RESEARCH ARTICLE

Proniosomal Nano carriers for Enhanced Oral Delivery of Efavirenz: Optimization, Characterization and Bioavailability Improvement

Swarupa Arvapalli, Anka Rao Areti*

Department of Pharmaceutics, KL College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh-522022, India

Received: 21st Jun, 2025; Revised: 28th Jul, 2025; Accepted: 24th Aug, 2025; Available Online: 25th Sep, 2025

ABSTRACT

This study aimed to develop and optimize Efavirenz-loaded proniosomal formulations to enhance its oral bioavailability, stability, and controlled release characteristics. Proniosomes were prepared using a slurry method incorporating Span 60, cholesterol, Efavirenz, and maltodextrin as a carrier matrix. A 2^4 factorial design was employed to systematically investigate the influence of formulation variables on critical quality attributes such as particle size, polydispersity index (PDI), and encapsulation efficiency (EE%). The optimized formulation exhibited a particle size of ~490 nm, PDI < 0.3, zeta potential of −19.76 mV, and EE% of 78.12 %, indicating a stable and uniform vesicular system. Morphological analysis using optical microscopy. *In-vitro* release studies showed a biphasic release pattern with an initial blast following a sustained release over 24 hours. Drug release kinetics best fit the first-order and Korsmeyer-Peppas models, suggesting diffusion-controlled mechanisms. Pharmacokinetic evaluation in rats revealed that the optimized formulation achieved higher Cmax (789.6 ng/mL), prolonged half-life (4.12 h), and significantly increased AUC (6051.83 ng·h/mL) than plain Efavirenz (Cmax 132.21 ng/mL, t½ 2.04 h, AUC 854.43 ng·h/mL), confirming improved bioavailability. These findings underscore the potential of proniosomal carriers as a great delivery platform for enhancing the therapeutic performance of Efavirenz via oral administration.

Keywords: proniosomes, encapsulation efficiency, factorial design, sustained drug release, pharmacokinetics

How to cite this article: Swarupa Arvapalli, Anka Rao Areti. Proniosomal Nano carriers for Enhanced Oral Delivery of Efavirenz: Optimization, Characterization and Bioavailability Improvement. International Journal of Drug Delivery Technology. 2025;15(3):1130-36. doi: 10.25258/ijddt.15.3.31

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Efavirenz, a non-nucleoside reverse transcriptase inhibitor, is a cornerstone drug in antiretroviral therapy for HIV infection. However, its clinical efficacy is declined by poor aqueous solubility and low bioavailability (typically 40–45%), which leads to variable plasma concentrations and suboptimal pharmacokinetics. This, in turn, increases the risk of therapeutic failure, development of drug resistance, and adverse effects due to the need for high doses[1,2]. Several formulation strategies, such as solid dispersions, nanoparticles, and lipid-based systems, have been investigated to overcome these limitations. Among these, vesicular systems like proniosomes have shown significant promise in the encapsulation and controlled delivery of hydrophobic drugs such as Efavirenz[3-8].

Proniosomes are dry, free-flowing powders composed of surfactants, cholesterol, and stabilizers that upon hydration spontaneously form niosomes non-ionic surfactant vesicles capable of encapsulating both hydrophilic and hydrophobic drugs[9-12]. Unlike conventional niosomes and liposomes, proniosomes are preferred for their enhanced physical and chemical stability, simplified storage and transportability, and capacity for scale[13-15]. The conversion of proniosomes to niosomes involves a simple hydration step under mild conditions, ensuring protection of labile drug molecules and maintaining a uniform vesicle size distribution[10,16]. This property is particularly valuable in the case of efavirenz because nanosized vesicles promote enhanced dissolution, increase the surface area available for absorption, and may facilitate drug transport across biological membranes, resulting in improved oral bioavailability[9,17].

Through systematic formulation optimization-particularly using factorial experimental designs proniosomal systems can be fine-tuned to achieve optimal encapsulation efficiency, particle size, and release behavior. For instance, a 2^3 full-factorial study revealed that modifying surfactant type, surfactant-cholesterol ratio, and TPGS content significantly improved oral bioavailability and cytotoxic activity of drug-loaded proniosomes[18]. Proniosomes offer advantages such as excellent physical stability, scalability, and ease of storage, while preserving drug-loaded vesicles during hydration[19].

Additionally, the incorporation of TPGS into vesicular systems (e.g., docetaxel-TPGS proniosomes) led to sustained drug release, spontaneous uptake enhancement, and up to a 7-fold increase in oral bioavailability compared to conventional formulations[20].

These studies emphasize proniosomes' ability to provide predictable, controlled drug release based on models like Higuchi and Korsmeyer-Peppas, zero-order while their stability profiles support their potential as viable pharmaceutical carriers.

