Application Note
No. 256/2017

Lyophilisation of mannitol and NaCl solutions in serum vials

Lyovapor™ L-200 Pro

1. Introduction

In this Application Note sodium chloride (NaCl) and mannitol are used for freeze drying experiments. The unambiguous crystal structure of NaCl renders this salt a model compound. In contrast, mannitol is well known to crystallize in different polymorphs[1] and it may form hydrates[2].
Nevertheless, mannitol is the most used bulking agent for freeze dried pharmaceutical formulations. The benefits of using mannitol are that it crystallizes during freezing, creates a beautiful cake and permits drying processes at higher product temperatures, thus with higher sublimation rates compared to purely amorphous systems[3].

2. Equipment

- BUCHI Lyovapor™ L-200 Pro
- BUCHI Lyovapor™ Software
- Deep Freezer -40°C, tritec HANNOVER
- Stainless steel tray
- Serum bottles, 5mL, WHEATON (223738) with caps (22 mm)
- Mettler Toledo HR73 Halogen Moisture Analyser

3. Chemicals and Materials

- D-Mannitol, Fluka (63560)
- NaCl, Fluka (71382), puriss, ≥ 99.5 %

4. Experimental

4.1. Sample preparation

3 mL of a 5 % aqueous mannitol or NaCl solutions (50 g/L) were transferred into the vials with a volumetric pipette (70 vials of each solution). Then, the samples were frozen overnight in a deep freezer at -40°C on a stainless steel tray. Alternatively, a -20°C freezer can be used.

4.2. Lyovapor L-200 settings

After 24 hours of deep freezing the vials were transferred on the tray to the Lyovapor™ L-200 for freeze drying. The general settings are listed in Table 1.
For unknown formulations it is recommended to determine the collapse temperature by means of a freeze drying microscope. Furthermore, a safety temperature margin can be programmed to protect the sample from collapsing.

Table 1: General settings for drying of 5 % aqueous mannitol and NaCl solutions in a Lyovapor™ L-200.

Drying chamber type	Cube	Safety temperature below collapse [°C]	0.0
Sample collapse temperature [C°]	30.0	Gas type	Ambient air

The shelf temperature itself was chosen such that it does not exceed 20 °C at the end of the primary drying and 25 °C at the end of the secondary drying. The steps of the primary and secondary drying process were programmed using the Lyovapor™ Software as listed in Table 2.

During the primary drying phase the bulk solvent, in this case water, is removed from the sample by sublimation. In the secondary drying phase the sample is dried by removing adsorbed solvent. The same programs were applied to freeze dry mannitol and NaCl.

Table 2: Parameters of the primary and secondary drying steps, set on the Lyovapor™ Software.

Step		1	2	3
Phase		Primary Drying	Primary Drying	Secondary Drying
Time	hh:mm	06:00	18:00	00:30
Temperature set point	°C	0.0	20.0	25.0
Temperature gradient	°C/min	0.11	0.02	0.17
Pressure type		Regulated	Regulated	Regulated
Pressure set point	mbar	0.200	0.100	0.050
Safety pressure	mbar	1.500	1.500	1.500
Safety pressure duration	sec	10	10	10

4.3. Halogen moisture analysis

After drying, the residual moisture contents of the samples positioned on the tray diameter were analyzed to assess the drying efficiency. Therefore, the samples were ground in a mortar and transferred to the moisture analyzer within 30 seconds. For residue moisture content analysis, a halogen moisture balance using parameters listed in Table 3 was applied. The switch-off criterion refers to a change of no more than 1 mg / 140 s.

Table 3: Moisture analyser settings.

Switch-off criterion	5
Drying temperature [C°]	110

4.4. Shelf temperature distribution

The temperature distribution on the shelf was controlled by thermocouples installed on the shelf as illustrated in Figure 1. To simulate a freeze-drying process the pressure was set to 0.2 mbar and the shelf heated to 35 °C and kept constant for two hours. Temperature and time were recorded.

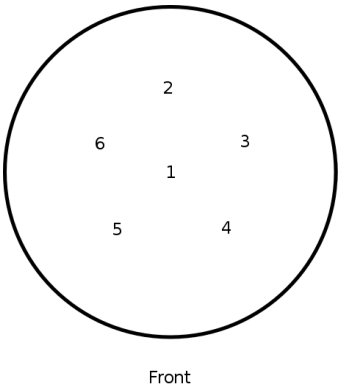

Figure 1. Positions of the thermocouples during the shelf temperature distribution test.

