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Freeze-drying of nanocapsules: Impact of annealing on the drying process

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Abstract

Freeze-drying process was recently applied to improve the long-term storage stability of nanocapsules. Thermal treatment by annealing is an interesting process to optimize a freeze-drying cycle of these colloidal vectors. The objective of this paper is to investigate the impact of annealing on primary and secondary drying characteristics and on nanocapsules (NC) properties. Nanocapsules were prepared from poly-ε-caprolactone (PCL) biodegradable polymer and stabilized by polyvinyl alcohol (PVA), and then freeze-dried with two cryoprotectants: sucrose and poly vinyl pyrrolidone (PVP). Freeze-dried nanocapsules were characterized by size measurement and transmission electron microscopy after reconstitution. The effect of annealing on the kinetics of sublimation, on the mass transfer resistance and on the porosity of the freeze-dried product has been studied in the case of PVP. Finally, the effect of annealing on the kinetic of secondary drying was studied and the results were coupled with the isotherm of sorption. Results showed that PCL nanocapsules could be freeze-dried without any modification of their properties in presence of the two cryoprotectants used. Annealing of nanocapsules suspensions could accelerate the sublimation rate without any modification of nanocapsules size in the case of the two studied cryoprotectants. Such improvement could be explained by the increase of ice crystals size after annealing and by the diminution of mass transfer resistance by the dried layer. The acceleration of sublimation rate seems to depend on the temperature of annealing. The annealing of sucrose solution slows down the secondary drying kinetic whereas no effect is observed in the case of PVP.

Keywords: Nanocapsules; Freeze-drying; Annealing; Sublimation rate; Drying process; Mass transfer resistance

1. Introduction

Freeze-drying is an industrial process used to ensure the long-term stability and to preserve the original properties of pharmaceutical and biological products. This process was recently applied to improve the long-term stability of nanoparticles (Abdelwahed et al., 2006a,b; De Chasteigner et al., 1996). Nowadays, such colloidal vectors have gained a considerable interest because of their ability to ensure a specific drug targeting, especially in the case of cancer therapy (Brigger et al., 2002).

Among the various types of vectors, nanocapsules present a specific problem on drying because of their fragile structure composed of a thin envelope encapsulating an oily or aqueous core. Nanocapsules cannot withstand the freeze-drying stress especially during freezing, the first step of the process, which involves the crystallization of water and the cryo-concentration of dissolved components in the formulation. Such freezing stress can destabilize nanocapsules and induces their aggregation (Abdelwahed et al., 2006a,b; De Chasteigner et al., 1995). Usually, cryoprotectants are added to the formulation to protect the submicronic particles.

Furthermore, the freezing step can impact the texture of the frozen matrix and the final morphological characteristics of the freeze-dried cake (Patapoff and Overcashier, 2002). Thus, the freezing process influences the ice crystal size and, consequently, the primary and secondary drying stages. The porosity and the specific surface of the final cake depend on these stages of the process. In general, the optimization of freeze-drying cycle is aimed at accelerating the sublimation which is the longest step of the whole process.

Chamber pressure and shelf temperature have been well studied as the main factors of the freeze-drying process which can improve the sublimation rate. However, these factors must be well adjusted in order to avoid the product overheating and its collapse.

Searles et al. (2001a) have reported a strong correlation between ice nucleation temperature and primary drying rate of

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freeze-drying. A slower sublimation rate was obtained with a high degree of supercooling which generates a larger number of spherulitic ice crystals and smaller pores in the dried cake allowing water vapor to flow. However, it is difficult to control the ice nucleation temperature because it is widely affected by the environmental particulates and there is a large heterogeneity between samples which induces primary drying rate heterogeneity.

The same researchers have reported that annealing can reduce the sample heterogeneity and accelerate the sublimation rate (Searles et al., 2001b). Annealing is a process in which samples are maintained at a specified subfreezing temperature above the glass transition temperature for a period of time.

The objective of this paper is to investigate the impact of annealing on primary and secondary drying characteristics and on nanocapsules (NC) properties. Nanocapsules were prepared from poly-ε-caprolactone (PCL) and stabilized by polyvinyl alcohol (PVA), and then freeze-dried with two cryoprotectants: sucrose and polyvinyl pyrrolidone (PVP). Freeze-dried nanocapsules were characterized by size measurement after re-dispersion in water. The glass transition temperature of maximally cryo-concentrated nanocapsules suspension was determined using differential scanning calorimetry for the two cryoprotectants. The effect of annealing on the rate and the kinetics of sublimation, on the mass transfer resistance, and on the pore size distribution of the dried cake has been studied in details for the case of PVP. Finally, the effect of annealing on the kinetic of secondary drying was studied and the results were interpreted with the help of the isotherm of sorption.

2. Materials and methods

2.1. Materials

Poly-ε-caprolactone (PCL) (M_w: 14 000 g/mol) was obtained from Sigma–Aldrich (France). Polyvinyl alcohol (PVA) Mowiol 4–88 (88% hydrolysed, M_w 31 000 g/mol) was purchased from Clariant (France). Miglyol 829 was supplied by Condea chemie (Germany). Ethyl acetate was obtained from Carlo Erba (France). D(+) sucrose from Prolabo (France). Polyvinyl pyrrolidone (povidon 25, PVP) was purchased from Merck (Germany). All other reagents were of analytical grade and water was purified by Alpha-Q ultra-pure water system (Millipore, Ireland).

