A Highly Concentrated and Taste-Improved Aqueous Formulation of Efavirenz for a More Appropriate Pediatric Management of the Anti-HIV Therapy

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Abstract: Pediatric HIV is scarce in developed countries; 90% of pediatric HIV patients are in developing countries. In contrast, children represent 15% of the new infections in poor countries. Approximately 90% of the HIV-positive children do not have access to antiretrovirals (ARVs). Without treatment, 50% of the patients die before the 2 years of age. Efavirenz (EFV, aqueous solubility ~4 μg/mL, 40-45% bioavailability), a non-nucleoside reverse transcriptase inhibitor (NNRTI), is a first-choice pediatric ARV. To assure therapeutic plasma concentrations, the low oral bioavailability demands the administration of relatively high EFV doses. Aqueous EFV irritates the oral mucosa, causing a Burning Mouth Syndrome (BMS). A triglyceride-based liquid formulation of EFV (30 mg/mL) is not commercially available worldwide, making the appropiate dose adjustment and the swallowing difficult. More importantly, clinical trials indicated that the oral bioavailability of this oily solution is lower than that of the solid one. Moreover, a relatively high inter-subject variability has been found. The present work reports the development and full characterization of a concentrated (20 mg/mL, 2%) and taste-masked aqueous formulation of EFV for a more appropriate management of the pediatric anti-HIV therapy. Formulations displayed high physicochemical stability over time under regular storage conditions. Release assays *in vitro* showed a burst effect (2 h) and zero-order kinetics later on (between 2 and 24 h), compatible with the oral administration route and release. Finally, taste tests performed by adult healthy volunteers indicated that the unique combination of flavors and sweeteners employed (i) reduced the intensity of the BMS and (ii) shortened its duration significantly. Overall results indicate that the cost-effective and scalable nanotechnological strategy proposed could enable the more covenient and compliant administration of lower EFV doses. Due to a better pharmacokinetic profile, this would result in similar plasma levels than higher doses administered in solid or triglyceride-soluble form. In this context, some reduction of the treatment cost can be envisioned. This could improve the access of less affluent pediatric patients to medication in poor countries.

Keywords: Efavirenz liquid formulation, Pediatric HIV, Taste masking test, Physicochemical stability, *in vitro* drug delivery.

1. INTRODUCTION

The last update on the global situation of the Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) shows that approximately 2.5 million children (<15 years) are among the more than 40 million HIV-infected patients [1]. A substantial progress was made since the implementation of the High Activity Antiretroviral Therapy (HAART) in 1996 [2, 3]. However, high doses and complex administration schedules affect patients´ lifestyle [4]. Epidemiology indicates that adherence levels lower than 95% dramatically constrain the therapeutic success to less than 50% [5].

Pediatric HIV is scarce in the developed world due to the effective prevention of mother-to-child-transmission (MTCT) [6]; 90% of pediatric HIV patients are in developing countries and represent approximately 15% of the new cases. More crucially, approximately 90% of the HIV-positive children do not have access to antiretrovirals (ARVs); without treatment, 50% of the patients die before the 2 years of age [7]. Only twelve ARVs have been approved by the regulatory agencies of U.S. and Europe for administration in children [8]. The number of liquid formulations commercially available is even more reduced [9]. In this context, the manipulation and the processing of original solid forms [10] to produce unlicensed medicines is the only alternative to treat HIV-infected neonates and infants [11]. Due to a serious lack of resources, the quality, safety and effectiveness of these extemporaneous formulations in countries with limited infrastructure and fragile health systems is highly doubtful [12-14]. In 2007, the World Health Assembly (WHA) proclaimed the right of children to access safe, effective and proven medicines [15].

Efavirenz (EFV, Sustiva®, Scheme **1**) is the first-choice non-nucleoside reverse transcriptase inhibitor (NNRTI) [16] recommended by the World Health Organization (WHO) for the initial treatment of children above the age of 3 [17, 18].

The extremely low aqueous solubility of the drug (4 μg/mL) leads to a limited oral absorption and low bioavailability (40-45%) [19, 20]. The inter- and intra-individual variability found are relatively high, these values

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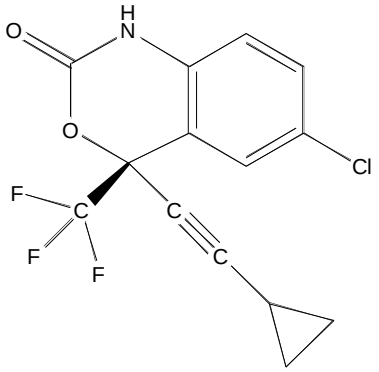
being approximately 55-58% and 19-24%, respectively [21, 22]. It should be stressed that these variability levels are significantly lower than those shown by protease inhibitors,



Scheme 1. Chemical structure of the antiretroviral efavirenz (EFV).

