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Enhanced oral bioavailability of the antiretroviral efavirenz encapsulated in poly(epsilon-caprolactone) nanoparticles by a spray-drying method

Aim: To encapsulate efavirenz (EFV) within poly(epsilon-caprolactone) (PCL) nanoparticles (NPs) and compare the oral pharmacokinetics with that of EFV-loaded micelles and pure EFV NPs. **Materials & methods:** EFV-loaded PCL NPs were produced by a double-emulsion/spray-drying method. **Results:** NPs displayed a hydrodynamic diameter of 200–250 nm. The encapsulation efficiency was 86–93% and the mass recovery was above 60%. X-ray diffraction indicated that drug and PCL underwent amorphization during the spray-drying process. Encapsulation within NPs significantly increased the maximum concentration in plasma and the bioavailability. **Conclusion:** EFV-loaded PCL NPs represent a promising platform to develop scalable pharmaceuticals with improved (bio)pharmaceutic performance.

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KEYWORDS: efavirenz ▪ HIV ▪ *in vitro* drug release ▪ oral bioavailability enhancement ▪ poly(epsilon-caprolactone) nanoparticle ▪ spray drying

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HIV/AIDS is the deadliest infectious disease [1]. Approximately 35 million people are living with HIV, with more than 25 million lives claimed since its emergence in 1981 [1–4]. A combination of antiretrovirals is administered on a chronic basis to prevent the progression towards AIDS [5]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) noncompetitively inhibit the activity of reverse transcriptase, the enzyme that transcribes the single-stranded HIV-1 RNA into dsDNA and enables the replication of HIV-1 in the host [1,6,7,101]. According to standard treatment guidelines, at least one NNRTI is included as a first-choice drug in the therapeutic cocktail [8,9]. NNRTIs usually display low aqueous solubility, which translates into poor and erratic oral bioavailability. This (bio)pharmaceutic drawback has posed major challenges to the pharmaceutical industry [10–13].

In recent years, nanomedicine has provided many technological platforms with the aim to develop strategies that can increase the solubility and bioavailability of poorly water-soluble drugs [4,7]. Nanoparticles (NPs) of biocompatible and biodegradable polymers have great potential as preferred candidates due to their ability to efficiently encapsulate hydrophobic drugs in amorphous form, thus increasing dissolution rates in biological fluids [14–16]. Aliphatic polyesters are of great value, mainly because they have been approved by the US FDA. Examples include poly(epsilon-caprolactone) (PCL), poly(lactic acid), poly(glycolic acid) and their copolymers (e.g., poly[lactic-*co*-glycolic] acid) [15,17]. For instance, PCL has found extensive biomedical applications [18] and it has been increasingly used in the development of drug-delivery systems [19].

Efavirenz (EFV) is the preferred NNRTI of the British HIV Association, the US Department of Health and Human Services and the International AIDS Society, and it is also the first choice in the treatment of children above the age of 3 years [8,20–22,102–104]. However, EFV is a hydrophobic drug displaying an aqueous intrinsic solubility of approximately 4 µg/ml, which leads to a limited oral bioavailability of 40–45% [21]. It was shown that the nano-encapsulation of EFV within polymeric micelles significantly increased the oral bioavailability of the drug relative to a conventional extemporaneous suspension and an oily solution [21,23]. The same platform improved the bioavailability of EFV in the brain after intranasal administration [24]. More recently, Katata *et al.* reported on the encapsulation of EFV within PCL NPs employing a double-emulsion (water-in-oil-in-water [w/o/w])/spray-drying method [25]. One of the main advantages of this technology is high encapsulation efficiency and drug cargo loading capacity, reproducibility and scalability. We envisaged that the bioavailability of the drug would be substantially improved due to a more prolonged residence time of the polymeric NPs in the gastrointestinal tract over the previously developed polymeric micelles. In this framework, the present work investigated for the first time

the oral pharmacokinetics of EFV-loaded PCL NPs coated, or not, with the polycationic mucoadhesive polysaccharide chitosan (CS), and compared it with that of pure EFV NPs produced by spray drying and EFV-loaded micelles.

Materials & methods

Materials

PCL (10 kg/mol), poly(vinylalcohol) (PVA; 13–23 kg/mol, 87–89% hydrolyzed), CS (low viscosity), lactose monohydrate (drying excipient), ethyl acetate and polyethylene glycol (PEG; 9 kg/mol) were obtained from Sigma-Aldrich (Johannesburg, South Africa). EFV was supplied by Aspen-Pharma (Johannesburg, South Africa). Pluronic® F127 (a linear poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) copolymer, molecular weight of 12.6 kg/mol, 70 wt% poly(ethylene oxide) [26]) was a donation from BASF (NJ, USA). Ultrapure water (resistivity higher than 18.2 MΩ) was used throughout the experiments. All other chemicals were of analytical grade and purchased from Sigma-Aldrich.

Preparation of EFV-loaded PCL NPs

EFV-loaded PCL NPs (PCL-EFV NPs) were produced using a modified w/o/w double-emulsion/spray-drying method [25,27]. Briefly, 2% PVA water solution (2 ml) was emulsified (3 min) with a solution of PCL (100 mg) and EFV (100 mg) in ethyl acetate (8 ml) using a high-speed homogenizer (5000 rpm; Silverson L4R GX-10 model; Silverson Machines Ltd, Chesham, UK) to form the primary water-in-oil emulsion (w_1/o). To produce CS-coated NPs, this emulsion was poured into an aqueous solution (w_2; 40 ml) comprised of 2% PVA, 0.5% PEG, 5% lactose and 0.3% CS, and the mixture was homogenized for 5 min (8000 rpm) to form the double water-in-oil-in-water (w_1/o/w_2) emulsion. To produce uncoated NPs, all of the components and the final volume were maintained, although CS was not added to w_2. The w_1/o/w_2 obtained was directly fed into a benchtop mini-spray dryer (Model B-290; BÜCHI Labortechnik AG, Flawil, Switzerland) and spray dried at 96°C, with an atomizing pressure between 6 and 7 bars and aspirator setting at 100%. The solid PCL-EFV NPs were collected and kept in a desiccator with silica gel at room temperature. Coated and uncoated NPs are named CS-coated PCL-EFV NPs and uncoated PCL-EFV NPs, respectively. The performance of the EFV-loaded NPs was compared with that of CS-coated pure EFV NPs (EFV-NPs) that were prepared by employing the same methodology without the addition of PCL into the first emulsion. In the same way, CS-coated drug-free NPs (PCL-DF NPs) were produced by the same protocol in the absence of the drug and were used as control. The percentage mass recovery was determined according to Equation 1:

$$Mass\ recovery\ (\%) = \frac{W_p}{W_{pol+EFV}} \quad \text{(Equation 1)}$$

Where W_P is the total weight of particles obtained after spray drying and $W_{pol\ +EFV}$ is the total initial amount of polymer, excipients and EFV employed in their production. Results are reported as mean ± standard deviation (SD) of three independent experiments.

