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ORIGINAL ARTICLE

Exploring the Solvent-Anti-solvent Method of Nanosuspension for Enhanced Oral Bioavailability of Lovastatin

Lovastatinin Gelişmiş Oral Biyoyararlanımı İçin Solvent-Anti-solvent Yöntemi ile Hazırlanan Nanosüspansiyon Formülasyonunun Araştırılması

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ABSTRACT

Objectives: Lovastatin is an antilipidemic drug that belongs to the class of statins that has poor oral bioavailability due to its low solubility and variable dissolution rate. The main aim of this study was to enhance the solubility and dissolution rate of the drug and understand its oral bioavailability.

Materials and Methods: Lovastatin nanosuspension was formulated using a solventanti-solvent method using a probe sonication technique. A nanosuspension was prepared, using hydroxypropyl methylcellulose (HPMC) K15M and pluronic F68 as stabilizers. The formulated nanosuspensions were characterized for particle size, polydispersity index (PDI) zeta potential, surface morphology, and *in vitro* release rate. Further, an *in vivo* bioavailability study and stability studies were also performed.

Results: Optimized formulation showed a particle size of 127±0.01 nm, a PDI of 0.492±0.001, and a zeta potential of -37.9 mV, which indicates good stability. Morphological study showed that the particles were in the nano range. The drug content was found to be in the range of 73-87%. *In vitro* release revealed much faster release of the drug in one hour compared to the pure drug and its marketed formulation. *In vivo* bioavailability study was carried out in Wistar rats, which showed improvement in bioavailability by approximately 2.5 folds compared with the marketed formulation. Stability studies indicated that the optimized formulation F2 was more stable at 4˚C±2˚C.

Conclusion: The prepared lovastatin nanosuspension showed improvement in solubility, dissolution rate, and oral bioavailability compared to the pure drug and its marketed formulation. Hence, lovastatin nanosuspension may be a potentially valuable tool for improving the oral bioavailability of lovastatin.

Key words: Lovastatin, oral bioavailability, solubility, nanosuspension

ÖZ

Amaç: Lovastatin, düşük çözünürlüğü ve değişken çözünme hızı nedeniyle oral biyoyararlanımı zayıf olan statinler sınıfına ait antilipidemik bir ilaçtır. Bu çalışmanın temel amacı, ilacın çözünürlüğünü ve çözünme hızını artırmak ve oral biyoyararlanımını belirlemektir.

Gereçler ve Yöntemler: Lovastatin içeren nanosüspansiyon, bir prob sonikasyon tekniği kullanılarak solvent-anti-solvent yöntemi kullanılarak formüle edildi. Stabilizatör olarak hidroksipropil metilselüloz (HPMC) K15M ve pluronic F68 kullanılarak bir nanosüspansiyon hazırlandı. Formüle edilen nanosüspansiyonlar, partikül boyutu, polidispersite indeksi (PDI), zeta potansiyeli, yüzey morfolojisi ve *in vitro* salım profilleri belirlenerek karakterize edildi. Ayrıca, *in vivo* biyoyararlanım çalışması ve stabilite çalışmaları da gerçekleştirilmiştir.

Bulgular: Optimize edilmiş formülasyonun, partikül boyutu 127±0,01 nm, PDI değeri 0,492±0,001 ve zeta potansiyeli -37,9 mV olarak belirlendi, bu da formülasyonun iyi bir stabiliteye sahip olduğunu gösterdi. Morfolojik çalışma, partiküllerin nano aralıkta olduğunu gösterdi. İlaç içeriği %73-87 aralığında bulundu. *İn vitro* salım, saf ilaca ve ticari formülasyona kıyasla ilacın bir saat içinde çok daha hızlı salım profili gösterdiğini ortaya çıkardı. *İn vivo* biyoyararlanım çalışması Wistar sıçanlarında gerçekleştirildi ve nanosüspansiyon formülasyonunda ticari formülasyona kıyasla biyoyararlanımda yaklaşık 2,5 kat iyileşme olduğunu gösterdi. Stabilite çalışmaları, optimize edilmiş F2 formülasyonunun 4˚C±2˚C'de daha stabil olduğunu gösterdi.