though they are still a matter of concern. To adjust the dose to body weight and prevent adverse effects and discontinuation, EFV usually would require Therapeutic Drug Monitoring (TDM) [23-25]. For example, the benefit of reducing the dose from 600 to 400 mg in adults has been recently reported [25]. Recommended daily doses in children are between 200 and 600 mg [26]. Regardless of the advantages of monitoring EFV plasma levels on patient compliance and adherence [27], TDM is not implemented everywhere as a routine. Another drawback is that soluble EFV may irritate the oral mucosa. The Burning Mouth Syndrome (BMS) [28] is a main cause of unplanned interruption of the ARV pharmacotherapy [29]. A concentrated EFV solution (30 mg/mL) of acceptable taste was developed using water-inmiscible triglyceride vehicles [30, 31]. However, this medicine is not commercially available worldwide. For example, liquid EFV is not registered by the regulatory agency in Argentina; this formulation is available only to a limited number of patients under a compassionate status (source: Program of Immuno-compromissed Patients, "Ramos Mejia" Hospital, Buenos Aires, Argentina). Noteworthy drawbacks of this formulation are significantly lower oral bioavailability than the capsules and high inter-subject variability. Also, administration of relatively large volumes of oily vehicles could produce profuse diarrhea in children and affect compliance and adherence seriously [32].

In general, a good correlation between solubility improvement and higher bioavailability has been found for most of the drugs classified into Class II of the Biopharmaceutic Classification System (BCS) (e.g., EFV). A few attempts to water-solubilize EFV using different carriers have been reported in the literature. Dutta *et al.* investigated the aqueous solubilization and cellular targeting of EFV by means of complexation with surface-modified polypropylene imine (PPI) dendrimers [33, 34]. Regardless the technological potential of this approach, the implementation of non-approved polymers is of relatively limited clinical relevance. Others investigated the solubilization of EFV in different types of cyclodextrins (CDs) and cyclodextrin-containing polymers [35, 36]; CDs are cyclic oligosaccharides that due to the combination of a hydrophobic cavity and a hydrophilic surface are capable of forming inclusion complexes with lipophilic drugs, enhancing their solubility in water [37]. Dissolution extents were increased 6- to 20-fold; apparent solubility in these carriers was about 100 μg/mL. However, to administer a 200 mg standard dose, large formulation volumes (approximately 2 L) might be demanded.

Prevalecence of the disease is extremely higher in resource-constrained countries [38]. Thus, there exists an urgent need to develop innovative, though cost-effective and scalable, antiretroviral medicines [39]; nanotechnologies can provide unique means to improve the effectiveness of the anti-HIV pharmacotherapy, also in constrained-setting countries [40].

Encapsulation of poorly water-soluble drugs within the hydrophobic core of polymeric micelles constitutes one of the most attractive nanotechnological strategies to improve their aqueous solubility of drugs [41]. Thermo-responsive poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) block copolymers are the most extensively investigated family of micelle-forming amphiphiles [42].

With the aim of improving the aqueous solubility and the oral bioavailability of EFV, our research group has recently investigated the encapsulation of EFV within polymeric micelles of linear and branched poly(ethylene oxide)-poly(propylene oxide) block copolymers [43-45]. These copolymers have proven good cell and biocompatibility and some of them were approved by the US FDA and EMEA as pharmaceuticals excipients [46]. The solubility of EFV was increased from 4 μg/mL to 33 mg/mL, representing up to 8250-fold [43]. Also, EFV-loaded nanocarriers remained physicochemically stable for more than two weeks after dilution in gastric-mimicking conditions [44]. Finally, EFV-loaded micellar systems (20 mg/mL) were administered by gavage to male rats (80 mg/kg) and the pharmacokinetics was compared to that of (i) a magistral suspension prepared by dispersing the content of a capsule in a 1.5% carboxymethylcellulose aqueous solution and (ii) a triglyceride solution, of identical concentration (20 mg/mL). Encapsulation into polymeric micelles leads to three outstanding findings: (i) significantly higher maximum plasma concentration (C_{max}), (ii) significantly higher oral bioavailability measured as the area-under-the-curve (AUC) and (iii) sharp decrease in the C_{max} and the AUC inter-individual variability [44]. However, the organoleptic properties of EFV represent a remarkable drawback. Moreover, the addition of pharmaceutical excipients could strongly affect the physicochemical stability of the formulation. The present study reports on the development and full characterization of a concentrated (2%) and taste-masked aqueous formulation of EFV for a more appropriate pediatric management of the anti-HIV therapy. Overall results indicate that the cost-effective and scalable nanotechnology strategy proposed could enable the more covenient and compliant administration of lower EFV doses. Due to a better pharmacokinetic profile, this would result in similar plasma levels than higher doses administered in solid form. Also, some reduction of treatment costs can be envisioned. This could improve the access of less affluent pediatric patients to medication in poor countries.

2. MATERIALS AND METHODS

2.1. Materials

Poloxamer Pluronic® F127 (F127, molecular weight 12.6 kDa) and poloxamines Tetronic® 904 (T904, molecular weight 6.7 kDa) and 1307 (T1307, molecular weight 18 kDa) were a gift of BASF (NJ, USA). Efavirenz (EFV) was donated by Richmond Pharmaceutical Laboratories (Buenos Aires, Argentina). All the other reagents and solvents were of pharmaceutical and analytical grade and they were used as received.

2.2. Preparation of Polymeric Micelles

Polymeric micelles (11% w/v) were produced by dissolving 10 g copolymer in buffer phosphate-citrate (90 mL, pH 5.0) at 4°C and equilibrating the system at 23°C at least 24 hours before use.