**Author for Correspondence: drankarao83@gmail.com*

Table 1: Full factorial design with observed responses

Run	A: Amount of Tween 80 (mg)	B: Amount of Cholesterol (mg)	C: Amount of Efavirenz (mg)	D: Amount of TPGS (%w/v)	Particle size (nm)	PDI	Encapsulation efficiency (%)
1	40	10	100	0.05	447.32	0.26	67.8
2	40	30	200	0.4	634.12	0.36	82.7
3	40	10	100	0.4	566.45	0.31	71.3
4	20	10	100	0.4	615.42	0.34	68.9
5	40	10	200	0.4	602.67	0.35	78.4
6	20	30	100	0.4	623.54	0.38	75.1
7	20	30	200	0.4	654.82	0.41	81.6
8	20	30	100	0.05	468.23	0.25	72.4
9	40	30	200	0.05	525.15	0.3	83.2
10	20	10	200	0.05	518.12	0.26	73.7
11	40	10	200	0.05	506.14	0.27	77.9
12	40	30	100	0.4	585.32	0.34	74.6
13	20	30	200	0.05	525.47	0.31	80.4
14	40	30	100	0.05	454.65	0.29	73.8
15	20	10	200	0.4	635.32	0.39	76.1
16	20	10	100	0.05	473.23	0.25	66.5

Therefore, it is hypothesized that efavirenz-loaded proniosomes particularly those incorporating TPGS will enhance the physicochemical stability, improve drug release profiles, and significantly increase the oral bioavailability of efavirenz compared to conventional formulations. The study aims to develop and optimize such proniosomal systems using a 2^4 factorial design by systematically varying key formulation components, namely Span 60, cholesterol, efavirenz, and TPGS. The overall objective is to determine whether these optimized, hydration-activated proniosomes can serve as an effective oral delivery system for efavirenz by improving drug absorption, stability, and therapeutic efficacy.

METHODOLOGY

Experimental Design and Optimization

A comprehensive 2^4 full factorial design was executed using Design-Expert® software (V13.0.5.0) to systematically investigate the effects of four critical process parameters on the characteristics of drug-loaded proniosomal systems. The independent variables were: Span 60 quantity (X_1), cholesterol quantity (X_2), drug quantity (X_3), and TPGS concentration (% w/v, X_4), studied at two levels each. These variables were chosen based on their known influence on proniosome formation, stability, and drug encapsulation.

The dependent variables, also termed Critical Quality Attributes (CQAs), were:

Y_1: Particle Size (PS)- ensures adequate bioavailability and efficient drug delivery.

Y_2: Polydispersity Index (PDI)- assesses uniformity of the size distribution.

Y_3: Encapsulation Efficiency (EE%)- measures successful incorporation of drug into vesicles.

Optimization was aimed at maximizing EE%, minimizing PDI, and obtaining nanosized particles, to achieve a stable and effective drug delivery system.

Preparation of TPGS-coated and Uncoated Proniosomes

Proniosomes were done using thin-film hydration method. Span 60, cholesterol, and Efavirenz were dissolved in ethanol, vortexed, and incubated at 70°C. The solvent was evaporated under decreased pressure, yielding a thin lipidic film. This film was added with deionized water at 60°C, followed by sonication to obtain a creamy proniosome powder.

For TPGS-coated proniosomes, hydration was performed with Sorensen's phosphate buffer (pH 7.4) containing 0.02% TPGS before evaporation. Uncoated proniosomes

