



Freeze-drying Cycle Development for Kogenate-FS

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Global Biological Development

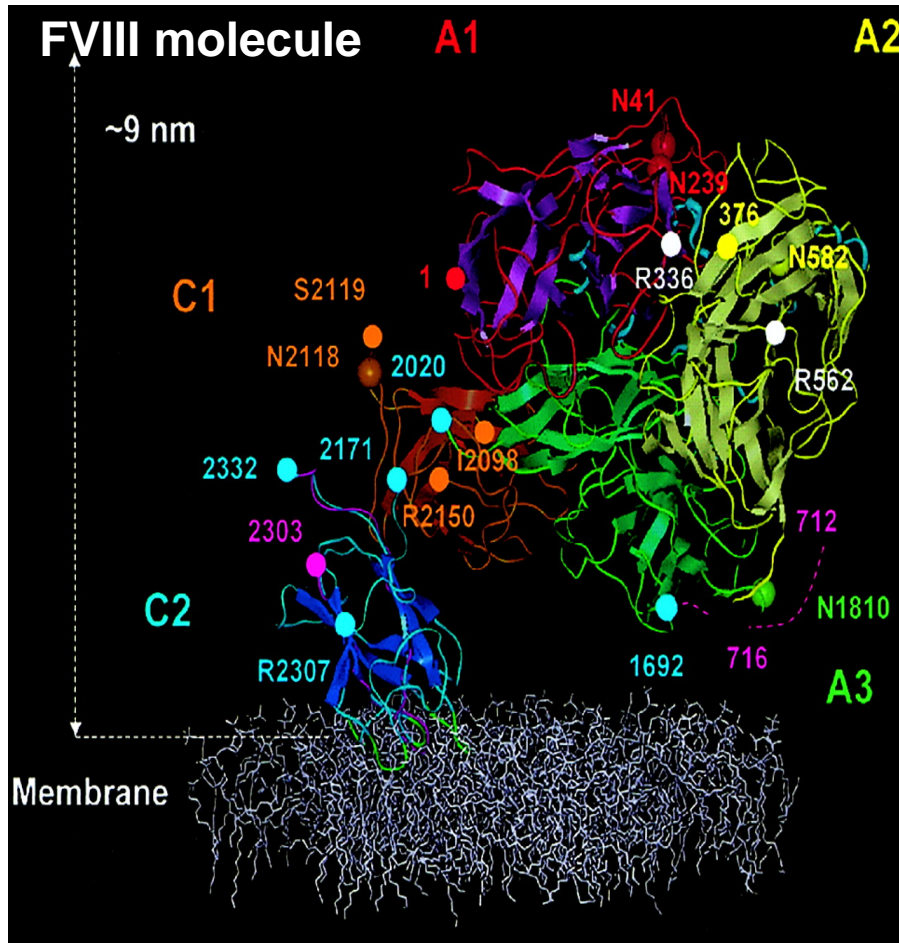


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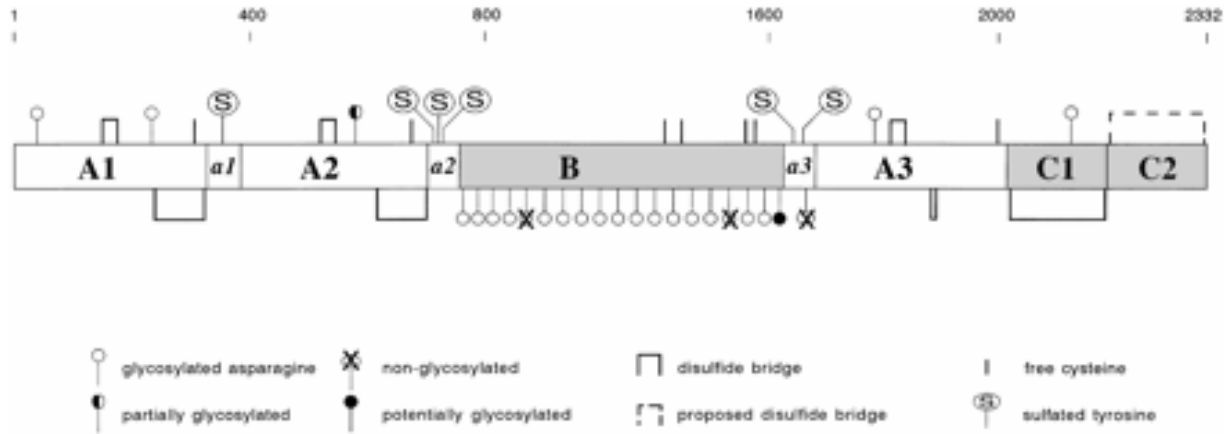
3-D structure of factor VIII -

Deficiency of FVIII results in Hemophilia A.

Replacement of FVIII is the treatment for Hemophilia A

Therapeutically FVIII is an ideal recombinant protein, but Factor VIII biochemistry is complex

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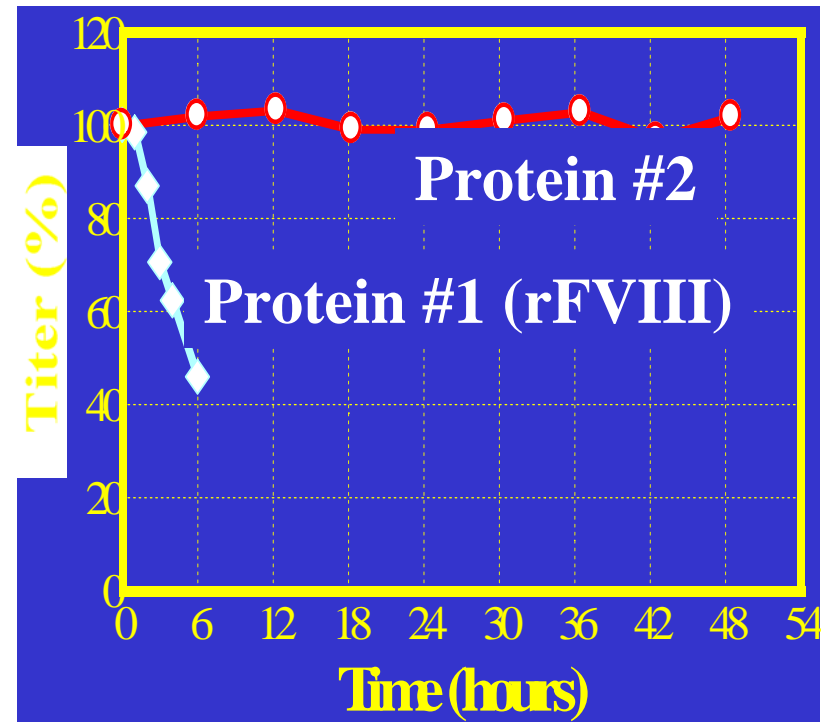
- FVIII is 2351 a.a. protein
- 25 N-linked glycosylation sites
- 6 tyr-sulfation sites
- Circulating form is a dimer with heavy (A1A2B) and light (A3C1C2) chains associated by a metal bridge
- The role of the B domain is unclear

(Lenting et. al. Blood, Vol. 92 No. 11 (December 1), 1998: pp. 3983-3996)



FVIII is an extremely challenging protein

- rFVIII is the largest protein made by biotech industry
- Multiple modifications required for proper function
- Cell Specific productivity is low
- Protein is very unstable





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The Formulation

Glycine 2.2%, Sucrose 1%, NaCl 30 mM, Histidine 20 mM, CaCl₂ 2.5 mM, Tween-80 80 ppm and 0.2-0.5 mg/mL rFVIII.

