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Research paper

Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins

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Abstract

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been applied to the imagery of solid lipid nanoparticles (SLNs) formulated from an amphiphilic cyclodextrin, 2,3-di-*o*-alkanoyl-β-cyclodextrin, β-CD21C6. Comparison of the results shows that the vacuum drying technique used in sample preparation for SEM causes shrinkage in the size of the SLNs, whereas the deposition method used for AFM causes the SLNs to form small clusters. The hydrodynamic diameter determined from photon correlation spectroscopy (PCS) is 359 ± 15 nm and the zeta potential is −25 mV.

Keywords: Amphiphilic cyclodextrin; Solid lipid nanoparticule; Atomic force microscopy; Scanning electron microscopy

1. Introduction

Solid lipid nanoparticles (SLNs) represent a rapidly growing class of colloidal transport system of considerable interest for pharmaceutical applications [1]. Compared to more 'classical' transporters such as liposomes, micelles, or polymeric nanospheres and nanocapsules, they possess numerous advantages including possibility of controlled drug release and drug targeting, increased drug stability, high drug payload, incorporation of lipophilic and hydrophilic drugs, low to non-existent biotoxicity of the carrier and few problems with respect to large scale production and sterilization [1].

Over the last few years, we have been developing SLNs based on amphiphilic supramolecular derivatives, starting with amphiphilic cyclodextrins [2–4] and more recently using amphiphilic calixarenes [5,6].

The characterization of SLNs can be achieved using photon correlation spectroscopy (PCS), zeta potential measurements, differential scanning calorimetry (DSC), X-ray powder diffraction and various other physical methods [7]. Evidently, imaging of SLNs is of considerable interest. Two types of high-resolution microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM), are available for clean imaging of the SLNs. Both systems present advantages and disadvantages; AFM allows imaging under hydrated conditions without pre-treatment of the samples, however, point–sample interactions may cause image distortion and non-contact mode imaging has a maximum resolution of around 2 nm. SEM imaging has no source–sample contacts and allows much higher resolution, however, imaging is carried out in high vacuum and samples require pre-treatment. The use of transmission electron microscopy (TEM) and SEM for SLN imaging has been previously reported by Sato [8]. AFM imaging of SLNs was first reported by zur Mülhen et al. [9]; we have carried out a number of AFM imaging studies on cyclodextrin based SLNs, [10,11] and more recently on calixarene based SLNs [6,12].

In this paper, we present SEM and AFM imaging of SLNs derived from the amphiphilic cyclodextrin, β-

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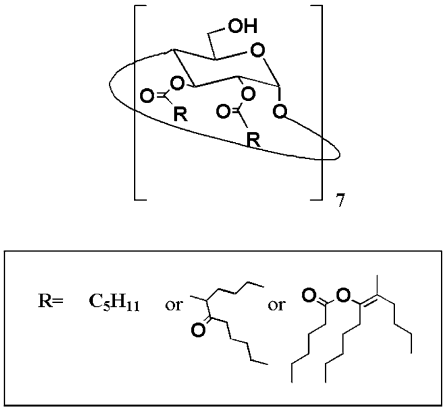

Fig. 1. Molecular structure of the amphiphilic cyclodextrin β-CD21C6.

CD21C6 (Fig. 1) and compare the information that can be derived from the two techniques.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Acros Organics (France) and used without further purification. β-Cyclodextrin was generously given by Wacker (France), and amphiphilic β-cyclodextrins were synthesized according to the general method related in the literature [4]. The chemical structure of β-CD21C6 is presented in Fig. 1.

2.2. Preparation of the SLNs

SLNs were prepared by the nanoprecipitation method [13]. Typically, 17 mg of amphiphilic cyclodextrins were dissolved in 10 ml of ethanol and this solution was slowly added, via a syringe equipped with a fitted capillary, to 20 ml of water under magnetic stirring at room temperature. The SLNs formed spontaneously. The ethanol was removed under reduced pressure and the total volume adjusted to 20 ml to yield a concentration of 1.75 mg/ml.

2.3. Sample preparation

2.3.1. SEM

Colloidal suspensions were deposited on a metallic probe then placed in liquid nitrogen during 10 min and evaporated under vacuum. SLNs were metallized with gold/palladium with a cathodic pulverizer Technics Hummer II (6 V–10 mA).