Preparation of EFV-loaded polymeric micelles

The pharmacokinetics of EFV nanoencapsulated within polymeric micelles was previously reported, although under different drug concentration conditions [21,23]. For a robust comparison of PCL-EFV NPs and EFV-NPs with that formulation, in this work, micelles were evaluated using an identical dose and drug concentration. Pluronic F127 polymeric micelles (10% w/v copolymer concentration) were produced by dissolving 1 g of the copolymer in phosphate–citrate buffer solution (10 ml; pH 5.0) at 4°C and equilibrating the system (25°C) at least 24 h before use [21,23]. EFV (60 mg) was added to the micelles (3 ml) and the mixture was shaken (MiniTherm-Shaker; Adolf Kuhner AG, Basel, Switzerland) at 25°C until total drug dissolution. The final drug concentration was 20 mg/ml (2% w/v) [21,23]. These EFV-loaded polymeric micelles showed a main size population of 27.3 nm (92.3% intensity) and a small fraction of 5.0 nm (7.7% intensity), as determined by dynamic light scattering (DLS) [23].

Particle size, particle size distribution & zeta-potential

The mean particle size (expressed as hydrodynamic diameter; D_h) and size distribution (expressed as polydispersity index; PDI) of all of the systems was determined by DLS (Malvern Zetasizer Nano ZS; Malvern Instruments, Malvern, UK) at 25°C, taking the average of at least three measurements. PCL-EFV NPs and EFV-NPs were resuspended in deionized water and sonicated (10 s) to ensure appropriate redispersion before analysis. To analyze the surface charge density, the zeta-potential of PCL-EFV NPs and EFV-NPs was measured using the same instrument. Each

sample was measured in triplicate and the results expressed as mean ± SD.

Drug encapsulation efficiency

The amount of EFV encapsulated within PCL NPs was determined using an indirect method. NP samples (20 mg) were dispersed in water (10 ml) and centrifuged to remove polymeric NPs. The concentration of free EFV in the supernatant was then determined by UV spectrophotometry (λ = 248 nm; Lambda 35; PerkinElmer, MA, USA). UV spectrophotometry has been shown to be sensitive enough to quantify EFV in different *in vitro* studies such as drug encapsulation efficiency, drug payload, release and physical stability [21,23,24,28,29]. The intrinsic solubility of EFV in pure water is 4 µg/ml. However, amorphization during spray drying and the presence of excipients such as PVA, PEG, CS and lactose in the formulation increased its aqueous solubility to 450 µg/ml (25°C), as determined in our laboratory. This enabled the solubilization of the nonencapsulated drug in a small volume of water. A stock solution of EFV in methanol (1 mg/ml) was diluted in distilled water to fit the concentration range between 0.005 and 0.035 mg/ml and analyzed by UV ($R^2 > 0.9954$). Each sample was measured in triplicate and the results expressed as mean ± SD. The encapsulation efficiency (%EE) was calculated using Equation 2:

$$\%EE = \frac{EFV_0 - EFV_{supernatant}}{EFV_0} \quad \text{(Equation 2)}$$

Where EFV_0 and $EFV_{supernatant}$ are the initial amount of drug used in the production of the NPs (100 mg) and the EFV concentration in the supernatant after the centrifugation, respectively.

Morphology

The morphology of the NPs was characterized by scanning electron microscopy (SEM; Field Emission Electron Microscope, JEOL JSM-7500F; JEOL Ltd, Tokyo, Japan) and transmission electron microscopy (TEM; JEOL JEM-2100; JEOL Ltd). For SEM, NPs were placed onto carbon stubs using a double-sided carbon adhesive tape and sputtered with conductive gold in a high-vacuum evaporator under an argon atmosphere prior to imaging, while TEM samples were prestained with uranyl acetate solution.

Attenuated total reflectance/Fourier transform infrared spectroscopy

Attenuated total reflectance/Fourier transform infrared spectroscopy was carried out using a PerkinElmer Spectrum 100 Fourier transform infrared spectrophotometer between 4000 and 650 cm^{-1}.

X-ray diffraction

X-ray diffraction analysis was conducted using an X'Pert PRO diffractometer (PANalytical, Almelo, The Netherlands) under reflection–transmission mode. Samples were placed in glass sample holders and scanned from 2θ = 5–60°, using a beam of Cu Kα radiation of λ = 0.1542 nm, operated at 45 kV, 40 mA. The scan speed and exposure time were 0.109419°/s and 17 min 27 s, respectively.

In vitro release studies

PCL-EFV NPs (30 mg) were suspended in phosphate-buffered solution (10 ml) under different pH conditions (1.3, 4.5 and 6.8) and placed in a water bath shaker (100 rpm) at 37°C. At predetermined time intervals, 1.5-ml supernatant aliquots were withdrawn and replaced by preheated fresh buffer. The solubility of EFV does not depend on the pH in the range between 2 and 10, becoming more soluble at more basic values due to the deprotonation of the cyclic carbamate [30]. The %EE was above 86%, and thus this amount of NPs (30 mg) contained a maximum of 2 mg of free EFV. Considering the solubility of the processed EFV in the presence of excipients that serve as solubilizers (450 µg/ml at 25°C) and the renewal of 60% of the volume of the release medium during the first 6 h of the assay, sink conditions were ensured. The concentration of drug released was monitored by UV spectrophotometry (see section titled 'Drug encapsulation efficiency'). Each sample of NPs

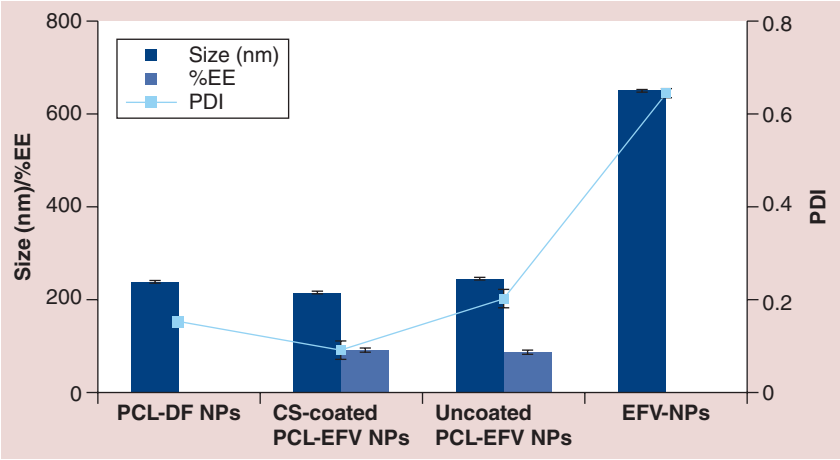

Figure 1. Properties of the different efavirenz systems. Data represent the mean ± standard deviation (n = 3).
CS: Chitosan; EE: Encapsulation efficiency; EFV-NP: Pure efavirenz nanoparticle; PCL-DF NP: Drug-free poly(epsilon-caprolactone) nanoparticle; PCL-EFV NP: Efavirenz-loaded poly(epsilon-caprolactone) nanoparticle; PDI: Polydispersity index.