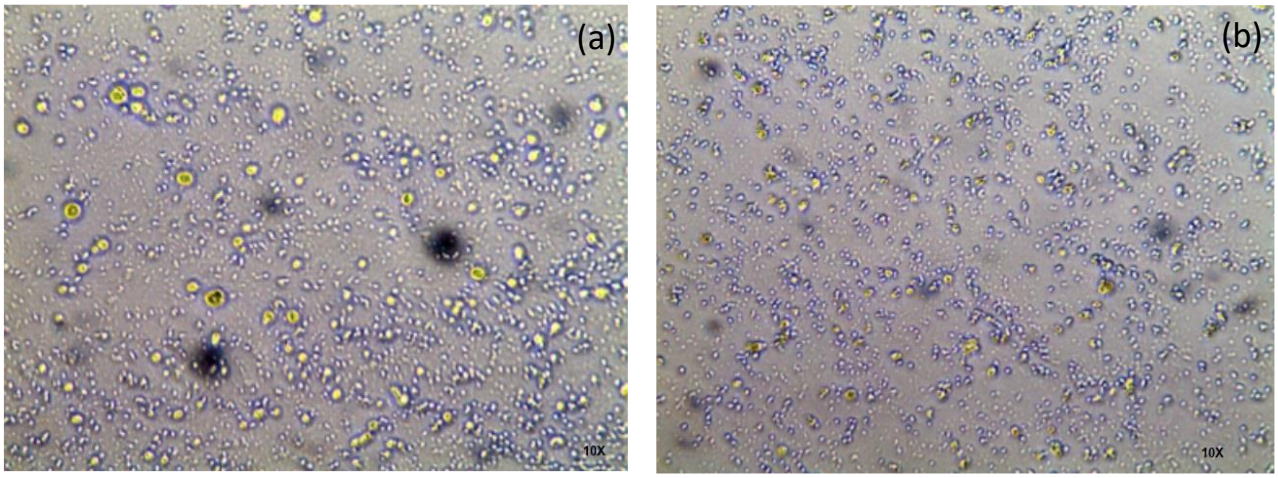

Figure 1: Optical microscopy images (a) Blank proniosomes (b) Efavirenz proniosomes

Table 2: ANOVA for selected factorial model for the responses Y_1, Y_2, and Y_3

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Particle size (Y_1)						
Model	75613.13	10	7561.31	12178.65	< 0.0001	significant
A-Amount of Tween 80	2311.93	1	2311.93	3723.71	< 0.0001	
B-Amount of Cholesterol	710.62	1	710.62	1144.57	< 0.0001	
C-Amount of Efavirenz	8447.91	1	8447.91	13606.65	< 0.0001	
D-Amount of TPGS	62418.78	1	62418.78	1.01×10^5	< 0.0001	
AB	136.25	1	136.25	219.45	< 0.0001	
AC	232.79	1	232.79	374.95	< 0.0001	
AD	492.29	1	492.29	792.9	< 0.0001	
BC	143.94	1	143.94	231.84	< 0.0001	
BD	151.6	1	151.6	244.17	< 0.0001	
CD	567.04	1	567.04	913.3	< 0.0001	
Residual	3.1	5	0.6209			
Cor Total	75616.23	15				
Polydispersity index (Y_2)						
Model	0.0393	5	0.0079	55.69	< 0.0001	significant
A-Amount of Tween 80	0.0008	1	0.0008	5.35	0.0432	
B-Amount of Cholesterol	0.0028	1	0.0028	19.51	0.0013	
C-Amount of Efavirenz	0.0033	1	0.0033	23.41	0.0007	
D-Amount of TPGS	0.0298	1	0.0298	210.66	< 0.0001	
AD	0.0028	1	0.0028	19.51	0.0013	
Residual	0.0014	10	0.0001			
Cor Total	0.0407	15				
Encapsulation efficiency (Y3)						
Model	394.08	4	98.52	106.3	< 0.0001	significant
A-Amount of Tween 80	14.06	1	14.06	15.17	0.0025	
B-Amount of Cholesterol	116.64	1	116.64	125.85	< 0.0001	
C-Amount of Efavirenz	252.81	1	252.81	272.77	< 0.0001	
D-Amount of TPGS	10.56	1	10.56	11.4	0.0062	
Residual	10.19	11	0.9268			
Cor Total	404.27	15				

were prepared using buffer without TPGS. Blank (drug-free) proniosomes were also prepared in parallel. All proniosome powders were then stored until further use.

Transformation to Niosomes by Hydration

To form niosomes, the proniosome powder was hydrated in phosphate buffer (pH 6.8) at 40°C under agitation. Hydration volume (5–25 mL) and time (5–30 minutes) were varied to optimize niosomal properties. The transformation process was assessed for its effect on particle size, encapsulation efficiency, and dispersion quality. Ideal hydration parameters were selected based on resultant niosomal homogeneity and stability.

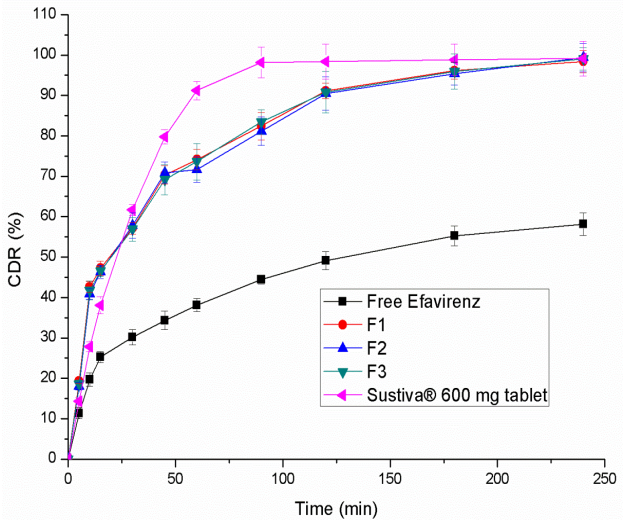

Figure 2: Dissolution profile of Efavirenz from proniosomes formulations

Characterization of Proniosomal Formulations

Entrapment Efficiency

Niosomes reconstituted from proniosomes were subjected to ultracentrifugation (15,000 rpm, 30 min, 0°C) to separate free drug from encapsulated drug. After centrifugation and resuspension, the free drug (in supernatant) was quantified by UV-Visible spectrophotometry[21], and encapsulation efficiency was calculated as:

$$Entrapment\ efficiency = \frac{C_t - C_r}{C_t} X\ 100 \quad \ldots..(5.1)$$

where C_t is the total drug and C_r is the free drug.

