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Material Behaviour

Surface-coated polycaprolactone nanoparticles with pharmaceutical application: Structural and molecular mobility evaluation by TD-NMR

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ABSTRACT

The development of nanosystems in the pharmaceutical field has been growing in recent years. Polymers in particular are attractive since they can enable controlled release. Furthermore, surface modification is essential to increase nanosystem stability and prolong the blood circulation time. In this context, the present study obtained polycaprolactone (PCL) nanoparticles coated with poly(ethylene oxide) −poly(propylene oxide) triblock copolymer using the nanoprecipitation method, aiming to evaluate the effect of PCL concentration in this type of pharmaceutical formulation on prolongation of drug release. Nanosuspensions were evaluated by the following techniques: FT-IR, DLS, FESEM, TEM, ZP, TGA, DSC, XRD and TD-NMR. With higher PCL concentration, the particle size also increased and negative surface charge was observed. The nanoparticles' spherical shape was confirmed by FESEM and TEM while XRD and TD-NMR revealed changes in Pluronic chains' organization around the PCL matrix. Although this kind of polymer combination is already known in the pharmaceutical field, structural and molecular mobility evaluation with new perspectives were performed, mainly by XRD and TD-NMR.

1. Introduction

Conventional drug delivery can cause high drug concentrations in the blood, exceeding the patient's toxicity level, which may result in the occurrence of adverse events as well as some therapeutic inefficiencies [1–3]. Aiming to promote both controlled drug release and targeted therapy, drug delivery systems have started to gain interest and, in this context, both carrier stability and a long blood circulation time are important [2–5].

Lately, nanocarriers have been studied in the pharmaceutical field [6], among them, polymeric nanoparticles, which are defined as submicron colloidal particles, usually composed of drug and polymer [3]. Polymeric nanoparticles can promote drug protection as well as avoid clearance from the body, resulting in a longer half-life. Furthermore, these nanocarriers can reduce the drug distribution through healthy tissues [7,8], promote controlled release, reduce adverse events, increase therapeutic efficiency and safety as well as overcome physiological barriers [3,7,9] and avoid efflux pumps [8].

Polycaprolactone (PCL), a biodegradable, biocompatible and nontoxic polymer, is promising for the development of nanoparticles [3,10,11]. Even although PCL has been widely used in the pharmaceutical field, its hydrophobic characteristic leads to fast removal from the body as well as poor stability of nanoparticles in water. To increase PCL nanoparticle stability, surface modifications are essential [11]. Due to their amphiphilic characteristic, poloxamers, also known as Pluronics, have been used to obtain surface-coated nanoparticles for drug delivery purposes. They are tri-block copolymers composed of hydrophobic and hydrophilic units of propylene oxide (PO) and ethylene oxide (EO), respectively [12].

Several techniques have been used to obtain nanoparticles from preformed polymers. Among them, the nanoprecipitation method, also known as interfacial deposition or solvent displacement, is widely employed [4,13,14] and enables obtaining colloidal nanosuspensions [4,7,13,14].

Regarding nanosuspension stability, nanocarriers must be stable and maintain their structural integrity in order to ensure drug delivery [9]. After long periods of storage, nanosuspension particles can fuse and aggregate [15], so obtaining dry particles became necessary. In this case, rehydration of particles to their original

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suspension is important [16,17]. However, nanoparticle irreversible aggregation can be caused by the stress induced by the drying process [15–19]. Thus, research groups have started to use cryoprotectants to protect colloidal nanoparticles [9,15–19], increase their stability during storage [9,15,18,19] and permit their resuspension in water. In this context, trehalose is a widely used cryoprotector [9,15,16,18].

This kind of polymer combination is already known in pharmaceutical field, however, this study focused on structural and molecular mobility evaluation with new perspectives by using TD-NMR. Since it provides information about material structure and properties, as well as allowing detailed study of crystalline and amorphous domains [20], ^{1}H relaxometry has been widely employed to evaluate polymer molecular dynamics [21,22], including for drug delivery applications [23].

This article evaluates the effect of PCL concentration on the development of polymeric nanoparticles coated with Pluronic F-68 using the nanoprecipitation method, and the possibility of drug release prolongation. Morphological, chemical and physical properties of this material were investigated as well as nanosystem stability regarding particle size and zeta potential.

2. Experimental

2.1. Materials

We used polycaprolactone (Mn 80.000 g/mol); Pluronic® F-68 (Mn 8.400 g/mol); acetone P.A.; D-(+)-trehalose dihydrate; and ethanol P.A. All materials were obtained from Sigma-Aldrich®.