2.2. Methods

2.2.1. Preparation of nanocapsules

Nanocapsules (NC) studied in this work were prepared by the emulsification–diffusion method (Quintanar-Guerrero et al., 1998). Briefly, 0.2 g of PCL and 0.5 g of Miglyol 829 were dissolved in 20 mL of ethyl acetate saturated with water. This organic phase was emulsified with 40 mL of aqueous phase, saturated with ethyl acetate, containing 2 g PVA using a high speed homogeniser Ultra-turax T 25, Ika (Germany) at 8000 rpm for 10 min. Thereafter, 200 mL of deionised water was added to the emulsion to induce the diffusion of ethyl acetate into the aqueous phase leading to the formation of nanocapsules. The organic solvent and a part of water were evaporated under reduced pressure, yielding 40 mL of a concentrated suspension.

An additional purification step was applied to eliminate the free stabilizer in solution. This step was carried out by washing of nanocapsules twice using deionised water after their separation via ultra-centrifugation at 50 000 rpm and 20 °C for 30 min (Abdelwahed et al., 2006a). The purified nanocapsules were re-suspended in deionised water.

2.2.2. Particle size measurement

The size of nanocapsules both before and after freeze-drying was determined by photon correlation spectroscopy (PCS) using Zetasizer 3000 HSa (Malvern, England) at 25 °C. Each measurement was performed in triplicate.

2.2.3. Freeze-drying of NC

The lyophilization of nanocapsules was performed using a pilot freeze-dryer Usifroid SMH45 (Usifroid, France). It consists mainly of three stainless-steel shelf plates ($3 \times 0.15\ m^2$), a coiled tube used as a condenser at -65 ± 5 °C and a vacuum pump. The operating pressure is regulated by air injection through a micro-valve. The apparatus is equipped with different control instruments: seven thermocouples to measure the product temperature and two platinum probes to measure the temperature of shelves and condenser. The total pressure is measured by a capacitive sensor within the range between 0 and 100 Pa. The partial pressure of water in the lyophilization chamber is measured by a hygrometer to determine the end of the sublimation step. The conditions applied during the present study were: freezing for 2 h at −45 °C with a temperature ramp of 1 °C/min, sublimation at −15 °C and 10 Pa for 15 h and finally the secondary drying was carried out at 25 °C and 5 Pa for 6 h.

The 0.5 mL of nanocapsules suspension and 0.5 mL of cryoprotectant solution were filled into 5 mL freeze-drying moulded vials (Fisher bioblock scientific, France) so as to reach a final concentration of cryoprotectant of 5% (w/v). Cryoprotectants used in this study were sucrose and polyvinyl pyrrolidone.

When the thermal treatment by annealing was applied, nanocapsules suspensions were first frozen at −45 °C with a temperature ramp 1 °C/min. Then annealing was applied by heating the frozen samples to different annealing temperatures: −10, −15 and −20 °C and the temperature was held for 1 h at this fixed temperature. Finally, the samples were cooled to −45 °C and the samples were freeze-dried under the same conditions as mentioned previously.

2.2.4. Transmission electron microscopy (TEM)

Morphology and structure of the freeze-dried nanocapsules after reconstitution were studied in a Topcon 002B microscope operating at 200 kV giving pictures with a 0.18 nm resolution. In order to perform the TEM observations, the concentrated suspension of nanocapsules was first diluted in water. After this a drop of the diluted NC suspension was directly deposited on a standard copper grid covered by a holy carbon film and dried at ambient temperature before observation.

2.2.5. Scanning electron microscopy (SEM)

Sample preparation for the observation of porous structure of freeze-dried plugs was performed as follows: the completely freeze-dried plug was extracted from the glass vial and rapidly cut into pieces using a single-edged razor blade. Either whole plugs or pieces of plugs were attached to SEM specimen mounts using silver paint. The specimens were coated with gold/palladium with a cathodic pulverizer technics Hummer II (6 V–10 mA). Imaging was realized on a FEG Hitachi S800 SEM at an accelerating voltage of 15 kV. The recorded images were analysed to estimate the pore area and the equivalent diameters.

2.2.6. Thermal analysis

The glass transition temperature of maximally cryo-concentrated suspensions (T'_g) was measured by thermal analysis performed by a differential scanning calorimeter DSC TA 125 (TA instrument, USA). A heating rate of 10 °C/min was applied throughout the analysis in the temperature range of −100 to 30 °C. The instrument was calibrated with indium for melting point and heat of fusion.

2.2.7. Determination of mass transfer resistance

The resistance to vapor flow through the dried layer in the vial was determined using a method published by Overcashier et al. (1999). The resistance to mass transfer was estimated as a function of the dried layer thickness using Eq. (1) and the measurable quantities sublimation rate, product temperature, and vial dimensions:

$$R_p = \frac{A_p}{\dot{m}} \times \left(2.7 \times 10^{13} \times \exp\left[\frac{-6145}{T_p - \frac{\Delta H_S \times \dot{m}}{A_v \times K_I} \times (L - l) + 273.15} \right] - P_C \right) \quad (1)$$

R_p is the normalized dried product resistance ($cm^2\,Pa\,h\,g^{-1}$), A_p the cross-sectional area of product in the vial (cm^2) $\dot{m}$ the sublimation rate (g/h), T_p the product temperature at the temperature sensor (°C), ΔH_s the heat of sublimation of ice (cal/g), A_v the cross-sectional area of vial base (cm^2), K_I the thermal conductivity of the product ($W\,m^{-1}\,K^{-1}$), Pc the chamber pressure (Pa), L the thickness of product, from temperature sensor to initial fill height (cm), and l is the thickness of dried layer (cm) determined by Eq. (2):

$$l = \frac{\dot{m}t + m_0}{pA_v} \quad (2)$$

t is the elapsed time, measured from the time at which the shelf temperature reaches the set point (h), m_0 the weight loss at $t = 0$ (g), p the frozen matrix density (g/cm^3), and A_v is the cross-sectional area of vial base (cm^2).

