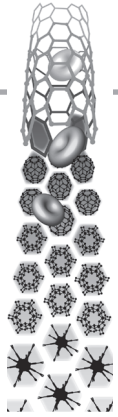

Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailaibility

Children constitute the most challenging population in anti-HIV/AIDS pharmacotherapy. Efavirenz (EFV; aqueous solubility 4 µg/ml, bioavailability 40–45%) is a first-line agent in the pediatric therapeutic cocktail. The liquid formulation of EFV is not available worldwide, preventing appropriate dose adjustment and more convenient administration. The bioavailability of liquid EFV is lower than that of the solid formulation. Improving the bioavailability of the drug would reduce the cost of treatment and enable less affluent patients to access this drug. **Aim:** To encapsulate EFV in polymeric micelles to improve the aqueous solubility and the the oral bioavailability of the drug. **Methods:** EFV was incorporated into the core of linear and branched poly(ethylene oxide)–poly(propylene oxide) block copolymer micelles. The size and size distribution of the drug-loaded aggregates were characterized by dynamic light scattering and the morphology by transmission electron microscopy. The bioavailability of the EFV-loaded micellar system (20 mg/ml) was assessed in male Wistar rats (40 mg/kg) and compared to that of a suspension prepared with the content of EFV capsules in 1.5% carboxymethylcellulose PBS solution (pH 5.0), and an EFV solution in a medium-chain triglyceride (Miglyol® 812). **Results:** This work demonstrates that the encapsulation of EFV, which is poorly water soluble, into polymeric micelles of different poly(ethylene oxide)–poly(propylene oxide) block copolymers significantly improves the oral bioavailability of the drug, and reduces the interindividual variability. **Conclusion:** This strategy appears a very promising one towards the development of a liquid aqueous EFV formulation for the improved pediatric HIV pharmacotherapy.

KEYWORDS: bioavailability ■ efavirenz solubilization ■ improved solubility ■ interindividual variability ■ pediatric HIV/AIDS pharmacotherapy ■ poloxamer and poloxamine polymeric micelles

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A recent report on the global situation of HIV/AIDS indicates that approximately 40 million infected people live worldwide [101]. Approximately 2.5 million patients are children (<15 years). Children show especially high mortality rates and represent a high-risk population [101]. HAART combines at least three different antiretroviral (ARV) drugs [1,2,102]. ARV doses are high and complicated regimens compromise the patient's lifestyle. According to epidemiological investigations, adherence levels below 95% dramatically reduce the chances of therapeutic success to approximately 50% [3].

Pediatric HIV has been almost eliminated in developed countries by limiting maternal-to-fetus and breastfeeding transmission [103]. By contrast, the situation in developing countries is dramatically different, where approximately 90% of infected children do not have access to medication. Pediatric HAART implementation is challenging owing to the reduced number of drugs approved for pediatric use [4]; pharmaceutical companies have only recently been compelled to conduct clinical trials in children by the US FDA and EMEA [104]. Adult-approved ARV drugs are available only in solid form [5]. To make adult medicines appropriate for children and enable dose adjustment and easy swallowing, solid forms are often processed to prepare magistral formulations [6]. Approximately 40 million children are administered unlicensed medicines every year in Europe [7,105]. There are serious quality, safety and effectiveness concerns over the use of these medicines [8–10]. However, when a liquid formulation is not commercially available, they remain the only alternative to treat HIV-infected neonates and infants [11].

The last World Health Assembly (WHA) recognized the right of pediatric patients to access safe, effective and proven medicines, approving the resolution 'Better medicines for children' [106] and launching the global campaign 'Make medicines child size' [106].

Efavirenz (EFV; Sustiva®) is a highly lipophilic non-nucleoside reverse transcriptase inhibitor classified in class II of the Biopharmaceutic Classification System [12]. EFV is a first-choice ARV in adult [13] and pediatric pharmacotherapy [14,15]. The low solubility of the EFV in aqueous medium hinders the absorption

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and biodistribution of the drug from the GI tract [16,17]. The oral bioavailability of the drug is around 40–45% and the interindividual variability observed demands therapeutic drug monitoring [18,19]. In general, a good correlation has been established between solubility improvement and higher bioavailability for most of the class II drugs [20,21]. For example, amprenavir, a poorly water-soluble protease inhibitor ARV, shows a dramatically low oral bioavailability [22]. To enhance the gastrointestinal absorption of the drug, two approaches were pursued: the chemical modification to the water-soluble phosphate ester prodrug, fosamprenavir, which is converted into the active molecule *in vivo* [22]; and solubilization in 23% D-α-tocopheryl polyethylene glycol 1000 succinate [23]. To make EFV more soluble and palatable, the drug was solubilized in a series of water-insoluble triglycerides [24,25]; however, this 30 mg/ml oily solution showed lower bioavailability than the solid formulation. Moreover, the intake of large oil volumes are expected to produce profuse diarrhea and affect patient compliance. To improve drug solubility and dissolution, Dutta *et al.* prepared EFV–dendrimer complexes [26,27]. However, the design of a drug-delivery system based on a non-approved carrier (e.g., dendrimer) constitutes, from a pharmaceutical perspective, a drawback by itself. Others have investigated the solubilization of EFV in different cyclodextrins (CD) and CD-containing polymers [28,29]. For instance, Sathigar *et al.* investigated the preparation of inclusion complexes of EFV with β-CD, hydroxypropyl β-CD and randomly methylated β-CD. Dissolution extents were between six- and 20-fold higher than the intrinsic solubility in water [28]. However, this solubilization improvement still appears to be insufficient for the development of a highly concentrated aqueous solution; a 200 mg dose of EFV would demand approximately 1 l of formulation.

Prevalence of the disease is extremely high in resource-constrained countries [30], where the development of scalable and cost-effective ARV medicines is a crucial access to enable patients to receive the appropriate medication [31,32]; owing to lower production costs, the administration of lower doses that are absorbed more efficiently after oral administration would represent a substantial improvement in the accessibility of more patients to EFV. In this framework, nanotechnologies can provide unique tools to improve the effectiveness of anti-HIV pharmacotherapy.

Inclusion into the hydrophobic core of polymeric micelles represents a very versatile nanotechnology approach to enhance the aqueous solubility of highly hydrophobic drugs [33]. The most broadly investigated polymeric micelle-forming materials are the poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) block copolymers [34]. Two families are commercially available: the linear PEO–PPO–PEO triblocks or poloxamers (Pluronic®) and the branched poloxamines (Tetronic®). Most works focused on the linear derivatives, although the four-arm counterparts have attracted increasing attention over the past few last years owing to their dually responsive behavior (temperature and pH) [35,36]. A remarkable advantage of the PEO–PPO-based systems over other experimental polymeric amphiphiles is the approval of some of the derivatives by the FDA and EMEA for use in pharmaceuticals and medical devices [37–39].