2.3. Preparation of the Formulation

Efavirenz (2 g, 20 mg/mL copolymer solution, 2% w/v final drug concentration) was added to 90 mL of the corresponding copolymer solution and shaken (48 h) in a temperature-controlled horizontal shaker at 23°C (Minitherm-Shaker; Adolf Kuhner AG, Switzerland) until total dissolution. Then, the different excipients (sweeteners, sorbitol, sodium ciclamate and potassium acesulfame; flavor, cherry; preservatives, sodium benzoate) were added and solubilized. Finally, menthol was dissolved in ethanol (1 mL) and added to the solution. Buffer was added to complete 100 mL.

2.4. Physicochemical Stability of the Formulations

To study the physicochemical stability of the formulation, samples were stored at 4ºC (refrigerator) and 24ºC (room temperature) and monitored over time (n = 3). The concentration of EFV in the different formulations and the appearance of degradation products were determined by liquid chromatography (HPLC) using a Phenomenex Luna 5 µm, C18, 150 mm x 4.60 mm column (Phenomenex, Torrance, CA, USA) with a UV detector (248 nm, UVIS 204, Linear Instruments, Reno, NV, USA) [44]. 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 range between 20 and 5000 ng/mL. Results of percentage of remaining EFV (%EFV) are expressed as mean ± S.D. (n = 3). To characterize precipitation products, crystals were isolated, thoroughly washed with distilled water, dried under vacuum at room temperature and analyzed by HPLC, differential scanning calorimetry (see below) and optical microscopy.

2.5. Physicochemical Stability of the Formulations Under Dilution

The systems under investigation are intended for the development of EFV oral formulations. In order to determine their ability to withstand dilution in the gastric environment, EFV-containing systems were diluted (1:10, 1:50 and 1:75) in a stomach-mimicking medium (HCl 0.1N, pH 1.5) [47], incubated at 37°C and the drug concentration monitored over time by HPLC (see above). The appearance of degradation products was also studied.

2.6. Thermal Analysis

To establish the meltin temperature (T_m) and enthalpy of melting of EFV (ΔH_m), a sample of drug (~5 mg) was analyzed by Differential Scanning Calorimetry (DSC, Mettler TA-400 differential scanning calorimeter) in a single heating ramp (25 to 200°C, 10°C/min.). A similar analysis was performed with precipitation products isolated from stability assays.

2.7. *In Vitro* Release Studies

To evaluate the EFV release profiles from the different drug-loaded micelles, original and diluted (1:10 in HCl 0.1N) F127, T904 and T1307-based formulations (10 mL) were placed within dialysis membranes (regenerated cellulose tubing, MWCO = 3500), immersed into an intestine-mimicking buffer (pH 6.8, 900 mL, 37°C) [48] and the drug concentration in the internal solution monitored over 24 hours by HPLC (see above). The medium was replaced every 6 hours [44]. The time point for medium release exchange was established in preliminary tests where the medium was exchanged every 3, 6 and 12 h. Differences in the results between time points 3 and 6 h were not statistically significant. Assays were carried out by triplicate and the results are expressed as the Mean ± S.D. To mimick intake conditions, an additional assay was conducted as follows: samples were primarily immersed in gastric-like medium (HCl 0.1N, pH 1.5, 900 mL) [47] and the release evaluated over 2 h, at 37°C. Then, the release assay was continued under intestine-like conditions for 22 h [48].

2.8. Taste Masking Tests

Taste masking properties were evaluated in blind randomized sensory experiments by ten healthy adult volunteers. Formulations (0.5 mL) were held in the center of the tongue (30 seconds) and then spat out. Flavor- and sweetener-free formulations were used as the control. The following parameters were measured: (i) time to the appearance of the BMS, (ii) intensity of the BMS and (iii) duration of the BMS. Volunteers let the irritation vanish before testing the next sample. The intensity of the BMS was scored using a numerical scale between 0 and 4, where 0, 1, 2, 3 and 4 were undetectable, threshold of detection, slight, moderated and strong BMS, respectively. A medium-chain triglyceride solution (20 mg/mL in Miglyol® 812) was also tested. Statistical differences ($p<0.05$) between the different samples and the original 2% EFV-loaded micelle sample (control), were analyzed using the Dunnett's Multiple Comparison Test.

The assay was performed following the Declaration of Helsinki guidelines and the local ethical regulations for human studies. Participants were previously informed and they expressed their consent prior to the test.

3. RESULTS AND DISCUSSION

3.1. Development of the Formulation

The limited commercial availability of the licensed liquid formulation of EFV (Sustiva® Oral Solution) still remains a hurdle towards a convenient pediatric anti-HIV pharmacotherapy in several countries. Moreover, its lipidic nature and non-miscibility with the gastrointestinal fluids constrains the oral absorption of the drug. In a previous work, we thoroughly investigated the encapsulation of EFV within PEO-PPO polymeric micelles as a nanotechnology strategy to increase the aqueous solubility of the drug and its oral bioavailability [44]. However, the unbearable taste of EFV in water remains a remarkable disadvantage that may affect patient compliance and adherence. Also, the addition of different pharmaceutical excipients that modify the properties of the medium (e.g., ionic strength) could affect the physicochemical stability of the drug-loaded polymeric micelles; dissociation of the micelles would lead to the irreversible precipitation of the drug.