A solution of PVP at 5% (w/v) was used in this study. One milliliter of this solution was filled into a 3 mL tubed freeze-drying vial. Samples were removed from the freeze-drier at time t using device that did not distributed the vacuum inside the chamber. Sublimation rate was calculated using the weight lost at time t. Sublimation was carried out at −30 °C and 6 Pa.

2.2.8. Secondary drying kinetic

Secondary drying kinetic was studied for the two cryoprotectants PVP and sucrose, respectively. For each excipient, two cycles were compared, one with annealing step and another without this thermal treatment. In the case of PVP a complete freeze-drying cycle was carried out, and the samples were subsequently stored at 25 °C and 15% of relative humidity for one week to bring the samples to uniform water content. In the case of sucrose, the kinetic of secondary drying was studied directly after the sublimation step because such storage might cause the collapse of the dried cake. At regular intervals, three samples were removed from the freeze-drier and the residual humidity was quantified by Karl Fischer titration (Metler Toledo titrator DL38, Suisse) using methanol as a solvent.

2.2.9. Sorption isotherms

The sorption isotherm of PVP and sucrose were carried out by equilibrating freeze-dried samples over saturated salts which generated different relative humidities at 25 °C. This study was performed during 1 month for PVP and during 3 days for sucrose to preserve the amorphous state of sucrose at low relative humidity. The used salts were: LiCl, $MgCl_2$, KCO_3, $MgNO_3$, NaCl, KCl, $BaCl_2$ which respectively gave the following values of relative humidity: 11, 33, 43, 53, 75, 84 and 90%.

2.2.10. Statistical analysis

Differences in primary drying kinetic, freeze-dried product porosity, and secondary drying kinetic between the different conditions of freezing were determined by Student's t-test (Microsoft Excel Software). Differences were considered significant if $P < 0.05$.

3. Results and discussion

3.1. Freeze-drying of nanocapsules

Poly-ε-caprolactone nanocapsules prepared by emulsion–diffusion of solvent had a diameter of about 311 nm. The polydispersity index of 0.06, indicated a monodisperse colloidal suspension. Freeze-drying process generates a variety of stresses which tend to destabilize unprotected nanocapsules. These nanocapsules have a fragile envelope that may not withstand these stresses. Nanocapsules aggregation and the formation of macroscopic particles were noticed after freeze-drying of nanocapsules without cryoprotectant (Table 1). The use of cryoprotectants seems to be necessary, two different types of cryoprotectants have been proposed: sucrose and polyvinyl pyrrolidone (PVP).

Measurements of nanocapsules diameter, polydispersity index and the ratio of nanocapsules size after and before freeze-drying confirmed the conservation of nanocapsules after rehydration when the two excipients sucrose and PVP were used (Table 1). This result indicated a successful freeze-drying of nanocapsules.

Fig. 1 shows a TEM observation of freeze-dried nanocapsules after reconstitution. It is clear from this image that nanocapsules

Table 1
Nanocapsules diameter size and polydispersity index measurements before and after freeze-drying without and with cryoprotectants

Cryoprotectant	Before freeze-drying		After freeze-drying		S_F/S_I
	Size ± S.D. (nm)	P.I. ± S.D.	Size ± S.D. (nm)	P.I. ± S.D.	
Without			Macroscopic particles	–	–
Sucrose	311.3 ± 2.2	0.06 ± 0.01	304 ± 2.42	0.05 ± 0.02	0.96
PVP			301 ± 3.92	0.05 ± 0.02	0.97

P.I.: polydispersity index; S_F/S_I: ratio of PCL NC size after and before freeze-drying; S.D.: standard deviation. PCL NC prepared with 5% (w/v) of cryoprotectant. Data are presented as the mean ± S.D. ($n = 3$).

were spherical in shape and well conserved after freeze-drying. The thin polymeric membrane was intact around the oily core of nanocapsules. An amorphous matrix of PVP could be observed at the outer surface of NC. The formation of such matrix could prevent the fusion and the aggregation of nanocapsules during the drying process (Abdelwahed et al., 2006b).

During the freezing step, there are dramatic changes of the frozen NC suspension. The ice crystallization leads to a phase separation and cryo-concentration of nanocapsules. Such modification can damage labile bioproducts and probably induce aggregation or fusion of nanocapsules. Suitable cryoprotectant can prevent such damage. It has been suggested that stabilization of liposomes and nanoparticles during freeze-drying, requires only that they be maintained in a vitrified state (Abdelwahed et al., 2006a,b; Crowe et al., 1994; De Chasteigner et al., 1996). Carbohydrates are widely used for the protection of nanoparticles and liposomes during freeze-drying. Such excipients can be easily vitrified during freezing and form a protective amorphous matrix around nanoparticles.

