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Note

Sequential atomic force microscopy imaging of a spontaneous nanoencapsulation process

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Abstract

Since hydrophilic matrices were proposed for controlled drug delivery, many polymeric excipients have been studied in order to make drug release fit the desired profiles. It has been pointed out that λ-carrageenan, a sulphated polymer from algae, can suitably control the release rate of basic drugs from hydrophilic matrices with no need for complex technological processes. In this work, we propose a method to monitor morphologically the interaction between λ-carrageenan and dexchlorpheniramine maleate (D-CPM), in order to find out how the release profiles can be so easily controlled. To this end, solutions of both polymer and drug were prepared at very low concentration. Solutions were mixed and samples were taken every hour over a period of 20 h. The characterization technique employed, atomic force microscopy (AFM), provides a high resolution, allowing to show the three-dimensional morphology of the samples within the nanometric scale. The results demonstrate that λ-carrageenan is able to nanoencapsulate spontaneously D-CPM molecules, which offers the possibility to easily control the release rate of the drug. This work has moreover demonstrated the suitability of AFM for the specific case of the on-time monitoring of interaction processes that happen in pharmaceutical systems.

Keywords: λ-Carrageenan; Dexchlorpheniramine maleate; Nanocapsules; Atomic force microscopy

Since hydrophilic matrices were proposed for oral controlled drug delivery (Christensen and Dale, 1962), many polymeric excipients have been studied in order to make drug release fit the desired profiles (Bonferoni et al., 1992, 1993, 1994; Caram-Lelham and Sundelöf, 1995, 1996; Bonferoni et al., 1997, 1998). In the case of polyelectrolytic polysaccharides, the presence of

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many reactive groups brings about a wide span of weak bonds, such as ionic, hydrogen or hydrophobic interactions. Since these interactions can lead to strong modifications of both conformation and solubility, it is plain that a complete characterization of the system is needed to understand drug–polymer interaction mechanism.

In a previous work, we have recently shown that λ-carrageenan, a sulphated polymer from algae, when in aqueous solution, is able to nanoencapsulate spontaneously anphiphilic basic drugs (Oliva et al., 1999). To assess that nanoencapsulation is spontaneous, the whole process has to be characterized.

The aim of this work is therefore to perform a complete morphological characterization of the nanoencapsulation process between λ-carrageenan and dexchlorpheniramine maleate (D-CPM). D-CPM has been chosen as a model of anphiphilic basic drug.

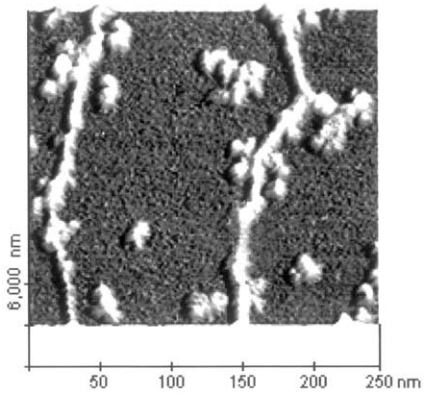

Fig. 1. AFM image of λ-carrageenan suspensions.

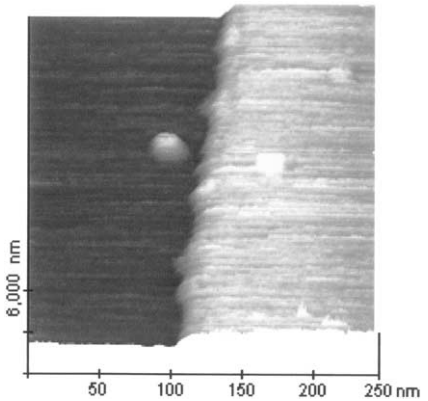

Fig. 2. AFM image of D-CPM solutions.

Here we use an atomic force microscope (AFM) to characterize morphologically the nanoencapsulation process and monitor its evolution with time. AFM allows to record the three-dimensional morphology of sample surfaces with nanometric or even atomic resolution (Binnig et al., 1986). In contrast to more widespread high resolution characterization techniques (SEM, TEM, …), AFM is a non-destructive technique that can be operated in practically any environment, including air and liquids and also in vacuum. Furthermore, in most cases no special sample treatment is needed.

The physical magnitude measured by the AFM is the force between the sample and a sharp tip placed very close to the sample surface (the probe). To this end the tip is attached to a microfabricated cantilever whose deflection is usually detected by a position-sensitive photodiode array. AFM topographic images are obtained by scanning the sample surface while keeping constant the force applied by the probe, between 0.1 and 10 nN.

Solutions of λ-carrageenan and D-CPM were prepared by sonication (60 min) at very low concentration (10^{-6}–10^{-8} mg/ml). Both solutions were mixed, and samples were taken every hour over a period of 20 h. All solutions were prepared with p.a. grade reagents and triply deionized water (18 MΩ resistivity: MilliQ, Millipore, Bedford, MA).

Sample substrate used is highly oriented pyrolytic graphite, (HOPG) (Advanced Ceramics, Cleveland, OH), which is oriented in the exfoliable plain, allowing to obtain atomically smooth surfaces.

For the AFM sample preparation, we deposit 1 μl of the sample on a freshly cleaved HOPG surface, which corresponds to an approximate surface concentration of 10 molecules/μm^2, to avoid covering completely the substrate, since it is the reference of the probe measurements. Afterwards, the sample is dried in a dry nitrogen flux chamber (RH < 0.1%, 20–30 min) until the liquid is completely evaporated.