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Sonuç: Hazırlanan lovastatin nanosüspansiyonu, saf ilaca ve ticari formülasyonuna kıyasla çözünürlük, çözünme hızı ve oral biyoyararlanım açısından gelişme göstermiştir. Bu nedenle, lovastatin nanosüspansiyonunun, lovastatinin oral biyoyararlanımını geliştirmek için potansiyele sahip olduğu sonucuna ulaşıldı.

Anahtar kelimeler: Lovastatin, oral biyoyararlanım, çözünürlük, nanosüspansiyon

INTRODUCTION

Oral route is the highly preferred route for administration of drugs as it provides high patient compliance.[1] A large number of drugs that are available in the market exhibit low oral bioavailability because of their low aqueous solubility and intrinsic dissolution rate. According to the Biopharmaceutical Classification System, drugs with poor aqueous solubility are classified either as class II or class IV drugs.[2] Poor aqueous solubility of the drugs results in low oral bioavailability, varying absorption rate, and inter/intrasubject proportionality.[3] Oral bioavailability of various drugs is also affected by another factor (i.e., poor gastrointestinal permeability). According to the literature, various techniques like solubilization, salt formation, micronization, change in physical form, use of prodrug and drug derivatives, addition of surfactants, and pH alteration have been utilized for improving the dissolution and bioavailability of the drugs having poor aqueous solubility.[4]

Nanotechnology has reshaped the field of drug delivery and research. Pharmaceutical nanoparticles are solid submicron sized (<100 nm in diameter) drug carriers that may or may not be biodegradable.[5] Types of nanoparticles that are applied in drug delivery include nanosuspensions, polymeric nanoparticles, and lipid nanoparticles. Nanosuspension is the colloidal dispersion of solid drug particles in a liquid phase having a particle size of <1 μm with an average particle size of 200-600 nm.[6] It consists of pure drug and stabilizers (surfactants or polymers). Their small particle size facilitates effective transportation of drug molecules to cells with an optimum therapeutic effect and reduced adverse effects.[7] The potential benefits of nanosuspension technology for poorly soluble drug delivery are increased drug dissolution rate, increased rate, and extent of absorption, and hence the bioavailability of drugs. The selection of a suitable stabilizer or surfactant as well as the manufacturing method may offer nanosuspension with highest stability for long-term storage. Nanosuspension may be formulated by a bottom-up or top-down approach.

Cardiovascular disease remains the leading cause of morbidity and mortality worldwide, and hyperlipidemia is a major factor contributing to its development.[8] Lovastatin is a cholesterol-lowering agent that has been isolated from a strain of *Aspergillus ferreus*. It is very effective and well tolerated by patients with moderate hypercholesterolemia. Lovastatin also manifests pharmacological activities of bone formation and chemoprevention. Due to its rapid metabolism in the gut and liver, lovastatin exhibits poor oral bioavailability of <5% and a shorter half-life of 2-5 h.[9] According to the literature, various attempts have been made to improve the aqueous solubility and bioavailability of lovastatin by preparing self-emulsifying drug delivery systems, nanostructured lipid carriers (NLC), and extended-release formulation by one-step melt granulation method. For instance, Sunil et al.[10], Jun and Daxin[11] and Gande et al.[12] prepared stabilized self-emulsifying drug delivery systems in the form of hydrogel, NLC, and solid lipid nanoparticles (SLN) of lovastatin, respectively, with an objective to enhance the solubility and bioavailability of lovastatin, but there are no comparative data of their formulation with the already existing marketed product to justify the enhancement in bioavailability of the drug.

Hence, attempts were made to improve solubility and oral bioavailability of lovastatin by formulating lovastatin nanosuspension via the solvent-anti-solvent method using probe sonication technique in this study. Further, nanosuspensions were evaluated for particle size, polydispersity index (PDI), zeta potential, drug content, an *in vitro* release study, *in vivo* bioavailability study, and stability study.

MATERIALS AND METHODS

Materials

Lovastatin was procured as a gift sample from Lupin Pharmaceuticals, Goa. Hydroxypropyl methylcellulose (HPMC) K15M was purchased from Yarrow Chem Products, Mumbai. Pluronic F68 was purchased from Ozone Pharmaceuticals, Mumbai. Acetone, Chloroform, Methanol, and Ethanol were purchased from Molychem, Mumbai. Dialysis membrane having a cutoff molecular weight between 12,000 and 14,000 Dalton was purchased from Hi Media.