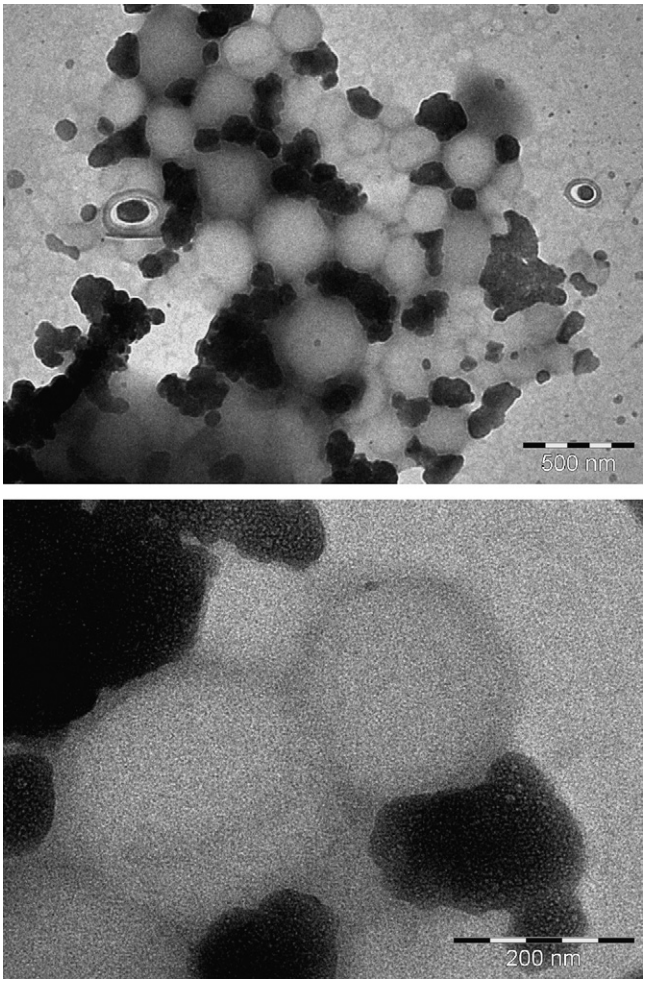

Fig. 1. TEM observation of freeze-dried nanocapsules with 5% (w/v) of PVP after reconstitution. This observation was performed without negativation.

3.2. Impact of annealing on primary drying characteristics

3.2.1. Effect on sublimation rate

The manner of freezing can have a significant impact on primary drying characteristics; In order to quantify this impact, PCL NC prepared with two cryoprotectants sucrose and PVP have been freeze-dried with applying four freezing protocols (see Section 2.2.3): freezing without annealing and freezing with annealing during 1 h at three different temperatures −10, −15 and −20 °C.

It has been found that annealing did not modify significantly nanocapsules size in the case of the two cryoprotectants used and the ratios of nanocapsules size after and before annealing at the different temperatures (S_F/S_I) were about 1 (data not shown).

The impact of annealing on the sublimation rate of nanocapsules using sucrose and PVP as protectants is presented in Table 2. It is clear that the process which did not include annealing produced the lowest sublimation rate: 87.9 mg/h for sucrose and 70 mg/h for PVP, whereas, annealing produces the highest sublimation rate. In the case of sucrose, the sublimation rate increases from 86.7 to 124.8 mg/h, whereas in the case of PVP, the sublimation rate was increased from 77 to 83.9 mg/h when increasing the temperature of annealing from −20 to −10 °C. Annealing at −10 °C could increase the sublimation rate to

Table 2
Sublimation rate of two suspensions of nanocapsules protected with: sucrose and freeze-dried in moulded vials and PVP freeze-dried in tubed vials without annealing and at three different annealing temperatures

Samples	Sublimation rate (mg/h)	
	Sucrose: moulded 5 mL vials	PVP: tubed 3 mL vials
Without annealing	87.9 ± 6.4	70 ± 3.1
−20 °C	86.7 ± 4.2	77 ± 7.3
−15 °C	101.3 ± 9.3*	73 ± 2.7
−10 °C	124.8 ± 6.2*	83.9 ± 2.5*

Data's are presented as the mean ± S.D. ($n = 3$); *$P < 0.05$ relative to the corresponding sample without annealing.

Table 3
Glass transition temperatures (T'_g) of cryoprotectant solutions with and without nanocapsules

Cryoprotectant[a]	T'_g (°C)	
	Aqueous solutions	Suspension of PCL NC
Sucrose	−30.78 ± 1.02	−32.26 ± 0.98
PVP	−21.67 ± 0.36	−21.52 ± 0.35

Data's are presented as the mean ± S.D. ($n = 3$).
[a] Concentration of cryoprotectant 5% (w/v).

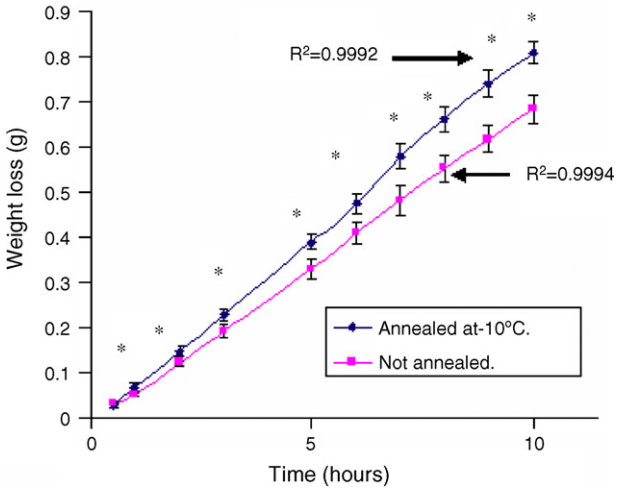

Fig. 2. Weight loss kinetic during primary drying of 5% (w/v) nanocapsules protected with PVP without annealing and with annealing at −10 °C. Data are presented as the mean ± S.D. ($n = 6$). $^{*}P < 0.05$ relative to the sample without annealing.