Our research group has embraced the spirit of the WHA resolution, Better Medicines for Children, and investigates different nanotechnological approaches to optimize the biopharmaceutical properties of anti-HIV drugs in the pediatric pharmacotherapy. In an attempt to improve the solubility and oral bioavailability of EFV, the present study explored the inclusion of the drug into polymeric micelles of linear and branched PEO–PPO copolymers. The solubilization profiles and properties of the drug-loaded micelles were thoroughly characterized. Pharmacokinetic studies in rats showed a significant increase in the bioavailability of EFV and supports the use of this strategy toward the development of a concentrated, stable and cost-efffective EFV aqueous formulation for the management of pediatric HIV/AIDS.

Materials & methods

Materials

Poloxamers Pluronic F68 (MW 8.6 kDa), F108 (MW 14.8 kDa) and F127 (MW 12.6 kDa), and poloxamines Tetronic® 1107 (T1107, MW 15 kDa) and 1307 (T1307, MW 18 kDa) were a gift from BASF (NJ, USA). EFV (Richmond Pharmaceutical Laboratories, Buenos Aires, Argentina), Na_2HPO_4, citric acid, KCl, NaOH, HCl and solvents were of analytical grade and they were used as received.

Preparation of poloxamine micelles

Polymeric micelles (1–17% w/v, depending on the polymer) were produced by dissolving the required amount of polymer in phosphate citrate buffer solution (pH 5.0) at 4°C and equilibrating the system at 23°C, at least 24 h before use.

Preparation of EFV-loaded polymeric micelles

Solubilization assays were conducted at pH 5.0; poloxamines display good solubilization capacity and the degradation of the drug is minimal (see later) under these conditions [40]. EFV (in excess, ~50 mg/ml polymer solution) was added to the different micellar systems in phosphate citrate buffer (pH 5, 3 ml) in glass vials (10 ml). Samples were shaken (48 h) in a temperature-controlled horizontal shaker at 23°C (Minitherm-Shaker; Adolf Kuhner AG, Switzerland). Suspensions were filtered (0.45 µm, cellulose nitrate filters) to remove insoluble EFV and dried in a vacuum oven at room temperature. Particles with sizes above this value were removed by the filter. This is a commonly used technique for the preparation of drug-loaded micelles. In general, PEO–PPO polymeric micelles display sizes less than 450 nm, even when micellar fusion occurred. Hence, we expect a minimal change of the size distribution after filtration. Dry specimens were redissolved in methanol and the samples diluted as required to fit the calibration curve range. EFV concentrations were determined by UV (247 nm, CARY [1E] UV-Visible Spectrophotometer Varian, USA) at 23°C using a calibration curve of methanolic solutions covering a range of 4–20 µg/ml (correlation factor was 0.9998–1.0000) [41]. Polymer solutions in methanol were used as a blank. Both the polymer and the drug are very soluble in methanol. Concentrations are expressed in µg/ml or mg/ml.

Solubility factors (f_s) were calculated according to the equation:

$$f_s = S_a / S_{water}$$

where, S_a and S_{water} are the apparent solubility of EFV in the different micellar systems and the intrinsic solubility of the drug in a polymer-free aqueous medium, experimentally determined at pH 5.0 (4 µg/ml), respectively.

Measurement of the micellar size

The average hydrodynamic diameter of 10% F127 and T1307 drug-loaded micelles was measured by dynamic light scattering (DLS; Zetasizer Nano-Zs, Malvern Instruments, UK) provided with a He–Ne (633 nm) laser and a digital correlator ZEN3600 at 25°C. Samples were filtered (0.45 µm) prior to the assay. Results are expressed as the average of five measurements. Standard deviations were 15 (polydispersity index 0.476 ± 0.003) and 22% (polydispersity index 0.493 ± 0.041) for F127 and T1307 measurements, respectively.

Morphology of EFV-loaded micelles

The morphology of EFV-loaded micelles was studied in 10% (w/v) T1307 and F127 systems by transmission-electron microscopy (TEM; Philips CM-12 TEM apparatus, FEI Company, Eindhoven, The Netherlands). Samples (5 µl) were placed on a grid covered with Fomvar film. After 30 s, the excess was carefully removed with filter paper and phosphotungstic acid (2% w/v, 5 µl) was added. After 30 s, the excess was removed and water (5 µl) was added and left for 30 additional seconds before removing. Samples were finally dried in a closed container with silica gel and analyzed. The diameter of the micelles/aggregates was measured using a calibrated scale.

Physicochemical stability of drug-containing micelles

No chemical degradation of EFV was expected under the mild conditions of the study [40]. To evaluate the physicochemical stability of the EFV-containing micelles, specimens previously prepared were stored at room temperature for different periods of time and the drug concentration monitored by high-performance liquid chromatography (HPLC; see later). Samples were processed as described earlier. The percentage of remaining EFV (%EFV) was calculated and expressed as mean ± standard deviation (n = 3). To characterize precipitation products, crystals were isolated, thoroughly washed with distilled water, dried under vacuum at room temperature and analyzed by HPLC (see later). Statistical differences ($p < 0.05$) between the concentration at different time points and the initial concentration were analyzed using the Dunnett's Multiple Comparison Test.

Physicochemical stability of drug-containing micelles upon dilution

Our investigation pursues the development of EFV oral liquid formulations. In order to determine their ability to withstand dilution in a gastric environment (and to estimate micellar disassembly rates), EFV-containing systems were diluted (1/50 and 1/75) in stomach-mimicking conditions (HCl 0.1 N, pH 1.5), incubated at 37°C and the drug concentration monitored by HPLC (see later). A similar assay was performed at pH 5.0. Polymer-free saturated drug solutions were used as controls.

In vitro release studies

To evaluate the drug-release profiles from the micellar reservoir, 10% F127 and T1307 efavirenz-containing systems were diluted (1/50

and 1/75) in buffer (pH 5.0) and specimens (50 ml) were placed into dialysis membranes (regenerated cellulose tubing, molecular weight cut off = 3500), immersed into an intestine-mimicking buffer (pH 6.8, 900 ml) and the drug concentration in the internal solution monitored over 24 h by HPLC (see later), at 37°C. The medium was replaced every 6 h. The time point for medium release exchange was established in preliminary tests where the medium was exchanged every 3, 6 and 12 h. No substantial differences in the results between time points 3 and 6 h were found. Based on this, we decided to exchange the medium every 6 h. Results are expressed as mean ± standard deviation (n = 3).