Crystalline Matrix formulation

Glycine crystallizes to form a crystalline matrix for the sucrose and others to collapse upon. The glycine provides excellent cake, good reconstitution characteristics, and ease in freeze drying. The sucrose stabilizes rFVIII during processing and storage.



Cycle Development Steps

- **Characterize the formulation.**
- **Optimize the process based on the understanding of the formulation.**
- **Scale-up the process and transfer the process to production.**
- **Validate the process.**

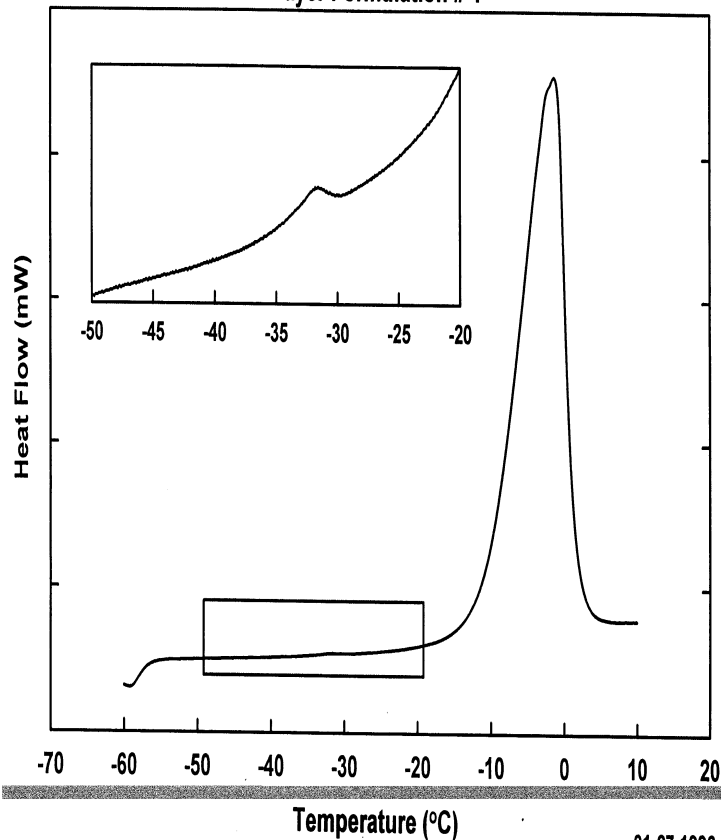
Characterize the Formulation

- **Utilize Differential Scanning Calorimetry (DSC) to determine Tg'.**
- **Confirm results with Lyo-microscope and determine the maximum allowable product temperature during lyophilization.**
- **Generate a water absorption/desorption curve for characterizing the secondary drying process.**
- **Conduct moisture optimization study for developing secondary drying.**

