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Influence of controlled ice nucleation on the freeze-drying of pharmaceutical products: the secondary drying step

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ABSTRACT

Vacuum Induced Nucleation is often discussed in the context of primary drying performances and its tunability, with the potential to tailor the nucleation temperature to the desired porous structure. Instead, here we investigate its influence on secondary drying dynamics and, in particular, on rate of desorption and vial-to-vial inhomogeneity. So as to track the evolution of residual moisture during secondary drying, vials were regularly collected through a vacuum-tight sampling device; the residual moisture and the morphology of the porous cake was then determined by Karl Fischer titration and Scanning Electron Microscopy, respectively. The control of freezing promotes the formation of larger ice crystals and, as a result, accelerates the sublimation of ice and slows down the desorption process. Overall, we found that it reduces the total (primary and secondary) drying time and produces much more uniform batches than those obtained by the conventional freezing, and this positive effect was observed since the end of primary drying. In conclusion, the control of freezing was beneficial to the total drying time reduction, vial-to-vial homogeneity and allowed a better control of product inhomogeneity.

1. Introduction

Freeze drying is widely used in pharmaceutical industry to stabilize labile drugs by solvent sublimation at low pressure; it encompasses three different stages: freezing, primary drying, and secondary drying. During freezing only a part of water freezes and is thus available for sublimation during primary drying; the remaining part is embedded into the solute matrix and is removed via desorption during secondary drying. Typically, secondary drying is carried out at higher shelf temperature than primary drying, and attention has to be paid to gradually increase the shelf temperature so as to avoid any damage to the lyophilized product such as shrinkage and/or collapse (Pisano et al., 2012). Although some textbooks advice to carry out secondary drying at the lowest pressure that can be achieved by the equipment, there is not any scientific-sound evidence supporting that the lowest pressure can actually speed up the desorption process (Pikal et al., 1990).

The final residual moisture of the lyophilized product needs to be precisely controlled, because many products may be damaged by overdrying, which promotes the protein activity loss upon storage, as well as by high values of residual moisture that speed up its degradation over time (Pikal, 1994; Pisano et al., 2013).

The rate of desorption is dramatically impacted by the freezing protocol, because it depends on the specific surface area and thus on the average pore size of the lyophilized product. In the last decade a number of publications focused on the control of the freezing process, showing that it is beneficial to primary drying performance both in terms of drying time reduction and improvement in batch uniformity (Kasper and Friess, 2011; Kramer et al., 2002; Liu et al., 2005; Oddone et al., 2014, 2016). Typically, the controlled freezing methods induce the nucleation event at higher temperature with respect to the conventional freezing, producing larger ice crystals that, on the one hand, speed up primary drying because of lower resistance to vapor flow and, on the other hand, should reduce the specific area of the lyophilized product and, thus, slow down the desorption step. However, it is not yet clear the impact of the controlled freezing on secondary drying even if it has been hypothesized that this technology can have a negative impact on the secondary drying time (Passot et al., 2009). The present work aims to examine in depth this aspect, using the Vacuum Induced Nucleation method (Oddone et al., 2014) as case study. In particular, we have investigated its impact on the rate of desorption, the average residual moisture and its distribution at the end of primary drying,

Abbreviations: SEM, Scanning Electron Microscopy.

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List of symbols

C°	Equilibrium value for the residual moisture (% kg_{water} kg_{dry}^{-1})
C_t	Target value for the residual moisture (% kg_{water} kg_{dry}^{-1})
$\tilde{C}_w$	Residual moisture as measured by Karl Fisher (% kg_{water} kg_{dry}^{-1})
C_w	Residual moisture of the lyophilized product (% kg_{water} kg_{dry}^{-1})
$C_{w,0}$	Residual moisture of the lyophilized product at the end of primary drying (% kg_{water} kg_{dry}^{-1})
E_a	Energy of activation for the desorption process (kJ mol^{-1})
k_0	Pre-exponential factor in Eq. (3), (s^{-1})
k_d	Rate constant for the desorption process (s^{-1})
R	Ideal gas constant (J mol^{-1} °C^{-1})
r_d	Rate of desorption (% s^{-1} kg_{water} kg_{dry}^{-1})
T	Temperature (K)
T_f	Temperature of the heat transfer fluid (K)
t	Time (s)
t_d	Secondary drying time (s)

and at the end of secondary drying as well, and finally on the design space curve for the secondary drying stage (Pisano et al., 2012).