Pharmacokinetic evaluation

The bioavailability of the EFV-loaded micellar system was assessed in male Wistar rats (220–250 g). Three 20 mg/ml EFV systems were evaluated: EFV-loaded 10% F127 micelles, a suspension of the content of EFV capsules in 1.5% carboxymethylcellulose phosphate citrate buffer solution (pH 5.0) and a solution in a medium-chain triglyceride (Miglyol® 812). Animal experiments were performed in accordance with the 'Principles of laboratory animal care' (NIH publication No. 85–3, revised 1985). Animals were maintained on a 12-h light/dark cycle and kept in a room at 22 ± 2°C with the air adequately recycled. All animals received a standard rodent diet (Asociación Cooperativas Argentinas, Buenos Aires, Argentina) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals, and 69% starch and vitamin supplements, containing the same amount of calories. Experiments were performed in rats fasted for 12 h (n = 10 for each group). Drug formulations were orally administered by gavage, where a stomach tube is inserted into the esophagus of conscious rats. The dose of EFV and volume of injection were 40 mg/kg and 0.2 ml/kg, respectively. After drug administration, blood samples (70 µl) were obtained at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 24 h by tail vein collection, as described elsewhere [42]. Blood samples were centrifuged (10,000 rpm, 10 min, 4°C) to collect the plasma. It is important to mention that blood sampling could alter pharmacokinetic and pharmacodynamic behavior of drugs due to fluid loss. In our experimental protocol, total blood volume extracted was approximately 700 µl during 24 h. This volume is significantly lower than the maximal recommended (3.5 ml), and therefore we assume that the blood loss during our experimental protocol did not affect the pharmacokinetic properties of EFV. Plasma (10 µl) was deproteinized with acetonitrile (20 µl) and the concentration of the drug determined by HPLC.

Chromatographic method for EFV analysis

Samples from stability studies and plasma samples were determined by liquid chromatography (HPLC) using a Phenomenex Luna 5 µm, C18, 150 × 4.60 mm column (Phenomenex, CA, USA) with a UV detector (248 nm, UVIS 204, Linear Instruments, Reno, USA). A previously described technique was used [43]. During the mobile phase distilled water, acetonitrile and triethylamine (60:40:0.2, pH 3) were pumped at a flow rate of 1.4 ml/min. The analytical method for quantification was validated in the range of 20–5000 ng/ml.

Evaluation of *in vivo* data

Noncompartmental analysis of EFV plasma concentrations was performed using the TOPFIT program (version 2.0, Dr Karl Thomae Gmbh, Schering AG, Gödecke AG, Germany) that uses a cyclic three-stage optimization routine (1D direct search; vectorial direct search/Hooke-Jeeves modified; Gauss-Newton/Marquadt modified). The following pharmacokinetic parameters were estimated for each formulation: area under the curve (AUC_{0-24}), maximum plasma concentration (C_{max}) and time to maximum plasma concentration (t_{max}). Pharmacokinetic parameters of EFV were log transformed for statistical analysis in order to reduce heterogeneity of the variance and further compared by one-way ANOVA and Bonferroni test as a *post-hoc* test. Statistical tests were performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, California, CA, USA). Statistical significance was defined as $p < 0.05$. Interindividual variability of pharmacokinetic parameters was assessed by comparison of the coefficient of variation.

Results

Solubilization of EFV into PEO–PPO polymeric micelles

The first goal of the study was to explore the ability of PEO–PPO polymeric micelles to host EFV molecules within the micellar core as a strategy to enhance the water solubility of the drug. Below the critical micellar concentration (CMC), the solubilization of drugs can be enhanced to a limited extent [44]. To maximize the solubilizing

properties of the amphiphile, copolymer concentrations above the CMC of each poloxamine and poloxamer were used (Table 1).

Poloxamines are pH-dependent molecules. At pH values less than pK_a (in the range of 2.0–7.4), repulsion of diprotonated or monoprotonated species hinders micellization and the CMC increases. As expected, the highest polymer concentration provided better solubilization of EFV (Figure 1). Copolymers with the highest weight percent (wt%) PEO content (~80%; F68 and F108) showed notably lower solubilization extents than more hydrophobic derivatives; for example, 1% F68 and F108 systems were below the CMC and the solubilization improvement was negligible.

For F68 and F108 copolymers, the gradual increase in the polymer concentration resulted in a slight increase in solubility; for example, 3% and 7% F68 solutions increased the solubility of EFV from 20 to 90 µg/ml representing 5- to 23-fold increments with respect to the reported intrinsic solubility of 4 µg/ml (Table 2). Above the CMC (~10%), a sharp increase in the solubilization ability of F68 was found, with S_a values of 2.90 (f_s = 725) and 10.13 mg/ml (f_s = 2533) for 12 and 17% F68 solutions, respectively. For F108, an increase in the solubilization ability was found at a lower polymer concentration (~3%) (Figure 1). Copolymers containing a lower PEO content (70%) demonstrate remarkably lower CMC values of approximately 0.5–1% (Table 1). Thus, some solubilization improvement was attained even at copolymer concentrations as low as 1%. At this concentration, S_a was 0.25 (f_s = 63), 0.68 (f_s = 170) and 1.70 mg/ml (f_s = 425) for T1107, T1307 and F127, respectively. The polymer concentration was increased 3%, which resulted in S_a values of 2.27 (f_s = 568), 3.64 (f_s = 910) and 5.30 mg/ml (f_s = 1325). Finally, 10% T1107, T1307 and F127 systems showed S_a values of 19.5 (f_s = 4870), 19.0 (f_s = 4750) and 21.5 mg/mLl(f_s = 5365), respectively (Table 3), representing up to 22 wt% drug load.

Table 1. Hydrophilic–lipophilic balance and CMC values of the different PEO–PPO block copolymers employed in this study*.

Polymer	HLB‡	CMC 25°C (% w/v)§	Ref.
Pluronic® F68	>24	~10	[56]
Pluronic F108	>24	4	[50]
Pluronic F127	18–23	0.1–0.5	[57]
Tetronic® 1107	18–23	0.5–1	[35,36]
Tetronic 1307	>24	0.2–1.0	[36,58]

*CMC ranges indicate different previously reported data.
‡HLB values provided by BASF.
§Polymer concentrations are in g/100 ml.
CMC values may differ depending on the technique used for the determination.
HLB: Hydrophilic–lipophilic balance; CMC: Critical micellar concentration; PEO: Poly(ethylene oxide); PPO: Poly(propylene oxide).