Methods

Optimization of process parameters

Selection of a suitable solvent-anti-solvent ratio

Before proceeding toward formulation of the lovastatin nanosuspension, a solvent and anti-solvent were selected based on the solubility studies of drugs in different solvents. In the solvent-anti-solvent method, the selected solvent should be a water-miscible solvent and capable enough to dissolve the drug to a greater extent so that a clear solution is obtained. Conversely, the solvent in which the drug was least soluble or was completely insoluble was selected as an anti-solvent. As the drug exhibits maximum solubility in methanol, it was selected as a solvent, and water was selected as an anti-solvent since the drug was least soluble in water. Different ratios like 1:1, 1:2, 1:3, 1:4, and 1:5 were tried for formulating a nanosuspension. The ratio of solvent:anti-solvent that resulted in a production of nanosuspension with the best reproducible particle size, and PDI was selected as an optimized ratio.

Optimization of sonication time

Optimization of sonication time was done by sonicating the formulation for 2, 5, 10, 15, and 20 mins. Based on the best and reproducible results of particle size and PDI, an optimized sonication time was selected.

Selection of polymer and surfactant

A suitable polymer was selected by screening various polymers like HPMC K15M, HPMC K100M, and PVPK30. Based on the best and reproducible results of particle size, PDI, and the ability to inhibit crystal growth, a suitable polymer was selected. Various surfactants like pluronic F68, pluronic F127, and polyethylene glycol 6000 were tested to determine the surfactant effective in reducing the particle size of the drug.

Effect of flow rate

Particle size of the obtained nanosuspension was measured at varied flow rates of drug solution into polymer solution at 2-8 mL/min to select the optimum flow rate during the formulation of nanosuspension. All the process optimization parameters are depicted in Table 1.

Formulation of nanosuspension by solvent-anti solvent technique

Six formulations of (F1-F6) lovastatin nanosuspensions were prepared by the solvent-anti-solvent method using a probe sonication technique. Briefly, a specified amount of drug was completely dissolved in the water-miscible solvent (methanol). In another beaker, polymer/surfactant was added to the water (anti-solvent) and further sonicated till a clear polymeric solution was formed. The prepared drug solution was then added to the polymeric solution at a rate of 2 mL/min and maintained in an ice bath to prevent particle collision till precipitation occurred.[13-16] The quantities of ingredients used in the formulation are mentioned in Table 2, and the solvent-anti-solvent technique is depicted in Figure 1.

Evaluation of lovastatin-loaded nanosuspensions

Particle size analysis

The particle size of the prepared nanosuspension was determined using a dynamic light scattering (DLS) particle size analyzer. For analysis, the nanosuspension was diluted with Millipore water at a ratio of 1:5 and further sonicated for 2 min. Samples were analyzed in triplicate.[17]

Polydispersity index

PDI is also measured using a DLS particle size analyzer. The obtained PDI values give an idea about the particle size distribution of nanoparticles. Their values range from 0.000 to 1.000, which demonstrate that the lower the value, the narrower the size distribution of nanoparticles and vice versa.[18]

Table 1. Optimization of process parameters and their evaluation

Optimization of the solvent:anti-solvent ratio and sonication time				
Solvent (methanol):anti-solvent (water) ratio	1:1	1:2	1:3	1:4
Sonication time (min)	05	10	15	20
Particle size (nm)	1595	623	407	379
PDI	1.6	1.5	0.04	0.4
Effect of polymer on particle size and PDI				
Polymer	**Particle size (nm)**	**PDI**		
HPMC K15M	483	0.5		
HPMC K100M	1054	0.5		
PVP K30	1064	0.5		
Effect of a surfactant on particle size and PDI				
Surfactant	**Particle size (nm)**	**PDI**		
PEG 6000	1205	1.9		
Pluronic F127	926	0.4		
Pluronic F68	442	0.6		
Effect of flow rate on particle size and PDI				
Flow rate	**Particle size (nm)**	**PDI**		
2 mL/min	487	0.4		
4 mL/min	604	0.5		
6 mL/min	798	0.5		
8 mL/min	1023	0.6		

PDI: Polydispersity index, HPMC: Hydroxypropyl methylcellulose, PVP: Polyvinylpyrrolidone, PEG: Polyethylene glycol

