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RESEARCH PAPER

Evaporative Drying of Aqueous Dispersions of Solid Lipid Nanoparticles

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ABSTRACT

Solid lipid nanoparticles (SLNs) have been proposed as alternative colloidal drug carriers. SLNs are obtained by dispersing warm oil-in-water microemulsions into cold water. The aim of this research was to investigate an evaporative drying process for aqueous dispersions of SLNs. For this purpose, a special apparatus, namely, a thermostatic minidesiccator having alumina as the drying medium, was designed to carry out the evaporative drying at a controlled temperature. Besides the water removal kinetics, the mean particle size and the size distribution of SLNs were measured during the drying with the aim of detecting the highest temperature at which the drying process can be carried out without significantly affecting the SLN average diameter. The SLN dispersions were evaluated with and without a hydrophilic excipient, commonly used as a cryoprotector (trehalose). The drying temperature of 10°C was found to be the most suitable for obtaining SLNs as a powder, maintaining almost the same size as that of the SLNs in dispersion.

Key Words: *Evaporative drying; Minidesiccator; Particle size; Solid lipid nanoparticles*

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INTRODUCTION

Solid lipid nanoparticles (SLNs) have been proposed as alternative colloidal drug carriers; different preparation techniques, such as hot and cold dispersion of lipids and high-pressure homogenization of melted lipids, have been reported (1–4).

In our laboratory, SLNs were prepared by dispersing warm oil-in-water microemulsions into cold water (5). These nanoparticles were formed by the rapid solidification of the microemulsion oil nanodroplets, and they had a spherical shape. The SLNs considered here had a small mean diameter and narrow size distribution and were able to incorporate both lipophilic and hydrophilic drugs.

Generally, SLNs can be stored at 4°C as aqueous dispersions over a long period of time; dry SLNs are more easily handled and stored.

The freeze-drying of SLN water dispersions has already been studied; as reported in previous research (6), the size of the SLN increased during freeze-drying to varying extents, depending on the lipid matrix, the presence of a cryoprotector in the dispersion, and the freeze-drying conditions. Similar results have also been obtained by Müller and coworkers using several cryoprotectors (7).

The size and shape of the SLN can affect certain parameters of the colloidal systems, such as the specific surface, thus influencing experimental results both in vitro and in vivo: A larger specific surface allows greater drug release from the SLNs.

Small nanoparticles are desirable for administration to laboratory animals; in particular, for intravenous administration, size is an important factor; it is also important for other administration routes, such as ocular (8) and oral (9–11).

We investigated evaporative drying at low temperatures, with the main aim being to obtain SLNs as a powder by controlling the growth of SLN size to achieve reproducible physical characteristics. In fact, preliminary results (12) suggested that this technique can be an alternative to freeze-drying, eliminating the freezing step and thus the agglomeration phenomena that can lead to a particle size increase.

For this purpose, an apparatus, namely, a minidesiccator, was designed to carry out the evaporative drying of SLN aqueous dispersions at a controlled temperature with activated alumina as the drying medium. The influence of the operating conditions on the process time and SLN characteristics was studied.

EXPERIMENTAL

Materials

Stearic acid, basic alumina (in the range of 80 to 200 mesh), and trehalose were purchased from Sigma (Milan, Italy); Epikuron 200 (soya phosphatidylcholine 95%) and taurocholate sodium salt were kindly provided by Lucas Meyer (Hamburg, Germany) and PCA (Basaluzzo, Italy), respectively. All other chemicals were analytical grade.

Description of the Apparatus

A specially designed minidesiccator (width 10 cm, height 12.5 cm) was manufactured in Plexiglas; in the first design, the two compartments were separated by a row of 6 mm OD stainless steel, which acted as a heat exchanger with the external environment. To reduce sealing and mechanical problems, a new prototype was realized in which channels for the cooling medium were placed in the basement of the upper compartment and perforated to allow the passage of the vapors to the desiccant (see Fig. 1). Basic alumina was placed in the lower compartment as a desiccant; stainless steel minitrays containing the SLN dispersions (or other samples to be dried) were placed in the upper compartment on the cooling tubes. The minidesiccator was immersed in