Effect of EFV on the size of the aggregates

Drug incorporation into the micelles could lead to an increase in the size of the aggregates due to the expansion of the micellar core [45,46] and the fusion of drug-containing micelles into larger ones (secondary aggregation) [35,47]. The latter phenomenon would stem from strong van der Waals interactions between the exposed cores in densely packed micellar systems. The size of 10% F127 and T1307 EFV-loaded micelles was measured by DLS (Figure 2). More than one size population was found. The presence of a limited fraction of small size aggregates (5 and 7 nm for F127 and T1307, respectively) was in agreement with the presence of micelles of unchanged size. Main size fractions comprised larger structures (~25 nm). These results were consistent with the enlargement of the micellar core upon incorporation of solute molecules. Samples remained transparent and indicated that micellar fusion was not taking place [35,47].

The morphology of F127 and T1307 drug-loaded micelles was investigated by TEM, as displayed for 10% EFV/T1307 micelles (Figure 3).

Solvent evaporation and shrinkage of the structures during sample preparation usually affect the size and size distribution; micelles appear smaller than they are. Conversely, since DLS measures the hydrodynamic radius, it

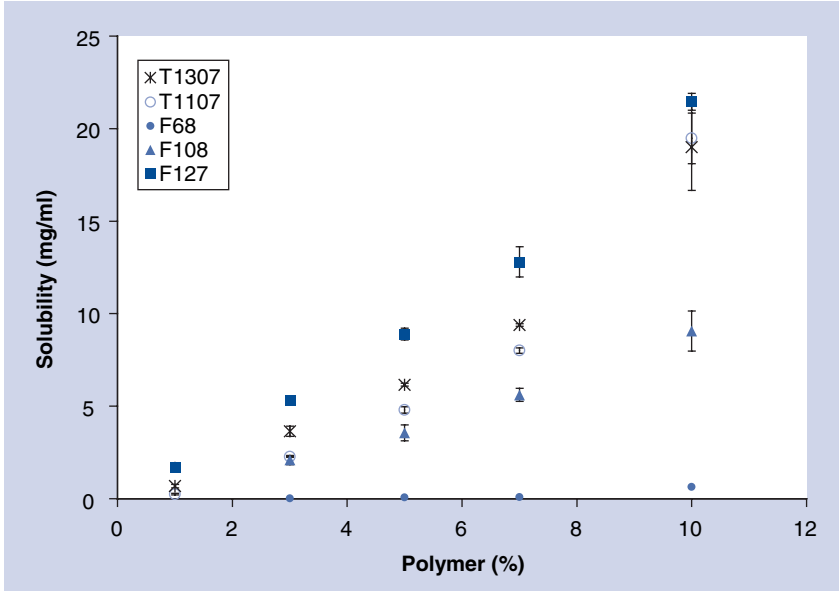

Figure 1. S_a of efavirenz versus polymer concentration of different poly(ethylene oxide)–poly(propylene oxide) block copolymers.

Table 2. Efavirenz solubility factors in poloxamine and poloxamer micellar systems at pH 5.0.

Polymer	Efavirenz solubility factor (f_s)							
	1	*3*	*5*	*7*	*10*	*12*	*15*	*17*
Tetronic® 1107	63	568	1200	2000	4870	–	–	–
Tetronic® 1307	170	910	1540	2348	4750	–	–	–
Pluronic® F68	–	5	15	23	160	725	1580	2533
Pluronic® F108	–	523	890	1403	2265	–	–	–
Pluronic® F127	425	1325	2223	3200	5365	–	–	–

results in particle size overestimation. Considering these aspects, the DLS and TEM results were in good agreement.

Physicochemical stability of the drug-containing micelles

Changes of the micellar concentration due to fluctuations in the temperature under uncontrolled storing conditions could lead to the dissociation of the aggregates and the irreversible precipitation of EFV; physical stability is crucial toward the development of a liquid formulation of the drug. Specimens were stored at room temperature and monitored for EFV content (Figure 4). F127 and F108 EFV-loaded micelles showed high stability over 1 month; titer levels remained almost unchanged at approximately 95–100% (Figure 4A) and no precipitates were apparent (Figure 4D). F127 micelles at 10% showed a slight, although not significant, decrease in soluble EFV titers before day 10. F68 systems were less stable than F127 ones (Figure 4B). Poloxamine systems appeared to be less stable than the poloxamer-based ones; among the poloxamines, T1307 was more stable than T1107 (Figure 4C). In general, soluble EFV titers decreased significantly between days 10 and 20 of the experiment in poloxamine formulations.

The instability of drug-loaded poloxamines was also evidenced by the gradual formation of needle-like crystals in the bottom of the vial (Figure 4D). According to HPLC and complementary UV data (not shown), degradation products of EFV were not found in the supernatant or the precipitate.

Physicochemical stability of drug-containing micelles upon dilution

To determine the behavior of drug-loaded micelles upon dilution in gastric-like medium, 10% F127 and T1307 EFV-containing micelles were diluted (1/50 and 1/75 dilutions) and the drug concentration monitored over time at 37°C. Two main phenomena could theoretically affect the stability of the system: the dissociation of the micelles due to final polymer concentrations below the CMC (for both poloxamers and poloxamines) and protonation of the central ethylenediamine molecule (only in the case of poloxamines); and chemical degradation of EFV. To evaluate the potential hydrolysis of EFV under the assay conditions, polymer-free EFV solutions of comparable concentration were used as controls. It is important to stress that CMC values substantially decrease upon heating from 25 to 37°C, thus favoring aggregation [48]; in other words, F127 displays CMC values around 0.001 and 0.1–0.5% at 37°C and room temperature, respectively. Pluronic F127 diluted systems were highly stable, regardless of the dilution and pH used (Figure 5).

A similar trend was observed for the 1/50 dilution of EFV-loaded T1307 micelles (pH 1.5, 37°C), which remarkably maintained the drug concentration in solution. By contrast, a further dilution of EFV-containing T1307 (1/75) showed a relatively fast drug concentration loss (20% at day 1) (Figure 5A). Drug concentrations then remained at approximately 80%. No signs of drug hydrolysis was apparent by HPLC analysis. Dilution media with a pH of 5.0 showed similar results with high stability over 2 weeks (Figure 5B). In this case, even the most diluted T1307 system (1/75) remained unchanged.

In vitro release studies

In order to characterize the release profiles, 10% F127 and T1307 EFV loaded-micelles with different dilution levels (1/50 and 1/75 at pH 5.0) were

Table 3. Pharmacokinetic parameters of the different 20 mg/ml efavirenz formulations administered orally.