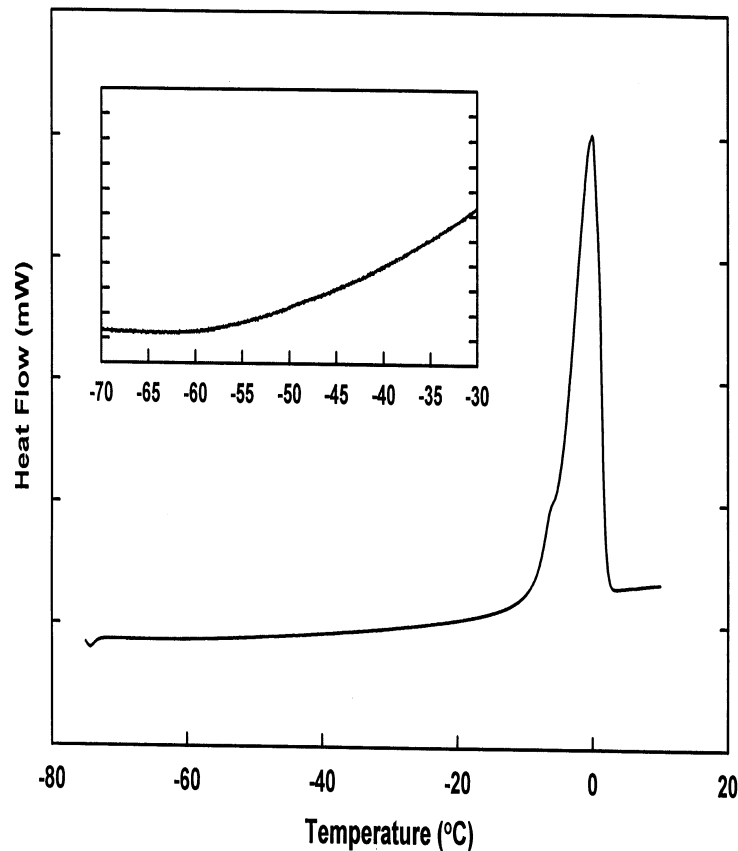


Characterize the Formulation

Bayer Formulation # 1

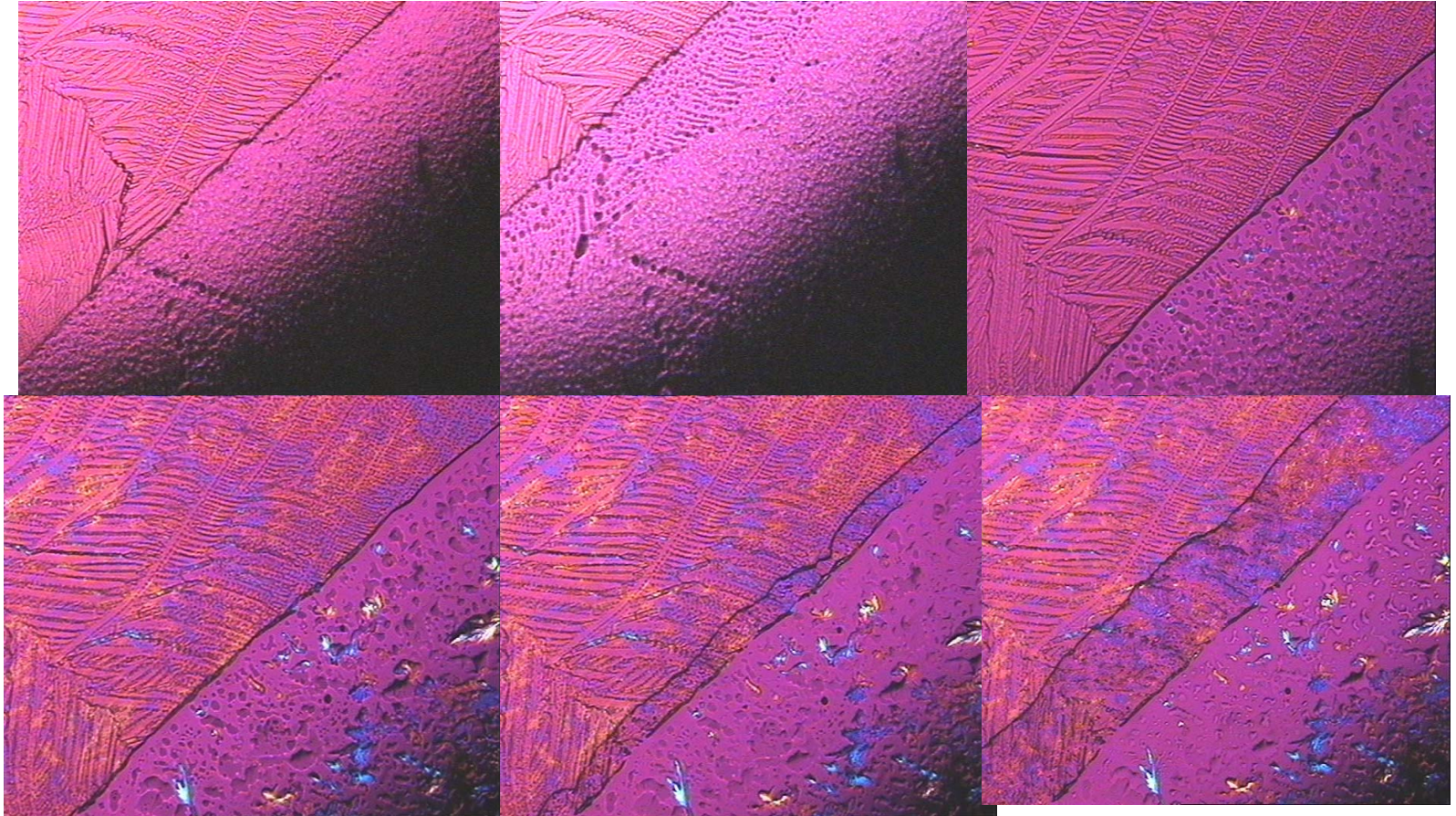


Bayer Formulation #1 (annealed at -20 °C for 1hr)

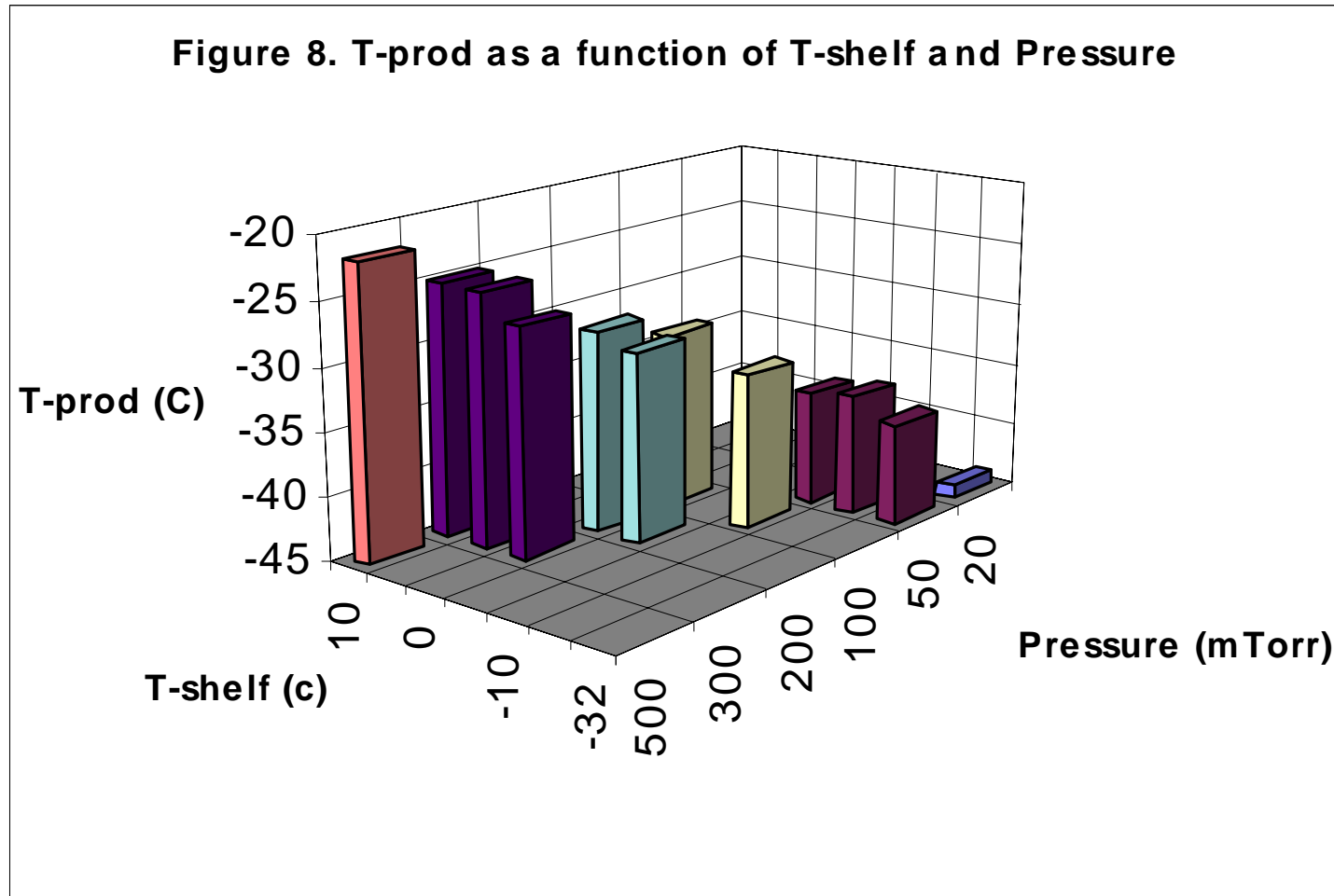


Characterize the Formulation (Cont.)

Freeze-drying micrographs of formulation showing collapse and retention of structure
(mag = 140x)



Characterize the Formulation (Cont.)