The goal of the present work was to develop and fully characterize a highly concentrated EFV aqueous formulation with enhanced taste. A previous work evaluated the combination of a broad spectrum of flavors and sweeteners to improve the taste of EFV [49]. The strategy relied on the addition of FLAVORx, a brand name flavoring system (FLAVORx Co., Bethesda, MD), commercially available only in the US. Menthol and cherry flavors have been previously described as effective masking excipients [50]; the colling effect of menthol relieves local irritations in the oral mucosa [51]. Similar beneficial properties have been ascribed to sorbitol and acesulfame [52]. Once developed, formulations were thoroughly evaluated to state the physicochemical stability and the organoleptic properties. Three PEO-PPO copolymers were used. Poloxamer F127 has been approved by the US FDA for pharmaceuticals and it is probably the most extensively investigated poloxamer [42, 46]. Also, previous results showed that EFV-loaded F127 micelles are physicochemically stable [44]. On the other hand, poloxamines present a unique structural feature (a central ethylenediamine group) that makes them pH-responsive and enable *N*-alkylation to modulate the drug delivery rate [45]; micelles are less stable under acid conditions and tend to disassemble releasing the drug faster. A goal of the present work was to comparatively investigate formulations based on both poloxamers and poloxamines. The selection of poloxamines T1307 and T904 relied on their structural properties. T1307 generates efavirenz-loaded micelles of similar size to F127 ones and displays a similar solubilization capacity [44], while T904 is more a hydrophobic amphiphile and solubilizes the drug much better; e.g., the maximum solubility attainable in 10% T904 solution is 33 mg/mL as opposed to about 20 mg/mL for 10% F127 and T1307 systems. This behaviour indicates a greater drug/micelle affinity.

3.2. Physicochemical Stability of the Formulations

Two mechanisms could affect the concentration of EFV in the formulation over time: (i) physical instability due to the disassembly of the micelles and irreversible drug precipitation and (ii) EFV hydrolysis. Both poloxamers and poloxamines display a reverse thermo-responsive behaviour: the lower the temperature, the higher the critical micellar concentration (CMC) found. Cooling can result in micellar disassembly and drug release and precipitation. At 4°C, a F127-based formulation displayed high stability, the %EFV being 92% of the initial drug concentration at day 28 (Fig. **1A**). Poloxamine-based systems were less stable. T1307 systems remained almost unchanged during the first week of the assay (%EFV 93.5%) and lost about 17% of the initial drug load after two weeks. Then, a gradual decrease to a 25% was found, at day 28. Contrary to this, T904 systems showed a much more pronounced decrease to levels below 30 and 20% at days 7 and 28, respectively. These findings were supported by the appearance of a crystalline precipitate (Fig. **2A**,**a**) that showed a thermal behavior identical to that of pure EFV (Fig. **2B**). This phenomenon stemmed from the higher CMC of T904. At 24°C, all the formulations showed high physicochemical stability, %EFV values being greater than 90-95% (Fig. **1B**). In addition, precipitates were not found (Fig. **2A**,**b**). HPLC analysis of both the formulation and the precipitates confirmed the chemical stability of EFV (and the absence of degradation products) under the working conditions, as opposed to previous reports indicating the hydrolysis under low pH and high T conditions [53] (Fig. **2D**, **E**). In addition, the gradual decrease in the area of the HPLC peak of EFV in a T904-based formulation due to precipitation was an additional evidence of the physical instability of poloxamine-based formulations stored in the refrigerator (Fig. **2E**,**c**).

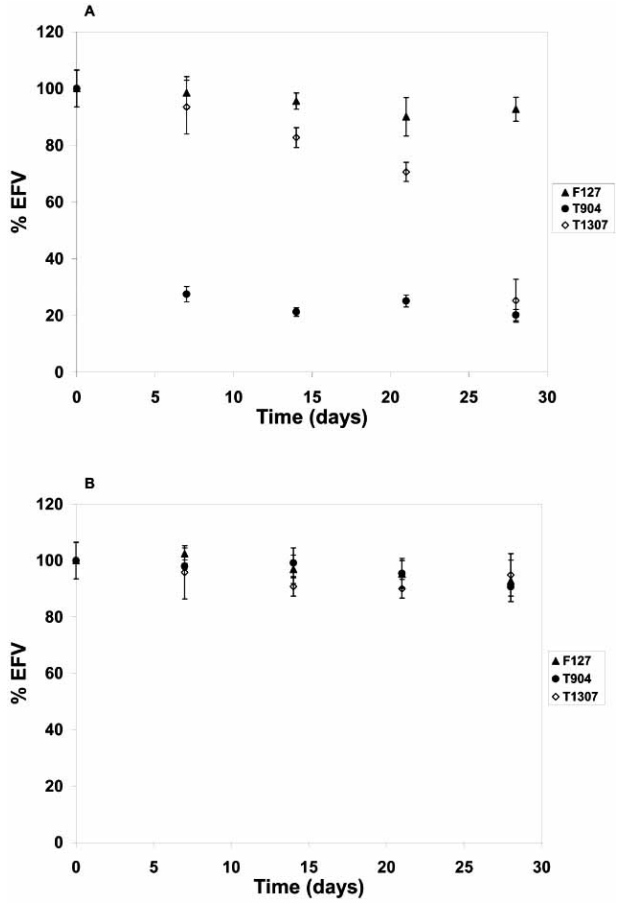


Fig. (1). Remaining percentage of efavirenz (% EFV) in solution in different formulations at **A**) 4°C and **B**) 24°C.