Particle Size, Polydispersity Index, and Zeta Potential

Particle size, PDI, and zeta potential were measured by dynamic light scattering (DLS) using a Nanotrac Wave II instrument (Malvern, UK). Analyses were conducted at room temperature using triplicate samples, with care to avoid bubble interference.

Microscopic and Electron Microscopy

Vesicle formation was confirmed by optical microscopy (100x magnification) and documented by digital photography.

In-vitro Drug Release Studies

Drug release from TPGS-coated and uncoated niosomes was evaluated in simulated intestinal fluid (SIF; pH 6.8 with 0.1% Tween-80) using a dialysis method. Niosomal dispersions containing 5 mg Efavirenz were placed in cellulose membrane dialysis bags and immersed in 100 mL SIF at 37°C with continuous stirring. At scheduled time points, the release medium was replaced, and drug concentration was analyzed by HPLC. Release data were fitted to various kinetic models Higuchi, and Korsmeyer-Peppas, Zero-order, First-order to elucidate mechanisms and characterize dosage form performance[22,23].

Flowability

The angle of repose was determined by the funnel method, measuring the height and base diameter of powder piles to assess the flow characteristics, which are crucial for processing and handling of proniosomal powders.

In-vivo Pharmacokinetic Studies

Male Wistar rats (250 ± 20 g) were randomly divided into four groups (n=6). Each group received a single oral dose (10 mg/kg) of either: pure drug in methylcellulose suspension, marketed formulation, TNF-coated proniosomal formulation, or non-coated proniosomal formulation. Blood samples were collected at multiple time points up to 72 hours' post-dose. Plasma was separated and analyzed by HPLC to determine pharmacokinetic parameters. All animal procedures were approved by the Institutional Animal Ethics Committee (Approval No. PGP/AF/CP-00181/07/2022).

RESULTS AND DISCUSSION

A 2^4 full factorial design was used to find the influence of four formulation variables Tween 80, cholesterol, Efavirenz, and TPGS on critical quality attributes (CQAs) of TPGS-coated Efavirenz proniosomes. Sixteen experimental runs were conducted to evaluate their impact on particle size, polydispersity index (PDI), and encapsulation efficiency (EE%) as shown in Table 1. Particle size of the formulations ranged from 447.32 to 654.82 nm, with a mean of 552.25 ± 71.00 nm. The goal was to optimize drug loading while achieving nanosized particles with narrow size distribution and enhanced stability for improved oral bioavailability of Efavirenz.

The results of ANOVA for all three responses- particle size (Y_1), PDI (Y_2), and encapsulation efficiency (Y_3)-are presented in Table 2. ANOVA analysis of the factorial model for particle size (Y_1) confirmed high significance, with an F-value of 12,178.65 and p-values < 0.0001 for all individual factors and their interactions, indicating a strong model fit. Among these, TPGS and Efavirenz emerged as the most influential, with TPGS showing the highest F-value (101,000), making it the primary predictor of particle size. Model reliability was supported by an R^2 of 0.99996, adjusted R^2 of 0.99988, and predicted R^2 of 0.99958, all in close agreement, confirming model robustness and absence of overfitting.

The low standard deviation (0.7879), coefficient of variation (0.14%), and high adequate precision value (318.742) further validated the model's precision and predictive capability. The resulting polynomial equation (Equation 1 describes how the input variables affect particle size:

$$Particle\ size = 552.25 - 12.02\,A + 6.66\,B + 22.98\,C + 62.46\,D + 2.92\,AB + 3.81\,AC - 5.55\,AD + 3.00\,BC + 3.08\,BD - 5.95\,CD \quad \ldots.(1)$$

Negative coefficients for Tween 80 (A) suggest it reduces particle size, whereas cholesterol (B) and Efavirenz (C) contribute to an increase. TPGS (D) has the most substantial positive effect on particle size. Notably, interactions between TPGS and both Tween 80 (AD) and Efavirenz (CD) produced a reduction in particle size, indicating potential counterbalancing effects. Residuals between observed and predicted values were minimal. However, runs 6 and 12 showed high externally studentized residuals and Cook's Distance values (0.436 and 0.425), suggesting possible outliers or influential points. Despite these, leverage and DFFITS values remained consistent, confirming the model's reliability.