Table 2. Formulation design of lovastatin nanosuspension using the solvent-anti-solvent method

Formulation number	Lovastatin (mg)	HPMC K15M (mg/mL)	Pluronic F68 (mg/mL)	Methanol (mL)	Water (mL)
F1	10	1.0	-	10	50
F2	10	1.5	-	10	50
F3	10	-	1.0	10	50
F4	10	-	1.5	10	50
F5	10	1.0	1.5	10	50
F6	10	1.5	1.0	10	50

HPMC: Hydroxypropyl methylcellulose

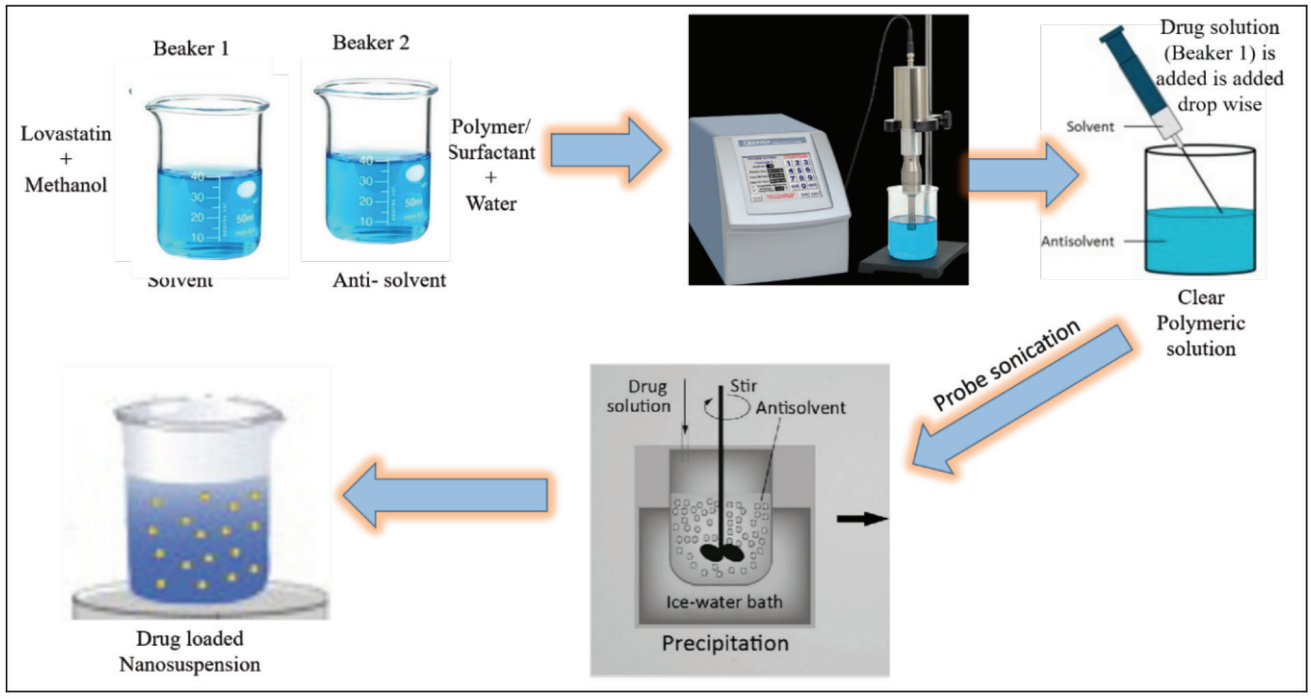

Figure 1. Formulation of nanosuspension by the solvent-anti-solvent technique

Drug content

Lovastatin nanosuspension was equivalent to 1 mg and was added to 10 mL of methanol; it was further diluted with a phosphate buffer up to 100 mL and stirred continuously for 2 h. It was subjected to ultracentrifugation at 15,000 rpm for 20 min. The supernatant was collected, suitably diluted, and analyzed spectrophotometrically at 238 nm.[19]

Zeta potential

A physical property exhibited by particles in suspension is zeta potential. It is used for the optimization of emulsions and suspensions as its knowledge reduces the number of trial formulations. It is also an important parameter to predict long-term stability.[20]

Transmission electron microscopy (TEM)