about 30 and 17% in the case of sucrose and PVP, respectively. These differences are due to the glass transition temperature of the excipients. The T'_g of PVP and sucrose are −21.67 and −30.78 °C, respectively (Table 3) which means that the three chosen temperatures of annealing are well above the T'_g of

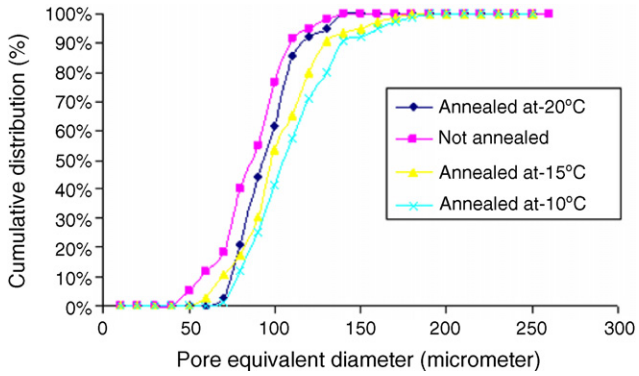

Fig. 4. Pore size cumulative distribution of freeze-dried PVP with applying four freezing protocols: freezing without annealing and freezing with annealing during 1 h at three different temperatures −10, −15 and −20 °C.

sucrose than to the T'_g of PVP. Our results are in agreement with those presented by Searles et al. (2001b). They have been found that annealing below the T'_g could not increase the rate of sublimation.

Fig. 2 presents the amount of sublimed ice during freeze-drying of NC suspensions containing PVP at 5% (w/v) without and with annealing at −10 °C. The amount of ice sublimed from vials was approximately linear with respect to time in the case of samples annealed at −10 °C and not annealed (coefficient of correlation is 0.999). The acceleration of drying kinetic by annealing is obvious from this figure.

3.2.2. *Effect on porosity of freeze-dried product*

Fig. 3 shows scanning electron micrographs of freeze-dried PVP without annealing and with annealing at −10 °C. It can be observed that the two plugs had a leafy amorphous appearance consisting of an irregular array of large pores which had been previously occupied by ice.

Fig. 4 presents the cumulative distribution of the pores size for a freeze-dried sample of PVP obtained without and with

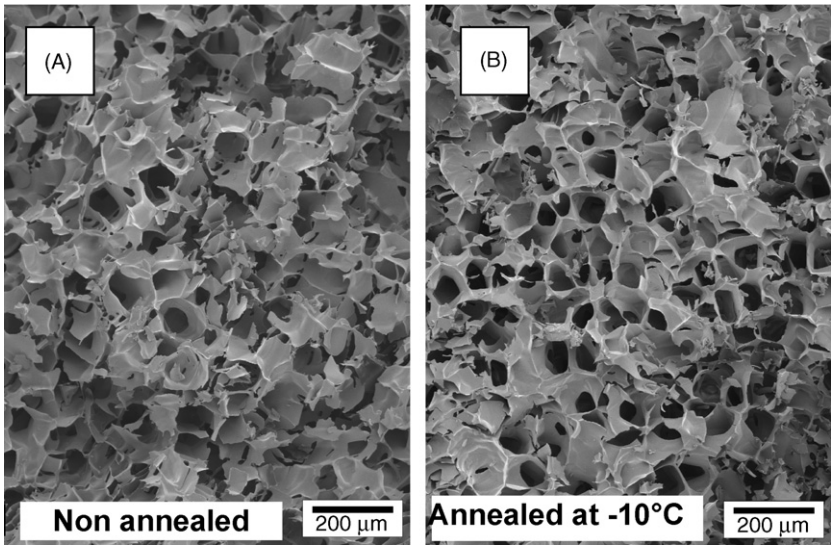

Fig. 3. Structure of freeze-dried PVP without annealing (left) and with annealing at −10 °C (right). Note that the two plugs had a leafy amorphous appearance consisting of an irregular array of large pores which had previously occupied by ice (magnification 60×).

Table 4
Pore equivalent diameter and rate of dried layer thickness progress of PVP solution freeze-dried without annealing and at three different annealing temperatures

Samples	D equivalent (μm)	Rate of dried layer thickness progress (m/s)
Without annealing	86.16 ± 19.9	$1.58 \times 10^{-7} \pm 8.2 \times 10^{-9}$
−20 °C	94.68 ± 16.6*	$1.75 \times 10^{-7} \pm 16.8 \times 10^{-9}$
−15 °C	101.84 ± 24.7*	$1.66 \times 10^{-7} \pm 7.02 \times 10^{-9}$
−10 °C	108.95 ± 25.6*	$1.89 \times 10^{-7} \pm 5.76 \times 10^{-9}$*

Data's are presented as the mean ± S.D. ($n = 75$ for pore size determination, $n = 6$ for the determination of the rate of dried layer thickness); *$P < 0.05$ relative to the corresponding sample without annealing.

