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Research paper

A study of the freeze-drying conditions of calixarene based solid lipid nanoparticles

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Abstract

In this note, we report a study of cryoprotectant carbohydrate (glucose, fructose, mannose and maltose) effects on the reconstitution of calixarene based solid lipid nanoparticle (SLN) suspensions after freeze-drying, studied by atomic force microscopy and photon correlation spectroscopy. All carbohydrates tested showed excellent cryoprotection and redispersion properties with the calixarene based SLNs.

Keywords: Calixarenes; Solid lipid nanoparticles; Carbohydrates; Cryoprotectants; Atomic force microscopy

1. Introduction

The use of solid lipid nanoparticles (SLNs) as colloidal transporters [1] represents an alternative to more traditional transporters such as liposomes [2], nanoparticles [3] and micelles [4]. Their advantages include high temporal and physical stability, high loading capacities, non-toxicity and ease of scale-up procedures.

The calix[n]arenes [5] are cyclic oligophenols (Scheme 1a) produced by the base catalyzed reaction of *para*-tert-butyl-phenol and formaldehyde. They are widely used in material and separation sciences because of their ease of chemical modification and their relatively low cost; the non-toxicity [6,7] and non-immunogenicity [8] of their derivatives are opening a new field of application in bio-medical and pharmaceutical sciences.

Recent work on supramolecular SLNs, based on amphiphilic cyclodextrins [9] and calixarenes [10], has shown these to be interesting alternatives to the classical SLNs in view of their capacity to include molecules both in the molecular cavities and also within the SLN matrix. A second point is their high temporal stability even when they are produced in the absence of co-surfactants [11].

It is well known that to avoid risks of embolism, the carriers used for drug delivery in intravenous administration have to stay in suspension as single, non-aggregated particles. In spite of the promising properties of calixarene based SLNs shown by our systematic investigation of their stability parameters, one weakness has been shown: the rate of redispersion of these systems following freeze-drying, in the absence of cryoprotectants [11].

Small sugars, generally monosaccharides, have been used in the cryoprotection of various colloidal dispersions, including Dynasan Imwitor 900 based SLNs [12], poly(DL-lactic acid) (PLA) based polymeric nanoparticles [13] and even highly fragile liposomes [14].

In this paper we report the effects of cryoprotectant carbohydrates (glucose, mannose, lactose and fructose) in freeze-drying/reconstitution cycles on calixarene based SLNs and their redispersion ability. The use of photon correlation spectroscopy (PCS) to measure the particle size of reconstituted suspensions and atomic force microscopy (AFM) imaging of dried films of calixarene based SLN/carbohydrate dispersions is described.

2. Material and methods

2.1. General

Solvents and chemicals were purchased from Acros Oganics (France) and used without further purification.

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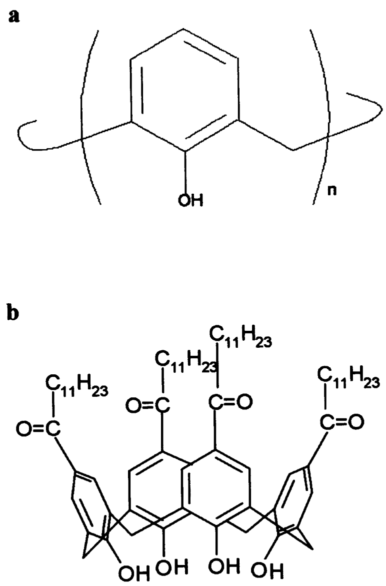

Scheme 1. General formula of calix[n]arenes (a) and formula of *para*-dodecanoylcalix[4]arene (b).

Para-dodecanoylcalix[4]arene (Scheme 1b), **1**, was synthesized as previously described [15].

2.2. SLN preparation

Para-dodecanoylcalix[4]arene was dissolved in tetrahydrofuran (THF) at a concentration of 5 mg/ml. To a volume of 2 ml of this solution, in a conical flask, under magnetic stirring (500 rev./min), was added 100 ml of pure water (resistivity >18 MΩ·cm) at a constant flow rate of 300 ml/min. The suspension was stirred for an additional minute. After evaporation of the solvent at 40 °C under reduced pressure, the final volume was adjusted at 100 ml, giving a colloidal suspension of 0.1 g/l.

To 1 ml of SLNs suspensions was added an equal volume of an aqueous solution of the relevant carbohydrate (glucose, fructose, mannose, maltose) in order to obtain a final carbohydrate concentration of 1, 2, 5, 10, 15, 20 and 25% of sugar in mass.

2.3. Freeze-drying/redispersion

The SLNs in suspension in an aqueous carbohydrate solution were frozen at −15 °C overnight in glass vials. The freeze-drying process was carried out at −55 °C (±3 °C) at a pressure of 10^{-4} Torr during 24 h. Suspensions were reconstituted in 2 ml of pure water under vortex agitation.