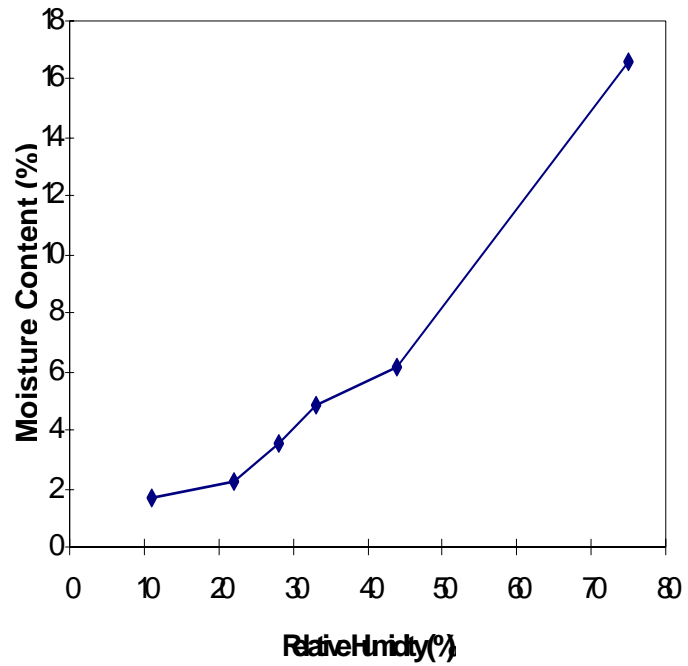


Characterize the Formulation (Cont.)

water absorption/desorption curve

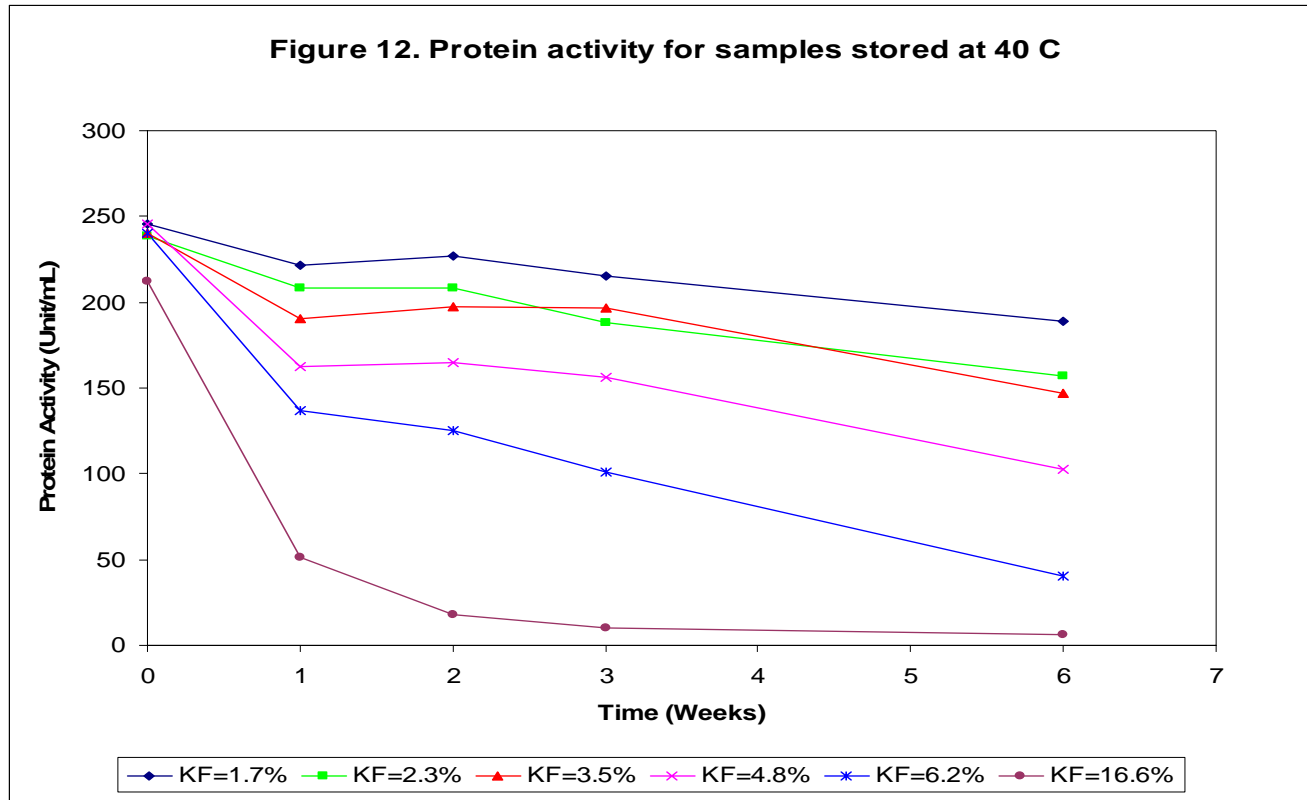


Figure 11 Water Absorption Curve



Characterize the Formulation (Cont.)

Results of a Moisture Optimization Study

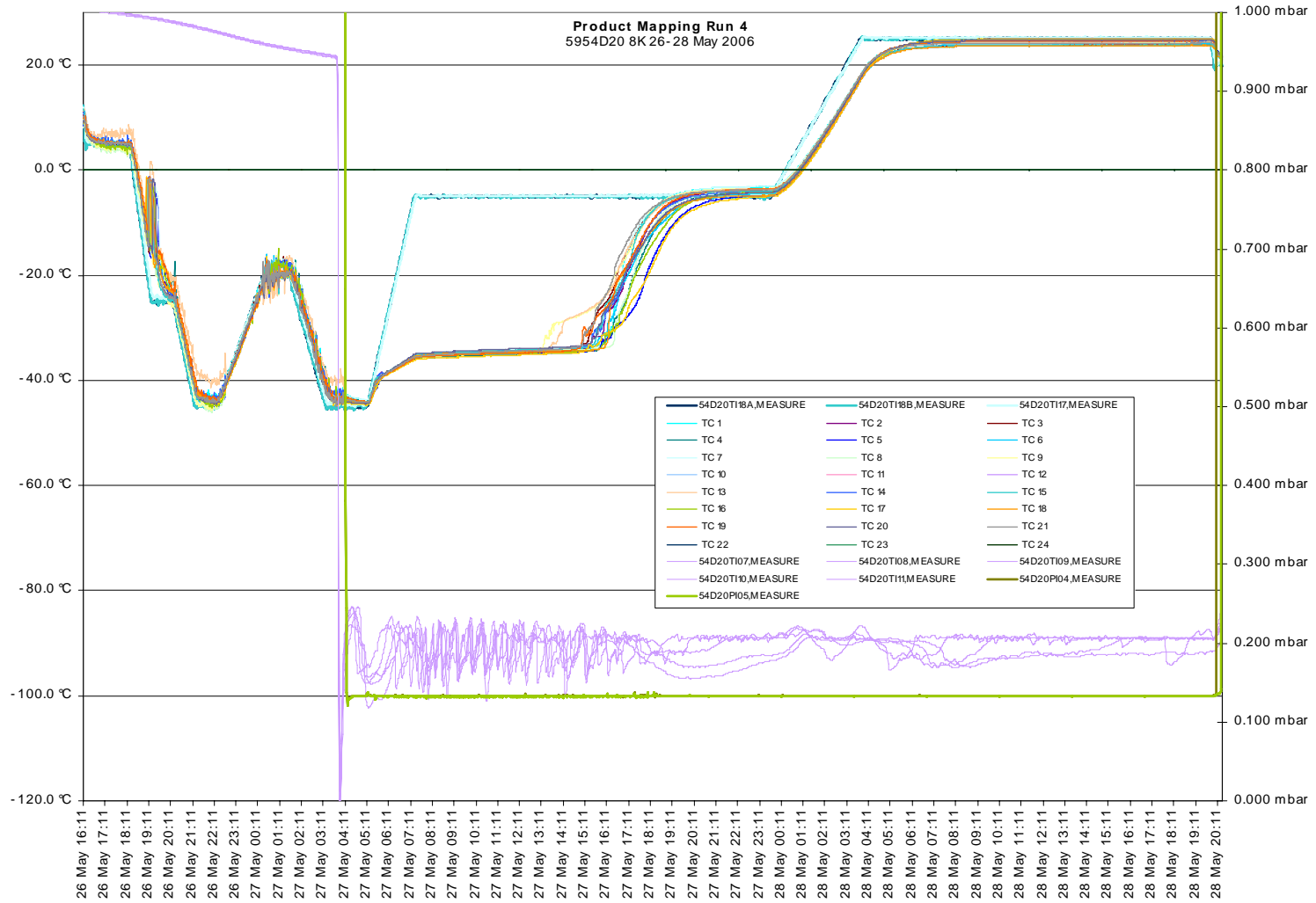


Optimize the process based on the understanding of the formulation:

- **Freezing:** Annealing is necessary to crystallize glycine.
- **Primary drying:** Product temperature must be < -15 C. Drying time is a function of shelf temperature and pressure.
- **Secondary drying:** “The drier, the better” make it easier to determine process parameters, such as shelf temperature, pressure and duration.



The Freeze Drying Cycle



Conclusion

With a thorough understanding of the formulation, we can reduce the amount of trial-and-error in cycle development.

- o An annealing step is necessary to crystallize a bulking component in the formulation.
- o Consequently, it allowed us not only to conduct the primary drying aggressively at a relatively high product temperature, but also to obtain a good cake appearance.
- o The characteristic of “the drier the better” for this formulation permitted us to easily optimize the secondary drying to provide uniform moisture content in the final containers.



Design Space I: Is Collapse OK?

- Produce non-collapsed, micro-collapsed and collapsed lyophilized cakes from the same formulation by varying the freezing and primary drying conditions
- Investigate the effect of collapse and micro-collapse on rFVIII.

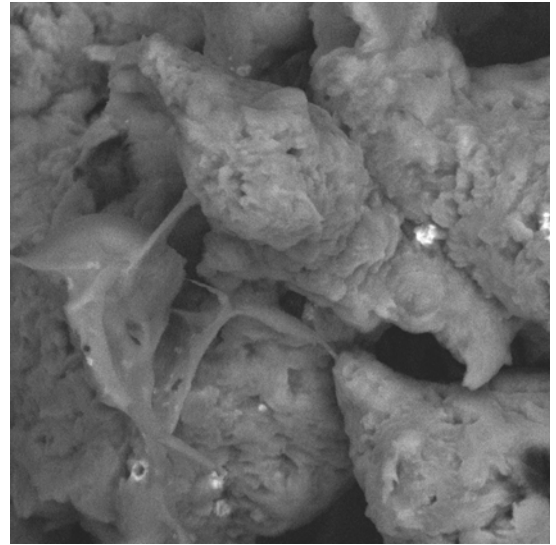
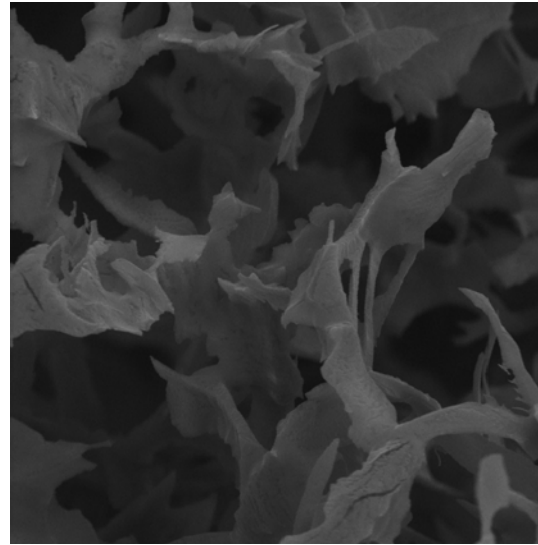
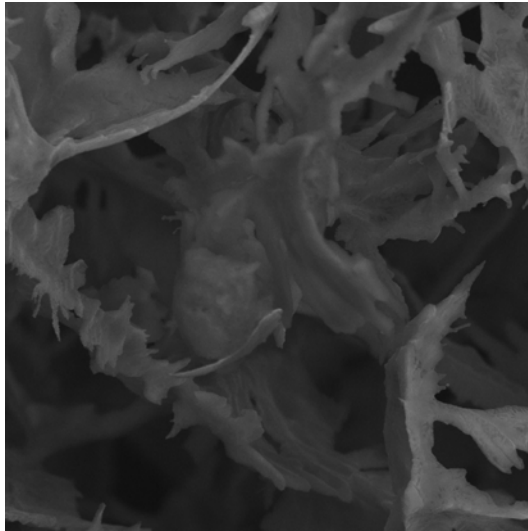


Freeze-drying Cycles Used:

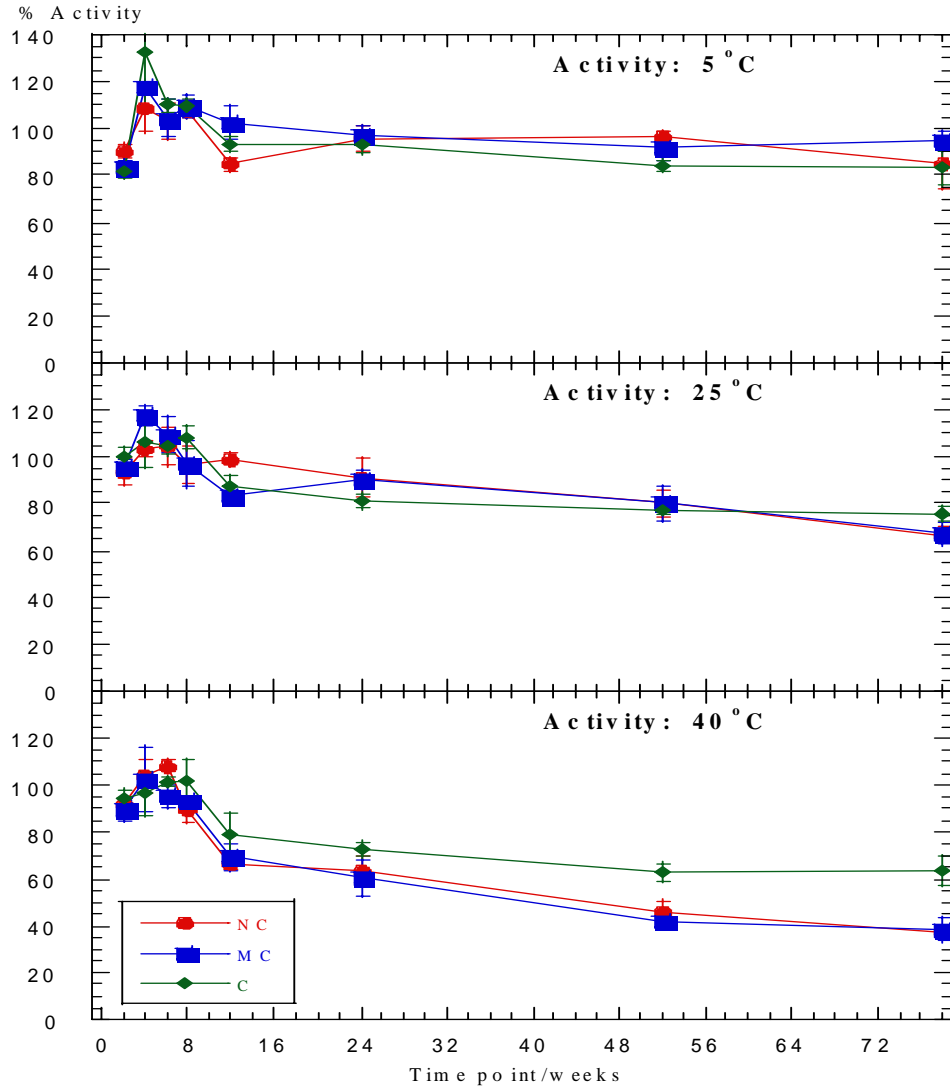
- Non-collapsed (NC): Freeze with annealing and lyophilize with a very conservative cycle.
- Micro-collapsed (MC): Freeze with annealing and lyophilize with a more aggressive drying cycle.
- Collapsed (C): Fast freeze (no annealing) and lyophilized with a very conservative cycle.

