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Development and Optimization of Phospholipid-Based Efavirenz Nanosuspension for Enhanced Solubility and Lipolytic Performance

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Abstract:

Efavirenz (EFV), a BCS Class II antiretroviral drug, exhibits poor aqueous solubility, resulting in limited oral bioavailability. The present study aimed to develop and optimize a phospholipid-based nanosuspension of EFV using the antisolvent precipitation method to enhance its solubility and dissolution characteristics. Various stabilizers, including phospholipids, surfactants, and polymers, were screened to obtain a stable nanosuspension. A 2^3 factorial design was employed to optimize the formulation by evaluating the effects of phospholipid load, surfactant concentration, and drug load on particle size and polydispersity index (PDI). The optimized formulation exhibited a particle size of 188.8 nm and a PDI of 0.115, indicating the formation of a stable and homogeneous nanosuspension. Morphological characterization was performed using transmission electron microscopy, while particle size analysis was conducted using dynamic light scattering. In vitro lipolysis studies demonstrated a significant improvement in EFV solubilization from the phospholipid–Tween 80 nanosuspension (PL-T-NS) compared to the conventional EFV dispersion. After 60 minutes of lipolysis, the optimized nanosuspension achieved 24.50 ± 0.42% drug solubilization, whereas the EFV dispersion showed only 5.58 ± 0.39% solubilization. The enhanced performance was attributed to the presence of phospholipids and the nanosized drug particles, which improved the formation of solubilizing colloidal structures during lipid digestion. The study demonstrates that phospholipid-based nanosuspensions represent a promising strategy for improving the solubility and oral delivery of poorly water-soluble drugs such as efavirenz.

Keywords: Efavirenz, Nanosuspension, Antisolvent Precipitation, Factorial Design, *In Vitro* Lipolysis

Introduction:

The oral delivery of poorly water-soluble drugs remains a significant challenge in pharmaceutical development. A large proportion of newly discovered drug candidates exhibit low aqueous solubility, resulting in poor dissolution rates and variable oral bioavailability. Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor widely used in the treatment of Human Immunodeficiency

Virus (HIV) infection, belongs to Biopharmaceutics Classification System (BCS) Class II and exhibits poor aqueous solubility, which limits its therapeutic performance.

Nanosuspension technology has emerged as an effective approach for improving the dissolution rate and bioavailability of poorly soluble drugs. Nanosuspensions consist of pure drug particles stabilized by surfactants or polymers and dispersed in an aqueous medium. Reduction of particle size to the nanometer range increases the surface area available for dissolution, thereby enhancing drug solubility and absorption.

Among various formulation approaches, phospholipid-based nanosuspensions have attracted considerable interest because phospholipids possess excellent biocompatibility and the ability to enhance drug solubilization through self-assembly and micelle formation. The selection and optimization of formulation variables are critical for achieving nanosuspensions with desirable particle size distribution and stability. Design of Experiments (DoE) and factorial design methodologies provide systematic tools for evaluating the influence of formulation factors and their interactions.

Therefore, the present study was undertaken to formulate and optimize a phospholipid–Tween 80 nanosuspension of efavirenz using the antisolvent precipitation technique. The formulation was characterized in terms of particle size, polydispersity index, and morphology, and its performance was evaluated using an in vitro lipolysis model to assess its ability to improve drug solubilization.

Materials and method

Phospholipon® 90 G was obtained as a gift sample from Lipoid, Germany. Gattefossé India Pvt. Ltd. kindly gifted the sample of Gelucire® 50/13. Kolliphor® P188, Kolliphor® P407 (Poloxamer 188 and Poloxamer 407), and Cremophor® EL were obtained as gift samples from BASF India. Tween® 80 was purchased from S.D. Fine Chemicals Ltd. Polyvinylpyrrolidone (PVP K-30) and Brij® 35 were purchased from Sigma-Aldrich.

