Research Article

Lyophilization and stability study of noscapine-loaded polycaprolactone nanoparticles

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ABSTRACT

Aim: Lyophilization or freeze-drying is a dehydration process used to overcome the instability of nanoparticles suspension, increasing its shelf-life, and simultaneously facilitating its handling and storage. This study aims to determine the effect of different cryoprotectants, lyophilization process on their physiochemical properties before and after lyophilization of noscapine-loaded polycaprolactone (PCL) nanoparticles and to evaluate the effect of lyophilization on storage of noscapine-loaded nanoparticles at real time, ambient temperature, and accelerated storage conditions. **Materials and Methods:** Noscapine-loaded PCL nanoparticles were prepared by double emulsion solvent evaporation method. Freeze-drying microscopy (FDM) used to evaluate the collapsed temperature of sucrose, dextrose, mannitol, and D-sorbitol. Freeze-drying cycle performed using benchtop lyophilizer for freeze-drying cycle optimization. Stability study performed for 6 months at real time, ambient, and accelerated conditions. **Results and Discussion:** Collapse temperature is important parameter in the development of freeze-drying cycle of nanoparticle and is evaluated using FDM for four cryoprotectants. The primary drying at −35°C and −30°C and secondary drying at +20°C were performed. The lyophilized noscapine-loaded PCL nanoparticles (CBY3H2) with sucrose as cryoprotectant were subjected to real time showed no change in particle size, zeta potential, and entrapment efficiency. Slight increase in particle size and decrease in entrapment efficiency at ambient and accelerated storage conditions for 6 months. **Conculsion:** The noscapine-loaded PCL nanoparticles successfully formulated, screened for cryoprotectants for lyophilization. The effect of cryoprotectant on lyophilization process studied and finalized the freeze-drying cycle. The optimized lyophilized noscapine-loaded PCL nanoparticle showed better stability in real-time stability than ambient and accelerated storage conditions.

KEY WORDS: Freeze drying microscopy, Lyophilization, Noscapine, Poly caprolactone, Stability study

INTRODUCTION

Lyophilization or freeze-drying is a dehydration process used to overcome the instability of nanoparticles suspension, increasing its shelf-life, and simultaneously facilitating its handling and storage.[1,2] Lyophilization is commonly used to improve the long-term stability of nanoparticles.[3] A good lyophilizate should have some important characteristics, namely, maintain the physical and chemical properties of the original product and obtain a cake with good aspect, short reconstitution time, low residual moisture content, and good long-term stability.[4]

Lyophilization has three main steps, namely, freezing, primary drying, and secondary drying. Thus, the water is removed from formulations by sublimation of ice and further desorption of unfrozen water under vacuum. The processing conditions at which formulations are subjected may generate freezing and desiccation stresses, with detrimental consequences to nanoparticles stability. Different excipients such as cryoprotectants and lyoprotectants may be used to minimize the lyophilization stresses and preserve the physical-chemical properties of nanoparticles. Sugars are the preferable cryo- and lyo-protectants mainly because they are chemically innocuous and can be easily vitrified during the freezing step.[5]

Another important property of sugars is that they also affect the glass transition temperature (Tg and Tg') of formulations, which have major importance on the optimization of the lyophilization cycle.[6] A higher cryoprotectant concentration and faster freezing rate lead to better nanoparticle redispersibility.[7,8] The selection of an adequate cryo- or lyo-protectant and freezing rate is not straightforward, and it may depend both in the formulation properties and in the lyophilization cycle.[1]

Nanoparticles are often produced in an aqueous suspension form. However, nanoparticles suspended in an aqueous

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medium are physically unstable, so particle aggregation and fusion are frequent phenomena during long periods of storage. The hydrolytic action of water on the polymer matrix can lead to drug leakage, hamper or eliminate the sustained release properties and lead to the formation of undesirable degradation products.[9] A nanoparticle suspension is also prone to the development and growth of microorganisms. To overcome all these problems, the transformation of the nanoparticle suspension into a solid dosage form is the obvious solution.

In most of the works focusing in nanoparticles lyophilization, the choice of a good cryo- or lyo-protectant and lyophilization cycle has been relied on empirical approaches or even performed by trial and error. Therefore, it is crucial to perform a systematic study in the optimization of nanoparticles lyophilization, by assessing the physical-chemical properties of formulations and understand the engineering principles inherent to lyophilization.

Furthermore, after lyophilization, the nanoparticles need to be easily resuspended, present no modification of particle size distribution, and preserve the activity of the loaded drug. To achieve all these purposes, it is crucial to optimize and focus in three main aspects involved in the stability of nanoparticles: the formulation, the lyophilization process, and the storage conditions.