Parameter	EFV-loaded micelles		EFV suspension			EFV triglyceride solution		
	Mean	*CV %*	*Mean*	*CV %*	*p-value*	*Mean*	*CV%*	*p-value*
C_{max} (ng/ml)	2863*	28.3	1526*	42.2	0.002	1789*	61.4	0.04
t_{max} (min)	2.85	40.5	2.38	50.0	–	2.42	53.0	–
AUC (mg/ml/h)	23.5‡	37.1	15.1‡	55.2	0.050	12.6‡	49.0	0.018

*C_{max} of EFV micellar system is significantly higher than the suspension and the oil solution.
‡AUC of EFV micellar system is significantly higher than the suspension and the oil solution.
AUC: Area under curve; CV%: Coefficient of variation; EFV: Efavirenz.

dialyzed against intestine-mimicking medium (pH 6.8) and the concentrations monitored over 24 h (Figure 6).

In general, EFV release profiles described a linear curve that was consistent with zero-order kinetics. For example, 1/50 dilutions of F127 and T1307 released 25.7% of the encapsulated drug at day 1 (note that R^2 was 0.945 and 0.994, respectively). The delivery rate increased in formulations with higher dilution (1/75) with a total release of 39.9% for poloxamers and 38.5% for poloxamines at day 1 (note that R^2 was 0.996 and 0.989, respectively).

Pharmacokinetic assays

This study aimed to investigate the encapsulation of EFV into PEO–PPO polymeric micelles as a strategy to develop a concentrated aqueous solution of the drug for the treatment of pediatric HIV patients. Thus, the evaluation of the bioavailability and interindividual variability in animals was a central goal. The *in vivo* performance of 10% F127 drug-loaded micelles was compared with that of two formulations currently being used: an extemporaneous suspension obtained by manipulation of commercial capsules and an oily solution. Three main parameters were measured: the C_{max}, the t_{max} and the AUC. EFV plasma concentration versus time plots after oral administration of different formulations is presented in Figure 7. Noncompartmental analysis of EFV plasma concentrations indicated that the encapsulation of EFV in polymeric micelles led to a significant increase of the C_{max} to 2863 ng/ml from 1526 and 1789 ng/ml for the suspension and the triglyceride solution, respectively. These values represented an 87.6 and 60.0% increase. A similar trend was found with the AUC, which increased from 15.1 (suspension) and 12.6 (oil) to 23.5 mg/ml/h for the micellar system, representing an 55.6 and 86.5% improvement, respectively. t_{max} values were similar for all the samples. Finally, the interindividual variability was estimated by the coefficient of variation of C_{max} and AUC obtained for each one of the animal groups.

The micellar system showed a pronounced decrease in the variability of both C_{max} and AUC data with respect to the suspension and the triglyceride solution (Table 3).

Discussion

Owing to the progress in ARV pharmacotherapy, HIV/AIDS has become a chronic disease. Therapeutic success largely depends on adherence to dose schedules. This study comprehensively investigated the inclusion of EFV, a first-line ARV that displays very low aqueous solubility, into polymeric micelles of linear and X-shaped PEO–PPO block copolymers as a strategy to optimize the solubility and bioavailability of EFV.

To comparatively evaluate the solubilization capability of the different amphiphiles, three parameters governing this phenomenon were anticipated: the molecular weight, the hydrophilic–lipophilic balance (HLB) and the

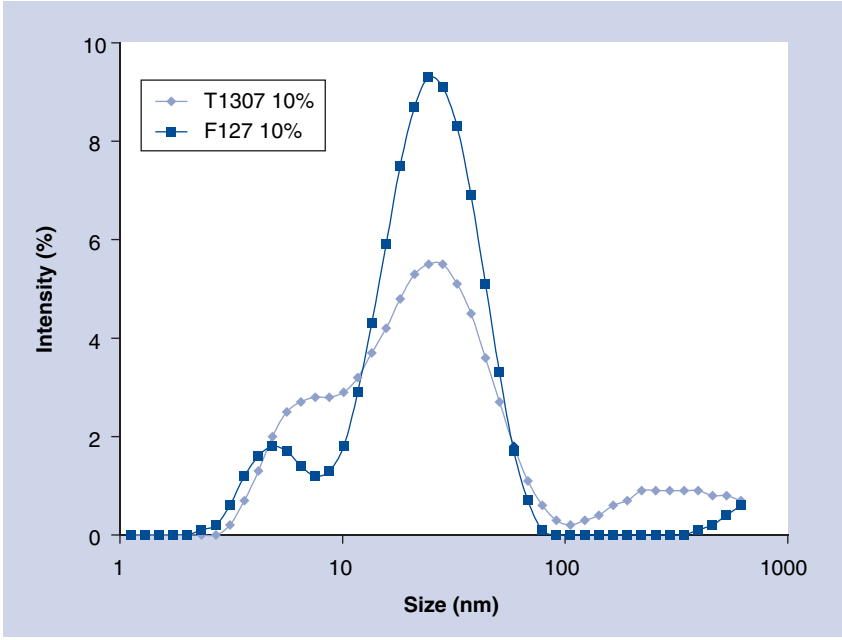

Figure 2. Size distributions of efavirenz-loaded 10% F127 and T1307 polymeric micelles. Standard deviations were 15 (polydispersity index 0.476 ± 0.003) and 22% (polydispersity index 0.493 ± 0.041) for F127 and T1307 systems, respectively.

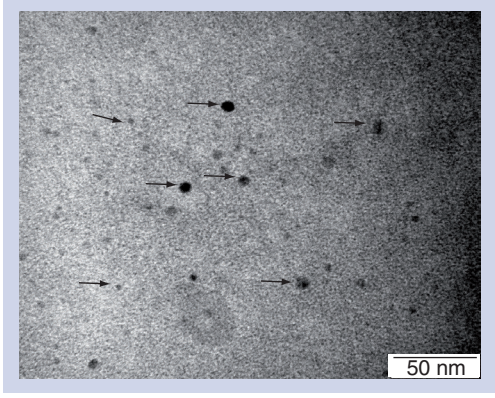

Figure 3. Transmission-electron micrograph of efavirenz-saturated 10% T1307 micelles prepared in buffer (pH 5.0) and negatively stained with 2% phosphotungstic acid. Arrows point out the presence of spherical micelles of variable sizes.