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was analyzed in triplicate and the results expressed as mean ± SD. Parameters were compared by two-way analysis of variance and the Bonferroni test as the *post hoc* test.

Oral pharmacokinetics

The oral pharmacokinetics of EFV was assessed in male Wistar rats (225–300 g). Animal experiments were in line with the 'Principles of laboratory animal care' [31] and local regulations (Argentina). Animals were maintained on a 12-h light/dark routine at 22 ± 2°C and received a standard rodent diet. Experiments were carried out in rats fasted overnight (12 h). Drug formulations were administered to conscious rats by gavage. The EFV dose and volume administered were 20 mg of EFV/kg and 4 ml/kg, respectively. For this, the exact amount of uncoated and CS-coated PCL-EFV NPs and pure EFV NPs were weighed to fit the individual rat weight, resuspended in the corresponding volume of water and vortexed (3 min). EFV-loaded Pluronic F127 polymeric micelles (10% final copolymer concentration, EFV concentration of 20 mg/ml) were diluted (1:3) to adjust the final administration dose (20 mg/kg) and volume (4 ml/kg). After administration, blood samples (70 µl) were collected from the tail vein at time points between 0.5 and 48 h [32], allowed to clot and centrifuged (10,000 rpm, 10 min, 4°C). The isolated serum (10 µl) was deproteinized with acetonitrile (20 µl) and the concentration of the drug was determined by HPLC.

Chromatographic method for EFV

The concentration of EFV in plasma was measured by HPLC, using a Phenomenex Luna® 5 mm, C18, 150 × 4.60 mm column (Phenomenex Inc., CA, USA) with a UV detector (248 nm; UVIS 204; Linear Instruments, Chennai, India) [21]. The mobile phase composed of distilled water: acetonitrile:triethylamine (60:40:0.2; pH 3) was pumped at a flow rate of 1.4 ml/min. The analytical method for quantification was validated in the 20–5000 ng/ml range.

Analysis of *in vivo* data

Noncompartmental analysis of EFV plasma concentrations was performed using the TopFit software (version 2.0; Boehringer Ingelheim, Ingelheim, Germany). The following pharmacokinetic parameters were estimated: maximum plasma concentration (C_{max}), time to the maximum plasma concentration (t_{max}), area under the curve between the administration time and 24 h (AUC_{0-24}), area under the curve between the administration time and 48 h (AUC_{0-48}) and elimination rate constant (k_e). Parameters were compared by one-way analysis of variance and the Bonferroni test, as the *post hoc* test. Statistical

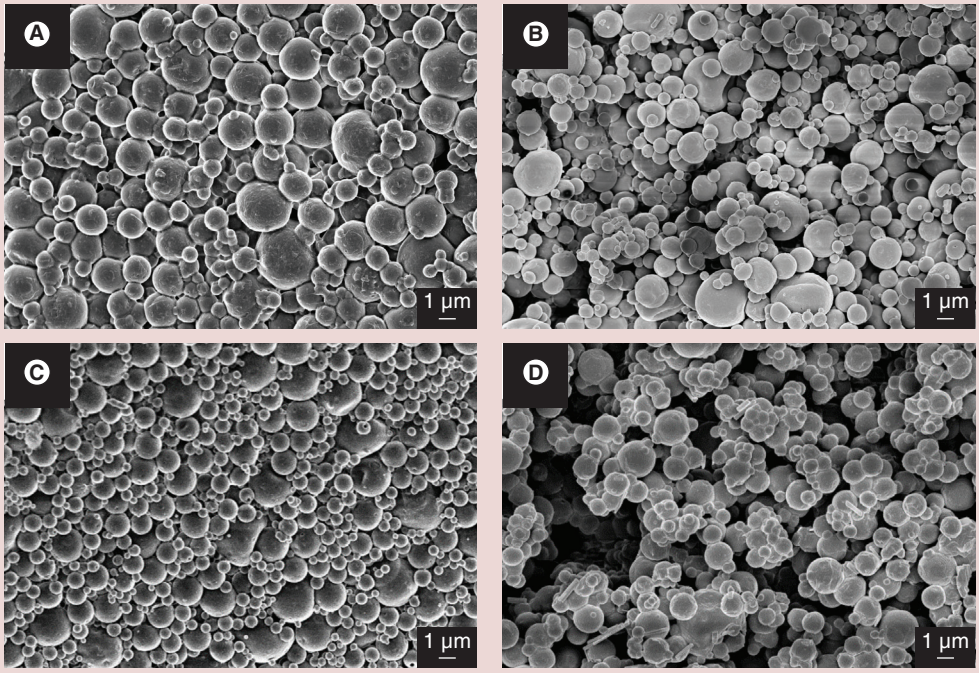

Figure 2. Morphology of the different nanoparticles by scanning electron microscopy. **(A)** Drug-free poly(epsilon-caprolactone) (PCL) nanoparticles (NPs), **(B)** chitosan-coated efavirenz-loaded PCL NPs, **(C)** uncoated efavirenz-loaded PCL NPs and **(D)** pure efavirenz NPs.

analysis was performed using GraphPad Prism® version 5.02 for Windows (GraphPad Software, CA, USA). Statistical significance was defined as $p < 0.05$. The variability between individuals was assessed by calculating the coefficient of variation (expressed in %), which is a normalized measure of the dispersion of a probability distribution. The coefficient of variation (expressed in %) is calculated as the ratio of the SD and the mean value multiplied by 100.

Results & discussion

Preparation & characterization of the NPs

Particle size is a key parameter that influences the dissolution rate of the drug in the gastrointestinal tract [33]. For instance, as the particle size drops to the nanometer scale, the surface area available for dissolution substantially increases, causing a decrease in the diffusion layer thickness of the drug material and increasing the dissolution rate [34,35]. EFV is classified into class II of the Biopharmaceutic Classification System (low aqueous solubility and high permeability), and thus encapsulation within polymeric NPs or 'nanonization' of pure EFV was expected to enhance its oral bioavailability.