Methods:

Nanosuspensions were prepared by the antisolvent precipitation method in which acetone was used as the organic solvent and water acted as the antisolvent. Phospholipon® 90 G, Brij® 35, Cremophor® EL, Poloxamer 407, Poloxamer 188, Tween® 80, Gelucire® 50/13, and Polyvinylpyrrolidone K-30 were screened at different ratios to formulate nanosuspensions with particle size and polydispersity index (PDI) within the desired range. To ensure the successful preparation of a stable nanosuspension, initial trials were conducted without the addition of the drug.

Briefly, the phospholipid was dissolved in acetone, while the other stabilizers were dissolved in water. The organic phase was added to the aqueous phase under constant vortexing until a uniform dispersion was formed. The resulting dispersion was stirred on a magnetic stirrer until complete evaporation of acetone occurred.

Among the various combinations tested, Phospholipon® 90 G and Tween® 80 formed a nanosuspension that did not show any instability immediately after preparation as well as after one week of storage. Hence, an Efavirenz (EFV) nanosuspension using a mixture of phospholipid and Tween® 80 (PL-T-NS) was prepared using the above-mentioned procedure, wherein EFV was dissolved in acetone along with the phospholipid.

Optimization of EFV Nanosuspension (PL-T-NS) Using a Factorial Design

A 2^3 factorial design was employed to optimize the particle size and polydispersity index of the PL-T-NS formulation. The preparation of nanosuspensions by the antisolvent precipitation method is influenced by several formulation and process variables, including stabilizer concentration, drug concentration, amount of organic solvent, and amount of antisolvent, all of which can affect particle size and polydispersity.

Preliminary studies were conducted to reduce the number of independent variables to three. Phospholipid load (A), stabilizer concentration (B), and drug concentration (C) were identified as the major contributing factors affecting particle size and polydispersity index. Other variables, such as the amounts of organic solvent and antisolvent, were kept constant throughout the study.

Each independent variable was evaluated at two levels: a low level (-1) and a high level (+1). The experimental conditions selected for the study are presented in Table 1.

Table.1. Factors and levels investigated

Factor Name	Symbol	Low Level (-1)	High Level (+1)
Phospholipid Load (mg)	A	300	500
Surfactant Concentration (mg)	B	150	250
Drug Load (mg)	C	25	75

Table.2 study design

Run	Phospholipid Load (mg)	Surfactant Concentration (mg)	Drug Load (mg)
1 (Centre Point)	400	200	50
2	300	150	75
3	300	250	25
4	500	250	25
5	300	250	75
6 (Centre Point)	400	200	50
7	500	150	75
8	500	150	25
9	500	250	75
10	300	150	25

Preparation of Ritonavir (RTV) Nanosuspension

Ritonavir has been reported to act as a pharmacokinetic enhancer for drugs such as lopinavir and darunavir. To investigate whether ritonavir could enhance the performance of Efavirenz when co-administered, a ritonavir nanosuspension was prepared. Based on preliminary trials conducted with Efavirenz, a combination of phospholipid and Tween® 80 was selected as the stabilizer system.

Briefly, ritonavir was dispersed in a mixture of phospholipid, Tween® 80, and water. The resulting dispersion was homogenized using a high-speed homogenizer (Ultra-Turrax®, IKA). The homogenized dispersion was then probe-sonicated for 4 minutes to reduce the particle size to the nanometer range.

Characterization of nanosuspensions

I. **Particle Size and Polydispersity Index (PDI)**

Particle size and particle size distribution are key parameters that influence the physical stability and biological performance of nanosuspensions. The particle size distribution of the formulations was determined using an N5 Submicron Particle Size Analyzer (Beckman Coulter, USA).

The instrument utilizes Photon Correlation Spectroscopy (PCS) based on the principle of Dynamic Light Scattering (DLS). Formulations were appropriately diluted with freshly prepared Milli-Q Water (Type I) before analysis. Measurements were performed at 25°C using a 25 mW Helium–Neon laser (632.8 nm) as the light source, with detection at a scattering angle of 90°. The obtained data were analyzed using PCS Software Version 3.02.

II. **Imaging by Transmission Electron Microscopy (TEM)**

The morphology of the nanosuspensions was examined using Transmission Electron Microscopy (TEM). The study was carried out at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Bombay, using a CM 200 microscope (Philips) with a resolution of 0.24 nm and an operating voltage range of 20–200 kV.