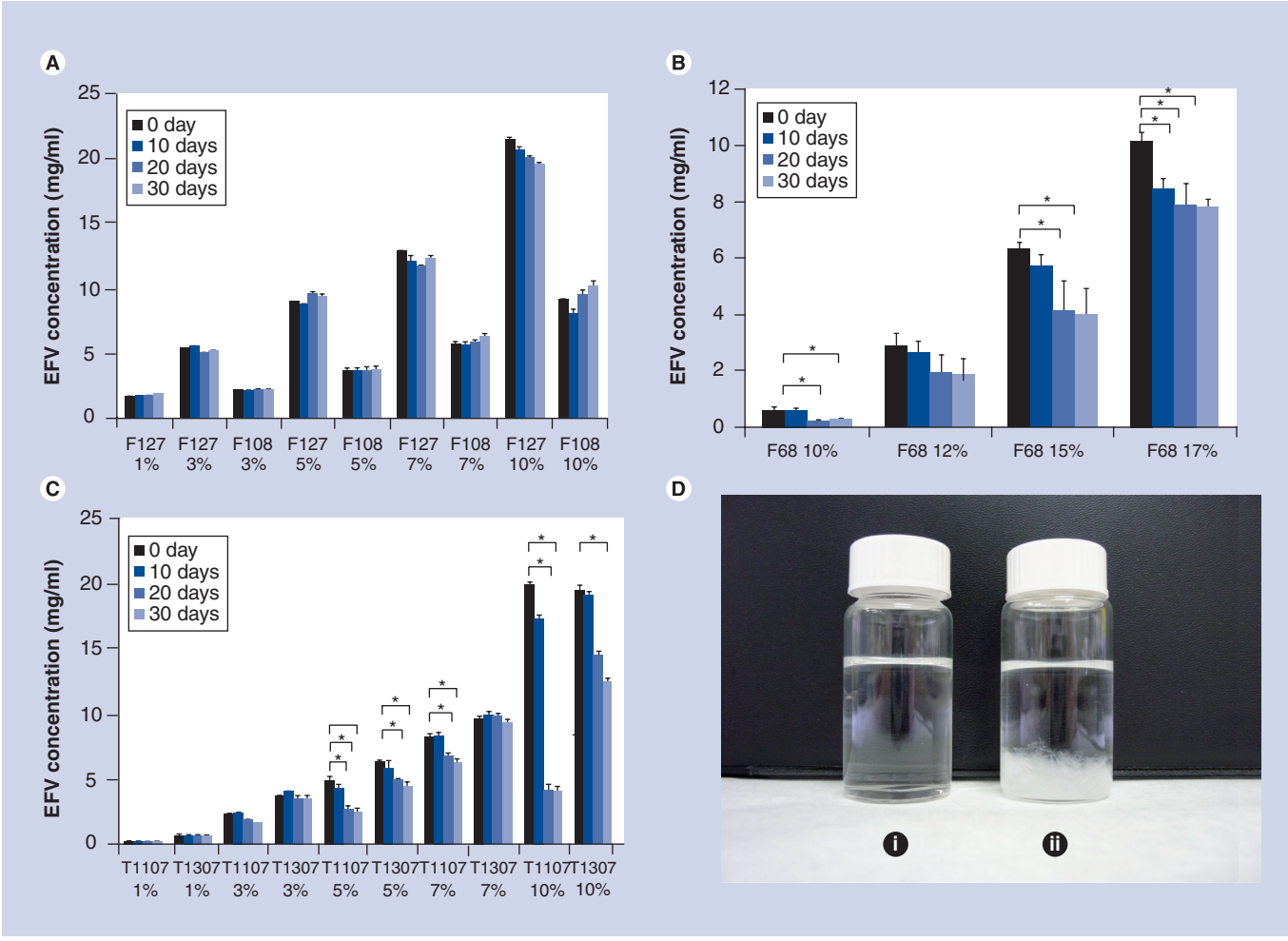

Figure 4. Physicochemical stability of efavirenz-loaded systems over time, under uncontrolled room temperature conditions. (A–C) show the percentage of EFV in solution of drug-loaded polymeric micelles stored under uncontrolled room temperature conditions over time. **(A)** Pluronic® F127 and F108, **(B)** Pluronic F68, **(C)** Tetronic® T1107 and T1307. **(D)** Appearance of 10% systems **(i)** F127 and **(ii)** T1307 EFV-loaded systems after 1 month.
*Statistically significant decrease ($p < 0.05$) when compared with the initial EFV concentration of the system.
EFV: Efavirenz.

molecular structure of the amphiphile. In general, for similar HLB values, higher molecular weight molecules generate larger cores with higher solubilizing capacity. For a similar molecular weight, a more hydrophobic molecule (lower HLB) displays a higher solubilizing ability. At the lowest polymer concentration evaluated (1%), F68 and F108 did not aggregate and a negligible solubilization was observed. Once the concentration of the amphiphile overcame the CMC, a linear increase in S_a was found; this behavior suggested that the increase in the amphiphile concentration led to the generation of additional micelles that solubilized additional drug molecules [46]. Owing to a higher molecular weight, F108 shows a lower CMC value (3–4%) than F68 (10%) and it effectively solubilized EFV at lower concentrations. Regardless the higher hydrophobicity of T1107 (HLB = 18–23) compared with T1307 (HLB >24), enabled the larger T1307 micelles (MW 18 kDa) to solubilize EFV more efficiently than T1107 (15 kDa) in the 1–7% polymer concentration range. These findings indicate that the size of the micellar core is likely the predominant parameter influencing solubilization. At a higher polymer concentration (10%), T1107 and T1307 solubilization data were almost superimposable.

The size of the drug-loaded aggregates is a key feature in their interaction with different cell types and a size-dependent cellular uptake behavior of nanoparticles has been reported [49]. The micellar morphology and size of drug-loaded poloxamines have been described elsewhere [35]. Drug-free PEO–PPO micelles with high %PEO contents usually display unimodal hydrodynamic size profiles in the 5–15 nm range [35,36]. For copolymers with similar molecular weights, linear PEO–PPO copolymers form aggregates of larger size than the

branched ones [36,50]. Based on this, F127 theoretically generates larger aggregates than T1307. On the other hand, a compensation effect due to the remarkably higher molecular weight of T1307 is expected. Overall, data indicated that F127 and T1307 EFV-containing micelles have very similar sizes (around 25 nm) and size distributions. In addition, TEM analysis confirmed the spherical morphology of the drug-containing micelles.