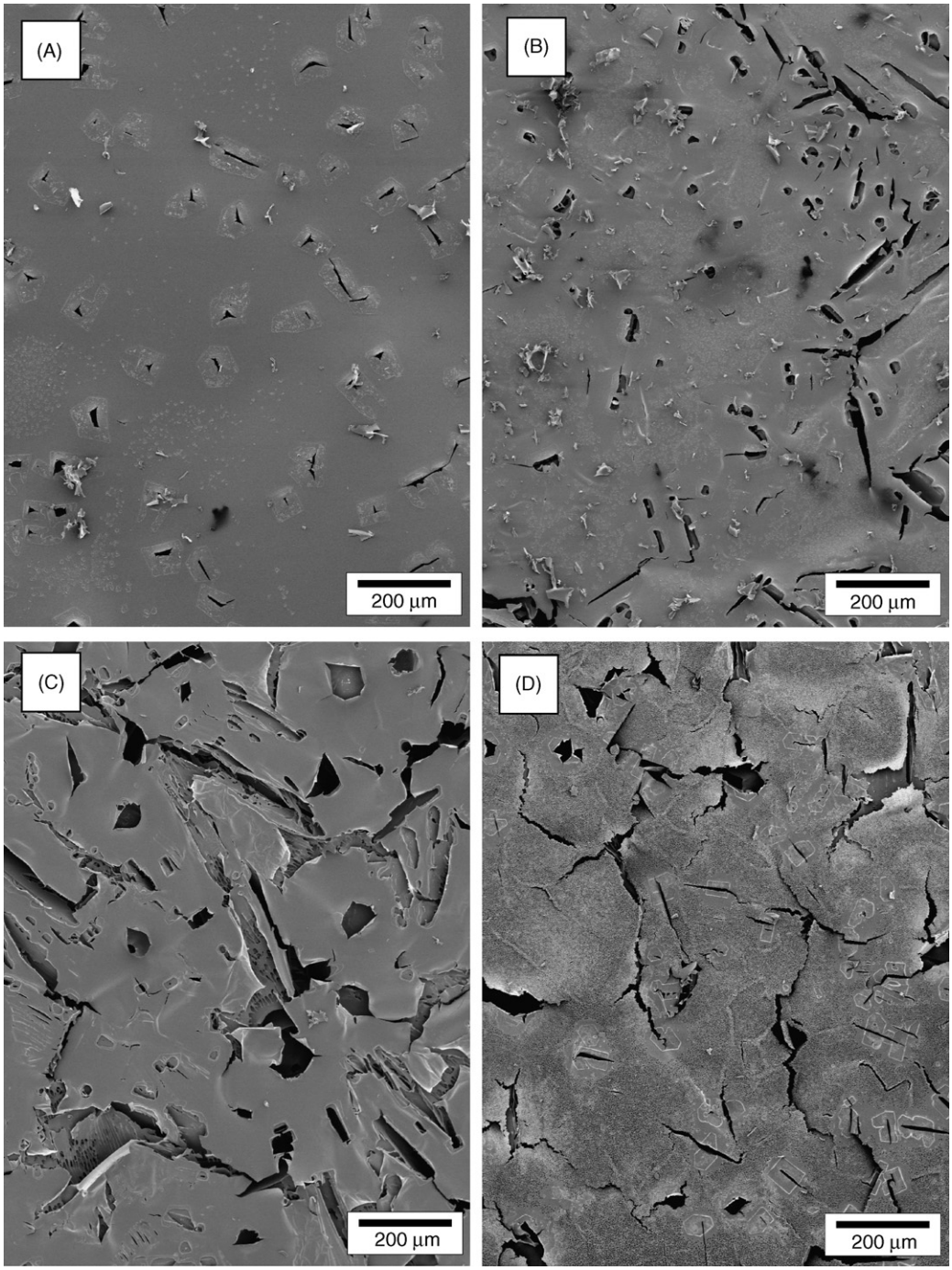

Fig. 5. SEM micrograph of the top surface of freeze-dried PVP with applying four freezing protocols: freezing with annealing during 1 h at three different temperatures: (A) −10 °C, (B) −15 °C, (C) −20 °C and (D) freezing without annealing (magnification 60×).

annealing at different temperatures. It can be seen that 90% of the population has an equivalent diameter less than 110 μm in the case of a sample freeze-dried without annealing whereas 90% of the population has an equivalent diameter less than 140 μm in the case of annealed sample at −10 °C.

Table 4 presents the values of equivalent diameter of pores of samples for each annealing process. According to the previous results, we can conclude that the annealing permits to increase the pore size in the freeze-dried product which improves the diffusion of water vapor flow across the dried mass. These results are in agreement with the results obtained by Hottot et al. (2004). Using an episcopic axial lighting microscope as a direct characterization method of the ice crystals size, these authors found that an annealing treatment of 3 h at −10 °C increased the average pore diameters (+40%) of a pharmaceutical protein formulation and decreased the primary drying time (−16%). Searles et al. (2001b) have reported enhancements in drying rate up to 3.5 fold after annealing; a significant rate enhancement could be gained with only 30 min of annealing.

A thin skin about 4 μm was present at the top surface of the plug of PVP freeze-dried with annealing at different temperatures and without annealing because of solute cryo-concentration. Cracks and pits in the top skin were presumably caused by the escape of water vapor from the preparation during ice sublimation (Fig. 5). Such results suggest that the thermal treatment by annealing does not cancel completely the formation of skin at the top of freeze-dried plug. However, these cracks and pits were larger and more numerous in the case of freeze-drying without annealing and with annealing at −20 °C (Fig. 5C and D), presumably because of the more important resistance to vapor escape from the cake by the top skin. Searles et al. (2001b) observed that annealing opened up holes on the surface of the lyophilized cake. This would occur via drainage of the amorphous film into adjacent junctions.

Annealing is often used in freeze-drying to induce the crystallization of active ingredients and excipients. Annealing has also important effect on the size distribution of ice crystals within a frozen sample. It was supposed that annealing increased the ice crystals size by Ostwald repining. This is a phenomenon by which dispersed crystals smaller than a critical size decrease in size whereas the larger grow. For amorphous solutes, annealing above T'_g will result in melting of ice into adjacent non-ice region as the system follows the freezing-point depression curve. The bulk mobility of the amorphous phase and diffusional mobility of all species in that phase is increased by the increased water content and the higher temperature. This phenomenon allows the system to relax into a physical configuration of lower free energy.