Polydispersity Index (PDI)

PDI values across formulations ranged from 0.15 to 0.41, with a mean of 0.31 ± 0.052, reflecting variability in particle size distribution. ANOVA results confirmed the model's significance for PDI (Y_2), with an F-value of 55.69 ($p < 0.0001$), indicating strong predictive reliability. Among the individual factors, TPGS (D) had the most significant effect ($F = 210.66$, $p < 0.0001$), followed by Efavirenz (C) and cholesterol (B), while Tween 80 (A) showed a milder influence ($p = 0.0432$). Notably, the interaction between Tween 80 and TPGS (AD) was also significant, suggesting combined effects on PDI modulation.

Statistical parameters further supported the model's robustness, with $R^2 = 0.9653$, adjusted $R^2 = 0.9480$, and predicted $R^2 = 0.9113$. The low standard deviation (0.01188) and coefficient of variation (3.75%) indicated minimal data spread, while an adequate precision value of 23.015 confirmed a gaint signal-to-noise ratio. These statistics validate the model's suitability for doing the design space and optimizing formulation parameters. The polynomial equation (Equation 2) describes the coded relationship between formulation factors and PDI:

$$PDI = 0.317 - 0.007\,A + 0.013\,B + 0.014\,C + 0.043\,D - 0.013\,AD \quad \ldots..(2)$$

Here, increased TPGS (D) significantly elevated PDI, while Tween 80 (A) slightly reduced it. Efavirenz (C) and cholesterol (B) had moderate positive effects. The AD interaction term indicated that simultaneous increases in Tween 80 and TPGS could slightly reduce PDI, countering TPGS's individual impact. Residual analysis showed good agreement between observed and predicted values, with most deviations below ±0.02. Run 8 displayed the highest residual (-0.0162) and Cook's Distance (0.299), but remained within acceptable limits, suggesting no undue influence. DFFITS and studentized residuals also confirmed that no single point significantly distorted the model.

Encapsulation Efficiency

Encapsulation efficiency (EE%) ranged from 66.5% to 83.2%, with a mean of 75.28 ± 5.19%. ANOVA results (Table 2) demonstrated that the model for EE (Y_3) was

Table 3: Physicochemical characteristics of Efavirenz - proniosomes

Formulation code/parameter	F1	F2	F3	Blank proniosomes
Vesicle size (nm)	493.42 ± 17.89	487.86 ± 13.52	486.39 ± 20.34	467.89 ± 11.34
Polydispersity index	0.28 ± 0.01	0.26 ± 0.01	0.29 ± 0.02	0.23 ± 0.01
Zeta potential (mV)	- 22.34 ± 0.34	-19.76 ± 0.49	- 21.42 ± 0.72	-19.34 ± 0.14
encapsulation efficiency (%)	77.56 ± 3.6	78.12 ± 4.3	76.18 ± 2.9	--
Volume of hydration (ml)	11.3 ± 0.4	10.9 ± 0.6	10.4 ± 0.8	9.8 ± 0.3
Time of hydration (min)	7.3 ± 0.7	6.9 ± 0.5	8.1 ± 0.3	7.8 ± 0.4
Drug content (%)	97.89 ± 1.62	99.54 ± 3.46	98.76 ± 2.92	--

Table 4: Drug release kinetics of Efavirenz optimized formulation

Formulation	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
Code	R^2	n	R^2	n	R^2	n	R^2	n
F2	0.6538	0.3401	0.9878	-0.0079	0.8912	6.3323	0.9874	47.2104

highly significant ($F = 106.3$, $p < 0.0001$), with all formulation variables—Tween 80 (A), cholesterol (B), efavirenz (C), and TPGS (D)—showing statistically significant effects ($p < 0.05$)[24]. Among these, efavirenz had the strongest influence, followed by cholesterol, while Tween 80 and TPGS contributed moderately. The low residual value (10.19) and high mean squares further support the model's accuracy.

Model diagnostics confirmed robust predictability, with $R^2 = 0.9748$, adjusted $R^2 = 0.9656$, and predicted $R^2 = 0.9466$, all in close agreement. The low standard deviation (0.9627) and coefficient of variation (1.28%) reflected high precision, and the adequate precision value of 31.310 indicated a strong signal-to-noise ratio, suitable for navigating the formulation design space. The final regression equation based on the factorial design is:

$$Encapsulation\ efficiency\ (\%) = 75.28 + 0.94\,A + 2.70\,B + 3.97\,C + 0.81\,D \quad \ldots..(3)$$

All coefficients were positive, indicating that increases in each factor enhanced EE, with Efavirenz (C) exerting the most significant effect, followed by cholesterol (B). Tween 80 (A) and TPGS (D) had smaller but still beneficial contributions. Residual analysis showed close agreement between observed and predicted EE values, with most residuals within ± 1.2%. Notable deviations, such as in Runs 6 and 12, remained within acceptable ranges. Cook's Distance, DFFITS, and studentized residuals indicated that no individual run unduly influenced the model's outcome, confirming its reliability.