Collapse was caused by changing the freezing process only

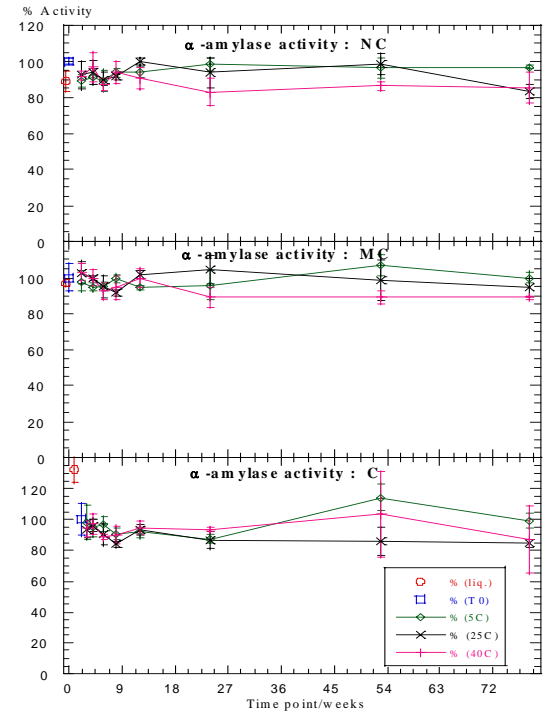




rFVIII stability at 5, 25 and 40°C for each of the three cake structures (separated by temperature).



α -amylase stability at 5, 25 and 40°C for each of the three cake structures.



Design Space II: Range Finding Study

- Use a lab scale freeze-dryer
- Use Kogenate FS formulation
- Group the critical process parameters into 10 extreme limit freeze-drying conditions (lower limits and upper limits)
 - Varying freezing temperature in freezing phase
 - Varying chamber pressure (vacuum) and shelf temperature in primary and secondary drying phases
 - Check ramp rates from one phase to another phase.



Table 1. Summary of “Limits Critical Process Parameters”.

Phase	Condition	Temperature limit (°C)	Ramping (°C / min)	Pressure limit (mTorr) ^{*1}
Freezing	Upper	-42, -17, -42	0.3	Atmosphere
	Licensed	-45, -20, -45	0.2	Atmosphere
	Lower	-48, -23, -48	0.1	Atmosphere
Primary-drying	Upper	-2	0.3	150
	Licensed	-5	0.3	100
	Lower	-8	0.3	50
	Upper – higher ramping rate	-2	0.4	150
	Lower – lower ramping rate	-8	0.2	50
Secondary-drying	Upper	28	0.1	50
	Licensed	25	0.1	100
	Lower	22	0.1	150
	Upper – higher ramping rate	28	0.2	50

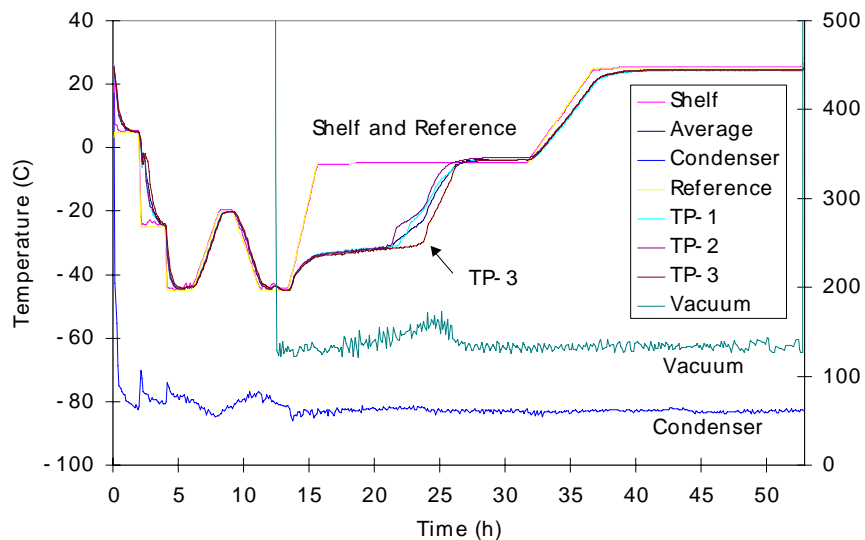


Assessment

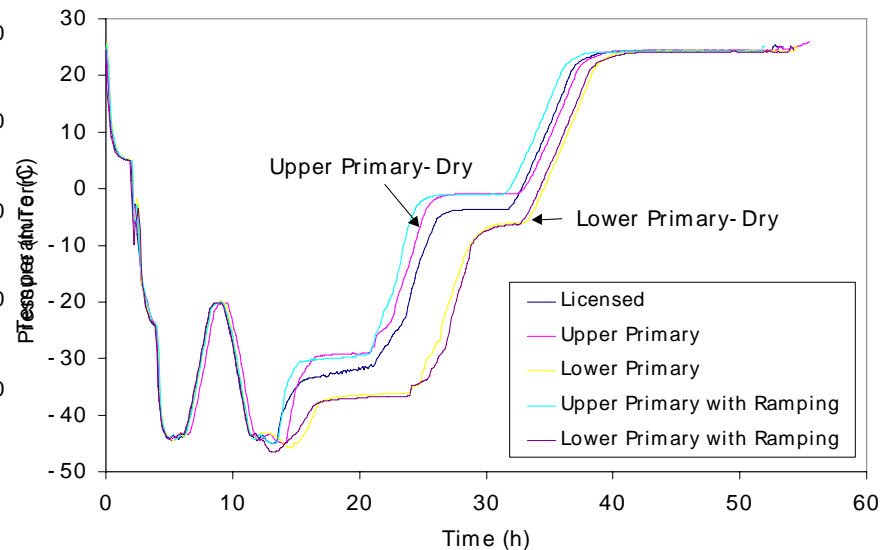
- Use thermocouples to monitor the completion of freeze-drying cycles
- Compare differences among 10 freeze-drying cycles (n=6) for:
 - Potency
 - Solubility and appearance
 - Residual moisture
 - Product temperature profile
- Perform an ANOVA statistical analysis to assess potency differences