The most common methods for the encapsulation of hydrophobic drugs within polyesters are based on simple oil-in-water emulsions. However, during the time of the spray-drying process, the emulsion needs to be highly stable without mechanical stirring to prevent droplet coalescence and size growth. The principle for the preparation of PCL-EFV NPs by the double-emulsion spray-drying method is that the organic phase and both aqueous phases are in thermodynamic equilibrium. Katata *et al.* showed that a double emulsion was stable enough to carry out a reproducible spray-drying process with minimum size growth

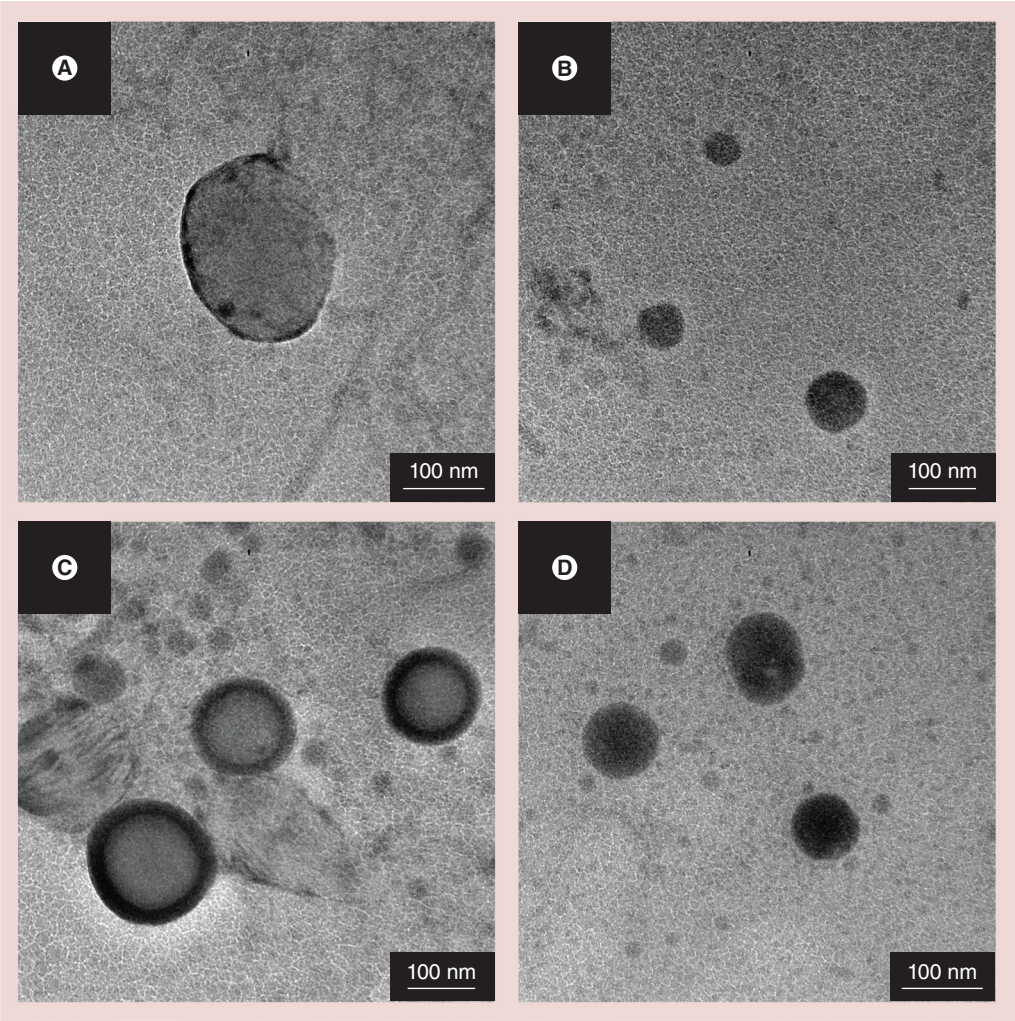

Figure 3. Morphology of the different nanoparticles by transmission electron microscopy. **(A)** Drug-free poly(epsilon-caprolactone) (PCL) nanoparticles (NPs), **(B)** chitosan-coated efavirenz-loaded PCL NPs, **(C)** uncoated efavirenz-loaded PCL NPs and **(D)** pure efavirenz NPs.

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and small size polydispersity [25]. The opposite was true for a simple emulsion that underwent relatively fast droplet size growth. In addition, partially water-soluble solvents such as ethyl acetate resulted in smaller and more homogeneous PCL NPs than the water-immiscible dichloromethane [25]. The incorporation of the polycationic polysaccharide CS into the formulation was aimed at providing electrostatic and steric stabilization, preventing particle agglomeration and promoting particle size reduction [36,37], as well as to generate a mucoadhesive NP coating that would prolong the residence time in the gut and increase the absorption of the NPs by the paracellular route due to the transient disruption of the tight junctions of the intestinal epithelium [38,39].

Figure 1 presents data of D_h, PDI and %EE. The PCL:EFV weight ratio during the production was maintained constant at 1:1. CS-coated PCL-EFV NPs displayed one size population with a D_h of 207 nm and a very low PDI of 0.09. In addition, the %EE was 93%, representing an encapsulated drug cargo of approximately 47% w/w. CS-coated blank NPs were slightly larger than those containing EFV, with D_h and PDI being 234 nm and 0.155, respectively. When CS was not included in the formulation, the size increased to 247 nm and the PDI to 0.203, while the %EE decreased slightly to 86% (equivalent to a drug cargo of approximately 43% w/w) (Figure 1). These findings supported the stabilizing effect of CS in the double emulsion. Electron microscopy analyses confirmed the spherical morphology of all of the NPs, including EFV-NPs (Figures 2 & 3). SEM showed the absence of free EFV in EFV-loaded NPs (Figures 2B & 2C), consistent with high %EE values (Figure 1). The larger size of the NPs by this technique with respect to DLS was probably related to the adsorption of excipients (PVA, PEG and lactose) used during the spray-drying process. Regardless of the effect of CS on the size of the NPs, PDI values by DLS were always below 0.3, which is an indication of the relatively homogeneous size distribution achieved with this technique. TEM analysis followed a similar trend (Figure 3). Interestingly, uncoated PCL-EFV NPs revealed the capsular structure of the NPs owing to the double-emulsion production method. EFV-NPs displayed a D_h and PDI of 651 nm and 0.645, respectively (Figure 1), which was in good agreement with previous reports on the production of pure drug NPs by this bottom-up method [40]. The size, as determined by SEM and TEM, was smaller, although of the same order of magnitude. Finally, the mass recovery was 60 ± 3%, 65 ± 5%, 73 ± 9% and 62 ± 2% for PCL-DF NPs, CS-coated PCL-EFV NPs, uncoated PCL-EFV NPs and EFV-NPs, respectively. These results stress the advantage of the spray-drying method for the production of NPs with narrow size distributions and high EFV encapsulation extents and cargos.