2.3.2. AFM

Samples of the SLNs formulated from $\beta CD21C_6$ were prepared by deposition of 10 μl of the colloidal suspension onto freshly cleaved mica plates, followed by drying during 24 h at 25°C.

2.4. SLNs characterization

2.4.1. Photon correlation spectroscopy

The particle size and the polydispersity index were measured on a Malvern 4700 spectrometer and 7132 256-channel correlater 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. Particle analysis was carried out using the Malvern software package using multiple mode analysis. All measurements were repeated five times and the variance of the measurements was less than 5%.

2.4.2. Zeta potential

The surface charge of SLNs was determined by measurement of the zeta potential of the particles extracted from their electrophoretic mobility. A Malvern Zetasizer 2C instrument was used. SLNs were suspended in 10^{-3} M KCl and measurements were made in triplicate at 25°C.

2.4.3. SEM imaging

Imaging was carried out on a FEG Hitachi S 800 SEM at an accelerating voltage of 15 kV.

2.4.4. AFM imaging

Imaging was carried out on a Thermomicroscope (Santa Clara, CA) Explorer Atomic Force Microscope using a linearized 100 μm scanner in non-contact mode using high resonant frequency ($F_0 = 260$ kHz) pyramidal cantilevers with silicon probes having force constants of 47 N/m. Scan speeds were set at 1 Hz. Scan sizes were taken from 50 to 5 μm. Image resolution was 500 × 500. Image analysis was carried out using the Thermomicroscopes SPML5.01 software package and images are presented unfiltered.

3. Results and discussion

SLNs were characterized by PCS and exhibit a diameter of 359 ± 15 nm. The polydispersity index of 0.2 indicates a polydisperse colloidal dispersion. The zeta potential was determined to be −25 mV. This supports the hypothesis that the primary hydroxyl groups of the amphiphilic cyclodextrin, β-CD21C6, are located on the surface of the SLNs. Imaging of SLNs by both SEM and AFM are expected to provide information on SLN morphology and size.

SEM and AFM images of the SLNs derived from β-CD21C6 are presented in Figs. 2 and 3, respectively. Both techniques confirm that the SLNs are circular in shape. In the case of the images obtained from AFM, it can be observed that the SLNs tend to be organized as clusters of 15–30 SLNs. This is probably due to the sample preparation

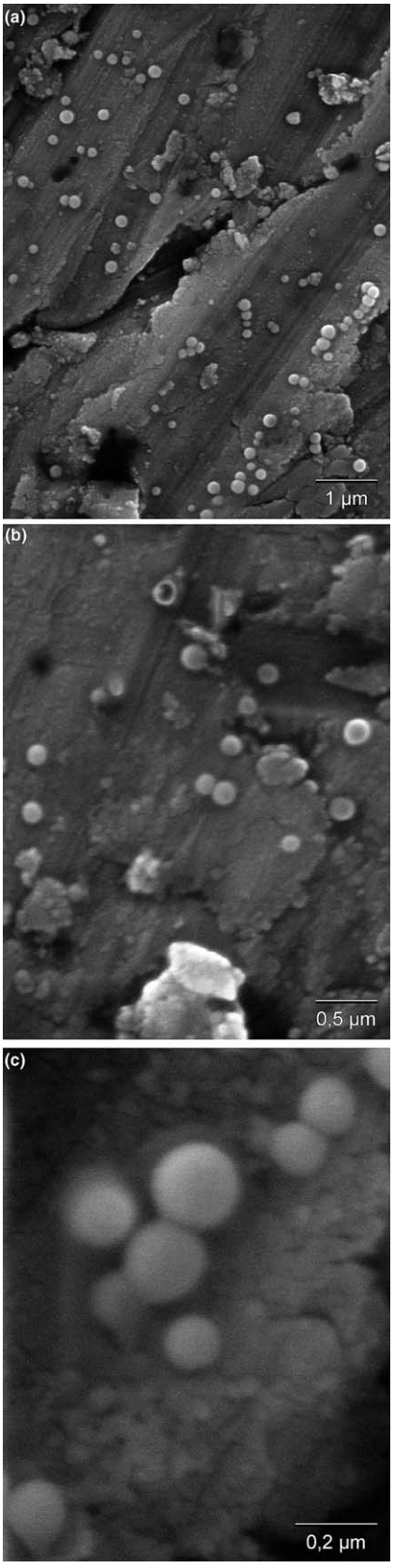

Fig. 2. SEM images of the β-CD21C6 derived SLNs, scale bar: (a) 1 μm, (b) 0.5 μm and (c) 0.2 μm.