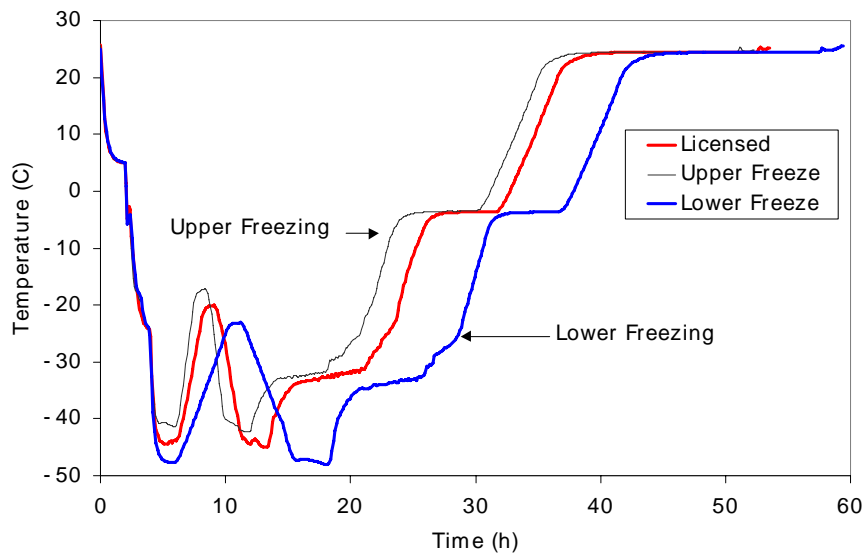
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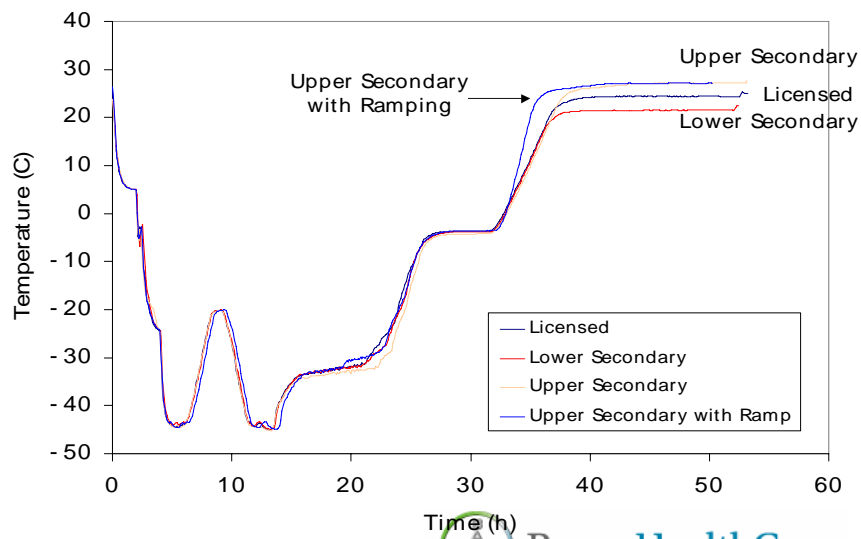
Primary-Dry Changes.



Annealing Changes.



Secondary-Dry Changes.



Results of the Rang Finding Study

Description	Lyophilized potency* N=6 (IU/mL)	Liquid potency * N=6 (IU/mL)	Appearance N = 6	Solubility N = 6 (min)	Residual Moisture N=3 (%)
Licensed	138.4 ± 7.2	131.7 ± 8.3	accept	0.1 ± 0.0	1.18
Upper Freezing	137.1 ± 7.3	141.3 ± 7.4	accept	0.1 ± 0.0	1.51
Lower Freezing	135.8 ± 7.4	133.6 ± 10.3	accept	0.1 ± 0.0	1.00
Upper Primary	136.3 ± 12.9	135.1 ± 8.7	accept	0.1 ± 0.0	1.40
Lower Primary	143.0 ± 8.9	132.3 ± 6.2	accept	0.1 ± 0.0	1.14
Upper Primary High Ramp	137.8 ± 4.3	132.5 ± 8.8	accept	0.1 ± 0.0	1.18
Lower Primary Decrease Ramp	134.0 ± 5.6	134.3 ± 9.5	accept	0.1 ± 0.0	1.20
Upper Secondary	136.2 ± 7.3	129.3 ± 4.9	accept	0.1 ± 0.0	1.04
Lower Secondary	133.4 ± 10.2	131.6 ± 6.9	accept	0.1 ± 0.1	1.38
Upper Secondary High Ramp	141.6 ± 10.1	132.0 ± 5.8	accept	0.1 ± 0.0	1.22

