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INTRODUCTION

The first phase in a freeze drying process is freezing the sample to initiate formation of ice which subsequently sublimes under the low pressure applied during primary drying. This can be achieved by several procedures, e.g. putting the sample into a freezer, immerse it in LN_2 or cooling it directly on the shelves of a freeze dryer. Before nucleation (= the initial formation of ice crystals) starts, the sample has usually super-cooled to a more or less extent. Super-cooling means that a solution is held at a temperature below its thermodynamic freezing point without showing nucleation. The degree of super-cooling is defined as the temperature differential between the equilibrium freezing point and the temperature at which ice crystals start to form [(1)]. Nucleation is a random process and the nucleation temperature (and time) may vary in a wide range, compromising batch homogeneity [(3)]. The degree of super-cooling determines the ice crystal size and therefore the pore structure and pore size distribution: greater super-cooling results in smaller ice crystals and vice versa [(1, 2)]. Ice crystal size directly impacts primary drying rate and hence the time required for the primary drying phase in a freeze drying process [(2)].

The criticality of the freezing step for the overall freeze drying process has been known for long time and several approaches have been applied to control nucleation, e.g. ice-fog [(1)], inducing freezing of the solutions from the surface by applying vacuum [(4)], or ultra-sound [(5)]. Drawback of many of the techniques is that they are only suitable in lab scale.
Praxair Inc. recently introduced a method for controlling nucleation which is commercially available for both, lab and production scale freeze drying: ControLyo™ Nucleation on Demand Technology [(2)] (picture 1).
This Technical Note gives a short introduction on how controlled nucleation can reduce primary drying time.

Picture 1:
Lyostar 3 incl. ControLyo™

Material and Methods

Material

5% (w/w) sucrose solutions were prepared by dissolving sucrose (Fisher Chemical) in deionized water (Labchem Inc.). Prior to filling of 10 mL serum tubing vials with 3 mL the solution was filtered through a 0.22 µm filter (Millipore Inc.).

Methods

One Lyostar™ 3 shelf was loaded per run with 166 (run #1) and 164 (run #3) product containing vials, respectively. The outer one to two rows were filled with empty 10 mL vials. Type T thermocouples (TCs) were placed inside and/or outside of center and edge vials (picture 2). Odd numbers represent TCs attached outside whereas TCs with even numbers were inside the vial.

The freeze drying recipes were as follows:

a) **Run #1 (with ControLyo™)**

Freeze / Thermal treatment
- Load at ambient temperature
- cool down to 4°C, equilibrate 60 min
- cool down to -3°C with 0.5°C/min, equilibrate 60 min
- pressurize to 28 psig, hold 45 min
- depressurize to 2 psig, hold 20 min
- cool down to -45°C, hold 180 min

Primary drying
- T_s -45°C, hold 10 min, 57 mTorr
- T_s -20.7°C, ramp rate 0.5°C/min, hold 1260 min, 57 mTorr

Pirani/CM Differential Control was activated with a setpoint of 5 mTorr and a retest time of 5 min.

Secondary drying
- T_s 40°C, ramp rate 0.1°C/min, hold 180 min, 400 mTorr, vacuum ramp time 120 min

b) **Run #3 (no controlled nucleation)**

Freeze / Thermal treatment
- Load at ambient temperature
- cool down to 5°C with 0.5°C/min, equilibrate 30 min
- cool down to -5°C with 0.5°C/min, equilibrate 30 min
- cool down to -45°C with 0.5°C/min, hold 60 min

Note that focus of this work was on primary drying time; therefore a SMART standard protocol was used for freezing and drying parameter setpoints of run #3. Further investigations need to be conducted to optimize the freezing / thermal treatment phase for overall process time reduction.

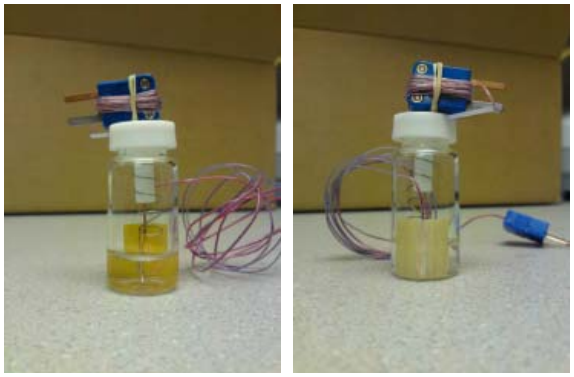
Primary drying
- T_s -45°C, hold 10 min, 57 mTorr
- T_s -20.7°C, ramp rate 0.5°C/min, hold 1260 min, 57 mTorr

Pirani/CM Differential Control was activated with a setpoint of 5 mTorr and a retest time of 5 min.