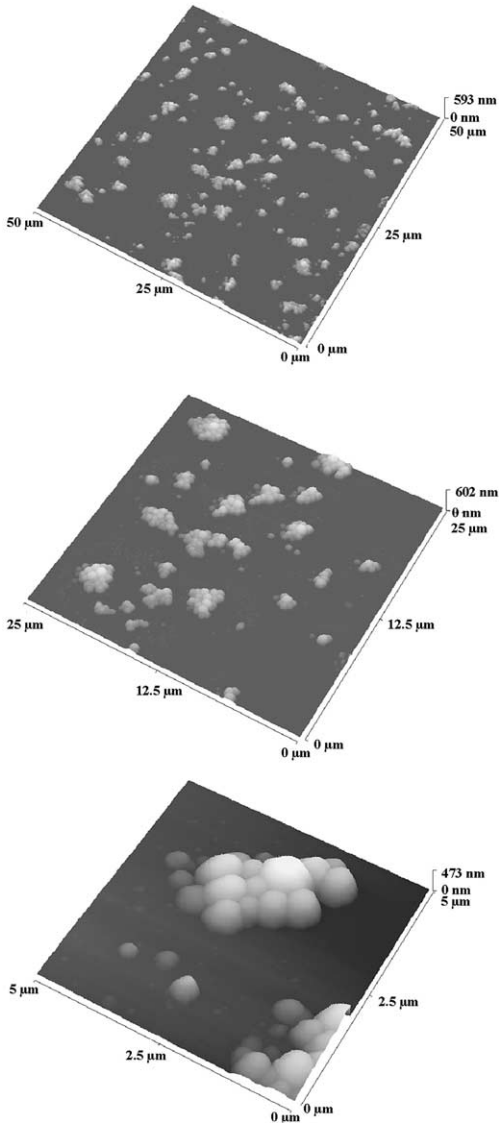

Fig. 3. Non-contact mode AFM images of the β-CD21C6 derived SLNs at scan ranges of: (a) 50 μm, (b) 25 μm and (c) 5 μm.

Table 1
Mean size (nm) of SLNs determined by PCS, AFM and SEM

	Diameter (nm)
PCS	359 ± 15
SEM	212 ± 12
AFM	
Diameter	359 ± 50
Height	140 ± 27

method where the colloidal suspension is slowly dried. In contrast, the SLNs imaged by SEM appear to be well separated on the surface.

The sizes of the SLNs as determined by PCS, SEM and AFM are presented in Table 1. While the mean SLN diameter determined by AFM is in agreement with the mean SLN diameter determined by PCS (359 ± 50 nm compared with 359 ± 15 nm), that determined by SEM is considerably smaller (212 ± 12 nm), corresponding to an apparent decrease of 147 nm.

In the case of AFM sample preparation, bulk water molecules are removed by evaporation at 25°C during 24 h. This implies that the SLNs are still hydrated. Furthermore, the SLN images obtained by AFM are in agreement with a matrix structure for the SLNs, which are sufficiently mechanically resistant to collapse. Nevertheless, there is a slight flattening of the SLNs giving a difference between the diameter and the height of the SLNs.

For the SEM sample preparation, both the water of the bulk phase and the water present in the SLN matrix are completely removed by freeze-drying. Such drying apparently causes shrinkage so that the mean diameter determined by SEM is significantly smaller than that determined by PCS and AFM.

Comparing the results of two methods, we find that the SLN size measured by SEM is smaller than that obtained for colloidal suspensions, but that single SLNs are often imaged. Whereas for AFM, the observed size is closer to that obtained from PCS, the sample preparation method generally, in this case, leads to clustering of the SLNs.

In conclusion, both of these methods of high-resolution microscopy are complementary and lead to access different information. This is due, in particular, to the different sample preparation methods used for these techniques. The advantage of AFM is the simple sample preparation as no vacuum is needed during operation and the sample does not need to be conductive. AFM allows observation of the SLNs in a hydrated state that is closer to that of the SLNs in suspension while SEM leads to observation of the SLNs in a less aggregated state.

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