The external morphology of lovastatin nanosuspension was studied using TEM, and it was performed at SAIF, Cochin. Samples for TEM were prepared by initially diluting with Millipore water. Then, a drop of nanosuspension was kept on a copper grid (carbon coated) and stained with phosphotungstic acid. The grid was air dried and observed at different magnifications under TEM.[21]

In vitro drug release study

The *in vitro* drug release of lovastatin was performed using the dialysis bag diffusion technique. An accurately weighed quantity of nanosuspension (equivalent to 10 mg of the drug) was placed in a dialysis bag, and the bag was sealed. Then, the bag was suspended initially for 1 h in a basket containing 900 mL of pH 6.8 phosphate buffer. Aliquots of 5 mL of the sample were withdrawn at pre-determined intervals from the compartment, and the same amount was replaced by the fresh buffer. The sample was analyzed spectrophotometrically after suitable dilution by determining the absorbance at 238 nm.[22]

In vivo bioavailability study

Healthy Wistar rats weighing 180-200 g were housed in polypropylene cages and maintained at room temperature for 12 h dark/light cycles. They were fed with standard pelleted diet and water. The animals were acclimatized for one week under laboratory conditions before experiments were done on the animals. Ethical clearance was obtained from the Institutional Animal Ethics Committee (resolution no: KLECOP/CPCSEA/ Re.no.221/ PO/RE/S/2000/CPCSEA Res.27-24/12/2018) prior to the beginning of the research. The *in vivo* study was aimed to mainly estimate the amount of drug in the blood withdrawn from rats at various time intervals.[23]

Twelve healthy male Wistar rats weighing 180-200 gms were selected and divided into two groups, each containing six rats. Group 1 received the marketed product of lovastatin, and group 2 received lovastatin nanosuspension equivalent to 0.108 mg in normal saline through the oral route. After 0.5, 2, 4, 6, 8, 10, and 24 h, 0.5-1 mL blood was collected from the tail vein into an Eppendorf tube containing 10-µL EDTA and centrifuged at 5000 rpm for 20 min. Supernatant plasma was collected, filtered through a 0.45 µm membrane into clean vials and analyzed spectrophotometrically to determine the concentration.

Short term stability studies

The optimized formulation was subjected to stability studies as per International Council on Harmonisation (ICH) guidelines. The formulation was exposed to varying conditions of temperature and relative humidity, i.e., 25°C/65% RH and 4°C/65% RH, for a period of three months in a humidity control oven. The samples were collected and evaluated for particle size and percent cumulative drug release at intervals of 0, 1, 2, and 3 months, respectively.

RESULTS AND DISCUSSION

Optimization of process parameters

For the drug lovastatin, maximum solubility was observed in methanol, and least solubility was observed in water. Hence, methanol was selected as a solvent and water as an anti-solvent. Based on the effect of the solvent: anti-solvent volume ratio on particle size, a 1:4 ratio was considered optimum as the particle size was obtained in the nano range. Based on the best and reproducible results, the optimized sonication time was found to be 20 mins, which produced particles in the nano range.

Polymer screening was performed using various polymers like HPMC K15M, HPMC K100M, and PVPK30. Particle size was measured immediately after precipitation. HPMC K15M was found to be more effective in inhibiting crystal growth as compared to particles prepared without the use of a polymer. Hence, HPMC K15M was selected as the stabilizer for the experiment. Pluronic F68 surfactant was found to have more potential as the obtained particle size of nanosuspension was observed in the nano range.

It was observed that an increase in flow rate (2-8 mL/min) increased the particle size from 487 nm to 1023 nm because of large crystal formations. Hence, a flow rate of 2 mL/min was found to be optimum to get the particle size in the nano range. The process optimization parameters and their evaluation results are depicted in Table 1.