The nanosuspensions were suitably diluted with water, and a drop of the diluted sample was placed onto a carbon-coated copper grid. The sample was allowed to stand for 30–60 seconds, after which excess liquid was removed using filter paper.

The samples were then negatively stained with 1% w/v phosphotungstic acid solution (pH adjusted to 7 using 1 N NaOH). Excess staining solution was removed by blotting, and the grid was allowed to air-dry. The prepared grids were subsequently examined under the transmission electron microscope at different magnifications.

III. **In Vitro Lipolysis Studies**

In vitro lipolysis studies are performed to evaluate the role of excipients that undergo hydrolysis in the presence of pancreatic lipase, an enzyme naturally present in the gastrointestinal tract. These studies help predict the digestion behavior of lipid-based formulations and their influence on drug dissolution and release.

Materials

Sodium chloride, calcium chloride dihydrate, and tris maleate were purchased from S.D. Fine Chemicals Ltd. Bile salts and pancreatic lipase were procured from HiMedia Laboratories. Lipoid GmbH provided a gift sample of Phospholipon® 90 G, while Colorcon India supplied Methocel® K4M (Hydroxypropyl Methylcellulose, HPMC) as a gift sample.

Preparation of Lipolysis Medium

The lipolysis medium used for the in vitro lipolysis studies was prepared according to the composition presented in Table 3.

Table 3. Composition of Lipolysis Medium Used for In Vitro Lipolysis Studies

Ingredients	Quantity Taken (g)
Sodium Chloride	0.351
Calcium Chloride Dihydrate	0.0294
Tris Maleate	0.0947
Bile Salts	0.086
Lecithin (Phospholipon 90G)	0.038
Water	36 mL

Preparation of EFV Dispersion

An Efavirenz (EFV) dispersion was prepared by triturating 50 mg of EFV with 25 mg of Methocel® K4M (HPMC). Small quantities of Milli-Q water were gradually added during trituration until a pourable dispersion was obtained. The final volume of the dispersion was adjusted to 10 mL with Milli-Q water. The resulting EFV dispersion contained 5 mg/mL of EFV.

Procedure for In Vitro Lipolysis Study

A volume of 36 mL of lipolysis medium was transferred into a beaker, and 2 mL of the formulation was added. The pH of the medium was adjusted to 6.5 using 0.1 N sodium hydroxide (NaOH). Subsequently, 40 mg of pancreatic lipase enzyme, previously dispersed in 3 mL of water, was added to the medium. The mixture was stirred continuously on a magnetic stirrer for 1 hour. During the experiment, the pH was regularly monitored and maintained at 6.5 by the addition of 0.1 N NaOH whenever required. Aliquots were withdrawn at 30 minutes and 60 minutes after the initiation of lipolysis. The collected samples were centrifuged at 40,000 rpm for 45 minutes using an ultracentrifuge (Beckman Coulter Allegra™ 64R Centrifuge, USA). Following centrifugation, the supernatant was carefully separated and analyzed using Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) equipped with a UV detector set at 247 nm. The amount of EFV present in the supernatant was quantified to assess the extent of drug solubilization during the lipolysis process.

Results and discussion

Optimization techniques employed in experimental design provide a systematic understanding of formulation and processing factors. Optimization is a valuable tool that enables the quantitative evaluation of formulations that have been qualitatively developed through a rational approach involving excipient selection and process variables. These techniques are widely used to implement the Quality by Design (QbD) approach in pharmaceutical product development.

Design of Experiments (DoE) is a systematic methodology used to determine the relationship between process variables (factors) and the corresponding output responses. DoE-based optimization techniques can be implemented using various experimental designs to evaluate how product and process characteristics vary as a function of input variables.

In recent years, DoE has been extensively applied in the pharmaceutical industry not only for the development of new products and processes but also for the optimization and modification of existing formulations.

In a factorial design, each factor is studied at two or more levels, and all possible combinations of factor levels are evaluated. The total number of experiments required is determined by the number of factors and levels investigated.