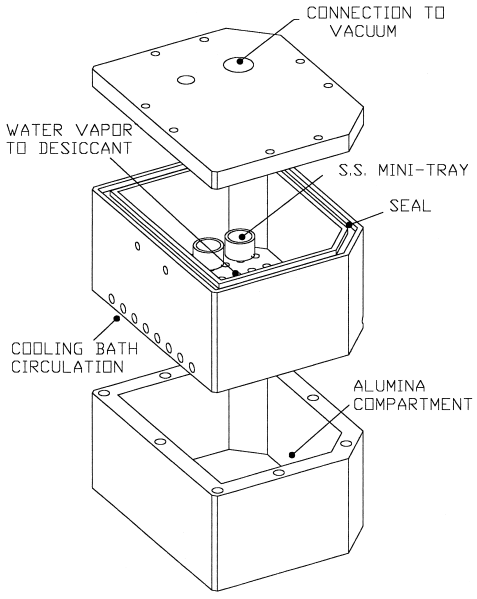

Figure 1. View of the minidesiccator.

a thermostatic bath to control the temperature; the cooling medium (a water/propyleneglycol solution) was made to flow through the minidesiccator tubes to guarantee a uniform internal temperature profile. The temperature was continuously monitored by a T-type thermocouple positioned in a reference tray containing distilled water. Similarly, distilled water was also used as a reference for water evaporation kinetics measurements.

Preparation of Solid Lipid Nanoparticles

Two different types of SLN dispersion were prepared: one without any excipient in the dispersion water and one containing 3% w/w trehalose.

To prepare the SLN, a warm oil-in-water (o/w) microemulsion was first prepared, consisting of stearic acid (0.70 mmol) as the internal phase, Epikuron 200 (0.40 mmol) as the surfactant, taurocholate (0.66 mmol) as the cosurfactant, and filtered water (111.11 mmol) as the continuous phase; SLN were obtained as previously described (6). In this research, the microemulsion : dispersion water ratio was 1 : 2 (v/v).

After washings, the SLN dispersions were concentrated using the TCF2 system (Amicon, Danvers, CT) to the selected value of 20% by weight (200 mg of solid SLN/ml dispersion). The washing solutions obtained from the two SLN washings and the solutions obtained in the step in which the dispersion was concentrated (concentrated solution) were also used as a reference in the drying experiments.

The SLN dispersions containing trehalose were obtained by dispersing the above microemulsion into a trehalose aqueous solution (3% w/w), again at a 1 : 2 ratio of microemulsion to aqueous solution (v/v). After washings (carried out as above, but using the trehalose solution), the SLN dispersion was concentrated as previously.

As a control, portions of these two SLN dispersions were freeze-dried using a Modulyo freeze-dryer (Edwards, Crawley, UK)

Characterisation of Solid Lipid Nanoparticles

Determination of the Sizes of Solid Lipid Nanoparticles

The average diameter and the polydispersity index of SLN were determined by photon correlation spectroscopy using a 90 PLUS particle size analyzer (Brookhaven Instruments, NY) at a fixed angle of 90° and a temperature of 25°C. The SLN water dispersions were diluted 1 : 20 with filtered water before analysis. Each reported value is the mean value of five measurements. The polydispersity index is a measure of the width of the size distribution of the nanosphere population (13).

Solid Lipid Nanoparticle Morphology

The morphology of the nanoparticles was observed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). TEM analysis was performed using a Philips CM10 instrument. Before analysis, the nonconcentrated SLN dispersions were diluted 1 : 10, stained with a 2% solution of osmium tetraoxide, and sprayed on copper grids. SEM analysis was performed using a 515 Philips instrument. The samples were prepared similar to those for TEM observation, that is, sprayed on copper grids; the grids were then fixed on the SEM stub and coated with a layer of gold.

Determination of Drying Rates

The drying experiments were performed using the two types of SLN dispersions, namely, with and without trehalose in the dispersion water; in both cases, the SLN dispersions were initially used either before or after washings. The washing solutions and the concentration solution were also tested in the minidesiccator to verify the effects of the cosurfactant and trehalose in the aqueous dispersions on the drying rate.

The evaporative drying experiments were performed over a temperature range between 2°C and 20°C, namely, at 2°C, 5°C, 8°C, 10°C, 15°C, and 20°C. For each experiment, 10 minitrays were placed in the minidesiccator. Of these, 6 minitrays contained 500 mg of the SLN dispersion (constituted of 100 mg of solid SLN and 400 mg of water), with 3 of these minitrays used to determine the drying rate and 3 minitrays to evaluate SLN size. Of the remaining 4 minitrays, 2 contained 500 mg of one of the two washing solutions; another minitray contained 500 mg of the concentrated solution; and the last minitray contained 500 mg of distilled water as a control.

During the drying experiments, the minitrays were weighed at a fixed time to determine the amount of water evaporated and thus to calculate the drying rate of the dispersion (minitrays 1–3). To determine the average diameter and the polydispersity index of

the SLN and to monitor the size variation during drying, 20 μL of SLN dispersion were withdrawn from minitrays 4–6 at the same time.