Formulation of nanosuspension by the solvent-anti-solvent technique

Lovastatin nanosuspension was successfully prepared by the solvent-anti-solvent method using a probe sonication technique. A total of six formulations were prepared using HPMC K15M and pluronic F68 as stabilizers. In the technique, the addition of the drug solution to the anti-solvent leads to higher supersaturation. This produces many nuclei because of a high nucleation rate, which in turn reduces the mass of the solute for subsequent growth. Submicron particles are thus produced, provided that the nucleating crystals' growth is arrested by the stabilizer via stearic or electrostatic mechanism. Because lovastatin is a hydrophobic drug, the most generally used anti-solvent is water. With respect to the solvent, it has more potential if it solubilizes a higher amount of drug and possesses a greater diffusion rate to the anti-solvent, whereas the stabilizer should possess good affinity toward the drug particles and result in a fast diffusion rate as well as sufficient adsorption onto the surface of the drug particles in the solvent-water mixture. Hence, a pair of solvent-stabilizer is crucial to achieve the submicron particles.[24]

Evaluation of lovastatin-loaded nanosuspension

Particle size analysis

Particle size of the prepared formulation was determined to confirm the production of the particles in the nano range. All formulations were found to be in the range of 127-401 nm (Table 3). It was observed that the particle size was decreased from 284 nm to 127 nm, as the HPMC concentration increased from 1.0 mg/mL to 1.5 mg/mL. This is attributed to the good affinity of the hydrophobic portion of HPMC for drug particles, which leads to an effective stearic barrier against growth. Similarly, when the concentration of pluronic F68 increased from 1.0 mg/mL to 1.5 mg/mL, the particle size decreased from 401 nm to 239 nm. This result may be due to the high affinity of lovastatin particles toward this stabilizer, which provided an effective stearic barrier against crystal growth. Additionally, particle size of the formulations containing the combination of both HPMC K15M and pluronic F68 (F5 and F6) were in the nano range.

Polydispersity index

PDI determines particle size distribution, which ranges from 0 to 1. The sample is said to be monodisperse when the PDI value is close to zero. When the PDI value is <0.2, it is regarded as a narrow size distribution. PDI of all the formulations are shown in Table 3. However, when the PDI value is >0.2, it is considered as polydisperse distribution. Among the prepared nanosuspension formulation, F1 and F3 showed monodisperse size distribution with PDI values of 0.28 and 0.048, respectively. F2, F4, F5, and F6 exhibited polydisperse-sizes distributions with PDI values of 0.492, 0.81, 0.61, and 0.30, respectively.

Zeta potential

The zeta potential was analyzed to determine the stability of the optimized formulation. The zeta potential of the optimized formulation was found to be -37.9 mV, which indicates good stability.

Transmission electron spectroscopy

TEM images of the optimized formulation F2 revealed that the particles were spherical in shape, evenly distributed, and were in the range of 64.62-157.82 nm, which is close to the value obtained from the particle size analyzer (Nanotrac). TEM images are shown in Figure 2.

Drug content

Drug content was calculated for the formulations F1-F6, and it was found to be in the range of 73-86.29%. Drug content was found to be maximum for the formulation F2 and least for the formulation F3.

In vitro drug release study

An *in vitro* drug release study performed in phosphate buffer (pH 6.8) for 1 h to check the release of drug is depicted in Figure 3. The results indicated that the formulation F2 with the least particle size showed a maximum release rate. Based on the drug content, particle size, and drug release profile, the formulation F2 was selected as an optimized formulation. The release profile of the optimized formulation was then compared to the release profile of the pure drug and its marketed formulation, as shown in Figure 4. The percent cumulative drug release obtained at the end of 1 h for optimized, marketed, and pure drug formulations were 92.83%, 60.47%, and 39.73%, respectively. This is because smaller the particle size, larger the surface area. Hence, the drug that is at or near the surface is easily released.

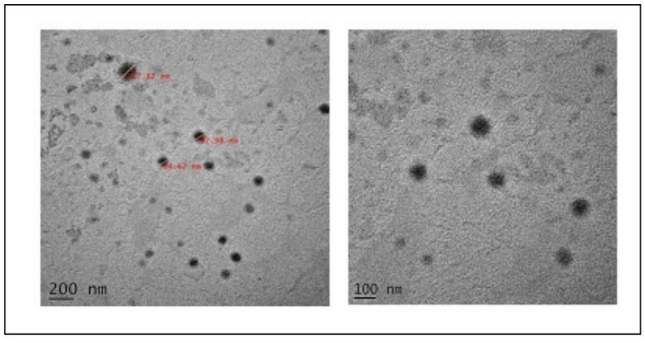

Figure 2. Transmission electron microscopy images of the optimized formulation F2