Secondary drying
- T_s 40°C, ramp rate 0.1°C/min, hold 180 min, 400 mTorr, vacuum ramp time 120 min

Results and Discussion

Figure 1 shows the difference in nucleation behavior when applying ControLyo™ compared to a standard freezing protocol without nucleation control. In controlled nucleation, all vials nucleate at the same time and at the pre-defined nucleation temperature (figure 1a), resulting in uniform temperature profiles of all TCs. In contrast, figure 1b shows a typical random nucleation; the first vial to nucleate super-cools to -7.4°C (TC04), the highest degree of super-cooling shows the vial with TC09 attached at -17.4°C, starting nucleation 27 min later than the first vial.



Picture 2: a) TC inside vial placed bottom center
b) TC fixed outside vial by adhesive tape

Nucleation at lower temperatures leads to smaller ice crystals which form separated and smaller pores, shifting the time needed to sublime all water towards higher values. This could be confirmed in these experiments. Figure 2 depicts the primary drying times determined by Pirani/CM differential. Using Pirani/CM differential control allows safe endpoint detection of primary drying. When activated, the freeze dryer does not advance to the next drying step if the differential of Pirani and CM reading is greater than the setpoint which was 5 mTorr in this case. When the number of water molecules in the chamber decreases, which is the case at the end of the sublimation phase, the Pirani reading decreases and aligns with the CM signal.

As long as sublimation takes place the product temperature does not increase due to the cooling caused by the phase transition. Upon completion of sublimation product temperature increases, resulting in an increase in thermocouple reading towards the shelf temperature.

1° time applying ControLyo™ was 30.5 h compared to 40.4 h using the traditional freezing approach without any control, giving a 10 h time saving (24%).

Pictures 3a and 3b show the lyophilized products. Both conditions result in acceptable cakes with the vials from run #3 (w/o controlled nucleation) showing slight shrinkage. This may be caused by locally high product temperatures due to the slower removal of water through smaller pores. Reconstitution time was <1 min for both products (data not shown).

