4). Only Bags 1 and 2 thaw a little faster than any of the 8 bags in Test 7. However, this is still relatively good thaw rate control regardless of load. In addition, the thaw rate was controlled independent of the bag position in the chamber.

Figure 12.



To further test the effect of load, thaw Test 9 was conducted with the same conditions using 1 bag (Figure 13). The bag reached +2ºC in 597 minutes, which is within the range of all 4 bags in Test 8. This shows that thaw rate was controlled independent of load.

Figure 13.



Conclusions

A method was successfully developed to control the desired freezing and thawing rate using a Farrar Scientific Model 4002 Controlled Rate Freeze/Thaw Chamber. It was validated with different loads (1, 4, and 8 bags) and product positions in the chamber. A program was developed that a client can use to reliably and uniformly bulk freeze and thaw their biological formulation.

References:

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