Incorporation of EFV molecules into polymeric micelles is the first stage toward the development of a concentrated solution. However, dissociation of the temperature-sensitive micelles under less favorable conditions (e.g., low ambient temperature) might lead to physical instability due to disassembly of the thermoresponsive micelles. As a consequence, the drug would be released from the micellar core to the aqueous medium, would further crystallize (due to the restitution of strong solute–solute interactions) and would finally precipitate irreversibly. Furthermore, EFV has been reported to be sensitive to hydrolysis [40]. F127 and F108 systems maintained the initial EFV concentration over 1 month. By contrast, F68 was less stable, probably due to the proximity of the polymer concentration to the CMC (~10%). Poloxamine systems also showed physical instability over time. This effect was more pronounced for T1107 than for T1307. Since the effective encapsulation into polymeric micelles depends on the generation of strong drug–core interactions that prevail over the drug intermolecular forces, our findings suggest that the less hydrophobic nature of the protonated poloxamine core led to a weaker EFV micellar core affinity, with a detrimental effect on preventing EFV nucleation, crystallization and precipitation.

The dilution of the micellar systems in the gastric medium after ingestion usually results in final polymer concentrations below the CMC and a gradual disassembly of the micelles is expected. In the particular case of poloxamines, acidification may also contribute to the physical instability of the micelles due to the electrostatic repulsion of diprotonated ethylenediamine central moieties [36]. One of the advantages of polymeric micelles over standard micelles (e.g., Cremophor® EL) is that they are kinetically stable and the dissociation process takes place slowly [51]. From this general perspective, a stronger disassembly propensity was anticipated for the branched derivatives as compared with the linear ones. Results confirmed the high physical stability of the EFV-loaded micelles under dilution, with poloxamer systems being more stable than poloxamine ones. As expected, T1307 showed pH-dependent behavior, especially for more diluted specimens. Remarkably, EFV was stable under low pH conditions and no degradation products were detected. According to these results, and despite the dilution and acidification in the gastric environment, EFV-loaded micelles are not expected to dissociate (and drug precipitate) substantially in their transit through the stomach. Once in

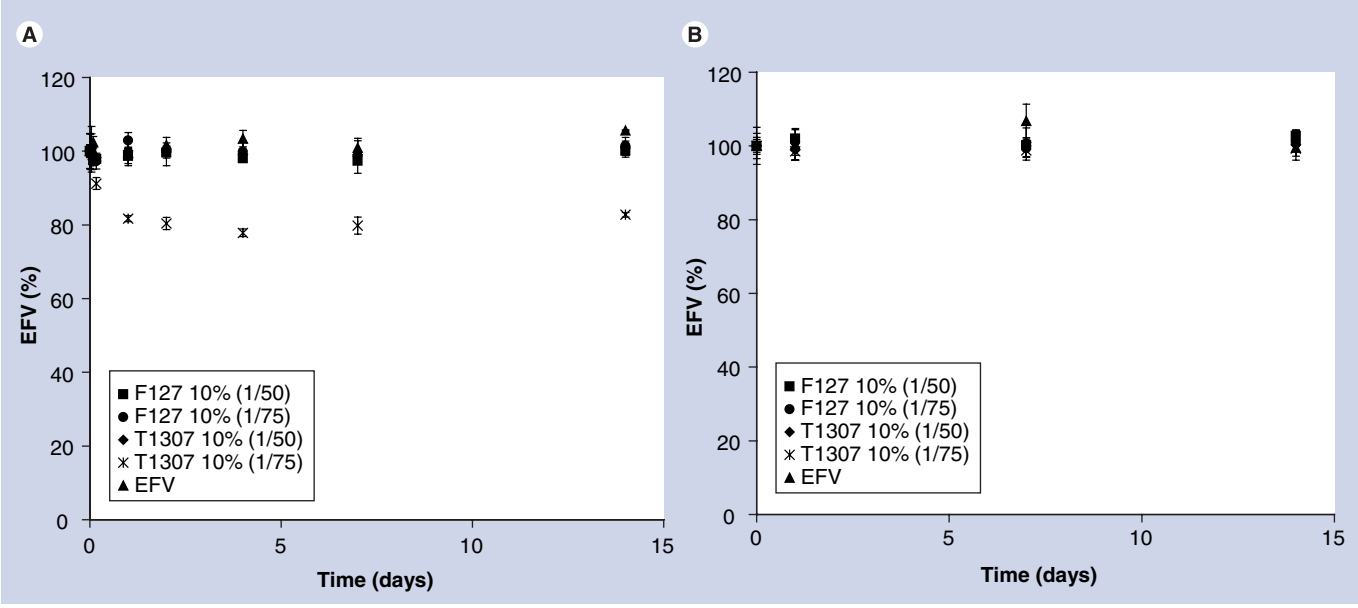

Figure 5. The percentage of efavirenz in solutions of diluted drug-loaded polymeric micelles over time. (A) pH 1.5 at 37°C and **(B)** pH 5.0 at 37°C.
EFV: Efavirenz.

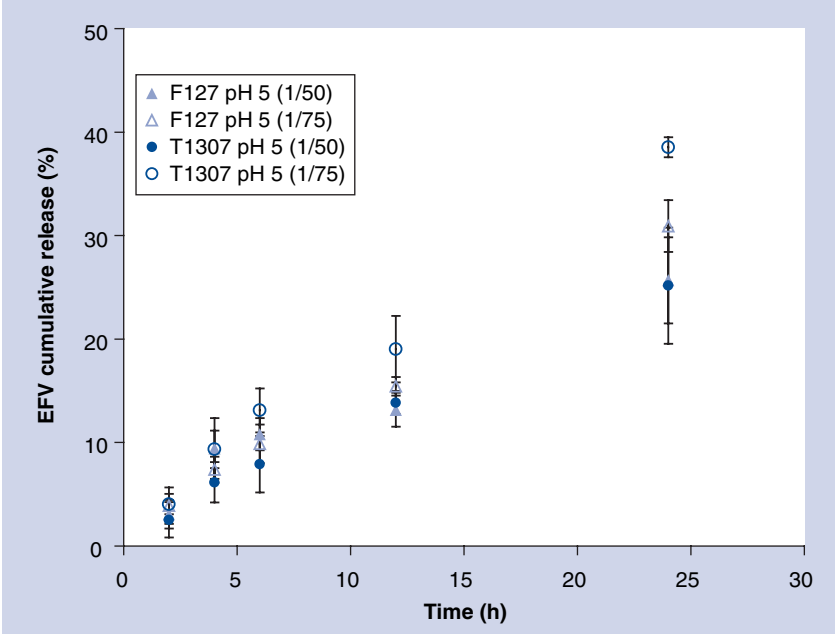

Figure 6. *In vitro* drug-delivery profiles of 1/50 and 1/75 diluted efavirenz-containing 10% Pluronic® F127 and Tetronic® T1307 under intestine-like conditions (pH 6.8).
EFV: Efavirenz.

the intestine, the system will undergo neutralization and the drug will be delivered from the micellar reservoir and absorbed. An extremely strong drug–core interaction would maintain the drug sequestered, constraining the delivery. Considering an oral administration, only the drug amounts released during the first 24 h are probably of relevance. *In vitro* assays indicated the controlled release (zero-order kinetics) of EFV from the micellar reservoir. Both drug-loaded polymeric micelles showed similar delivery profiles. The *in vitro* release was compared with that of an EFV solution in a medium-chain triglyceride (20 mg/ml). A 1/50 aqueous dilution in water formed a relatively stable oil-in-water emulsion that released extremely low drug levels, approximately 8.7% after 24 h. The F127 system appears to be a better alternative owing to its higher physical stability under any condition. In addition, F127 has been approved by the FDA for pharmaceutical products. Accordingly, the oral bioavailability of the drug-containing 10% F127 micellar system was evaluated in rats after a single 40 mg/kg dose.