2. Materials and Methods

2.1. Materials

All the tests were carried out using an aqueous solution of mannitol, having 5% by weight as solid content. Mannitol was purchased by Sigma Aldrich (Milan, Italy) and water for injection by Fresenius Kabi (Isola della Scala, Italy). A given volume of this solution (3 mL) was poured into 180 tubing vials (tubing vial 10R, Vidrio Soplado Manuel Perez, Rubì, Spain) that were then loaded directly on temperature-controlled shelves and arranged in clusters of hexagonal arrays.

2.2. Freeze-drying protocol

Two freezing protocols were investigated, the shelf-ramped freezing and the Vacuum Induced Nucleation method. In the case of shelf-ramped freezing, shelf temperature was lowered from room temperature to −45 °C. The cooling rate ranged from 0.5 to 0.8 °C/min. In the case of Vacuum Induced Nucleation, the solution was cooled down to −5 °C and hold at that temperature for 30 min; then, the pressure inside the drying chamber was reduced to 900 Pa and hold for 1 min. During this time, the drying chamber was isolated from the condenser as described in Oddone et al. (2014). The low pressure favored slight evaporation of the solution and its subsequent subcooling that promoted the nucleation of ice. Once nucleation occurred, shelf temperature was lowered to −10 °C and hold for 30 min, promoting the growth of ice crystals (Kramer et al., 2002; Oddone et al., 2014, 2016), and finally decreased to −45 °C and hold for 2 h.

During primary drying, shelf temperature was increased up to −10 °C and hold at the temperature till the completion of ice sublimation, while the pressure inside the drying chamber was set to 10 Pa. The endpoint of primary drying was determined by comparative pressure measurement; an additional soak time of 1 h was used to equilibrate the batch of vials. Secondary drying was carried out at 10 Pa, while shelf temperature was varied from 20 °C to 40 °C. Its duration was 7 h for all the runs.

2.3. Equipment and Instrumentation

Lyophilization cycles have been carried out in a laboratory freeze-dryer (LyoBeta 25, Azbil Telstar, Terrassa, Spain) equipped with capacitance (Baratron type 626A, MKS Instruments, Andover, MA, USA) and thermal conductivity gauges (Pirani type PSG-101-S, Inficon, Bad Ragaz, Switzerland), T-type miniature thermocouples with 0.5 mm diameter wire (Tersid S.p.A., Milano, Italy), and the automatic system, described in Pisano et al. (2010), for the control of the product temperature.

2.4. Residual moisture determination

Various methods have been proposed to control/monitor the residual moisture within a lyophilized product (Kan, 1962; Nail and Johnson, 1992). A simple and effective way to track residual moisture involves the use of a sampling device (Azbil Telstar, Terrassa, Spain), which allows the regular sampling of vials during secondary drying, coupled with one of the several analytical methods available to characterize the lyophilized samples such as: Karl Fischer titration, gravimetric analysis (Tang and Pikal, 2004), NIR spectroscopy (Last and Prebble, 1993), and the equilibrium water vapor measurement (Rey, 2004; Pisano et al., 2013, 2015). Here, in order to track the evolution of residual moisture of the lyophilized product, three vials were collected every 60 min using a sampling device. The sampling device is made of a rod, and a gripper, that makes it possible to pick vials up from the drying chamber and leave them inside a sampling chamber that can be isolated, though a butterfly valve, from the freeze-dryer. Because of the low solubility of lyophilized mannitol in methanol, 3 mL of formamide were added to each vial and then sonicated for 5 min. The water content of these samples was finally measured by Karl Fischer titration (KF Coulometer type DL32, Mettler Toledo, Novate Milanese, Italy). In order to determine the amount of water in formamide, a titration blank has been carried out. The net amount of water in the sample was thus found by subtracting the blank from the sample result.

2.5. Product morphology

The morphology of lyophilized samples was analysed by Scanning Electron Microscopy (FEI Quanta Inspect 200, Eindhoven, Netherlands) at 15 kV under high vacuum. The average pore diameter, and its distribution, within the lyophilized product was examined by using ImageJ software.

2.6. Determination of rate constant for the desorption process

According to literature the desorption process can be described by a first order mechanism (Pikal et al., 1990),

$$\begin{cases} \dfrac{dC_w}{dt} = -k_d(C_w - C^\circ) \\ C_w(t=0) = C_{w,0} \end{cases} \quad (1)$$

where $C_{w,0}$ is the residual moisture of the lyophilized product at the end of primary drying, while C° is the equilibrium water content and was determined from the moisture sorption isotherm of mannitol. The value of $C_{w,0}$ was measured by Karl Fischer over 20 vials collected as soon as secondary drying was started using the "sample thief". This model was initially proposed for an amorphous system, but then has been found to be adequate for crystalline solutes as well.