CHARACTERIZING THE THREE-DIMENSIONAL TOPOGRAPHY OF FREEZE-DRIED BIODEGRADABLE NANOSPHERES BY FORCE MICROSCOPY AND INTERFEROMETRIC PROFILOMETRY.

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Introduction

The development of colloidal systems for drug transport is of main interest due to its capability to release the drug in a controlled and continuous fashion when administered by intravenous route.
Among the most used polymers for this purpose, polylactic acid (PLA), polyglycolide acid (PGA) and their derived co-polymers such as polylactide-co-glycolide acid (PLGA) are to be highlighted owing to their biocompatibility and low toxicity. (1)
Different approaches including submicron emulsions, liposomes and micro and nanoparticulated systems have been extensively investigated in recent years for drug targeting. Today, biodegradable nanoparticles are probably the most used system to control the drug delivery of biomolecules.
Morphology and size distribution of the particles intended for drug targeting, has to be perfectly characterized to assure their ability to deliver the drug in the specified site.
Dynamic Light Scattering is perhaps the most widespread technique for precise particle size measurement within the nanometric scale. (2) But this high resolution technique has to be performed in aqueous solution, and often polymeric nanoparticles are poorly soluble.
Two rather new topographic characterization techniques are studied in this work, with the aim of achieving reliable size characterization of nanospheres in solid-state.
The purpose of this study is therefore to compare two different nanometric techniques adapted to characterize topographically solid nanoparticulate pharmaceutical systems.

Materials and methods

Nanospheres preparation:
Nanospheres were prepared by the solvent evaporation-precipitation method (3,4) from solutions of methoxypoly(ethilene glycol)-poly(lactide-co-glycolide) (mPEG-PLGA). (5,6).
The nanospheres were isolated by centrifugation and washed several times with water. Nanospheres were freeze-dried and stored in a dessicator at a room temperature below 5ºC.

Nanospheres characterization:
Here we use an atomic force microscope (AFM) for measuring lateral dimensions of nanospheres, which will be correlated with vertical measurements obtained by interferometric profilometry.
Sample preparation is equal for both tecniques. We used aggregates of freeze-dried nanospheres in solid state. A thin slice of the sample (2 x 1 x 0.5 mm) was adhered on a freshly cleaved muscovite mica substrate.

Atomic Force Microscopy (AFM):

Topographic imaging and particle size measurements were carried out by means of AFM operated in Tapping Mode (TMAFM). (7)
Surface topography images were acquired with an Extended-Multimode Nanoscope IIIa AFM system and processed with a Nanoscope v5.12.r3 software (Digital Instruments, Santa Barbara, CA).
Details on operation principles as well as measurement methodlogy have already been reported. (8)
Interferometric profilometry:
The interferometric surface analysis microscope uses scanning light interferometry to image and measure sample surfaces and provide surface structure analysis without contacting the surface. Measurements are three dimensional. Vertical measurements, normal to the surface, are performed interferometrically, whereas lateral measurements, in the plane of the surface, are performed by calculating the pixel size from the field of view of the objective in use. Depths up to 100 μm, with 0.1 nm resolution, are imaged independent of objective magnification. (9)
Interferometry is a traditional technique in which a pattern of dark and bright bands, called fringes, result from an optical path difference between a reference and a sample beam. Incoming light is split inside the interferometer, one portion reflects from the test surface and another portion reflects from an internal, high quality reference surface. After reflections, the beams recombine inside he interferometer, undergo constructive and destructive interferences, and produce the light and dark fringe pattern. Both beams are then directed onto a solid-state camera.
The sample surface is scanned by vertically moving the objective with a piezoelectric transducer (PZT). A series of interferograms are generated as the objective is scanned, while recording detector data in digital memory. The data acquired in this way consists of an array of interferograms, representing the variation in intensity as a function of scan position. The interferograms stored in the computer are processed individually by frequency domain analysis (FDA), a mathematical method for processing complex interferograms in terms of phases and spatial frequencies.
Fourier analysis is used to extract a range of phases for each color or wavelength in the spectrum of the white-light source. The source spectrum together with the corresponding phases is a frequency domain representation of an interferogram. The particular combination of phases found by FDA uniquely defines the surface height map. The resulting phase measurements would be different for each wavelength.
When particle size is rather homogeneous, freeze-dried nanospheres are generally deposited in a layered fashion. By measuring layer heights, particle size is determined.
All measurements were performed with a New View 100 (Zygo Corporation, Middlefield, CT).