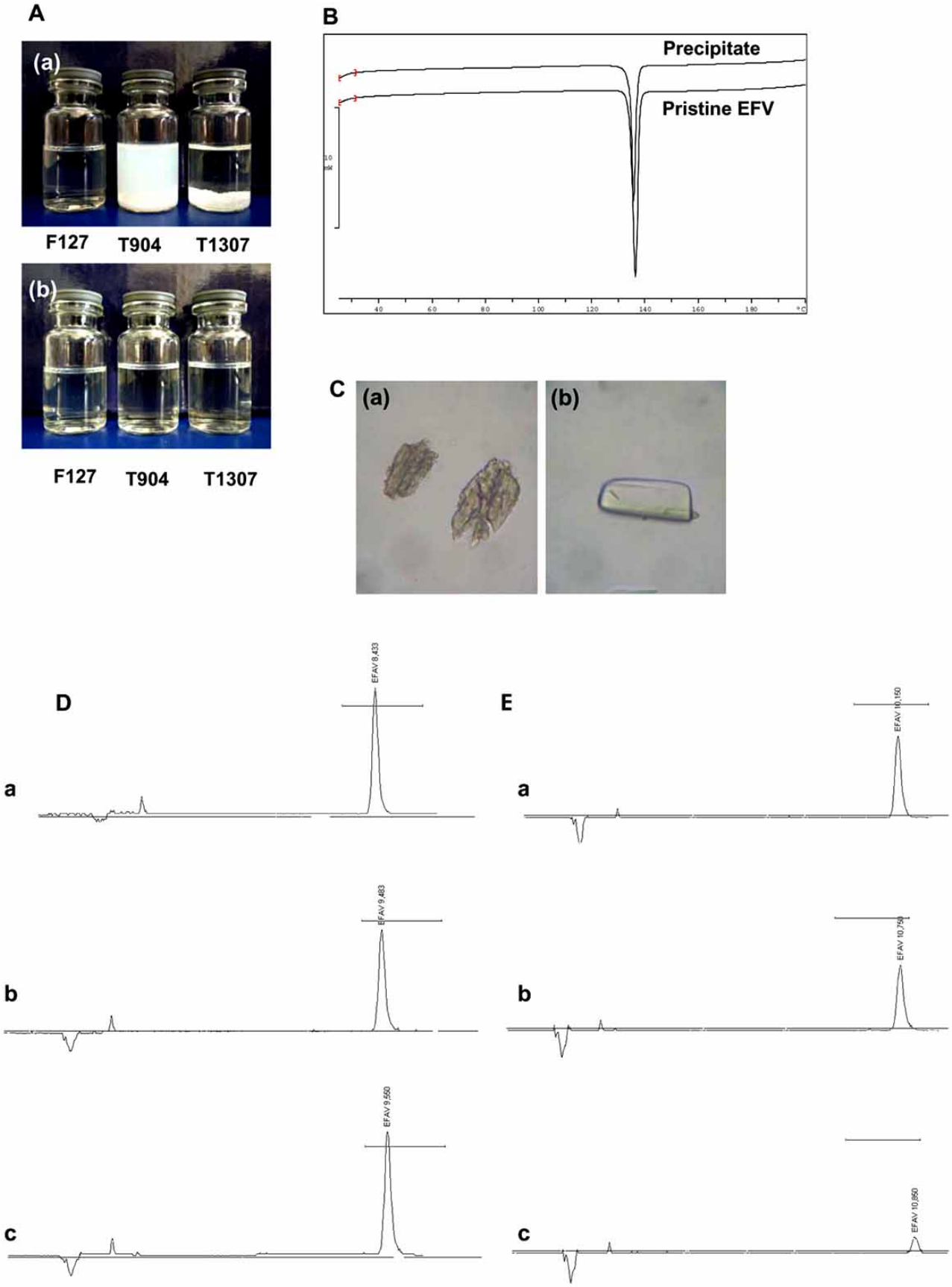


Fig. (2). Physicochemical stability of EFV formulations. (**A**) Aspect of different formulations after 28 days at (**a**) 4°C and (**b**) 24°C. (**B**) Thermogram of pristine EFV and the needle-like precipitate isolated from the EFV/T1307 formulation at day 28. (**C**) Optical micrographs of (**a**) pristine and (**b**) precipitated EFV. (**D**) Liquid chromatograms of the F127 formulation. (**a**) Day 0, (**b**) 24°C at day 28 and (**c**) 4°C at day 28. (**E**) Liquid chromatograms of the T904 formulation. (**a**) Day 0, (**b**) 24°C at day 28 and (**c**) 4°C at day 28.

