PREPARATION AND CHARACTERIZATION OF $\lambda$-CARRAGEENAN NANOSPHERES CONTAINING DEXCHLORPHENIRAMINE MALEATE

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Introduction
In recent years micro and nanoparticles have attracted a great deal of interest to control the drug delivery. Several techniques have been used to prepare nanoparticles, such as coacervation, (1,2) solvent evaporation, (3) interfacial polymerization, (4) spray-congealing, (5) etc. A previous work performed in our laboratory (6,7) demonstrated that $\lambda$-carrageenan, a sulphated polyelectrolytic polysaccharide, spontaneously self-assembles to amphiphilic basic drugs. Taking advantage of $\lambda$-carrageenan properties, an interesting alternative to produce nanoparticles is presented in this work.

The purpose of this study was to prepare and characterize $\lambda$-carrageenan nanoparticles containing dexchlorpheniramine maleate (D-CPM) as a model of amphiphilic basic drug. Surface chemical analysis of nanospheres was performed by means of X-ray photoelectron spectroscopy (XPS). Particle size and morphology were studied using atomic force microscopy (AFM).

Materials and methods
Materials:
D-CPM was obtained from Shering-Plough (Barcelona, Spain) and $\lambda$-carrageenan was purchased from Sanofi (Paris, France). All solutions were prepared with p.a. grade reagents and triply-deionized water (18 MO resistivity; MilliQ, Millipore, Bedford, MA).

Nanoparticle preparation:
Suspensions of $\lambda$-carrageenan were prepared at a concentration of 0.1 mg/mL by stirring and heating (60°C, 30 min). Afterwards were sonicated (200 w, 60 min) in order to avoid aggregation among polymer chains. Solutions of D-CPM (0.1 mg/mL) were prepared by sonication (200 w, 30 min). The nanoparticles were formed spontaneously after mixing the solutions of both polymer and drug (50:50). The resulting nanosuspensions were stirred for 1h at room temperature and ambient pressure, and then were sonicated (200 w, 60 min). Nanospheres were isolated by centrifugation and dried first in a stove at 40°C for 1h and after at room temperature overnight.

Nanospheres characterization:
XPS:
Samples of polymer, drug and nanospheres were analyzed in powder form. X-ray photoelectron spectra were obtained using a Physical Electronics PHI-5500 ESCA system, with a standard, non-monochromatic X-ray Al source operated at 300 W. For all samples, a survey spectrum was recorded over a binding energy range from 0 to 1100 eV, using a pass energy of 180 eV.
Determination of nanoencapsulation efficiency is done by evaluating the presence of Cl atoms in nanosphere surface, since such element is only present in D-CPM molecules. Its absence suggests that the outer layer of nanospheres is composed only of ω-carrageenan, which reveals that D-CPM is completely encapsulated. (8)

AFM:
Morphological characterization and particle size measurements were carried out by means of AFM operated in Tapping Mode (TMAFM). (9) Isolated nanospheres were re-suspended by stirring for 1 h. and diluted to a concentration of $10^{-8}$ mg/mL, with a dilution factor of 1:10. Briefly, 1 µL of the sample was deposited on a freshly cleaved HOPG surface. 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). For precise particle size measurements, a simple deconvolution model is employed. (7,10) Further details on sample preparation, deposition onto HOPG substrate and drying protocol have already been described in previous works. (6,7).

Results and Discussion
AFM studies:
AFM images showed rather homogeneous particles with a mean size about 50 nm. In Fig. 1, nanospheres' morphology is presented. Some spheroidal isolated particles were found. Figure 1. AFM image of nanospheres.

The width of the nanospheres was in the order of 50 nm, whereas heights were around 8 nm. The difference observed between $xy$ and $z$ dimensions, does not mean that nanoparticles are not spherical. Measured heights ($z$) are sometimes lower than the real values, because the sample can be subject to vertical compression by the tip during imaging. The drying stage could also contribute to this effect as a result of the water removal. To accurately characterize particle size, a frequency histogram exhibiting nanospheres diameter distribution is presented. (Fig. 2)

Figure 2. Frequency histogram exhibiting particle size distribution of nanospheres: convolution subtracted particle diameter ($x$) against number of particles ($y$).

XPS analysis:
In Fig. 3 the survey spectrum corresponding to ω-carrageenan/D-CPM nanospheres is given. The peak at 200 eV of binding energy corresponds to Cl 2p, which represents a 0.3% of the total surface composition. Considering that D-CPM spectrum exhibits a higher peak (3.4%), it is clear that a high nanoencapsulation rate was achieved.

Figure 3. XPS survey spectrum of nanospheres.

References