The aim of the present study was to determine the effect of different cryoprotectant, lyophilization process on their physiochemical properties before and after lyophilization of noscapine-loaded polycaprolactone (PCL) nanoparticles and to evaluate the effect of lyophilization on storage of noscapine-loaded nanoparticles at real time (2–8°C), room temperature (23–27°C), and accelerated storage conditions (33–39°C).

MATERIALS AND METHODS

Materials

Noscapine hydrochloride from Biological E Limited as a gift sample and PCL provided by Evonik Limited as a gift sample. Dichloromethane, polyvinyl alcohol, and poloxamer procured from Sigma-Aldrich Limited. Sucrose, mannitol, D-sorbitol, and dextrose procured from Merck Ltd. The following equipment has been used for the study, Lyostat – freeze-drying microscopy (FDM), Virtis from Spinco Biotech, KF titrator from Metrohm, and stability and cooling chamber from Newtronics Limited.

Preparation of Noscapine-Loaded PCL Nanoparticles

Noscapine-loaded PCL nanoparticles are prepared by double emulsion solvent evaporation as described by Ramesh and Kumar.[10]

FDM

Collapse temperatures (Tc) were determined by FDM (Lyostat-4, SP Scientific) equipped with a Duo 2.5 vacuum pump (Pfeiffer Vacuum GmbH, Asslar, Germany) and an AxioImager A1 microscope used with a 200-fold magnification (Carl Zeiss AG, Oberkochen, Germany). Four microliter of sample (1:1 cryoprotectant (5%):nanoparticles) was placed on the sample holder together with a spacer and covered by a glass slide. Samples were cooled down to −50°C with 1°C/min. A pressure of 0.1 mbar was applied and the samples were heated to −30°C with 5°C/min. The sample was held at these conditions to achieve a sufficient thickness of the sublimation front. Tc was detected as onset of collapse in the following drying step at 1°C/min up to 25°C. Pictures were taken every 10 s.[11]

Lyophilization

Optimized noscapine-loaded PCL nanoparticle (CBY3H2) was freeze-dried without cryoprotectant and with four different cryoprotectants, namely, D-mannitol, sucrose, D-sorbitol, and dextrose. These were added at 5 mL of 5% (w/v) stock solution which was added to 5 ml of nanoparticle dispersion and mixed completely before subjecting to freeze-drying cycle using benchtop freeze dryer (Virtis, SP Scientific, USA).[12] After freeze-drying (48 h-Table 1), the lyophilized cakes were characterized for their physical texture. These were reconstituted in original volume of purified water and characterized for particle size, zeta potential, residual moisture, and entrapment efficiency as per the method described in Sathesh Kumar *et al*.[10] Based on freeze-dried cake properties, reconstitution behavior and particle size after reconstitution, and residual moisture content, the cryoprotectant was finalized.

Estimation of Residual Moisture Content

Freeze-dried formulations were analyzed for residual moisture content by Karl Fischer titration method. The instrument operational parameters for KF Coulometer are given in Table 2.

Blank determination was done using three vacuum-sealed vials and their mean was taken for calculation. The flip-off seal of the vial was removed and the vial was placed in the sample holder in KF coulometer. Average cake weight was entered in the software for the calculation of percentage moisture content. Post-analysis, the vial weight was taken (for the vials, in which vacuum was released with nitrogen) and the cake weight was determined by measuring the empty vials and lyo cake containing vial, after removing lyo cake using water for injection. The process was repeated with required number of vials.[13]

Table 1: Optimized freeze-drying cycle based on FDM data

Step no.	Step type	Temp (°C)	Vacuum (mTorr)	Step time (HH: MM)	Ramp (R)/hold (H)
Thermal treatment steps					
1	Freezing	−40	-	01:00	H
2	Freezing	−45	-	00:30	R
3	Freezing	−50	-	00:30	R
4	Freezing	−50	-	00:30	H
5	Evacuation	−50	1000	00:00	-
Condenser set point: −40°C					
Primary drying steps					
1	Drying	−50	200	00:10	H
2	Drying	−40	200	00:30	R
3	Drying	−40	200	00:30	H
4	Drying	−35	200	00:30	R
5	Drying	−35	200	20:00	H
6	Drying	−30	200	05:00	R
7	Drying	−30	200	02:00	H
8	Drying	−20	200	01:00	R
9	Drying	−20	200	01:00	H
10	Drying	−10	200	01:00	R
11	Drying	−10	200	01:00	H
12	Drying	0	200	01:00	R
13	Drying	0	150	01:20	H
14	Drying	20	150	02:00	R
15	Drying	20	150	01:00	H
16	Drying	25	150	01:00	R
17	Post-heat	25	50	06:00	