A two-level factorial design (2^n design) is one of the simplest and most widely used experimental designs for screening studies and factor influence assessment. In this design, each factor is evaluated at two levels:

1. Low level (-1)
2. High level (+1)

Optimization Design for PL-T-NS

The optimization of the Phospholipid–Tween 80 Nanosuspension (PL-T-NS) was carried out using a 2^3 factorial design.

Independent Variables (Factors)

Factor	Variable
A	Phospholipid Load
B	Stabilizer Concentration
C	Drug Concentration

Particle Size

The polynomial equation obtained for particle size is as follows:

Particle Size=272.81−20.51A−23.36B+12.21C−18.09AB+45.24AC−40.86BC

Table 4 shows the particle size of PL-T-NS in the factorial design. The particle size of the nanosuspension varied from 188.8 nm, where the phospholipid, surfactant, and drug load were 500 mg, 250 mg, and 25 mg, respectively, to 386 nm, where the phospholipid, surfactant, and drug load were 500 mg, 150 mg, and 75 mg, respectively.

If the phospholipid and drug load were kept constant, an increase in surfactant load increased the particle size. This observation was unusual but can be attributed to an increase in the viscosity of the nanosuspension with an increase in surfactant concentration, which affects the particle size reduction process, as reported by Patel et al. for EFV nanosuspensions stabilized by SLS.

With the phospholipid and surfactant load kept constant, an increase in drug load increased the particle size. An increase in phospholipid load decreased the particle size when surfactant and drug load were kept constant. This may be attributed to the property of phospholipids to form micelles by self-assembly.

Polydispersity Index

The polynomial equation describing the polydispersity index (PDI) is as follows:

Polydispersity Index=0.41−0.086A−0.049B+0.045C+0.029AB+0.094AC−0.081BC

Table 4 shows the polydispersity index of PL-T-NS in the factorial design. The polydispersity index (PDI) varied from 0.115, when the phospholipid, surfactant, and drug load were 500 mg, 150 mg, and 25 mg, respectively, to 0.599, when the phospholipid, surfactant, and drug load were 300 mg, 150 mg, and 75 mg, respectively.

When the phospholipid and surfactant load were kept constant, an increase in drug loading increased the PDI. With the drug and phospholipid load kept constant, an increase in surfactant concentration decreased the PDI due to the ability of the surfactant to stabilize the nanosuspension, thereby yielding a more monodisperse system.

Felodipine nanosuspensions also showed a lower PDI with an increase in stabilizer concentration in an optimization study using a factorial design.

A decrease in PDI was observed when the drug and surfactant load were kept constant and the phospholipid concentration was increased.

Table.4. Particle size Poly dispersity index of PL-TNS in the factorial design

Run	Phospholipid Load (mg)	Surfactant Concentration (mg)	Drug Load (mg)	Particle Size (nm)	Polydispersity Index (PDI)
1	400	200	50	265.4	0.366
2	300	150	75	311.9	0.599
3	300	250	25	367.4	0.54
4	500	250	25	188.8	0.245
5	300	250	75	208.7	0.287
6	400	200	50	227.4	0.345
7	500	150	75	386.6	0.562
8	500	150	25	200.9	0.115
9	500	250	75	232.9	0.353
10	300	150	25	285.3	0.54

In Vitro Lipolysis Studies

A vast majority of the new chemical entities (NCEs) being developed in recent years are hydrophobic in nature and exhibit poor aqueous solubility. These drugs often show poor and variable bioavailability, which is further influenced by the dietary status of the patient, such as the fed or fasted state. To improve the delivery of poorly soluble drugs, various formulation approaches have been investigated and developed over the past decades. Among these, lipid-based drug delivery systems have gained considerable attention due to their ability to maintain the drug in a solubilized state throughout gastrointestinal transit. In addition, lipid-based systems offer several advantages, including sustained drug release, biocompatibility, and enhanced bioavailability of poorly soluble drugs.