2.4. PCS

PCS experiments were carried out on the undiluted suspensions described above using a Malvern 4700 spectrometer (Malvern, UK) and 7132 256-channel correlator with a 40 mW He-Ne laser (633 nm). All values were measured at an angle of 90° in 10 mm diameter cells. The system was thermostated at 25 °C. All measurements were repeated five times and the variance of the measurements was less than 5%. Viscosity differences at high sugar concentrations were taken into account in the Contin size calculation program.

2.5. AFM

Imaging was carried out using an Explorer AFM (Thermomicroscopes Inc., Santa Clara, USA) equipped with a 100 μm tripod scanner, in non-contact mode, using high resonant frequency ($F_0 = 320$ kHz) pyramidal cantilevers with silicon probes at a scan frequency of 1 Hz. Images were processed with the SPM Lab 5.01 software package and are presented unfiltered.

Samples were prepared by depositing 10 μl of the samples after redispersion in water on freshly cleaved mica plates, followed by drying overnight at 20 °C.

3. Results and discussion

The calixarene based SLNs were prepared as aqueous colloidal dispersions from *para*-dodecanoylcalix[4]arene, **1** (Scheme 1b), by the solvent diffusion method. The hydrodynamic diameter as measured by PCS was 145 nm with a high degree of monodispersity. The SLNs were imaged as dried suspensions on mica plates by non-contact mode AFM.

The dispersions were diluted into solutions of four sugars, glucose, fructose, mannose and maltose, to give a final fixed SLNs concentration of 50 mg/l and sugar concentrations of 1, 2, 5, 10, 15, 20 and 25%. No change in the size of the SLNs was observed by PCS. Then 2 ml of each of the SLNs suspensions was freeze-dried at −54 °C overnight to yield white finely dispersed powders. Reconstitution of the SLNs suspensions was carried out by the addition of 2 ml of water, followed by vortex treatment to yield the final SLN suspensions. This technique differs slightly from the previously published work by Zimmerman et al. on the reconstitution of Dynasan Imwitor 900 based SLNs, where manual shaking was performed [12].

The results of PCS measurements are presented in Fig. 1. No variations in the size of the SLNs were observed for all sugars tested, even at high concentrations. The cryoprotectant effect of these sugars, observed here, may arise from the formation of a protective capping layer around the SLNs, sustained by hydrogen bonds between the polar function of the calixarene molecules, exposed at the surface of the SLNs, and the hydroxyl functions of the sugar. The formation of a hydrosoluble matrix, where the particles are embedded in, may facilitate the reconstitution of the suspensions.

AFM non-contact mode images at 5 and 10 μm scan ranges, of a dried suspension of the nanoparticles, are

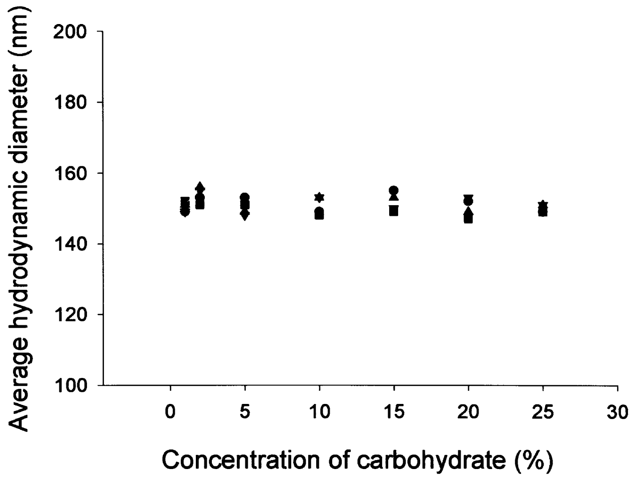

Fig. 1. Average hydrodynamic diameter measured by PCS of reconstituted **1**-based SLNs suspensions after freeze-drying in function of sugar concentrations (▲, mannose; ▼, glucose; ■, fructose; •, maltose).

presented in Fig. 2. The SLNs are present on the surface as aggregated and slightly flattened circular objects with an average height of 250 nm and an average diameter of 55 nm; the volume calculated from these values correlates well with the volume obtained from the hydrodynamic diameter obtained from PCS. AFM images of a dried suspension of SLNs after freeze-drying and reconstitution in an aqueous solution of glucose (2%) are presented in Fig. 3. The SLNs are present as individual spherical objects of 16 nm in height and 270 nm in diameter. An approximate calculation of the thickness of the film formed by 10 μl of a 2% solution of glucose, with a droplet diameter of 5 mm on the surface, gives a value in the range of 100–200 nm. The difference between the volume calculated from the PCS experiments and the approximation from those calculated from AFM images could be explained by the fact that the SLNs are embedded in the gel formed by the glucose and the topographic investigation only shows the part of the SLN protruding from the surface. The improvement of the mechanical resistance of these particles must be due to the presence of a protective layer of sugar on the surface of the SLNs. It must be noted that imaging of these samples is relatively difficult because of the highly hygroscopic character of the carbohydrate containing films causing the presence of a contaminant water layer.