Table 2: Operational parameters for KF Coulometer

Parameter	Set value	Remarks
Oven temperature	73°C	Value selected based on product melting point determined at QC
Nitrogen flow rate	60 mL/min	Optimal flow rate according to manufacturer
Extraction time*	300 s	Default extraction time. Changed to required value as per the study planz

Table 3: Screening of cryoprotectant for noscapine-loaded PCL nanoparticles

Cryoprotectant	Formulation code: CBY3H2			
	Cake appearance	Moisture content (%)	Particle size before lyophilization (nm)	Particle size after lyophilization (nm)
Negative control (no cryoprotectant)	Collapsed	5.21	258±26	400±48
Dextrose	Collapsed	2.27	264±23	385±22
D-mannitol	Intact	1.23	246±18	289±15
D-sorbitol	Intact	3.47	249±32	274±31
Sucrose	Intact	1.36	260±28	266±17

PCL: Polycaprolactone

Table 4: Entrapment efficiency of freeze-dried noscapine-loaded PCL nanoparticles (CBY3H2) stability sample stored at different stability conditions

Stability conditions	Entrapment efficiency (%)			
	Initial	1st month	3rd month	6th month
2–8°C	71.03±1.5	70.78±1.6	70.01±2.3	69.76±3.1
Room temperature (25°C±2/65RH)		71.15±1.7	69.80±2.8	65.97±5.4
Accelerated temperature (37°C±2/75RH)		69.98±1.7	68.85±3.3	62.01±5.1

Moisture content (%) in vials was calculated using the formula:

$$\%\text{Moisuture content} = \frac{\text{Moisture content in sample } (\mu g) - \text{Blank} - (\mu g)}{\text{Sample weight (mg)} \times 1000}$$

Stability Study

The physical stability of the freeze-dried formulation was evaluated for a period of 6 months under three different conditions: refrigerated condition (2–8°C), ambient condition (25°C±3°C/65 ± 5% RH), and accelerated condition (37 ± 2°C/75 ± 5% RH).[14]

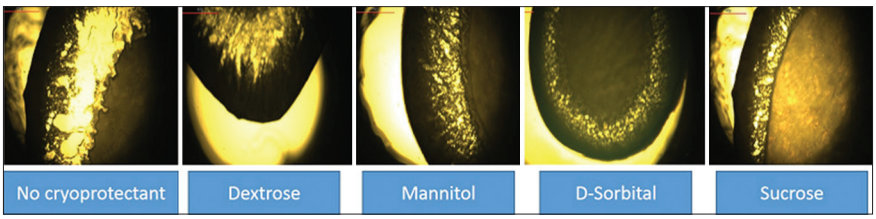

Figure 1: Images of freeze-drying microscopy of noscapine-loaded PCL nanoparticles

Figure 2: Appearance of noscapine-loaded polycaprolactone nanoparticles with various cryoprotectants

Formulations were stored in closed glass bottles at all three temperatures. At specified intervals of time (0, 1, 3, and 6 months), the samples were withdrawn and evaluated to determine particle size, PDI, zeta potential, and entrapment efficiency as per the method described by Ramesh and Kumar.[10]

Statistical Analysis

The results are reported as mean ± standard deviation of triplicate measurements. Statistical analysis of the results was performed in terms of particle size, size distribution, zeta potential, and entrapment efficiency of noscapine-loaded PCL nanoparticles.

RESULTS AND DISCUSSION

FDM

Freeze-drying above the product critical temperature can lead to loss of physical structure, incomplete drying (high moisture content), decreased solubility (reconstitution time), and reduced activity/or stability. Freeze-drying too far below the product critical temperature can lead to poor efficiency, high running costs, and longer cycles than necessary. Collapse temperature (Tc) is the temperature at which the material softens to the point of not being able to support its own structure. Eutectic temperature (Teu) is the temperature at which the solute material melts, preventing any structure from forming after the solvent has been removed. Glass transition (Tg) is the temperature at which the frozen glass first exhibits a change in viscosity from a brittle solid into a soft solid. Higher molecular weight components such as polymers tend to have higher critical temperatures. Lower molecular weight components such as salts and sugars tend to have lower critical temperatures. Crystalline/amorphous mix can have a major impact on critical temperature.

Many cycles use Tg as the critical temperature for freeze-drying cycle development. However, Tg can be significantly lower than Tc. Lower primary drying temperatures mean slower processing and more expensive cycles. Although Tg is significant, many formulations can be dried safely above this point. Knowledge of Tc is strongly recommended for formulation and cycle development.