Characterization of Efavirenz Loaded Proniosomal Formulations

Table 3 summarizes the physicochemical properties of Efavirenz-loaded proniosomes (F1–F3) and a blank formulation. Vesicle sizes ranged from 486.39 to 493.42 nm, with F1 showing the largest size (493.42 ± 17.89 nm). PDI values (0.26–0.29) indicated moderate size distribution, while the blank proniosomes had a lower PDI (0.23 ± 0.01), suggesting more uniform particles. Zeta potential ranged from –19.76 to –22.34 mV, with F1 displaying the highest negative charge (–22.34 ± 0.34 mV), implying better colloidal stability. Blank proniosomes had a ZP of –19.34 ± 0.14 mV. Encapsulation efficiency (%EE) varied between 76.18% and 78.12%, with F2 achieving the highest EE (78.12 ± 4.3%), indicating efficient drug entrapment. Hydration volume ranged from 10.4 to 11.3 mL, and hydration time varied between 6.9 and 8.1 minutes, with F2 exhibiting the shortest hydration time (6.9 ± 0.5 min) and F3 the longest (8.1 ± 0.3 min). Overall, F2 demonstrated the most favorable characteristics, including high encapsulation efficiency, smaller size, and moderate PDI and ZP, making it the most promising formulation for further development. As expected, the blank formulation showed lower size and PDI but lacked drug encapsulation.

Table 5: Different pharmacokinetic parameters

PK parameter	Plain Efavirenz	T-LPnf-3	T-LPnf-5
C_{max} (ng/ml)	132.21 ± 7.4	789.6 ± 43.21*	487.68 ± 29.16*
$AUC_{(0\text{-}24)}$ (ng.h/mL)	782.2 ± 38.56	5823.27 ± 143.28*	3818.39 ± 104.28
$AUC_{(0\text{-}\infty)}$ (ng. h/mL)	854.43 ± 42.45	6051.83 ± 243.27*	4012.67 ± 189.21*
T_{max} (h)	2.04 ± 0.08	4.12 ± 0.13	2.13 ± 0.16
K_{el} (h^{-1})	0.088 ± 0.012	0.115 ± 0.017	0.105 ± 0.021
$t_{1/2}$ (h)	7.87 ± 0.43	6.03 ± 0.36	6.61 ± 0.37

Optical microscopy images (Figure 1a & 1b) show well-formed vesicles in both blank (a) and efavirenz-loaded (b) proniosomes. The blank formulation exhibited uniform vesicle distribution with no aggregation, indicating good structural stability. The drug-loaded formulation showed similar morphology with slight variations in vesicle size, suggesting successful drug incorporation without compromising vesicle integrity. These findings are consistent with the observed particle size and encapsulation efficiency data, validating the integrity and performance of the proniosomal systems.

The dissolution profile of Efavirenz-loaded proniosomes (F1–F3), shown in Figure 2, demonstrated significantly enhanced release compared to free Efavirenz. All proniosomal formulations exhibited rapid initial release (>70% within 60 minutes) versus ~35% for free drug. While the marketed product (Sustiva® 600 mg) showed the fastest release (>90% at 60 min, ~100% at 240 min), proniosomes achieved comparable cumulative drug release (98–99%) over a slightly extended duration, indicating improved dissolution behavior and potential for enhanced bioavailability.

Drug release kinetics of the optimized formulation (F2), shown in Table 4, were evaluated using established models. The release followed First Order kinetics ($R^2 = 0.9878$), suggesting concentration-dependent release. The Higuchi ($R^2 = 0.8912$) supported a diffusion-controlled mechanism, while the Korsmeyer-Peppas ($R^2 = 0.9874$, $n = 0.4721$) indicated a combined diffusion and erosion mechanism. The Zero Order model showed a poor fit ($R^2 = 0.6538$), confirming that release was not constant over time. These results highlight the controlled and sustained release potential of Efavirenz from proniosomal formulations.

The angle of repose for the efavirenz physical mixture was 39.72° ± 1.59°, indicating poor flow. In contrast, the F3 formulation and blank proniosomes showed significantly improved flowability, with angles of 25.28° ± 1.18° and 23.16° ± 1.02°, respectively. This enhancement is likely due to morphological changes during proniosome formation, which improve powder handling and processing properties.