Ostwald ripening controlled by bulk diffusion for nonionic species has been characterized by Eq. (3) (Searles et al., 2001b):

$$\frac{dr}{dt} = \frac{D\upsilon}{r}\left[(C - C^*) - \frac{2\upsilon\gamma C}{RTr}\right] \quad (3)$$

where r is the ice crystal radius, t the time, D the diffusion coefficient of water through the amorphous phase, υ the molecular volume of water, γ the interfacial tension between ice and the amorphous phase, and C and C^* are the average bulk amorphous phase water concentrations at the current time and under equilibrium conditions, respectively.

3.2.3. Effect on mass transfer resistance

The resistance to mass transfer can be defined as the ratio of driving force to flow rate. The driving force for the diffusion of water vapor through the small pores of a dried product is the difference between the equilibrium vapor pressure of subliming ice and the partial pressure of water above the dried product (Pikal et al., 1984). In general, the flow of water vapor is impeded by three resistances during the sublimation step: resistance of the dried product layer, resistance of the semi-stoppered vial and resistance of the chamber of lyophilization. The dried product resistance seems to be the most important factor then its decrease will increase the rate of sublimation and hence the time for the primary drying phase of the cycle of freeze-drying.

Fig. 6 shows the resistance to mass transfer as a function of dried layer thickness of a solution of PVP freeze-dried without annealing and with annealing at three different temperatures. It can be seen that the measured resistance to mass transfer increases with increased dry layer thickness as reported by several authors (Overcashier et al., 1999; Hottot et al., 2005). The resistance increased linearly at the beginning of the sublimation, which indicated non-significant skin resistance at the product surface. It could be clearly seen that the resistance to mass transfer is significantly reduced after the application of annealing. The lower resistance was observed with annealing at −10 °C. This result was in agreement with the pore size distribution described previously. It is clear that the thermal treatment by annealing leads to an increase in pore size and reduces the resistance to mass transfer during ice sublimation. Similar results have been obtained by several authors who freeze-dried sucrose (Chouvenc, 2004) and proteins (Hottot et al., 2005). They used the pressure rise analysis method to determine the resistance to mass transfer and found that annealing decreased significantly this resistance.

Moreover, the dried layer thickness progressed more rapidly in the case of a freeze-drying cycle with an annealing treatment at −10 °C where the rate is of 1.89×10^{-7} m/s against 1.58×10^{-7} m/s without annealing (Table 4).

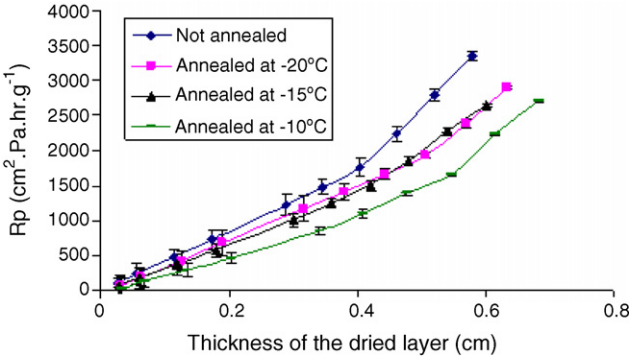

Fig. 6. Resistance to mass transfer as a function of freeze-dried layer thickness for formulated PVP at different annealing temperatures. Data are presented as the mean ± S.D. ($n = 6$).

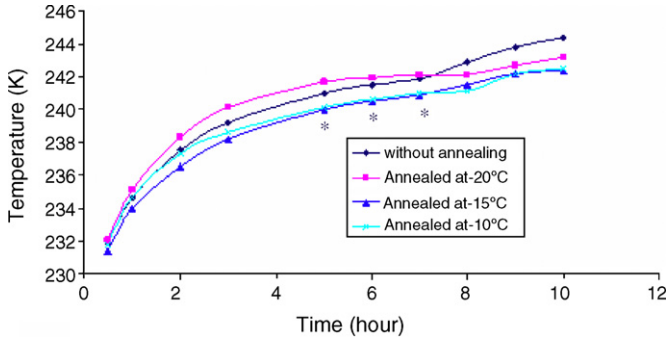

Fig. 7. Product temperature during primary drying of formulated PVP at different annealing temperatures. Data are presented as the mean ± S.D. ($n = 3$). $^{*}P < 0.05$, a significant differences have been observed between samples obtained without annealing compared to samples annealed at −10 °C.

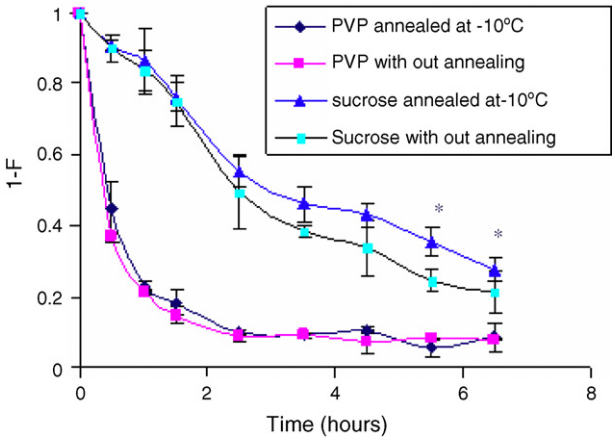

Fig. 8. Secondary drying kinetic of PVP and sucrose with annealing at −10 °C and without annealing. Data are presented as the mean ± S.D. ($n = 6$). $^{*}P < 0.05$ relative to the samples prepared without annealing using sucrose as protectant agent.