In vivo bioavailability study

The study was performed in Wistar rats to compare the plasma concentration of the optimized formulation F2 with that of the marketed product given orally in a normal saline. The average concentration was obtained at regular intervals for both the formulations. A comparative graph of plasma concentration vs. time of the optimized formulation F2 and the marketed product is shown in Figure 5. This graph revealed that the formulation F2 showed a greater bioavailability than that of the marketed product. Area under the concentration (AUC) of 29.34 µg h/mL, C_{max} of 4.9 µg/mL, and T_{max} of 4 h was observed for the marketed product when given orally. However, the optimized formulation showed AUC of 63.05 µg h/mL, C_{max} of 6.5 µg/mL, and T_{max} of 1 h, which was calculated by the trapezoidal method (Table 4). Thus, the lovastatin nanosuspension was able to improve the bioavailability by approximately 2.5 folds when compared to the marketed product.

Few more studies have been conducted with respect to the bioavailability enhancement of lovastatin. For instance, Roshan

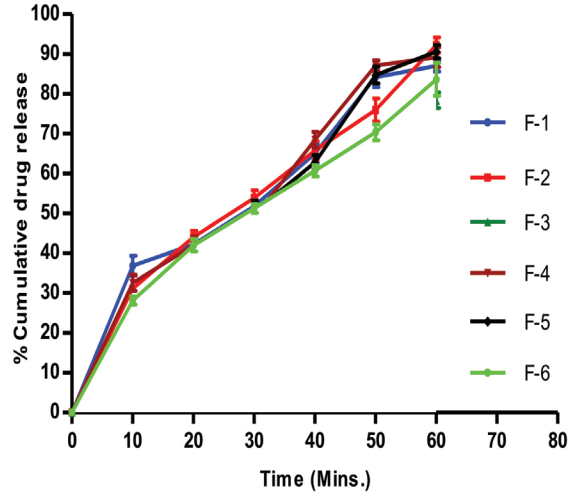

Figure 3. *In vitro* drug release profile for all the formulations

Table 3. Particle size, polydispersity index, percent drug content, and percent CDR of the nanosuspension formulations F1-F6

Formulation code	Particle size (nm)	PDI (Mv)	Drug content %	CDR %
F1	284±0.04	0.28±0.022	76.90±08	87.44±06
F2	127±0.01	0.492±0.001	86.33±07	92.83±08
F3	401±0.06	0.048±0.004	73.25±06	78.19±07
F4	239±0.002	0.81±0.003	78.02±06	89.53±05
F5	224±0.02	0.61±0.005	82.51±08	90.33±06
F6	319±0.03	0.30±0.02	74.00±05	80.25±05

Data are expressed as the mean ± SD (n=3). CDR: Constant default rate, PDI: Polydispersity index, SD: Standard deviation

Table 4. Pharmacokinetic parameters of marketed formulation and optimized formulation F2

Formulation	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-t} (µg/mL/h)
F2	6.5	01	63.05
Marketed product	4.9	04	29.34

C_{max}: Maximum plasma concentration, T_{max}: Time of maximum plasma concentration, AUC_{0-t}: Area under the concentration-time curve from dosing (time 0) to time t

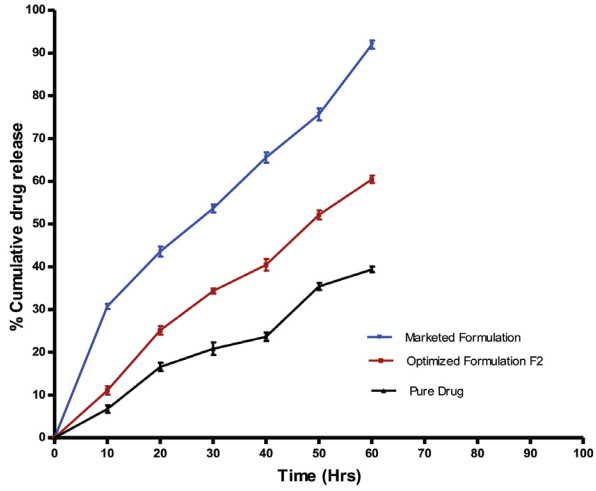

Figure 4. Comparison of *in vitro* drug release profile of the optimized formulation (F2) with that of a pure drug and marketed formulation