3.3. Physicochemical Stability of the Formulations Under Dilution

EFV-containing formulations are planned for oral administration. Thus, evaluating their behaviour under dilution in gastric-like conditions was of interest. The fast disassembly of the micelles and drug precipitation in the stomach would preclude the effective drug release in the intestine. In general, drugs undergo dilution in the total gastric volume (~600 mL) [54]. Considering that the volume of a single dose of EFV is between 10 and 30 mL, 10% F127, T904 and T1307-based formulations were diluted (1/10, 1/50 and 1/75) and the drug concentration monitored over two weeks, at 37°C. Previous results indicated the high physicochemical stability of the EFV-containing micelles upon dilution, the poloxamer-based system being more physically stable than the poloxamine-based ones [44]. Neverthelss, incorporation of pharmaceutical excipients could strongly affect the performance of the formulations as compared to excipient-free solutions. The results are exemplified for F127 and T904 in Fig. (**3**). All the formulations showed high stability, regardless the dilution extent; %EFV values were approximately 100% at day 28. Diprotonation of the central ethylenediamine group of poloxamines under low pH-values (e.g., pH 1.5) and the resulting electrostatic repulsion of positively-charged copolymer molecules favour the gradual dissociation of the micelles and the drug precipitation [55]. Remarkably, also a 1/75 dilution of a T904 formulation remained stable. EFV did not undergo hydrolysis under low pH conditions. Accordingly, formulations will remain physicochemically stable in the stomach and, due to a longer residence time in the intestine, they will release the drug and enable the absorption. The high stability of the diluted formulations would allow the dilution in water or soft beverages without affecting the stability of the system.

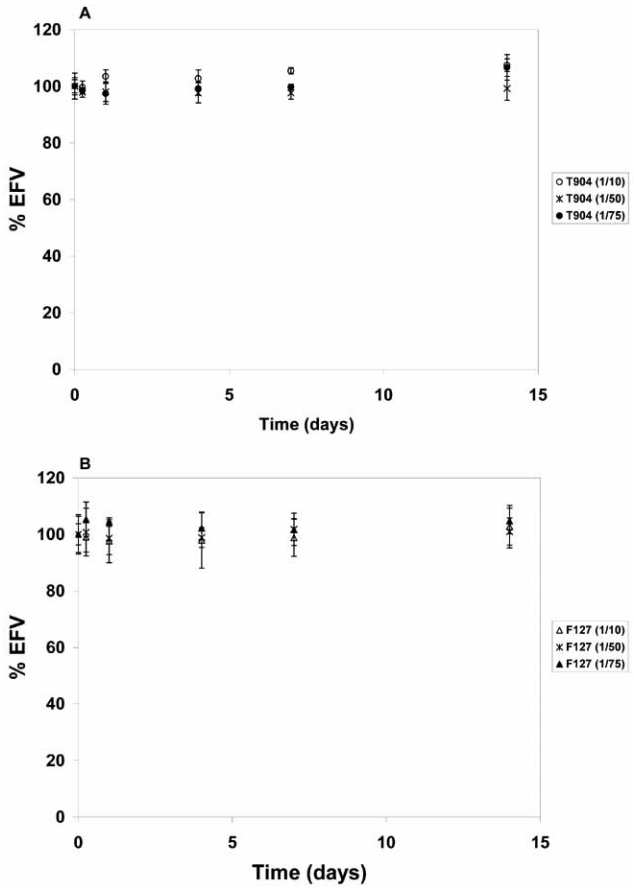


Fig. (3). Percentage of EFV remaining in solution of T904 and F127 formulations diluted in HCl 0.1N and incubated at 37°C over 2 weeks.

3.4. *In Vitro* Release Studies

A key parameter governing the encapsulation process is the drug/core affinity. Conversely, an extremely strong drug/micelle interaction would hamper the release and the absorption of the drug in the intestine. The *in vitro* release is exemplified for F127 and T904 formulations in Fig. (**4**). First, original and diluted (1/10 and 1/50) specimens were exposed to an intestine-like release medium (24 h) (Fig. **4A**, **B**). Undiluted samples showed similar burst effects (34-37%, at 2h). Then, a zero-order-kinetics was observed, the total released being 52.7 (R^2 = 0.9858) and 48.5% (R^2 = 0.9641) for the poloxamer and the poloxamine, respectively. The lower release rate of T904 stemmed from the higher hydrophobicity of the nanocarriers and the stronger drug/micelle affinity. Dilution of the samples reduced the concentration gradient between the formulation and the release medium. Thus, a gradual decrease in the burst effect was apparent as the dilution increased; i.e., 1/10 and 1/50 dilutions of a T904 formulation showed bursts of 17 and 12%, respectively. Then, zero-order profiles were observed, the total released amounts being approximately 30%. To mimick the *in vivo* intake conditions, a similar assay was carried out, though the primary release (2 h) was evaluated in stomach-like medium (Fig. **4C**). Burst releases remained almost unchanged around 35 and 15% for undiluted and diluted samples, respectively. In contrast, a slight increase in the total amount released from 28-30 to 35-39% was apparent.