The cryoprotectant effect of four carbohydrates (glucose, fructose, mannose and maltose) on *para*-dodecanoylca-

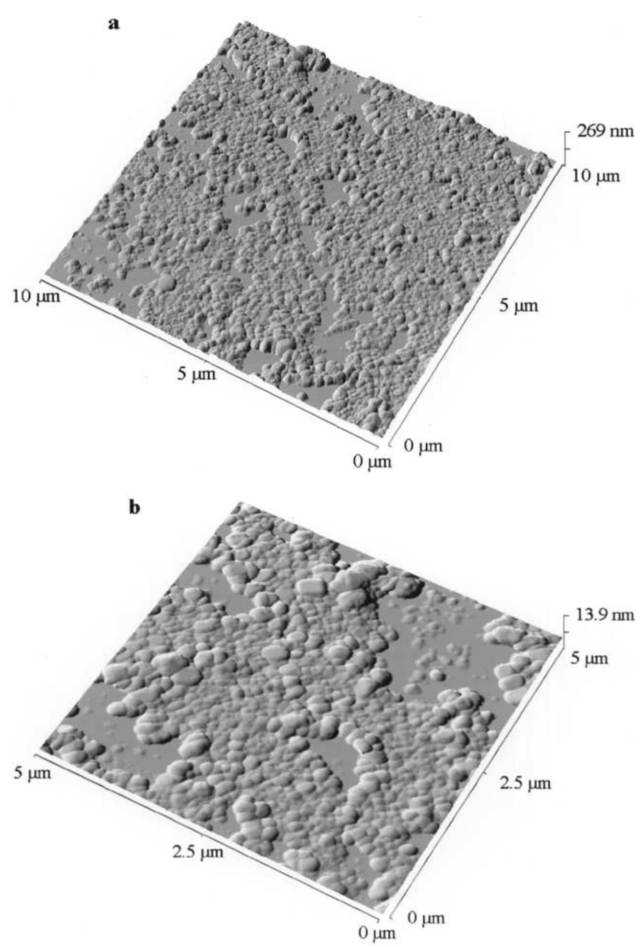

Fig. 2. Non-contact mode AFM images of **1**-based SLNs dried suspension in pure water at 10 (a) and 5 μm (b) scan range.

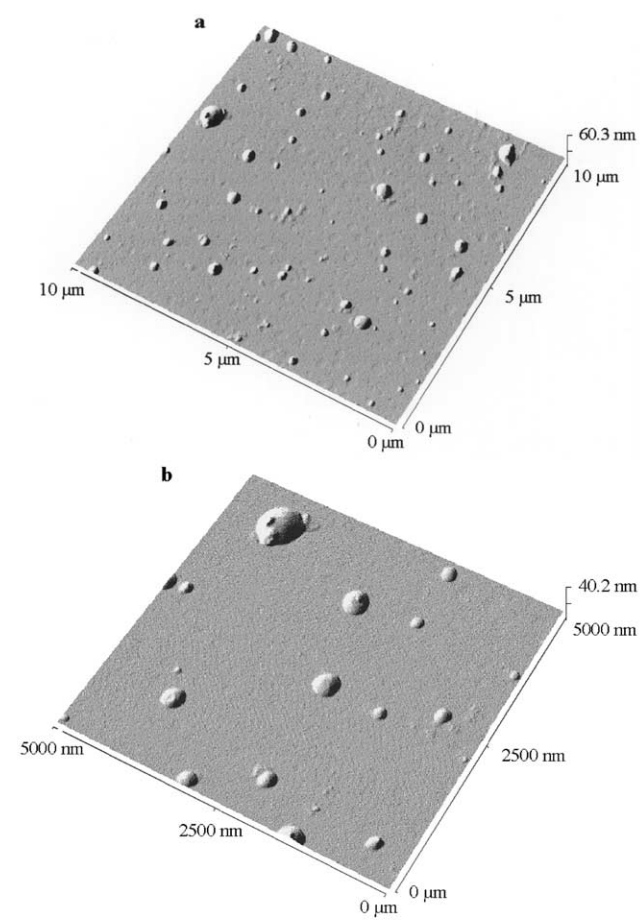

Fig. 3. Non-contact mode AFM images of **1**-based SLNs reconstituted after freeze-drying in a solution of glucose (2%) at 10 (a) and 5 μm (b) scan range.

lix[4]arene based SLNs has been investigated by PCS and AFM. These four carbohydrates have been shown to act as good cryoprotectants, allowing reconstitution of the suspensions after the freeze-drying process.

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