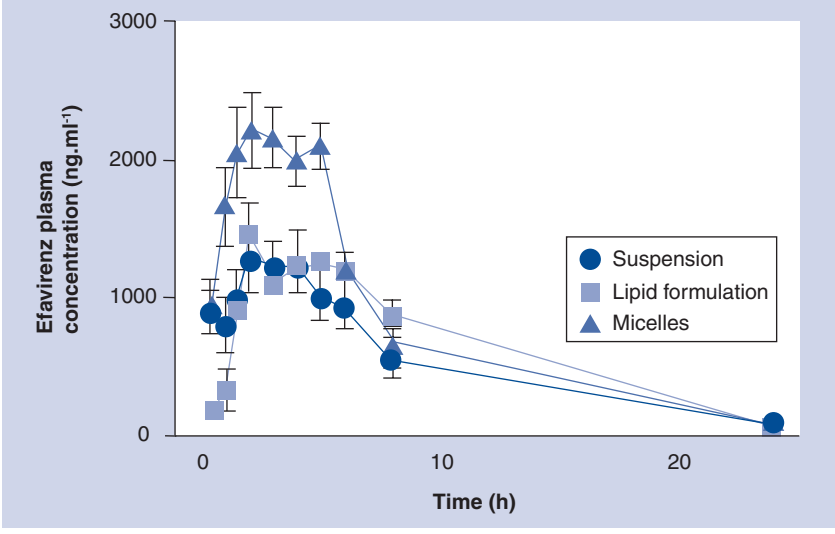

Figure 7. Plasma concentration profiles of 10% F127 efavirenz-loaded polymeric micelles, an extemporaneous suspension and a triglyceride solution after oral administration of a 40 mg/kg dose in rats (n = 10).

Pharmacokinetic analysis of EFV plasma levels after oral administration demonstrated that inclusion of EFV into polymeric micelles greatly improved the bioavailability of the drug; both the AUC and C_{max} of EFV-loaded F127 micelles were significantly higher than the suspension and lipid solution. The fact that the t_{max} parameter was similar for all the tested formulations suggested that EFV-loaded F127 micelles increased the amount of EFV absorbed without changing the rate of absorption. Another advantage of EFV-loaded F127 micelles compared with the suspension and triglyceride solution is the reduction in intersubject variability. Both the AUC and C_{max} for EFV-loaded F127 micelles showed significantly lower variability with regards to the suspension and lipid formulation. Moreover, lower intersubject variability of EFV-loaded F127 micelles is also demonstrated by the fact that the difference between the maximum and minimum parameter estimation was substantially lower with these micelles. For instance, the ratio between the highest and lowest AUC value was 2.9, 5.9 and 3.2 for the EFV-loaded F127 micelles, suspension and lipid formulation, respectively. Additionally, the ratio between the highest and lowest C_{max} was 2.0, 2.8 and 4.3 for the EFV-loaded F127 micelles, suspension and lipid formulation, respectively. Our studies confirmed the lower bioavailability of the lipid solution compared with that of an extemporaneous suspension. Finally, the administration of higher doses of nanoencapsulated EFV is expected to result in the exponential increase of the oral bioavailability of the drug, regardless of the formulation used [52], and more remarkable differences between the C_{max} and AUC induced by the nanotechnological formulation and the suspension and triglyceride solution. While the former phenomenon has been depicted elsewhere [52], the latter has been suggested in additional experiments currently ongoing in our laboratory [53].

Conclusion

The solubilization of the poorly water-soluble drug EFV in PEO–PPO-made polymeric micelles was investigated as a simple strategy for the development of anti-HIV/AIDS liquid pediatric formulations. Remarkable advantages of these block copolymers that robustly support their application as drug carriers are commercial availability in a broad spectrum of molecular weights and HLB values, and approval by the US and European regulation agencies of several derivatives for pharmaceutical products and medical devices. Solubility values were increased from 4 µg/ml to more than 20 mg/ml in the micellar systems. These improvements represent up to 5365-fold higher solubility. Moreover, solubility values are compatible with the volume required to administer a clinically relevant dose of 200 mg (~10 ml). It is also remarkable that the polymer concentrations used are in accordance with the regulations [54]. The comparative preclinical evaluation of the EFV/F127 micelle formulation with a suspension and an oily solution after oral administration confirmed the improved bioavailability of the novel nanoencapsulated drug and opens a cost-effective therapeutic alternative towards a more accessible pediatric anti-HIV pharmacotherapy. This approach could be especially relevant for less affluent patients in poor countries.

Future perspective

Nanotechnology has become a useful tool in developing effective medicines, and could be used to treat infectious diseases [55]. HIV/AIDS incidence is significantly higher among poor populations and countries. This discourages pharmaceutical companies from attempting to enhance the pharmacotherapy of current ARVs, which could increase therapeutic success, particularly in children. Contributions can range from the improvement of simple organoleptic and technological properties (e.g., solubility and stability) to the design of sophisticated delivery systems that target specific cellular and anatomical reservoirs. In any event, the ethical and scientific challenges faced in HIV require the implementation of nanotechnologies at reasonable costs to enable less affluent patients to access novel medications with improved features that increase the chances of therapeutic success.

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No writing assistance was utilized in the production of this manuscript.

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 animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Encapsulation of efavirenz in poloxamer and poloxamine polymeric micelles improved the aqueous solubility of this first-line antiretroviral by more than three orders of magnitude.
- Efavirenz-loaded micelles remained physicochemically stable under dilution in gastric-mimicking conditions.
- Water-solubilized efavirenz resulted in significantly higher plasma C_{max}, oral bioavailability and lower interindividual variability than a magistral suspension and a lipidic solution in a preclinical animal model.

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