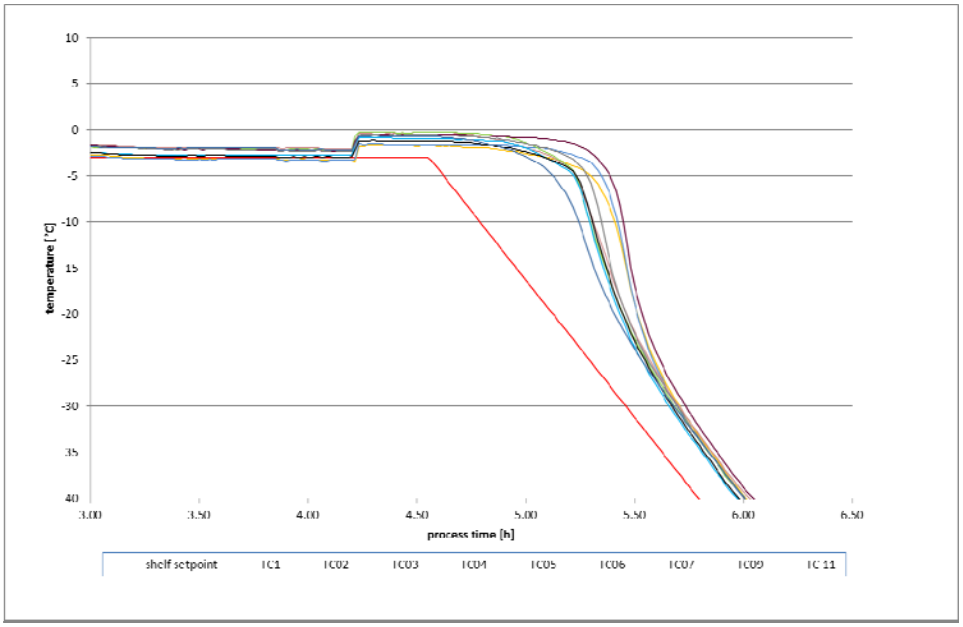


Figure 1a: controlled nucleation of 5% sucrose solution

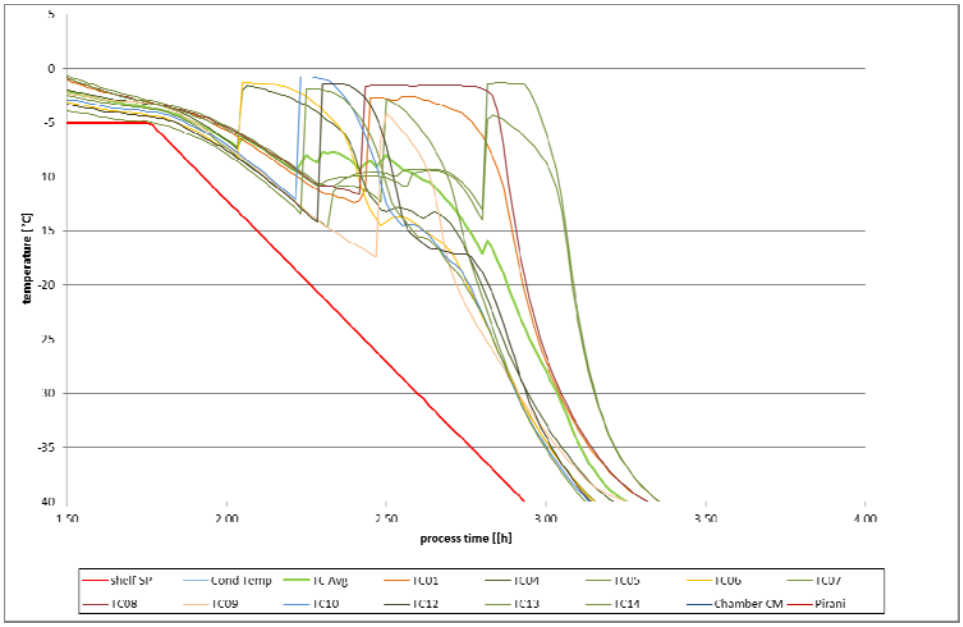


Figure 1b: uncontrolled nucleation of 5% sucrose solution

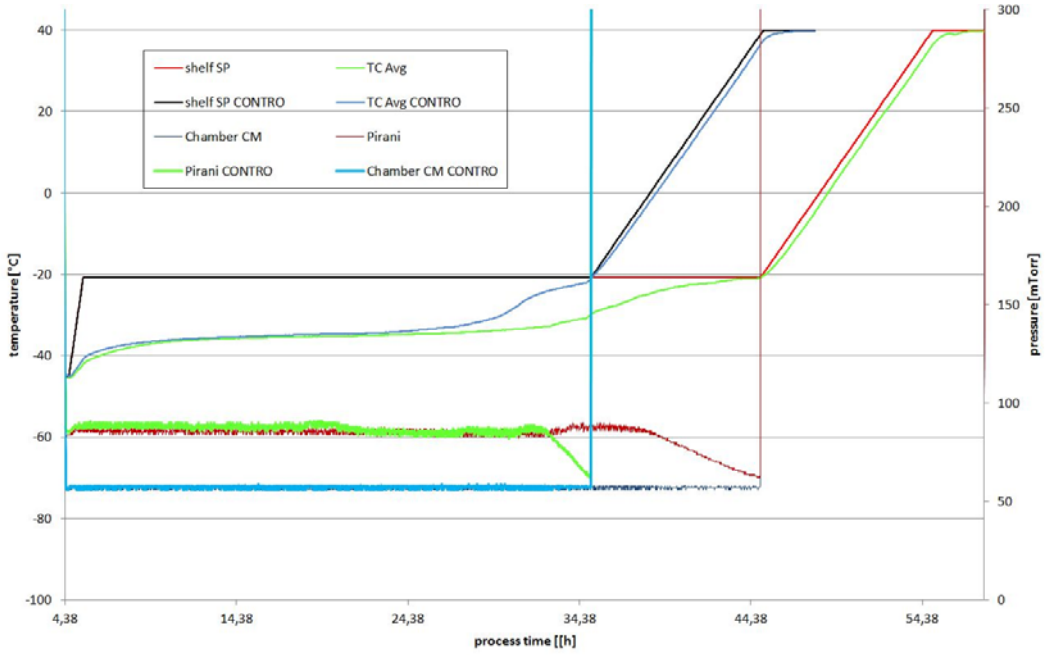


Figure 2: Primary drying times of 5% sucrose with and w/o controlled nucleation

Picture 3a: lyophilized cakes run #1 (ControLyo™)

Picture 3b: lyophilized cakes run #3 (uncontrolled nucleation)

Conclusion

With ControLyo™ Nucleation on Demand Technology for the first time nucleation can be controlled during a freeze drying process both in laboratory and production scale. The experiments introduced in this technical note show how controlled nucleation can shorten primary drying time. Further trials will be conducted to confirm these results also for other products.

References:

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