Due to the complexity of the events occurring after the ingestion of lipid-based formulations, simple dissolution testing alone is insufficient for their evaluation. Therefore, a more comprehensive in vitro assessment method is required. Drug solubilization from oral lipid-based delivery systems depends on the solubilization capacity of the intestinal environment and the colloidal species formed during digestion of the formulation.

To address these factors, an in vitro lipolysis model was developed that incorporates enzyme activity and pH monitoring to simulate physiological digestion conditions.

The lipolysis medium contains bile salts and phospholipids to mimic intestinal contents, and these components also influence lipase activity. Buffering agents such as tris maleate are included to maintain the pH of the medium between 6.0 and 7.5, thereby simulating physiological conditions of the fed state. The addition of calcium ions is essential because calcium forms insoluble salts with free fatty acids generated during lipid digestion. This prevents the accumulation of free fatty acids, which may otherwise inhibit lipase activity.

Briefly, when pancreatic lipase is added to the lipolysis medium containing the formulation, the lipids present in the formulation are hydrolyzed to produce free fatty acids. The generation of free fatty acids causes a decrease in pH, which is continuously monitored and maintained at pH 6.5 using sodium hydroxide solution. Maintaining the pH ensures that the pH-sensitive lipid digestion process proceeds efficiently throughout the experiment.

Samples withdrawn during the lipolysis process are centrifuged, and both the pellet and supernatant fractions are analyzed for drug content to determine the extent of drug solubilization during digestion.

The lipolysis model used for the evaluation of EFV nanosuspensions was developed by our research group and has previously been applied for the assessment of lipid nanoparticles containing quercetin and nelfinavir mesylate.

The percentage solubilization of EFV after 30 minutes and 60 minutes of lipolysis. The lipolysis profile demonstrated a marked improvement in the solubilization of EFV from PL-T-NS formulation compared with the EFV dispersion, which exhibited only 6% solubilization after 1 hour.

The enhanced solubilization observed with the nanosuspensions can be attributed to the presence of phospholipids, which improve the solubilization capacity of the formulation during digestion. In contrast, nanosuspensions containing Gelucire® as one of the stabilizers exhibited comparatively poor performance during the lipolysis study.

Previous studies by Subramanian et al. have reported that certain lipid excipients can inhibit pancreatic lipase activity. Inhibition of lipase by Gelucire® may have reduced lipid digestion, thereby limiting the formation of solubilizing colloidal structures and decreasing the solubilization of EFV. Consequently, the PL-G-NS formulation showed inferior performance compared with the other nanosuspensions.

Non-ionic surfactants are generally susceptible to digestion by lipases, which helps prevent drug precipitation following formulation digestion. However, formulations containing high concentrations of surfactants combined with lower amounts of glycerides have been reported to reduce drug solubilization. Therefore, the ratio of lipids to surfactants within the formulation plays a critical role in determining the solubilization capacity and overall performance observed during in vitro lipolysis studies.

Table 5. Percentage Solubilization of EFV During In Vitro Lipolysis Study

Formulation	% EFV Solubilization at 30 min	% EFV Solubilization at 60 min
EFV Dispersion	3.70 ± 0.14	5.58 ± 0.39
PL-T-NS	5.49 ± 0.25	24.50 ± 0.42

The present study successfully developed and optimized a phospholipid-based efavirenz nanosuspension using the antisolvent precipitation technique. A 2^3 factorial design effectively identified the influence of phospholipid load, surfactant concentration, and drug load on particle size and polydispersity index. The optimized formulation exhibited nanosized particles with low polydispersity, indicating good physical stability and uniformity.

The in vitro lipolysis study demonstrated a substantial enhancement in efavirenz solubilization from the phospholipid–Tween 80 nanosuspension compared with the conventional drug dispersion. The improved performance can be attributed to the nanoscale particle size and the presence of phospholipids, which facilitate the formation of solubilizing colloidal structures during lipid digestion. The findings suggest that phospholipid-based nanosuspensions are an effective strategy for improving the solubility and potential oral bioavailability of poorly water-soluble drugs such as efavirenz. Further in vivo studies may be conducted to establish the correlation

between enhanced solubilization and bioavailability.

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