FDM is a technique provides a real-time observation of our formulation during freeze-drying. The exact point of collapse or eutectic melt can be determined, enables rapid determination of collapse, melting, and eutectic event temperatures, archives data and image capture for future reference, and provides essential data to develop cycles on a rational scientific basis.

Based on the freeze-drying microscopy data, the collapse temperature was found and tabulated in Table 3 and Figure 1.

Cryoprotectant	Collapse temperature (Tc)	Glass transition temperature (Tg)
Dextrose	−32.2°C	26°C
Mannitol	−37.5°C	18°C
D-sorbitol	−28.5°C	44°C
Sucrose	−30.1°C	60°C

Various trials have been taken based on collapse temperature (data not shown), the lyo recipe is finalized [Table 1].

Screening of Cryoprotectant Based on Physical Appearance, Mean Particle Size, Entrapment Efficiency, and Residual Moisture Content

Optimized noscapine-loaded PCL nanoparticles (CBY3H2) successfully lyophilized using D-mannitol, glucose, D-sorbitol, and sucrose. The physical appearance, mean particle size, and residual moisture content of lyophilized nanoparticles with various cryoprotectants are shown in Table 3.

Cryoprotectant is usually added before freeze-drying to the formulation, which protects the product from freezing and drying stress and also to increase

Table 5: Particle size and zeta potential of freeze-dried noscapine-loaded PCL nanoparticles (CBY3H2) stability sample stored at different stability conditions

Stability conditions	Particle size (nm)				Zeta potential (mV)			
	Initial	1st month	3rd month	6th month	Initial	1st month	3rd month	6th month
2–8°C	252±14	256±12	244±18	252±11	−15.8±0.9	−15.0±0.7	−16.2±0.6	−15.6±0.8
Room temperature (25°C±2/65RH)		256±12	250±10	262±18		−15.2±0.8	−14.8±0.7	−14.4±0.8
Accelerated temperature (37°C±2/75RH)		258±14	262±16	268±22		−15.0±0.6	−14.4±0.9	−13.8±0.7

PCL: Polycaprolactone

formulation stability on storage. Cryoprotectants immobilize the nanoparticles within a glassy matrix and thus prevent their aggregation and protect them against the mechanical stress of ice crystals. Four widely used sugars as cryoprotectants such as D-mannitol, dextrose, D-sorbitol, and sucrose at a concentration of 5% w/v for the optimized nanoparticles.

Nanoparticles without cryoprotectant (negative control) have collapsed cake and particle size increased significantly after freeze-drying as compared to the formulation containing cryoprotectants [Figure 2]. Nanoparticles with D-Sorbitol and without cryoprotectant (negative control) shown more than 3% of limit residual moisture. (Limit: NMT 3%). Residual moisture for other cryoprotectants sucrose, dextrose, and mannitol remain will within limit. Mean particle size of nanoparticles slightly increased after freeze-drying with all the four cryoprotectants containing formulations. Sucrose was selected as cryoprotectant for lyophilization of noscapine-loaded PCL nanoparticles (CBY3H2) based on better cake appearance, within limit residual moisture, particle size before and after lyophilization. The lyophilized formulation (CBY3H2) was subjected to stability studies.

Stability Study of Noscapine-Loaded PCL Nanoparticles

Effect of storage time on lyophilized noscapine-loaded PCL nanoparticles (CBY3H2) on particle size, zeta potential, and entrapment efficiency at different conditions is summarized in Tables 4 and 5.

There is no significant change in particle size, zeta potential and entrapment efficiency of nanoparticles after 6 months of storage at 2-8°C. At accelerated conditions (37°C/75 RH) and ambient conditions, entrapment efficiency is decreased significantly after 6 months of storage.

CONCLUSION

In our study, noscapine-loaded PCL nanoparticles prepared by double emulsion solvent evaporation and subjected to FDM study for the estimation of collapse temperature. Collapse temperature of sucrose, mannitol, dextrose, and D-sorbitol was determined. Lyo recipe finalized based on the collapsed temperature for primary drying and glass transition temperature for secondary drying. The lyophilized formulation with sucrose subjected to real time, ambient temperature, and accelerated conditions, shown no change in particle size, zeta potential, and entrapment efficiency for nanoparticles stored in real time for 6 months. For ambient and accelerated storage, there is a slight increase in particle size and zeta potential, decrease in entrapment efficiency due to leakage.

The noscapine-loaded PCL nanoparticles successfully formulated, screened for cryoprotectants for lyophilization. The effect of cryoprotectant on lyophilization process studied and finalized the freeze-drying cycle. The optimized lyophilized noscapine-loaded PCL nanoparticle showed better stability in real-time stability than ambient and accelerated storage conditions.

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