The pharmacokinetic data highlight significant differences in the plasma drug profiles of Plain Efavirenz, the optimized proniosomal formulation (F2), and Sustiva® 600 mg tablets. Among the three, F2 consistently demonstrated higher and more sustained plasma drug concentrations over the 24-hour study period, indicating enhanced bioavailability. As summarized in Table 5, the Cmax of F2 (789.6 ± 43.21 ng/mL) was markedly higher than that of Plain Efavirenz (132.21 ± 7.4 ng/mL) and Sustiva® (487.68 ± 29.16 ng/mL), confirming superior absorption. The $AUC_{0–24}$ for F2 (5823.27 ± 143.28 ng·h/mL) was also significantly greater than that of Plain Efavirenz (782.2 ± 38.56 ng·h/mL) and Sustiva® (3818.39 ± 104.28 ng·h/mL), demonstrating enhanced overall drug exposure. F2 exhibited a delayed Tmax (4.12 ± 0.13 h) compared to Plain Efavirenz (2.04 ± 0.08 h) and Sustiva® (2.13 ± 0.16 h), suggesting a more controlled release profile. Despite this, F2 maintained higher plasma levels for a longer duration. The elimination rate constant (Kel) for F2 (0.115 ± 0.017 h^{-1}) was slightly higher than that of the other two, indicating a faster elimination rate. Correspondingly, F2 showed a shorter half-life ($t_{1/2}$) (6.03 ± 0.36 h) compared to Plain Efavirenz (7.87 ± 0.43 h), and was slightly lower than Sustiva® (6.61 ± 0.37 h). Overall, the proniosomal formulation demonstrated superior pharmacokinetic performance with higher Cmax, extended AUC, and sustained plasma levels, making it a promising system for enhanced oral delivery of Efavirenz.

CONCLUSION

The current study successfully made and optimized a Efavirenz-loaded proniosomal delivery system using a 2^4 factorial design. The optimized formulation exhibited desirable physicochemical properties, including nanoscale particle size, low PDI, high encapsulation efficiency, and good zeta potential, indicating a stable and uniform vesicular system. *In-vitro* drug release studies showed a sustained release profile, while release kinetics followed Korsmeyer–Peppas models and first-order indicating diffusion-controlled drug release. The pharmacokinetic evaluation in rats revealed significantly improved bioavailability, prolonged half-life, and higher systemic exposure for the optimized formulation. These results collectively demonstrate that proniosomal encapsulation is an effective strategy for enhancing the oral delivery and therapeutic performance of Efavirenz. The formulation shows potential for further preclinical development and future clinical translation as a robust platform for the controlled oral delivery of low bioavailable antiretroviral agents.

REFERENCES

1. Bastos MM, Costa CC, Bezerra TC, da Silva FD, Boechat N. Efavirenz a nonnucleoside reverse transcriptase inhibitor of first-generation: Approaches based on its medicinal chemistry. European journal of medicinal chemistry. 2016 Jan 27;108:455-65.
2. Yasuda N, Tan L. Efavirenz®, a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI), and a Previous Structurally Related Development Candidate. The Art of Process Chemistry. 2010 Oct 27:1-43.
3. Fitriani L, Haqi A, Zaini E. Preparation and characterization of solid dispersion freeze-dried efavirenz–polyvinylpyrrolidone K-30. Journal of Advanced Pharmaceutical Technology & Research. 2016 Jul 1;7(3):105-9.
4. Lavra ZM, Pereira de Santana D, Ré MI. Solubility and dissolution performances of spray-dried solid dispersion of Efavirenz in Soluplus. Drug development and industrial pharmacy. 2017 Jan 2;43(1):42-54.
5. Jain S, Sharma JM, Agrawal AK, Mahajan RR. Surface stabilized efavirenz nanoparticles for oral bioavailability enhancement. Journal of biomedical nanotechnology. 2013 Sep 1;9(11):1862-74.
6. Hari BV, Narayanan N, Dhevendaran K, Ramyadevi D. Engineered nanoparticles of Efavirenz using methacrylate co-polymer (Eudragit-E100) and its biological effects *in-vivo*. Materials Science and Engineering: C. 2016 Oct 1;67:522-32.
7. Kamble RN, Mehta PP, Kumar A. Efavirenz self-nano-emulsifying drug delivery system: *in vitro* and *in vivo* evaluation. AAPS PharmSciTech. 2016 Oct;17(5):1240-7.
8. Mukubwa GK, Safari JB, Walker RB, Krause RW. Design, Manufacturing, Characterization and Evaluation of Lipid Nanocapsules to Enhance the Biopharmaceutical Properties of Efavirenz. Pharmaceutics. 2022 Jun 21;14(7):1318.
9. Moghassemi S, Hadjizadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. Journal of controlled release. 2014 Jul 10;185:22-36.
10. Khatoon M, Shah KU, Din FU, Shah SU, Rehman AU, Dilawar N, Khan AN. Proniosomes derived niosomes: recent advancements in drug delivery and targeting. Drug delivery. 2017 Nov 1;24(2):56-69.
11. Asghari N, Houshmand S, Rigi A, Mohammadzadeh V, Dizaj MP, Hiagh ZSM. PEGylated cationic nano-niosomes formulation containing herbal medicine curcumin for drug delivery to MCF-7 breast cancer cells. J Med Pharm Chem Res. 2023;(January):556–68.
12. Beletkhanova MAM |Daniil DK |Amir S, Andreevna |Akhmedbek Ruslanovich Osmanova |Anzor