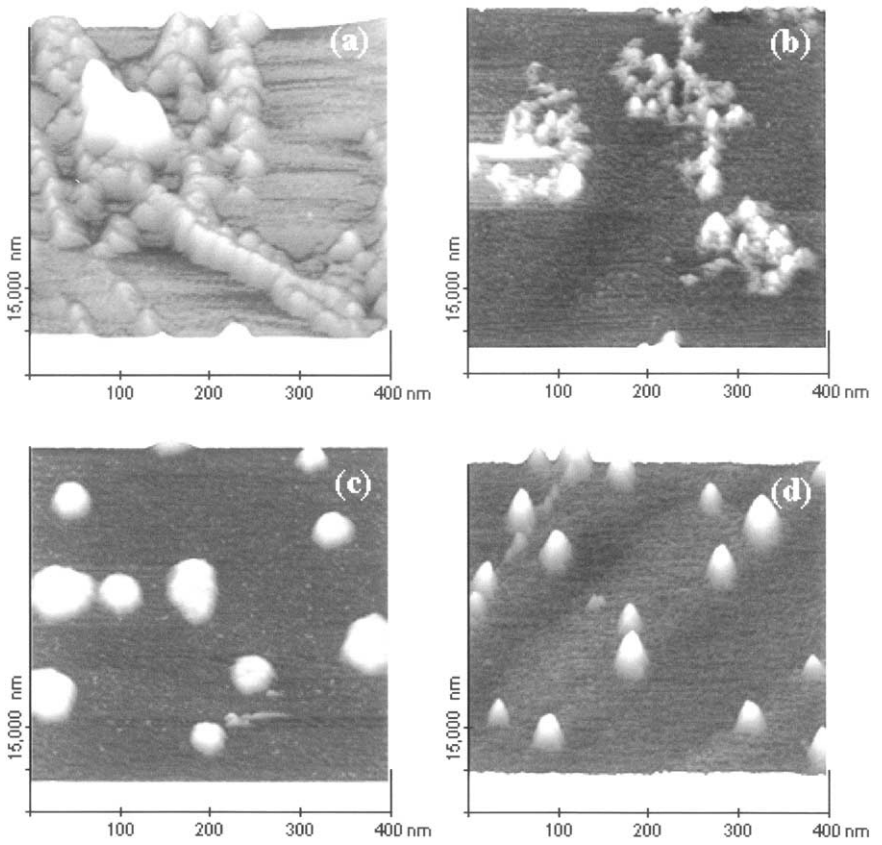

Fig. 3. AFM images at different stages of the nanoencapsulation process. Samples were taken from a mixed λ-carrageenan and D-CPM solution at different times: (a) $t = 0$, (b) $t = 6$ h, (c) $t = 10$ h and (d) $t = 17$ h.

Surface topography images were acquired in an Extended Multimode Nanoscope IIIa AFM system and processed with a NANOSCOPE V4.22 software (Digital Instruments, Santa Barbara, CA). The cantilevered tips were NCH Pointprobes (Nanosensors, Norderfriedrichskoog, Germany), manufactured in monocrystalline silicon with a spring constant of approximately 35 N/m and a resonant frequency of approximately 300 kHz.

Imaging was carried out in *tapping mode*, which is an intermittent-contact technique used to reduce lateral and frictional forces. Although this imaging mode has a slightly lower resolution, it has the advantage of reducing imaging artifacts and possible modifications of the surface by the tip. These aspects are critical in our case, since we image soft materials.

Preliminary studies were performed in order to characterize the topography of both polymer and drug, as shown in Figs. 1 and 2. Fig. 1 shows the morphology of λ-carrageenan suspensions. Typical chains already described for polymeric molecules were observed. Chains were distributed in an organized way and had a mean diameter of 2.5 nm (xy). Fig. 2 shows the morphology of D-CPM solutions, which exhibited some round-shaped isolated structures with a calculated xy molecule diameter of about 13.5 nm.

When characterizing the time evolution of the nanoencapsulation process, noteworthy differences were found among samples, mainly concerning the compactation degree: whereas imaging was difficult in the first samples due to the softness of the molecules, in the last stages almost no artifacts were found, and high quality images were

obtained, which explain the clear increase in the compactation. In Fig. 3 a set of images following the nanoencapsulation process is presented.

The Fig. 3(a) shows the system immediately after mixing the solutions of both polymer and drug (0 h). Some new structures about 95 nm wide and 6 nm high were observed, although polymer chains were still present.

In Fig. 3(b) the interaction between polymer and drug after 6 h is shown. Significant morphological differences were observed, mainly concerning size and hardness, although some artifacts were still registered, due to a movement of some structures by the tip interaction.

From the 10th h of the process, as represented in Fig. 3(c), images showed separated structures, although were still exhibiting an obvious trend towards conglomeration. Calculated dimensions for the present structures were in the order of 5–7 nm (z) by 20–80 nm (xy).

Fig. 3(d) shows completely formed nanocapsules, found after 17 h. Calculated diameter ranged from 5 to 30 nm. The high compactation degree and the homogeneity of the samples revealed that nanoencapsulation was finished.

The results described here demonstrate that nanoparticles were spontaneously nanoencapsulated over a period of 17 h. The observed capability of λ-carrageenan to nanoencapsulate spontaneously basic drugs offers the possibility to control the release rate of the drug from hydrophilic matrices with no need of complex technological processes.

Moreover, we have demonstrated the suitability of atomic force microscopy for the specific case of the on-time monitoring of interaction processes that happen in pharmaceutical systems.

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