2.2. Preparation of polymeric nanoparticles (Nps)

PCL nanoparticles coated with Pluronic F-68 were obtained using nanoprecipitation according to [13]. Different amounts of PCL were dissolved in acetone and three different concentrations were obtained – 0.2%; 0.4% and 0.6% (m/v) (Table 1). Solutions were stirred for 20 min under heating (40 °C) to solubilize the PCL in a sealed system. Meanwhile, 50 ml of aqueous phase was prepared, using Pluronic F-68 (0.5% m/v). After that, each organic solution was added to the aqueous phase under constant stirring and the organic solvent was evaporated over 72 h at 25 °C. Then, final suspensions were filtered and the nanosystems' final volume was completed to the original aqueous phase volume. For the freeze-drying process, trehalose was added at 5% (m/v). Formulations were frozen (−68° to −72 °C) for 3 h and lyophilized (−49 °C and 72 mmHg) for 96 h. This process was performed in the presence or absence of cryoprotectant.

2.3. Characterization

2.3.1. Dynamic light scattering (DLS) and colloidal stability of particles

Particle size distribution (PdI) was determined by DLS using a Malvern Instruments zetasizer Nano S90. A suitable amount of lyophilized particles with cryoprotectant was dispersed in distilled water at a total concentration of 50 mg/ml. Hydrodynamic diameter was also analyzed after three and six months of storage to evaluate mean diameter (MD) stability.

2.3.2. Zeta potential (ZP) and colloidal stability of particles

Surface charge of nanoparticles was characterized in terms of ZP using a Brookhaven Instruments NanoBrook ZetaPALS. All measurements were performed with 10 runs at 25 °C and results are reported in terms of ZP ± SD. Analyses were also performed after three and six months storage to evaluate ZP stability.

2.3.3. Field emission scanning electron microscopy (FESEM)

Nanoparticle morphology was examined by FESEM using a Zeiss Auriga 40 microscope operating at a 1.5 kV acceleration voltage and working distances of 3, 3.1 and 6.5 mm. Magnification of the electron micrographs were 20000× and 60000x.

2.3.4. Transmission electron microscopy (TEM)

The morphology and size of PCL coated nanoparticles were also observed by TEM. Nanosuspensions were diluted in water (1:10) and a drop of each was placed on a formvar-coated copper grid and allowed to dry. Images were obtained using a FEI Tecnai G2 Spirit microscope operating at 120 kV and captured using an Olympus Soft Imaging Solutions Veleta camera.

2.3.5. Fourier-transform infrared spectroscopy (FTIR)

FTIR was performed with a PerkinElmer FTIR, Spectrum version 10.4.2 spectrometer, using a ZnSe crystal (Pike Technologies, Madison) at 25 °C. The scanning range was 4000 to 600 cm^{-1} with resolution of 4 cm^{-1} and 60 scans.

2.3.6. Thermogravimetric analysis (TGA)

TGA was performed using a TA Instruments Q50 analyzer. Samples were heated from 0 to 700 °C under nitrogen flow at a rate of 10 °C/min.

2.3.7. Differential scanning calorimetry (DSC)

DSC measurements were carried out under nitrogen atmosphere at a rate of 50 ml/min using a Rigaku TAS 100 thermal analyzer. Samples were heated from −80 °C to 200 °C at a rate of 10 °C/min.

2.3.8. X-ray diffraction (XRD)

XRD analysis, performed with a X-ray Shimadzu LabX XRD-6100. Samples were exposed to CuKα radiation (λ = 1,5418 Å) at room temperature and data were recorded over the 2θ range from 10° to 30° using a scan rate of 0.02°/second. The crystallinity degree (Xc) was calculated using the Fytik software according to equation (1).

$$Xc = \frac{Crystalline\ phase\ area}{Crystalline\ phase\ area + amorphous\ phase\ area} \times 100 \quad (1)$$

2.3.9. Time-domain nuclear magnetic resonance (TD-NMR)

Relaxometry was employed to evaluate the samples' molecular dynamics through measurement of the spin-lattice and spin-spin relaxation times. NMR experiments were performed with a Maran Ultra spectrometer from Oxford Instruments, operating at 0.54 T (23.4 MHz for ^{1}H), with 18 mm magnet bore, at 30 °C./

The inversion-recovery pulse sequence ($p180°_x$ - τ - $p90°_x$) was employed to investigate samples' spin-lattice relaxation time

Table 1
Composition of nanoparticles.

Sample	PCL concentration (%m/v)	Cryoprotectant
PCL1	0.2%	Absent
PCL2	0.4%	Absent
PCL3	0.6%	Absent
PCL1WT	0.2%	With trehalose
PCL2WT	0.4%	With trehalose
PCL3WT	0.6%	With trehalose