Zeta-potential is an important surface property that can govern the physical stability of colloidal dispersions and also the interaction with living cells. High positive or negative zeta-potential values have shown to prevent agglomeration due to electrostatic repulsion and they increase physical stability. CS-coated PCL-EFV NPs, PCL-DF NPs and EFV-NPs displayed zeta-potential values of +26 ± 1, +29 ± 1 and +18 ± 1 mV, respectively, due to the adsorption of CS on the surface [41]. Conversely, uncoated PCL-EFV NPs showed a zeta-potential of -16 ± 5 mV, characteristic of pure PCL NPs [42]. This low value also conferred on the colloidal system good physical stability.

Attenuated total reflectance/Fourier transform infrared spectra of pure PCL, uncoated PCL-EFV NPs, CS-coated PCL-EFV NPs and PCL-DF NPs presented the characteristic peak of PCL due to the stretching vibration of C=O between 1721 and 1726 cm^{-1} (Figure 4). These results were in agreement with the literature [43,44]. Spectra of raw EFV and EFV-NPs presented a band at 2249 cm^{-1}, characteristic of the stretching of the exocyclic triple bond [45]. This band was much weaker in the processed than in the unprocessed

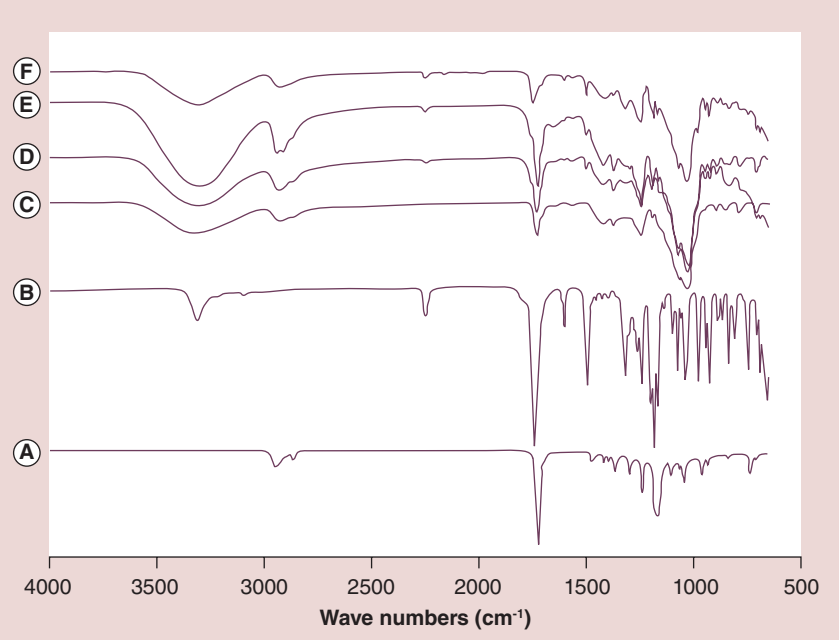

Figure 4. Fourier transform infrared analysis. (A) Pure poly(epsilon-caprolactone) (PCL), **(B)** efavirenz (EFV) drug, **(C)** drug-free PCL nanoparticles (NPs), **(D)** chitosan-coated EFV-loaded PCL NPs, **(E)** uncoated EFV-loaded PCL NPs and **(F)** pure EFV NPs.

drug, suggesting that the intensity drop was associated with the low degree of crystallinity after processing (see next paragraph). Uncoated and CS-coated PCL-EFV NPs also showed a very weak band at 2249 cm^{-1}. These results would also support that the drug was mainly encapsulated within the NPs. However, since EFV-NPs showed a similar result, this band was inconsequential. The same was valid for the band of EFV in 1740 cm^{-1} that corresponds to the stretching vibration of C=O [46] and overlapped with the strong C=O band of PCL. On the other hand, two additional characteristic bands of EFV at 1602 and 1496 cm^{-1} due to stretching vibrations of C=C bonds were still visible in EFV-NPs, while they almost disappeared in the EFV-loaded PCL NPs. These data, together with complementary %EE, SEM and TEM, confirmed the relatively high nanoencapsulation extent of EFV.

X-ray diffraction analysis was used to determine the nature of the encapsulated drug (crystalline or amorphous). The diffractogram of pure EFV presented multiple peaks, confirming that the unprocessed drug was crystalline (Figure 5). Pure PCL polymer presented two sharp peaks at 21.3 and 23.9° due to scattering of the crystalline region and a 'hump' due to the scattering from the amorphous one [43,47]. Uncoated and CS-coated PCL-EFV NPs and PCL-DF NPs were characterized by the absence of peaks of crystalline drug and extremely weak peaks of crystalline PCL (Figure 5). EFV-NPs yielded similar results, indicating the complete amorphization of the free drug [40]. Drug molecules on the surface are more weakly bound and less constrained in their thermal movement than molecules within the crystal lattice, this phenomenon leading to a decrease of the degree of crystallinity due to the pronounced growth of the surface area [48]. These results are very relevant and stress the potential of this processing method to also produce pure drug NPs.

In vitro release

Encapsulation of EFV within PCL NPs was envisioned to disperse the drug at the molecular level and increase dissolution rates in aqueous media, and to prolong the residence time of the nanocarrier in the gut with respect to polymeric micelles [21,23] and the free drug. These two phenomena were expected to further increase its oral bioavailability.

It is worth stressing that conventional *in vitro* drug release assays are of limited relevance for the prediction of *in vivo* performance of systems intended for oral administration. This is due to the complex structure of the gastrointestinal tract and the difficulty in mimicking the cascade of events that a drug-delivery system has to withstand after oral administration. In fact, they have been developed based on dissolution tests routinely used in the pharmaceutical industry for quality control purposes. For example, we previously showed that EFV-loaded Pluronic F127 and Tetronic® 1307 (BASF) polymeric micelles released between 20 and 40% of the total drug payload after 24 h, even when sink conditions were ensured [21]. This profile was less remarkable than the one *in vivo*. Thus, to correlate *in vitro* dissolution to *in vivo* pharmacokinetic data, an *in vitro–in vivo* correlation needs to be developed and validated. This was beyond the scope of the present work.