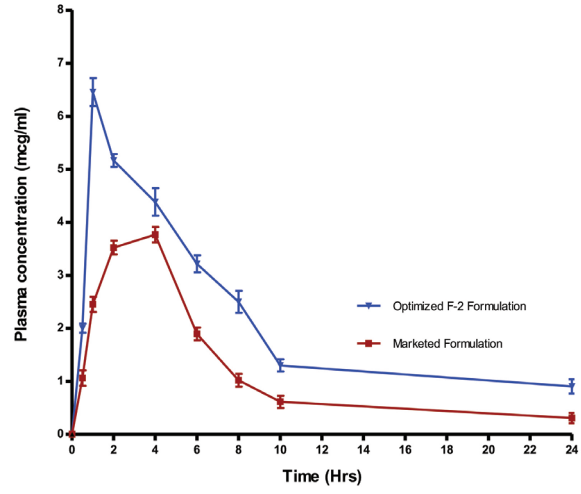

Figure 5. *In vivo* plasma drug concentration time curve of the optimized formulation (F2) and marketed formulation

et al.[25] performed comparative *in vivo* pharmacokinetic studies among solid lipid nanoparticle SLN carrier and NLC and concluded that the lovastatin-encapsulated NLC presented increased bioavailability (10.56%) compared to SLN (7.5%). Keerthi and Bhikshapath[26] developed a self-nanoemulsifying drug delivery system (SNEDDS) of lovastatin to increase its solubility and bioavailability. This study concluded that the optimized formulation of SNEDDS significantly improved the oral bioavailability of lovastatin as compared with the pure drug (no comparison with the marketed product).[26] Recently, Gaber[27] prepared and optimized nanoparticles by an ultrasonication-assisted precipitation method. *In vivo* studies confirmed that there was a 1.45-fold enhancement in C_{max} of lovastatin nanoparticles as compared to a marketed tablet.[27] These studies have also led to enhanced bioavailability of the drug, but not to the extent of a 2.5-fold increment compared with the marketed product. Thus, a developed nanosuspension by the solvent-anti-solvent method may be a potential technique to produce submicron particles of poorly water-soluble drugs, thereby enhancing oral bioavailability.

Short-term stability studies

Stability studies were conducted for the optimized formulation F2 as per ICH guidelines for a period of 3 months. Physical appearance of F2 changed slightly when samples were stored at room temperature 25˚C±2˚C/RH 65%±5% for 3 months. A sediment of thin layer was observed. However, it disappeared immediately with slight shaking. No change in the physical appearance was observed when nanoparticles were stored in the refrigerator at 4˚C±2˚C/RH 65%±5% for 3 months. On comparing the stability study data with the initial data, it was observed that there was not much change in the particle size and the *in vitro drug* release for the formulation stored at 4˚C±2˚C/RH 65%±5% to that stored at room temperature. Thus, the formulation stored at 4˚C±2˚C/RH 65%±5% showed better stability as compared to the formulation stored at 25˚C±2˚C/RH 65%±5%.

CONCLUSION

In the present study, the solvent-anti-solvent method was employed to formulate nanosuspension of lovastatin for enhancing the solubility, dissolution rate, and thereby its oral bioavailability. Lovastatin nanosuspension was formulated using methanol as a solvent, water as an anti-solvent, and HPMC K15M and Pluronic F68 as stabilizers. Fourier transform infrared spectroscopy and differential scanning calorimetry studies showed no interaction between the drug and the excipients that were used in the formulation (results not shown). On the basis of drug content, particle size, and drug release profile, formulation F2 was selected as an optimized formulation. TEM images suggest that the particle size of all the formulations were in the nano range. Zeta potential of optimized formulation F2 was found to be -37.9 mV, which showed a good stability. The dissolution rate depends upon the particle size. The smaller the particle size, the faster will be the dissolution. Formulation F2 showed a maximum dissolution rate of 92.83% in 1 h when compared with the pure drug and the marketed formulation, which showed 39.73% and 60.47% dissolution in the same time. A pharmacokinetic analysis of *in vivo* bioavailability data

indicated a 2.5-fold increase in comparison with the marketed formulation. Stability studies were conducted according to ICH guidelines for optimized formulation F2, and it was more stable at 4˚C±2˚C. Thus, it may be concluded that the solvent-anti-solvent method is a simple and potential technique to produce submicron particles of poorly water-soluble drugs, thereby enhancing oral bioavailability for commercial production.

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