A sample of SLN dispersion prepared from the same batch was stored at 4°C and was used as a reference for the particle size and polydispersity index of SLN over time.

RESULTS AND DISCUSSION

Figures 2a and 2b show the weight loss of water versus time in a series of identical samples of SLN aqueous dispersions at the lowest (2°C) and the highest (20°C) temperatures tested. Reproducibility was good for all samples over the whole temperature range; small differences in the evaporation rate among the different samples might be due to lower temperature gradients in the minidesiccator.

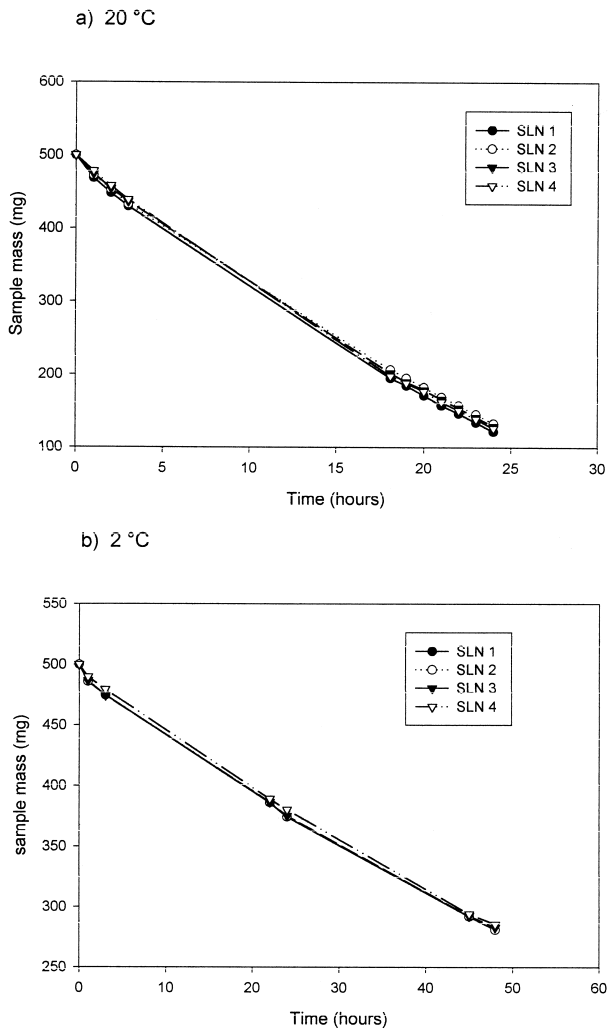

Figure 2. Reproducibility tests; weight loss versus time of different samples obtained from the same SLN aqueous dispersion at two temperatures: (a) 20°C and (b) 2°C.

The first experiments in water and in the trehalose solution showed that the evaporation rate of SLN dispersions and that of the washings and concentrated solutions was nearly the same. No difference in the drying kinetics was observed between unwashed and washed SLN dispersions either, confirming that the presence of dissolved molecules in the dispersion, such as taurocholate or trehalose, did not influence kinetics and particle growth.

Figure 3 shows the mass of evaporated water from an SLN dispersion at the temperatures studied. The drying rate markedly increased with temperature as a consequence of the increase in water vapor pressure, which acts favorably on the driving force of evaporation. In fact, in these experiments, only surface evaporation occurred: Water vapor diffused into the gaseous phase from the evaporating surface to the desiccant (which has a water vapor pressure of 0.4 Pa at 20°C). The process rate is thus controlled to a considerable extent by the vapor pressure driving force, which is only affected by temperature. The evaporation rate reduces only slightly with time; as a first approximation, the dependence of weight loss on time can be considered linear over a long time interval and is thus of approximately zero order with respect to the water content (12).

The drying process of SLN dispersions appeared to be slow at the lowest temperatures (2°C and 5°C). To obtain complete desiccation, more than 85 h were necessary at both temperatures; indeed,

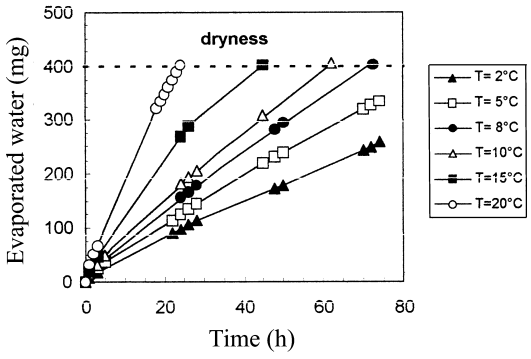

Figure 3. Effect of temperature on the water evaporation rate from washed aqueous dispersions of SLN (500 mg of dispersion, 20% w/w SLN).

after 72 h at 2°C, only about 75% of water had evaporated. At higher temperatures, the process was faster: At 15°C, complete dryness was achieved after 42 h, while at 10°C, the time required was 54 h; 24 h were sufficient to desiccate the SLN colloidal system completely at 20°C.