Design Space III: Product Mapping

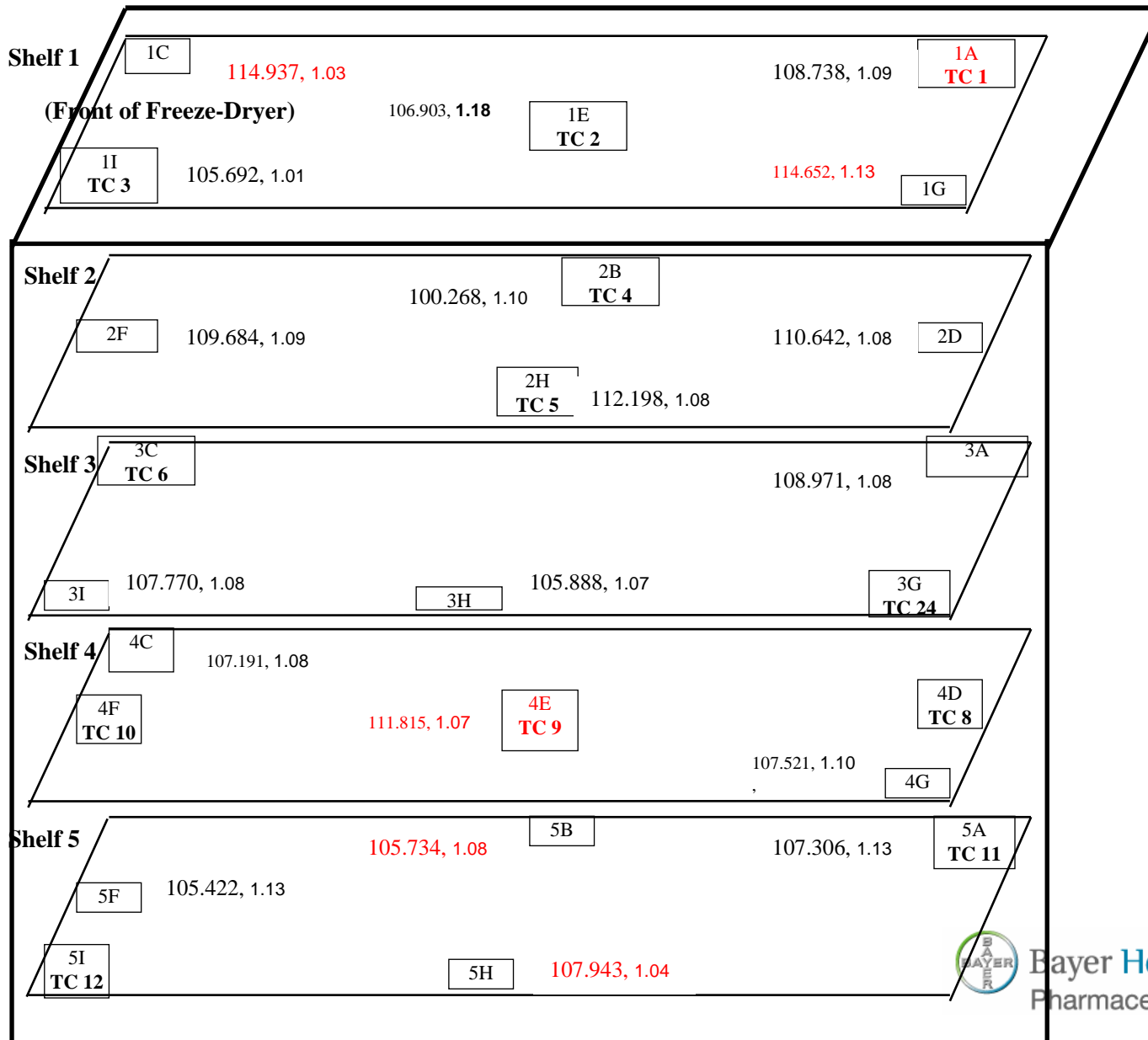
- At Production scale (220 sq. ft. dryer)
- Use thermocouples to monitor the completion of freeze-drying cycles
- Evaluate uniformity of a batch:
 - Potency
 - Residual moisture
 - Product temperature profile
- Perform 4 lots, 2 full loads (40,000 vials) and 2 min. loads (8,000 vials).
- Each load have approximately 1000 vials of real products for sampling and stability test; the rest of load were excipient fills.



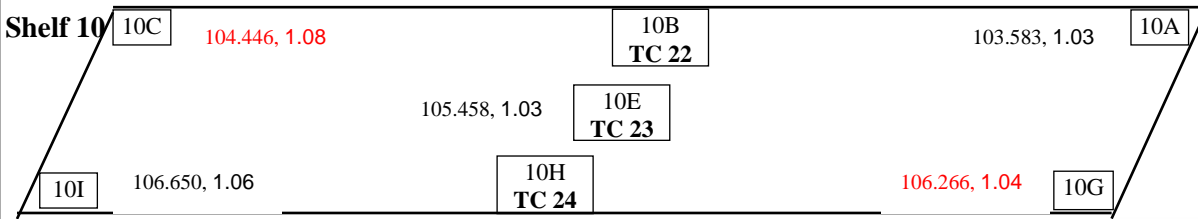
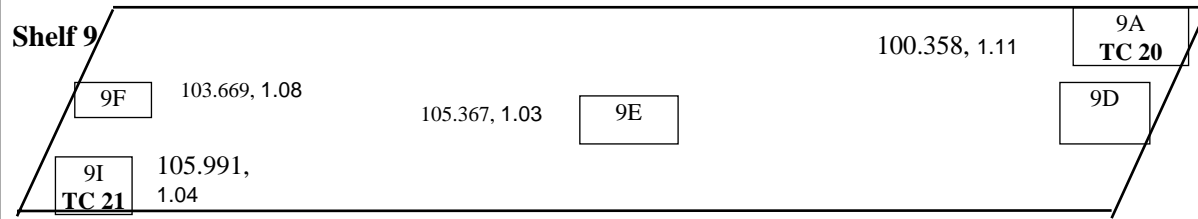
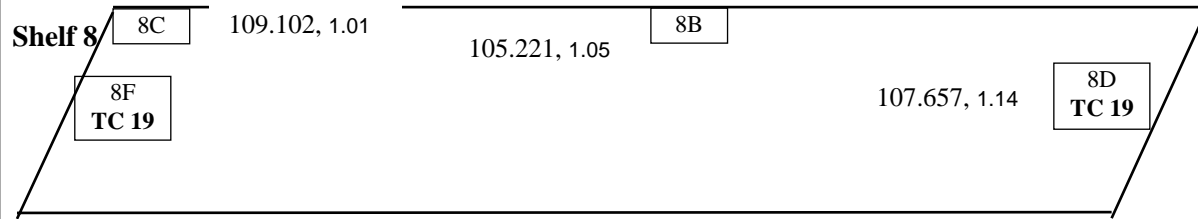
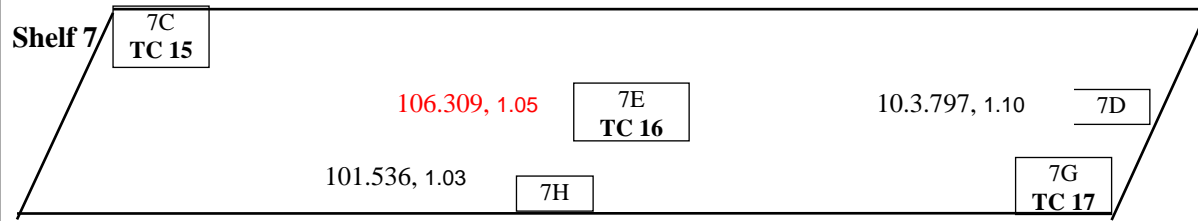
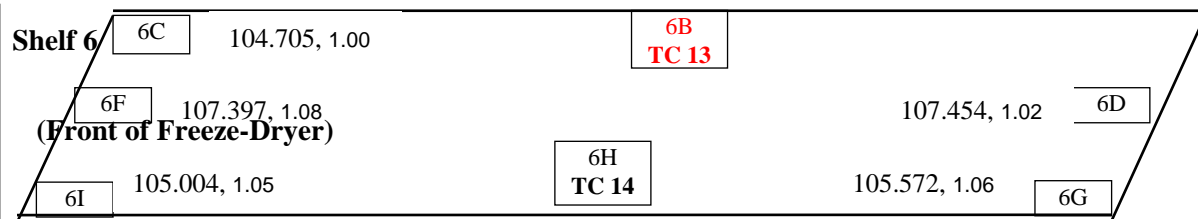
RUN 3 – D20 (MAX Load)

Figure 1 - Sample sector and TC locations

(Rear of Freeze-Dryer)



(Rear of Freeze-Dryer)



Conclusions

- The stability of freeze-dried rFVIII is not adversely affected by micro-collapse, and even total collapse, although the products are not “pharmaceutically acceptable”.
- A “rang finding” study allows us to define the limits of the critical process parameters that should be controlled in large scale commercial production.
- A “product mapping” study at production scale provides the understanding and validation of:
 - Different loads, Max vs Min.
 - Scalability, from Lab to Production
 - Consistency within multiple lots,
 - Uniformity within a dryer
 - The worst case locations.



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