3.5. Taste Masking Tests

A central goal of the present study was to improve the organoleptic properties of the aqueous formulation and to minimize the BMS caused by the ingestion of water soluble EFV; this phenomenon is more noticeable in the last portion of tongue and the throat. The syndrome is intimately associated with treatment interruption. In addition, vomiting and spitting might result in EFV underdosing and erratic plasma concentrations, owing to the partial intake of the administered dose. Pluronic® F127 has been approved by the FDA and it is available in National Formulary grade. In addition, the F127-based formulation showed the highest physicochemical stability among the investigated systems. In this context, a 2% EFV/10% F127 formulation and a similar drug-loaded system deprived of flavors and sweeteners were evaluated by ten healthy adult volunteers. Results are presented in Table **1**. In general, the addition of flavors and sweeteners had remarkable beneficial effects by significantly reducing the intensity of the BMS and also its duration. Even though a delay in the appearance of the phenomenon was observed, differences were not statistically significant. For example, the addition of excipients delayed the appearance

from 35 sec to 2.13 min and reduced the intensity from strong (score 3.9 for original EFV-loaded micellar systems) to slight-moderate (score 2.8 for the original formulation). The duration was shortened from 60.0 to 36.4 min.

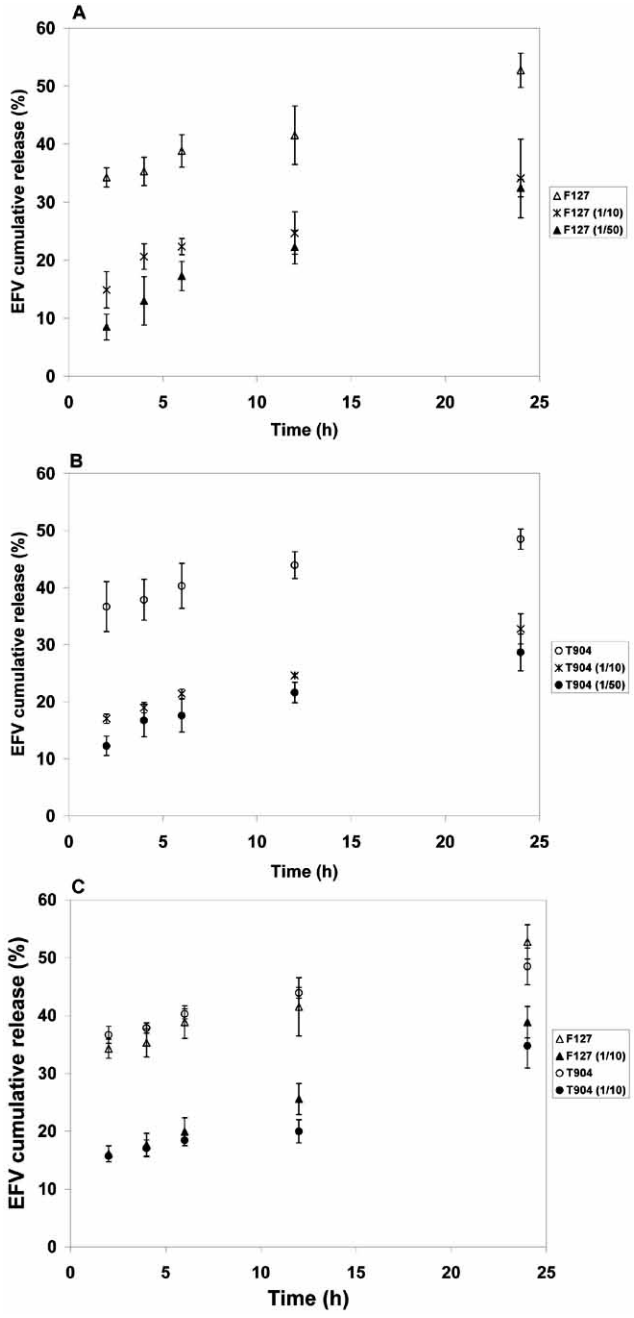


Fig. (4). (**A**, **B**) *In vitro* EFV release profile in intestine-mimicking (PBS, pH 6.8) at 37°C from different formulations: (**A**) Original and diluted (1/10 and 1/50) Pluronic® F127 formulation. (**B**) Original and diluted (1/10 and 1/50) Tetronic® T904 formulation. (**C**) *In vitro* EFV release profile in gastric-mimicking conditions (HCl 0.1N, pH 1.5) the first two hours and then in PBS (pH 6.8) from original and diluted (1/10) Pluronic® F127 and Tetronic® T904 formulations. Each point represents mean ± SD (n = 3).