The release of a drug encapsulated within polymeric NPs can be influenced by several factors such as drug–polymer interactions, the size and surface charge of the NPs, and the crystallinity of the polymer and the drug [49]. PCL in the NPs was almost completely amorphous after spray drying, as shown by x-ray diffraction analysis

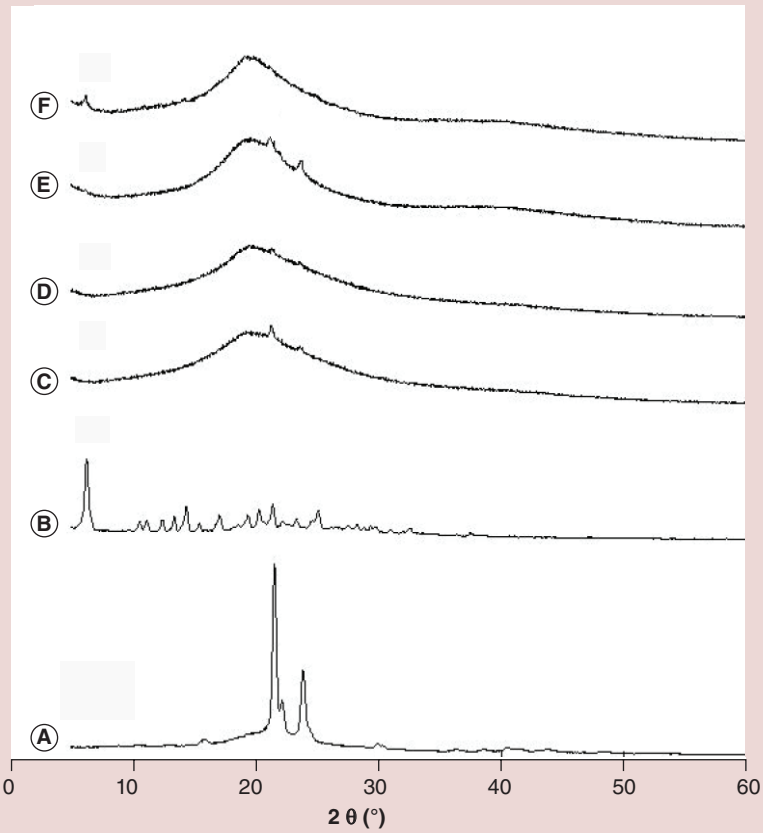

Figure 5. X-ray diffraction analysis. (A) Pure poly(epsilon-caprolactone) (PCL), **(B)** efavirenz (EFV) drug, **(C)** drug-free PCL nanoparticles (NPs), **(D)** chitosan-coated EFV-loaded PCL NPs, **(E)** uncoated EFV-loaded PCL NPs and **(F)** pure EFV NPs.

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(Figure 5). The polymer molecular weight is also a key parameter affecting the drug release, since this process is mediated by the erosion of the polymer matrix [18]. The hydrolysis kinetics of PCL *in vitro* is quite slow and hydrolysis was not expected to occur under the conditions of this assay, regardless of the relatively low molecular weight of PCL [50]. In this context, the release was assessed under three pH conditions: 1.3 and 6.8, which mimic the gastric and the intestinal pH, respectively, and under an intermediate value, 4.5. For CS-coated PCL-EFV NPs, the release was the fastest in pH 6.8, with approximately 27.0% of the total drug released after 24 h (Figure 6A). These findings were in good agreement with other EFV-loaded PCL NPs containing Eudragit® RS 100 (Evonik Industries, Essen, Germany), which released 10–30% in the same time interval [42]. A decrease in the pH of the medium to 4.5 and 1.3 led to a slight decrease of the release to 22.9 and 20.5%, respectively, at the same time point; differences between data at pH 6.8 and 1.3/4.5 were statistically significant in the time range between 1.5 and 20–24 h. Conversely, the difference at pH values 1.3 and 4.5 was not significant in the entire study. Differential EFV solubility in this pH range was not expected because the drug undergoes deprotonation only at pH values >10.2 [30]. The effect of pH probably relied on the repulsion of H^+ of the medium and protonated CS chains adsorbed on the surface of the NPs. The effect of CS was supported by data of uncoated PCL-EFV NPs that followed the reverse trend (Figure 6B); the rate was the fastest at pH 1.3, with 32.9% cumulative release. An increase in the pH to 4.5 and 6.8 led to a significant decrease of the release in the whole release range, values at 24 h being 25.7 and 24.2%, respectively. Here, the difference in release data at pH values of 4.5 and 6.8 was not significant for the whole study with the exception of the last two time points. In addition, the release from PCL NPs was substantially slower than release determined for polymeric micelles [21], owing to their greater stability under dilution; micelles tend to disassemble. In summary, regardless of the presence (or not) of CS coating, all of the systems combined an initial burst of EFV due to the release of nonencapsulated drug or drug adsorbed on the surface of the NPs, with a more sustained profile later on. On the other hand, the coating (or lack thereof) of the surface with CS could be capitalized on to fine tune the release kinetics.