Results and Discussion

In Fig. 1 an AFM topographic image s presented, which exhibits the spheroidal morphology of the aggregated nanospheres.

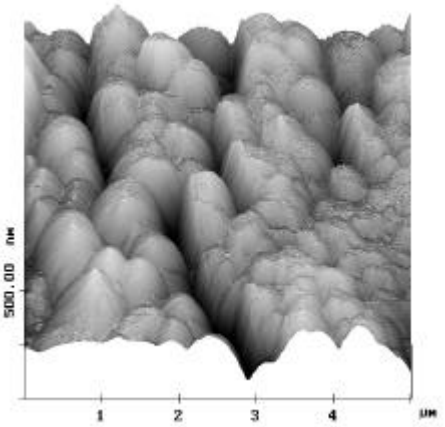

Figure 1. *AFM topographic image of nanospheres.*

Fig. 2 shows the section analysis of the sample. The dimensions of two single units are measured. Mean particle diameter was found to be in the order of 350 nm.

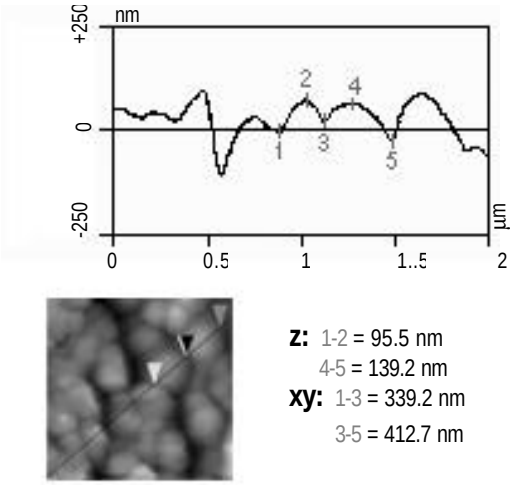

Figure 2. *AFM section analysisof tte nanospheres marked in the image below.*

It has to be pointed out that AFM is not a realiable technique for measuring vertical dimensions in aggregate samples, since resolution is affected by large height differences. The great vertical resolution of the perfilometer allowed us to precisely measure nanospheres' heights. In Fig. 3, a three dimensional projection of the sample is observed. The image exhibits the spatial disposition of nanosphere layers.

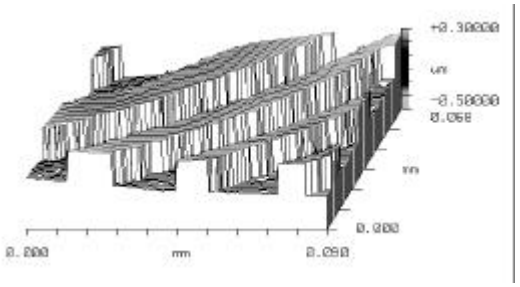

Figure 3. *Interferometric three dimensinal projection of a layered sediment of nanospheres.*

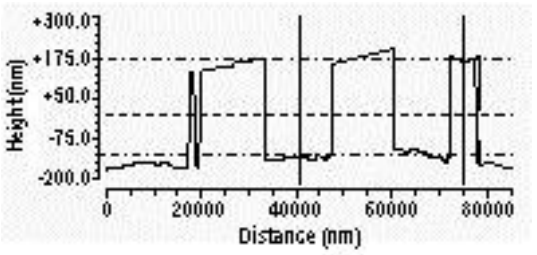

Figure 4. *Surface profile of a layered sediment of nanospheres.*

The surface profile of the analyzed surface shown in Fig. 4 highlights the homogeneity of particle heights, which were found to be in the order of 350 nm.

The good agreement of the results obtained by both techniques, demonstrate that atomic force microscopy and interferometric profilometry are highly reliable complementary techniques for the study of nanoparticulate pharmaceutical systems in solid state.

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