The product temperature profiles are similar except at the end of sublimation when the temperature increased slowly in the case of annealing but increased faster when annealing was not applied. Thus, the homogenization of ice crystal size through annealing treatment prevented the overheating of the product since the product temperature stabilized all along the sublimation around 241 K (Fig. 7).

It has been found that annealing could increase the primary drying time and the resistance of dried layer to mass transfer in mannitol–trehalose–sodium chloride based formulations (Xiaofeng and Pikal, 2004). Such phenomenon was explained by the fact that annealing induced both mannitol and NaCl to crystallize and prevented the partial collapse observed without annealing. The highly crystallized mannitol blocked the pathway for water vapor escape, contributing to the increase in the dry layer resistance and thus the longer times for primary drying.

3.3. Impact of annealing on secondary drying

Secondary drying refers to removal of unfrozen water which is adsorbed on the surface of the crystalline product or dissolved in an amorphous solid as a solid solution. It is well known that the secondary drying rate increases with an increase of product specific surface area (Pikal et al., 1990). As annealing increases the pore size in dried products, it decreases the specific surface area of pores and consequently a slower secondary drying is expected.

Fig. 8 shows the kinetic of secondary drying for PVP and sucrose samples with annealing at −10 °C and without annealing. Water content is expressed in terms of fractional attainment of equilibrium, F. Thus, the ordinate, $1 - F$, is a normalized measure of residual water and is defined as the fraction of initial water remaining in the sample at the time indicated (Pikal et al., 1990). For PVP sample and during the first hour, there is a sharp decrease in residual humidity indicating a rapid removal of unfrozen water. After that, a slower drying can be seen and finally the curve reaches a plateau. The plateau level of residual water decreases sharply as the drying temperature increases, and the attainment of low levels of residual water may require high secondary drying temperatures (Pikal et al., 1990). It could be observed from Fig. 8 that annealing did not slow down the drying kinetic in the case of PVP.

In the case of sucrose, the sharp decrease in the residual humidity during the first hour has not been found. A slower secondary drying rate was observed with sucrose compared to PVP. Furthermore, a significant difference (after 4.5 h of drying) between the secondary drying kinetics of annealed and non-annealed sucrose has been observed, which was related to the decrease in the specific area of annealed cake. It seems that annealing slow down the secondary drying kinetic of sucrose.

Sorption isotherm of water study was realized in order to determine on the one hand the degree of hygroscopicity of the formulation and on the other hand to assess the ease in secondary drying. In general, the easier the water adsorption, the easier the water desorption (Wang, 2004).

For PVP, the sorption isotherm was of S type which indicated a very hygroscopic product (Fig. 9). Therefore, the desorption of water during the secondary drying was easy and fast and the effect of surface area was possibly negligible. This could explain that the two curves of drying kinetic were similar.

The sorption isotherm of sucrose exhibited a different profile. At relative humidity below 43% sucrose absorbs less water

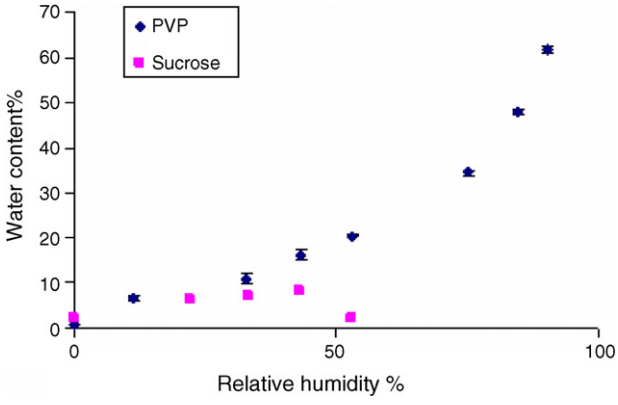

Fig. 9. Water vapor sorption isotherms of freeze-dried PVP and sucrose. Data are presented as the mean ± S.D. ($n = 3$).

vapor than PVP. However, desorption of moisture was observed when sucrose was stored at a relative humidity above 43%. It was reported in the literature that the observed desorption of moisture was accompanied by the crystallization of sucrose (Fakes et al., 2000). The conversion of amorphous sucrose to its crystalline form could be caused by moisture as well as by heat. It is clear from this isotherm that sucrose is less hygroscopic than PVP. Such a difference of hygroscopic property may explain the difference in the secondary drying kinetics of the two products. More rapid water desorption kinetic was obtained with PVP. Also, the effect of annealing on this kinetic was more obvious in the case of sucrose as explained above.

4. Conclusion

In conclusion, this study demonstrated that the freeze-drying of fragile structure like nanocapsules is possible, the use of cryoprotectants (sucrose and PVP) was necessary. After freeze-drying, nanocapsules conserve their physicochemical properties. The impact of annealing on primary and secondary drying was studied. The results showed that annealing has an impact on primary drying by accelerating the sublimation rate without any modification of nanocapsules size in the case of the two cryoprotectants used. Such improvement could be explained by the increase of the size of ice crystals after annealing and by the diminution of mass transfer resistance by the dried layer. The acceleration of sublimation rate depended on the temperature of annealing. The influence of annealing on secondary drying is dependent on the type of cryoprotectant used. We demonstrated that annealing of sucrose solution slows down the secondary drying kinetic whereas no effect is observed in the case of PVP.

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