5. Results and Discussion

5.1. Visual evaluation of the mannitol cake in the vials

Figure 2 shows the steel tray with the 70 vials containing freeze dried mannitol after the process. For visual comparison, the vials of the plate diameter are shown in Figure 3. All 70 vials contained a homogenous freeze dried mannitol cake.

Figure 2: Steel shelf with the freeze dried mannitol samples.

Figure 3: Samples analyzed for its residual moisture. The shelf position of the vials is indicated in Figure 2.

In this experiment, mannitol was used in pure form as a model compound to simulate a pharmaceutical formulation. Because freeze dried, mannitol-based pharmaceuticals typically contain other excipients to fulfill the stabilization and release needs of the active pharmaceutical ingredient, the extent of crystallization could be different than for pure mannitol[4].

5.2. Moisture analysis of the freeze dried mannitol and NaCl cakes

To determine the drying efficiency of the Lyovapor™ L-200, the residual moisture content of nine mannitol samples, positioned as indicated in Figure 2, were analyzed using a halogen moisture analyzer. The results of the measured moisture contents and drying efficiencies are shown in Table 4.

Table 4: Results of the residual moisture analysis after freeze drying with the Lyovapor™ L-200.

Vial	Starting weight of freeze dried sample [g]	Weight at end of analysis [g]	Residual moisture content [%]	Drying efficiency [%]
1	0.107	0.105	1.87	98.13
2	0.100	0.098	2.00	98.00
3	0.113	0.112	0.88	99.12
4	0.122	0.121	0.82	99.18
5	0.111	0.110	0.90	99.10
6	0.116	0.115	0.86	99.14
7	0.121	0.120	0.83	99.17
8	0.108	0.107	0.93	99.07
9	0.116	0.114	1.72	98.28

All samples, independent of their position on the shelf, contained no more than 2.0 % moisture after the freeze drying process. Maximum weight loss during residual moisture content determination was 0.002 g which is close to the detection limit of the balance.
We found that the mannitol samples positioned in the middle of the shelf contained marginally less residual moisture than the samples placed on the outer radius of the shelf. For NaCl this pattern was not observed. The residual moisture contents for NaCl varied randomly between 0.79-1.59 %. This may indicate that during the freezing process the fraction of mannitol hydrate formed is greater on the outer than on the inner shelf.
Strategies to remove more moisture are i) increasing the drying time, ii) increasing the temperature during secondary drying and iii) annealing[2].

Furthermore, it is likely, that some water is adsorbed on the sample while preparing it for the residual moisture content analysis. Hence, the drying efficiency is probably higher than reported.

5.3. Shelf temperature distribution

Average temperatures measured during the last 60 minutes of the experiments are given in Table 5. The set value was 35 °C and observed temperatures ranged from 34.7-35.1 °C.

Table 5: Results shelf temperature distribution measurement.

Position on the shelf	Average temperature
1	35.1
2	35.0
3	34.7
4	34.7
5	34.8
6	34.9

6. Conclusion

With the Lyovapor™ L-200, a high drying efficiency was achieved for the water removal of a mannitol model formulation and a NaCl solution. For both compounds, the optical appearance of the dried cake was uniform, and no collapsed cakes were observed. Furthermore, the temperature distribution on the shelf was found to be homogeneous.

7. References

1. Kim, A.I; Akers, M.J.; Nail, S.L. *J. Pharm. Sci.* ***1998****, 87 (8), 931-935.*
2. Yu, L.; Milton, N.; Groleau, E.G.; Mishra, D.S.; Vansickle, R.E. *J. Pharm. Sci.* **1998**, 88 (2), 196-198.
3. Gieseler, H.; Schneid, S.; Kramer, T. *Pharm. Tech.* [Online] **2008**, 32 (3), http://www.pharmtech.com/peer-reviewed-technical-note-quality-design-freeze-drying?id=&sk=&date=&pageID=4 (accessed Jan 31, 2017).
4. Liao, X.; Krishnamurthy, R.; Suryanarayanan, R. Pharm. **2007**, 24 (2), 370-376.