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Elaboration and structural analysis of aquasomes loaded with Indomethacin☆

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ABSTRACT

The aim of this study was to prepare nanoparticles in form of aquasomes with Indomethacin as a low solubility drug mode. Aquasomes charged with Indomethacin were obtained through the formation of an inorganic core of calcium phosphate covered with a Lactose film and further adsorption of the Indomethacin. Structural analyses, particle size, and morphology were evaluated by X-ray powder diffractometry, transmission electron microscopy, and scanning electron microscopy. The X-ray analysis of the samples and their observation through electronic microscopy allowed us to identify the inorganic calcium phosphate nucleus formation, as well as the layers of Lactose and Indomethacin. The particle size analysis of the aquasomes obtained with the Lactose layer and charged with the drug indicated an average particle size in the range of 60–120 nm, with a media of 90 nm. Standard deviation was 18.0234 and the standard error of the media 4.1348. The method was reproducible under the conditions used to prepare the aquasomes, such as ultrasound frequency and the moment of sonication for the formation of inorganic cores.

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1. Introduction

Within the last decade diverse technological strategies have been proposed in order to obtain nanoparticles, of a distinct nature, charged with drugs which in turn have revolutionized the systems of drug administration, particularly those of controlled liberation and the ones oriented at the vectoring of the active principle for liberation of target tissue or organs (Chow and Gonzalves, 1996; Alleman et al., 1993; Ramos-Picos et al., 2001; Kossovsky et al., 1996). Various methods used for the obtention of nanoparticles use polymers and encounter difficulties such as the compatibility of solvents and other constituents and the polymers and co-polymers with the active principle and biological fluids and factors of the collection system (Kim et al., 2000; Quintanar-Guerrero et al., 1998). Kossovsky proposed a system to prepare nanoparticles transporting the so-called aquasomes (Kossovsky et al., 1995), whose particle size (lower than 1000 nm) is appropriate to parenteral administration because it prevents the obstruction into the bloodstream capillaries (Banker and Rhodes, 1990).

Aquasomes differentiate from other nanoparticles systems by their conformation and the water absorbent nature which not only makes their aqueous transport permissible but also confers the possibility of establishing non-covalent links with

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distinct molecules and macromolecules promoting a major stability compared than liposomes; advantages that offer a particularly favourable environment for proteins thereby avoiding their denaturalization (Kim and Kim, 2002; Masatoshi and Yongning, 1998; Khopade et al., 2002). Those properties are possible because aquasomes are inorganic cores, which are coated with polyhydroxyl compounds and these are responsible for their hydrophilic behaviour.

The present study had the objective of preparing nanoparticles in the form of aquasomes. They were charged with Indomethacin, a model drug of low aqueous solubility that presents polymorphism (Del Río, 2002) and has lately been used as a study model in micro- and nanoparticulates systems (Charcosset and Fessi, 2005; Pinon-Segundo et al., 2006; Barichello et al., 1999). The structural analysis of these nanoparticles was carried out by diffraction of X-rays and electronic microscopy.

2. Materials and methods

2.1. Materials

Calcium chloride (J.T. Baker), monobasic sodium phosphate (J.T. Baker), acetone (J.T. Baker), ethanol (J.T. Baker), chloroform (J.T. Baker), Butvar's reactive for scanning electronic microscopy (SEM) and bi-distilled water were analytical grade. Lactose (Lactochem) and Indomethacin 99% (Auzohu-Konch Pharmaceutica) were pharmacopeial quality.

2.2. Methods

2.2.1. Preparation of aquasomes

The general procedure consists of an inorganic core formation, which will be coated with Lactose forming the polyhydroxylated core that finally will be loaded by Indomethacin, our model drug.

2.2.1.1. *Inorganic cores*. The inorganic cores obtained from calcium phosphate were prepared from the precipitation of a 0.25 M monobasic sodium phosphate solution and a 0.75 M calcium chloride solution agitated mechanically. The process variables were the level of ultrasound frequency (UF) and the influence of the sonication on the particle size of the inorganic cores.

Table 1 describes the experimental design 2^2 where the inorganic cores of samples one and three were sonicated at 30 and 90 pulses of frequency (UF) after the precipitation took place, respectively. Whereas samples two and four were sonicated at 30 and 90 (UF) during the precipitation of the core.

Table 1 – Factorial design 2^2 applying different sonication conditions for inorganic cores

Variable	Samples			
	1	2	3	4
Sonication	A	D	A	D
UF (pulses)	30	30	90	90

A: after precipitation, D: during precipitation. UF: ultrasound frequency.

A sonicator Vibracell Sonios VC 600-2 was employed during a period of 90 min each at 4 °C. After these treatments the dispersion containing the inorganic cores was filtered through a membrane filter (pore size 0.22 μm) of nitrocellulose. A freeze drying technique was applied over the filtered volume using a Labconco Lyophilizator, serie 67974.

2.2.1.2. *Lactose coating*. A sample of 1.0 mg of the inorganic cores was resuspended into a 1.0 ml bi-distilled water and was added to a 100 ml solution of Lactose, whose concentrations were 0.03, 0.06 and 0.09 M each. After a period of 90 min of mechanical agitation the dispersion was filtered through a membrane filter (pore size 0.22 μm) of nitrocellulose, and then lyophilizised producing the polyhydroxylated nanoparticles.

2.2.1.3. *Drug loading*. A solution 0.06 M of Indomethacin in acetone was added at a dispersion of 1 mg/ml of the polyhydroxylated cores after a mechanical agitation, which was maintained for 90 min. The dispersion was filtered and freeze-dried.

2.3. Identification, particle morphology, and distribution size analysis of nanoparticles

The analysis and characterization of the nanoparticles in each step were determined by transmission (TEM) and scanning electron microscopy (SEM) and X-ray powder diffractometry (XRPD).

The morphology and the size distribution were obtained through images of secondary electrons in (SEM) using a JEOL JSM-200 instrument. The samples were placed on the surface of a bronze-sample SEM-holder using a double-sided adhesive tape. The images were digitalized and the calculation of size distribution was done from these images using the Analysis Image Processor software (Soft Imaging System) as well as the statistical SPSS software, version 10.0.

The chemical composition and the crystalline structure of all samples were obtained through X-ray diffraction powder using a Booker AXS DP Advance diffractometer with Cu Kα radiation at 40 kV/40 mA. To do this, after the diffractograms were taken, their indexation indicated their corresponding power diffraction file (PDF number) that includes the chemical composition and crystalline structure as data of the information that it contains. For TEM observation and images the samples were placed on copper grids of 3 mm in diameter and 100 mesh, which have been previously covered with a plastic film of fomvar and coated with a carbon thin film. These TEM observations were carried out with the JEOL-100CX and JEM-1200 EX microscopes.

The TEM images of the samples were obtained both in a clear and dark field mode and the photographic films were digitalized using Adobe Photoshop software. The images were colored with the pStyler software, applying the false color technique.