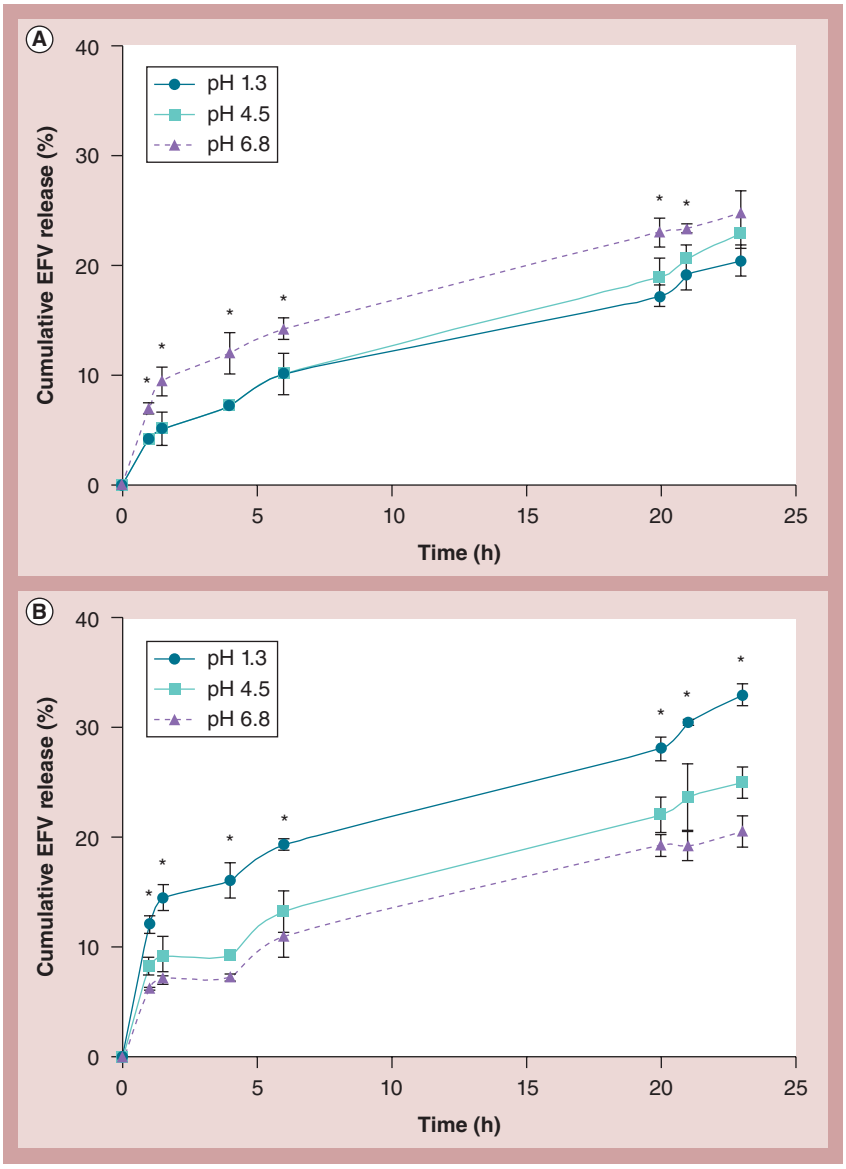

Figure 6. Drug release rate of efavirenz-loaded poly(epsilon-caprolactone) nanoparticles under three pH conditions (pH 1.3, 4.5 and 6.8). (A) Chitosan-coated nanoparticles and **(B)** uncoated efavirenz-loaded poly(epsilon-caprolactone) nanoparticles. Data represent mean ± standard deviation (n = 3).
*Statistically significant difference between values at **(A)** pH 6.8 with respect to 1.3 and 4.5 and **(B)** pH 1.3 with respect to 4.5 and 6.8 ($p < 0.05$).
EFV: Efavirenz.

Oral pharmacokinetics

The therapeutic efficacy of a drug administered by the oral route strongly depends on the administered dose, the gastrointestinal absorption and the bioavailability. We expected the EFV-loaded NPs to increase the oral bioavailability and attain plasma concentrations above the IC_{50} with lower EFV doses. In this framework, we comparatively assessed the pharmacokinetics of the different samples at a single EFV dose of 20 mg/kg. Previous studies indicated that EFV displays nonlinear oral pharmacokinetics [51]. C_{max} values were between 1.203 µg/ml (3.8 µM) for EFV-NPs

and 1.748 µg/ml (5.5 µM) for uncoated PCL-EFV NPs, and between 1.250 µg/ml (4.0 µM) and 1.287 (4.1 µM) for the polymeric micelles and the CS-coated PCL-EFV NPs, respectively (Figure 7 & Table 1). Differences were not statistically significant. These values were within the therapeutic window defined for EFV [52,53] and greater than those observed with an extemporaneous EFV suspension produced by dispersing the content of standard 600 mg EFV capsules in an aqueous vehicle (0.515 µg/ml, 1.6 µM) and a medium-chain triglyceride solution (Miglyol® 812; Peter Cremer Holding GmbH & Co. KG, Hamburg, Germany) that is similar to a commercially available pediatric formulation (0.687 µg/ml, 2.2 µM) [23]. These findings indicate that the dose could be adjusted to attain similar pharmacokinetics but with a significantly smaller dose. For all of the solid suspensions, t_{max} values ranged between 1.8 and 2.1 h, and were similar to those observed for the suspension and the oily solution [23]. Micelles showed a slight decrease to 1 h, differences not being statistically significant. The three nanosuspensions showed an increase in the AUC with respect to the polymeric micelles; for example, AUC_{0-24} values increased from 8.7 (micelles) to 10.2 (EFV-NPs), 11.0 (CS-coated PCL-EFV NPs) and 17.1 µg/ml/h (uncoated PCL-EFV NPs), representing a 17.2, 26.4 and 96.6% increase of oral bioavailability, respectively. On the other hand, it should be stressed that this increase was statistically significant only for uncoated NPs with respect to the polymeric micelles and EFV-NPs. When the AUC_{0-24} of uncoated PCL-EFV is compared with that of the extemporaneous suspension (AUC_{0-24} = 3.53 µg/ml/h) or the oily solution (AUC_{0-24} = 4.79 µg/ml/h) reported elsewhere [23], the AUC increased 4.84 and 3.57 times, respectively. This trend was similar for AUC_{0-48}, suggesting that polymeric NPs are retained in the intestine for a more prolonged time than the other systems. Furthermore, k_e showed differences that were not statistically significant. These data suggested that the elimination pathway was not altered by the different formulations because PCL NPs were not absorbed, as opposed to

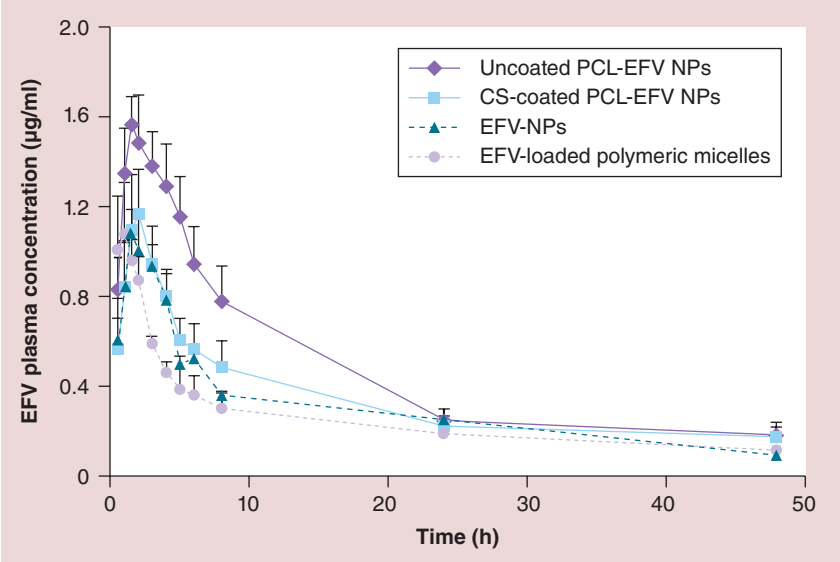

Figure 7. Plasma concentration profiles of efavirenz after oral administration of poly(epsilon-caprolactone) nanoparticles, poly(epsilon-caprolactone) nanoparticles coated with chitosan, efavirenz nanoparticles and efavirenz-loaded polymeric micelles between 0 and 48 h.
Data represent mean ± standard deviation (n = 6).
CS: Chitosan; EFV: Efavirenz; EFV-NP: Pure efavirenz nanoparticle;
PCL-EFV NP: Efavirenz-loaded poly(epsilon-caprolactone) nanoparticle.