The SLN diameter variations were measured during the drying process to determine a critical time of SLN growth. The mean SLN diameter increased to different extents during drying, depending on the process temperature. The diameter increase was moderate at temperatures of 10°C or lower (less than 15%). Increases in mean SLN diameter and polydispersity index at 2°C and 5°C were comparable or even lower than those of the reference SLN dispersions stored at 4°C.

Relatively high process temperatures (15°C and 20°C) caused a marked increase of SLN size (to 400 nm from about 80 nm); this was probably due to agglomeration phenomena, which appeared to occur at the higher process temperatures when the SLN dispersions were close to dryness, as shown below.

Figure 4 shows the relative variation of the mean diameter of SLN as a function of the process temperature after 24 and 48 h of evaporation. The results indicate that 10°C is the maximum temperature suitable for drying SLN and avoiding excessive increase of both size and polydispersity index.

The SLN dried completely at 10°C showed an average diameter of 80 nm and a polydispersity index of 0.04, which are not very different from the values before drying. (average diameter 70 nm and polydispersity index of 0.08). TEM analysis confirmed the spherical shape, small diameter, and narrow size distribution of dried SLN obtained at 10°C, while the SEM analysis confirmed that the size increase of SLN at the higher temperatures was due to aggregation of several spherical nanoparticles.

The freeze-dried SLN either in water or in trehalose solution showed a marked increase after freeze-drying; the average diameter and the polydispersity index were about 200 nm and 0.28, respectively, for SLN in water, while for SLN in trehalose solution, the average diameter and the polydispersity index were about 250 nm and 0.3, respectively.

The time required to achieve dryness and the variation of mean diameters and polydispersity indices before and after evaporative drying at different process temperatures are given in Table 1. The polydispersity index remained low after drying. Conversely, the polydispersity values increased after freeze-drying, as previously shown for the same SLN formulation (6); the cause can be sought in the

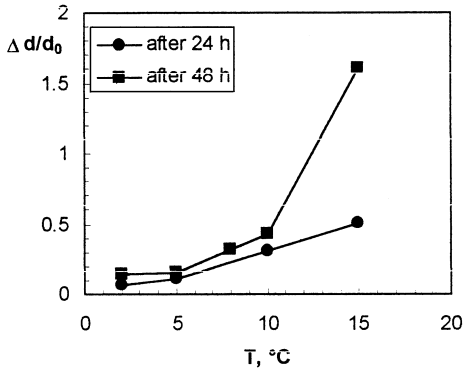

Figure 4. Relative variation of the diameter during drying as a function of process temperature.

Table 1

Average Diameter and Polydispersity Index of Desiccated SLN Dispersions (200 mg/ml of Washed SLN in Water)

		Average Diameter		Polydispersity Index	
Process Temperature (°C)	Time to Dryness (h)	Initial Dispersion (nm)	Dried SLN (mm)	Initial Dispersion	Dried SLN
2	96	65.0	72.5	0.18	0.12
5	88	69.0	76.5	0.20	0.12
8	70	72.5	84.0	0.15	0.10
10	56	70.6	80.0	0.08	0.04
15	48	72.0	223.8	0.20	0.20
20	24	70.0	390.0	0.15	0.38

volumetric increase of frozen water, which might exert pressure on the SLN.

This research also evaluated whether the presence of trehalose (a glucose disaccharide commonly used as a cryoprotector) altered the mean diameter of SLN and the evaporation rate compared to water dispersions. No difference was found in SLN size due to trehalose in the dispersions; diameter and polydispersity index were 64 nm and 0.07, respectively, in the dispersion with trehalose; they were 73.5 nm and 0.09, respectively, after drying at 5°C and 80.0 nm and 0.10, respectively, after drying at 10°C.

In conclusion, this evaporative drying process appears promising and inexpensive to obtain powders from SLN dispersions, even though longer times are required when working at the lower temperatures.

Work is in progress to scale-up the bench scale equipment by designing a special apparatus suitable for larger amounts of SLN dispersions and to optimize the process.

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