To evaluate the effect of sweet food and beverages on the BMS, grape jelly and chocolate milkshake were ingested immediately after the test. This a common practice to improve the taste of antiretrovirals and other chronic medicines in pediatric pharmacotherapy [49, 56]. Findings indicated the additional improvement of the taste profile; the intensity decreased to 2.20 and 1.00 with chocolate milkshake and grape jelly, respectively, while the duration was shortened to about 15 minutes. It could be correctly argued though, that these foods are most probably unavailable in extremely constrained settings. The goal of testing them was to show the potential of combinig the formulation with food ingestion to further assure compliance and adherence. According to the test results, the formulation displays an intrinsically acceptable taste. In this context, dilutions in tap water were also evaluated with even better results; the intensity of the BMS decreased from moderate to slight and from slight to threshold levels for 1/5 and 1/10 dilutions, respectively. In all the cases, the formulation performed better than the system without flavors and sweeteners

Moreover, the BMS lasted 7.4 min as opposed to 36.4 min. Finally, a triglyceride EFV solution (20 mg/mL) was also tested. Even though this formulation does not cause BMS, the oily nature of the formulation caused nausea sensation in more than 50% of the volunteers. Having expressed this, the taste of the formulation appears as a less concerning feature than the lower oral bioavailability and higher inter-individual variability found for this formulation [44]. Considering these facts, the developed formulation appears as more advantageous alternative for the pediatric therapy than the available ones. It is worth remarking that there are not previous reports describing the impact of (i) the time to the appearance of the BMS and (ii) the duration of the BMS on patient compliance. However, it is expected that a shorter duration of any adverse effect in a chronic pharmacotherapy would result in better patient compliance and adherence to the regimens. Thus, we decided to also report on these parameters and probably instate this more comprehensive approach for future taste tests conducted in volunteers. In this context, the implications of the combination of any formulation with food in the oral bioavailability need to be thoroughly investigated.

4. CONCLUSIONS

Nanotechnolgy has become a key player in the design of more effective medicines [41]. However, as opposed to cancer that does not recognize physical and socioeconomic boundaries, epidemic infectious diseases such as tuberculosis, HIV/AIDS and malaria are significantly more incidential among poor populations. This fact disencourages pharmaceutical companies to dedicate efforts to improve, at least minimally, the properties of the formulations. Even though nanotechnology appears as less affordable in developing countries owing to higher production costs, the ethical and scientific challenges are to apply them at reasonable costs [40]. Contributions can range from the improvement of simple organoleptic and technological (e.g., solubility and stability) properties to the design of sophisticated delivery systems that target specific cellular and anatomical reservoirs. Pediatric patients constitute a high-risk group. More than 90% of the children are in sub-Saharan Africa. In this global context, it is surprising that a

Table 1. Blind Taste Test of EFV-Containing Different Micellar Solutions and Formulations in Healthy Human Volunteers (n = 10)

	Sample (20 mg/mL)	*Appearance of BMS (min) (S.D.)*	*BMS intensity* (S.D.)*	*Duration of BMS (min) (S.D.)*
Blank	***Water***	-	0	-
Original 20 mg/mL sample	***Micelles (control)***	0.58 (0.17)	3.90 (0.32)	60.0 (8.2)
	Formulation	2.13 (0.67)	2.80** (0.63)	36.4** (13.7)
Ingestion of 5 mL grape jelly	***Micelles***	0.92 (0.41)	3.20 (0.92)	24.9** (10.8)
	Formulation	1.73 (0.93)	2.20** (1.03)	16.8** (3.9)
Ingestion of 50 mL chocolate milkshake	***Micelles***	0.71 (0.22)	2.20** (0.92)	24.9** (19.8)
	Formulation	5.03** (5.15)	1.00** (1.05)	15.3** (11.5)
1/5 dilution of original micellar formulation	***Micelles***	0.86 (0.29)	2.30** (1.16)	21.1** (13.7)
	Formulation	1.72 (1.11)	1.60** (0.52)	18.4** (11.9)
1/10 dilution of original micellar formulation	***Micelles***	0.67 (0.26)	2.20** (0.92)	19.1** (16.4)
	Formulation	3.95** (2.74)	1.10** (0.57)	7.4** (4.4)

Statistical differences ($p<0.05$) between the different samples and the original 2% EFV-loaded micelle sample (control), were analyzed using the Dunnett's Multiple Comparison Test.

*Note: 40 percent of volunteers reported no BMS after the test of the formulation followed by chocolate milkshake; score of BMS intensity was 0.

**Statistically significant difference with the control.

licensed aqueous formulation of efavirenz, a first-choice antiretroviral with low documented bioavailability, is not yet available worlwide. The only choice in many countries is to prepare extemporaneous magistral formulations of unproven efficacy; i.e., adsorption of drugs to excipients in the formulation may reduce the bioavailability with respect to the solid form. Previous preclinical investigations conducted in our laboratory showed a dramatic improvement in the oral bioavailability and the decrease of the inter-subject variability by means of EFV encapsulation into PEO-PPO polymeric micelles [44]. In this context, the present work reported for the first time on the development of an aqueous liquid formulation of EFV. To assure a better access worldwide, the formulation was developed to (i) enable easy and cost-effective preparation and (ii) fit the pediatric patient needs of dose adjustment and easy swallowing in a broad age range. The copolymers used for the generation of the nanocarriers are (i) commercial available, (ii) biocompatible, (iii) US FDA- and EMEA-approved and (iv) relatively cheap. In pediatric regimens, EFV doses are often in the 100-300 mg range. Remarkably, the formulation developed would demand the intake of an acceptable volume between 5 and 15 mL.

Ongoing investigations are being dedicated to (i) preclinically evaluate the formulations in at least two animal models and under a variety of conditions (e.g., concentrated *vs* diluted formulations, food, etc), (ii) conduct clinical trials in adult healthy volunteers and infected children and (iii) estimate the costs for the production of a pilot-scale batch.

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