Table 1. Efavirenz oral pharmacokinetic parameters upon oral administration of different formulations in rats.

PK parameter	Uncoated PCL-EFV NPs		CS-coated PCL-EFV NPs		EFV-NPs		Polymeric micelles	
	Mean	*CV (%)*	*Mean*	*CV (%)*	*Mean*	*CV (%)*	*Mean*	*CV (%)*
C_{max} (µg/ml)	1.748	19.9	1.287	41.1	1.203	34.3	1.250	23.3
t_{max} (h)	2.1	57.6	1.8	37.3	1.9	34.7	1.0	40.8
AUC_{0-24} (µg/ml/h)	17.1*	29.9	11.0	40.5	10.2	12.4	8.7	33.0
AUC_{0-48} (µg/ml/h)	21.8*	22.8	15.4	40.2	14.4	13.2	13.9	7.9
k_e (h^{-1})	0.053	53.9	0.042	36.1	0.035	27.4	0.042	65.5

Data represent the mean of six animals (n = 6). The variability between individuals was estimated by the CV (expressed in %) calculated as the ratio of the standard deviation and the mean value multiplied by 100. The EFV dose was 20 mg/kg.
**Statistically significant increase of C_{max} and AUC with respect to EFV-NPs and EFV-loaded polymeric micelles ($p < 0.05$).*
AUC_{0-24}: Area under the curve between the administration time and 24 h; AUC_{0-48}: Area under the curve between the administration time and 48 h; C_{max}: Maximum plasma concentration; CS: Chitosan; CV: Coefficient of variation;
EFV-NP: Pure efavirenz nanoparticle; PCL-EFV NP: Efavirenz-loaded poly(epsilon-caprolactone) nanoparticle; k_e: Elimination rate constant; PK: Pharmacokinetic; t_{max}: Time to the maximum plasma concentration.

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poly(lactic-*co*-glycolic) acid counterparts reported elsewhere [54].

Finally, the interindividual variability was estimated by calculating the coefficient of variation (expressed in %) of the different parameters in each animal cohort. PCL NPs showed a pronounced decrease in the variability of C_{max} with respect to other formulations (Table 1).

Overall, pharmacokinetic studies of EFV plasma levels after the oral administration demonstrated that the encapsulation of EFV within PCL NPs improved the bioavailability of the drug with respect to polymeric micelles and pure EFV-NPs. As mentioned above, the increase was even more remarkable when this performance is compared with common pediatric formulations such as a suspension or an oily solution in the same dose of 20 mg/ml [23].

A more thorough comparison of both PCL-EFV NPs indicated that the CS coating slightly reduced both C_{max} and AUC values, albeit not significantly. These results were unexpected because CS usually confers mucoadhesiveness and increases the paracellular absorption pathway of different nanocarriers in the intestine [38,39]. However, in a recent article that assessed the interaction of CS-coated solid lipid NPs with cells of the phagocytic system, results demonstrated that this polysaccharide prevented the uptake of the nanocarriers by macrophages [55], probably by a steric shielding effect. This behavior stresses the complexity of predicting the performance of drug-delivery systems and the relevance of conducting comprehensive *in vivo* studies to assess the effect of surface modifications on the pharmacokinetics.

Pure drug NPs are claimed to be one of the simplest, cheapest and scalable nanotechnology approaches to improve the oral bioavailability of poorly water-soluble drugs [37,56]. In this study, EFV-NPs showed a performance that was less optimal than EFV-loaded NPs, probably due to a slower dissolution rate when compared with the polymeric NPs and a shorter residence time. Finally, it seems that the performance *in vivo* was better than the one that could have been predicted by the *in vitro* results, where the EFV cumulative release did not overcome 30–40% of the total drug cargo after 24 h. These divergent data probably stemmed from the limited ability to mimic the conditions of the gastrointestinal tract *in vitro* and the greater sensitivity of PCL to hydrolysis in the presence of gastrointestinal enzymes that might increase drug release and absorption with respect to *in vitro* assays [57,58].

Conclusion

This work studied the pharmacokinetics of EFV-loaded PCL NPs and compared it with that of pure EFV, NPs and polymeric micelles. Data indicated that these polymeric NPs significantly increased the oral bioavailability of the drug with respect to all of the other formulations, independently of the CS coating. Furthermore, if this comparison is performed with two conventional liquid formulations (a suspension and an oily solution), this improvement increased 4.84 times. In this context, the dose could be fine tuned in future experiments to achieve therapeutic concentrations in plasma with a smaller dose. This dose adjustment could also reduce the cost of the treatment and eventually increase patient affordability to the medication [59], which by itself represents a challenge in HIV. At the same time, a successful bench-to-bedside translation strongly depends on the ability to fulfill two fundamental and interrelated conditions: scalability and cost–effectiveness. In this context, a careful evaluation of the pros and cons of each nanotechnology platform should be undertaken to ensure the appropriate access of the HIV-infected patients to these innovative formulations. PCL is a commercially available polymer of relatively low cost. Moreover, it would enable the coencapsulation of various antiretrovirals in the same NP, which would simplify drug administration by the oral route and increase patient compliance. On the other hand, the parenteral administration of antiretrovirals is being increasingly explored [60]. In this context, EFV-loaded NPs represent a valuable technology for depot drug-delivery systems that would sustain the release, and by doing so facilitate some therapeutic regimens.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Executive summary

Preparation & characterization of nanoparticles

- Efavirenz (EFV) was encapsulated within poly(epsilon-caprolactone) nanoparticles (NPs) with an efficiency of 86–93% and underwent amorphization during the process. EFV-loaded NPs showed sizes between 200 and 250 nm and narrow size distribution.

In vitro *release studies*

- All of the EFV-loaded NPs combined an initial burst with a subsequent more sustained release profile, with cumulative release amounts between 20 and 33% after 24 h.

Oral pharmacokinetics

- The encapsulation of EFV in poly(epsilon-caprolactone) NPs significantly increased the oral bioavailability of the drug when compared with pure drug NPs and EFV-loaded micelles.

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