Vadimovich Pirmagomedova |Arina, Rybalkoc K |Julia A, Samokhinc |Nikita Alexandrovich, |Angelina, Timaeva OD |Milana K. Unlocking the therapeutic potential of tetrandrine : Structural modifications and pharmacological insights. J Med Pharm Chem Res. 2025;7(March):2630–62.

13. Akhilesh D, Faishal G, Prabhu P, Kamath J. Development and optimization of proniosomes for oral delivery of glipizide. Int J Pharm Pharm Sci. 2012;4(3):307-14.
14. Jammal A Al, Raad MT, Said H El. Comparative Thermodynamic and Chemical Characterization of PEGylated Dendrimers and Aptamer-Guided Niosomes for Oleuropein Delivery to Brain Metastases. Chem Methodol. 2025;9:922–37.
15. Singh LP, Patro LR, Sayeed M, Mandadi R, Pant NC, Panda C, et al. Polymeric Drug Delivery Systems : Chemical Functionalization , and Biomedical Applications Design ,. J Chem Rev. 2025;7(3):421–51.
16. Witika BA, Bassey KE, Demana PH, Siwe-Noundou X, Poka MS. Current advances in specialised niosomal drug delivery: Manufacture, characterization and drug delivery applications. International journal of molecular sciences. 2022 Aug 26;23(17):9668.
17. Radwan Y, Karaly AH, El-Sherbiny IM. Nanovesicles for delivery of antiviral agents. InViral Infections and Antiviral Therapies 2023 Jan 1 (pp. 493-518). Academic Press.
18. Anwer KE, El-Sattar NEAA, Shamaa MM, Zakaria MY, Beshay BY. Design, Green Synthesis and Tailoring of Vitamin E TPGS Augmented Niosomal Nano-Carrier of Pyrazolopyrimidines as Potential Anti-Liver and Breast Cancer Agents with Accentuated Oral Bioavailability. Pharmaceuticals (Basel). 2022 Mar 9;15(3):330. doi: 10.3390/ph15030330.
19. Liu, H., Tu, L., Zhou, Y. et al. Improved Bioavailability and Antitumor Effect of Docetaxel by TPGS Modified Proniosomes: *In vitro* and *In vivo* Evaluations. Sci Rep 7, 43372 (2017). https://doi.org/10.1038/srep43372
20. Wong CN, Lee SK, Lim YM, Yang SB, Chew YL, Chua AL, Liew KB. Recent advances in vitamin E TPGS-based organic nanocarriers for enhancing the oral bioavailability of active compounds: a systematic review. Pharmaceutics. 2025 Apr 7;17(4):485
21. Preeti N. Yadav, Chhalotiya Usmangani K, Patel Kesha M, Tandel Jinal N. Quantification of A β Adrenergic Receptor Drug Mirabegron by Stability Indicating LC Method andUv–visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form. Chem Methodol. 2020;4(53):340–58. Available from: http://chemmethod.com
22. Rapolu K, Muvvala S. Optimization and Characterization of Brinzolamide Loaded Biodegradable, Amphiphilic Poly-caprolactone-Polyethylene Glycol-Poly-Caprolactone (5000-1000-5000) Tri-block Co-polymeric Carriers as Long-Acting Intravitreal Drug Delivery Vehicle for Glaucoma Therapy. Adv J Chem Sect A. 2025;8(3):639–66.
23. Prohit PV, Pakhare PS, Pawar VB, Dandade SS, Waghmare MS, Shaikh FA, et al. Formulation and Comparative Evaluation of Naproxen-Based Transdermal Gels. J Pharm Sci Comput Chem. 2025;1(2):83–105.
24. Soumya P, Sofi SI, Vignanandam S, Aishwarya B, Kholi CB, Anusha K, et al. A Study to Assess the Efficacy of Various Therapeutic Strategies Used in the Treatment of Psoriasis. J Pharm Sci Comput Chem. 2025;1(1):38–49.