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Original Article

Design, Optimization, and Characterization of Atorvastatin-Loaded Lipid-based Carriers for Enhanced Oral Bioavailability

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ABSTRACT

Aim: This study presents a dose reduction with sustained action and increased bioavailability. Atorvastatin (ATV) is the drug of choice for the management of hyperlipidemia, and the commonly available marketed product of ATV is tablets. ATV is a poorly water-soluble drug showing with a low oral bioavailability (approximately 12%). The first-pass metabolism of ATV is another cause of its low bioavailability. Nanoliposome of ATV increases bioavailability, hypothesizing the reduction of metabolism by reducing free exposure of the drug and protecting it from metabolizing enzymes) and increase their solubility due to the greater surface area of tiny nanoliposome of ATV. Nanoiposomes are tiny spherical vesicles with one or more lipid bilayers composed of physiological lipids. **Materials and Methods:** In this study, Nanoliposome of ATV were prepared by the thin film hydration Method: In analytical grade Atorvastatin (ATV), Cholesterol (CHL), Soybean Phosphatidylcholine (SPC), Tween, Acetonitrile purchased from Merck and characterized by vesicle size, size distribution, and drug loading efficiency. The preliminary investigation of batches was designed using a 3^2 full factorial design, with the size and drug loading as dependent variables. **Results:** The formulation batch 3 (NL3) showed liposomes 110.3 ± 14.6 nm in size with PDI value 0.28 ±0.03 and zeta potential -58.7 ± 7.1 with drug loading more than 60% and this batch was considered the optimized one. Further, this batch will be studied for its shape by FESEM and lamellarity by cryo-TEM analysis, and the justification of the increase in bioavailability was carried out in the selected animal model by HPLC. Thus, it is possible to administer drugs via experimentally generated nano liposomes, which could be an effective method to enhance bioavailability. **Conclusion:** New Study presents few folds higher drug bioavailability using nano carriers. In conclusion, the fabricated Nano liposomes of ATV may be a useful strategy for enhancing the bioavailability of ATV.

Keywords: Atorvastatin, Nano-liposome, Bioavailability Enhancement, Liposomal Formulation, *In vitro*-Study, Statistical Analysis, *In vivo*-Study.

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INTRODUCTION

Nanoliposome, a new type of formulation, are work well with human physiology. Compared to all other macromolecules for numerous pharmaceutical drugs with poor bioavailability in the previous years, lipid-based nanocarriers have been recognized as excellent carrier and delivery systems. Additionally, unique qualities will be demonstrated by nanocarriers such as high biocompatibility, inexpensive scale-up production, nominal toxicity, and loading efficiency of high drug.[1-3] To encapsulate and deliver bioactive compounds, adaptable platforms were provided by lipid-based nanocarriers, along with overcoming associated challenges. These novel carriers can improve the stability, drug retention enhancement, solubility, and therapeutic efficacy[4-6] of designing formulations, the bioavailability of nutrients is the key aspect to be considered when designing formulations. The amount and speed at which a drug remains the same and enters the bloodstream is called bioavailability. Through food meals when nutraceuticals are consumed, the compounds pass through the mouth, stomach, and intestine before reaching the bloodstream, as well as major barriers such as degradation and uncontrolled release in the stomach and small intestine, as well as poor bloodstream penetration from the intestinal epithelium.[7,8] Bioavailability will increase if these complications are resolved. Furthermore, bioavailability will also increase when greater bioaccessibility of nutrients results from protection against the gastrointestinal trac (GIT) environment, such as pH, by enhancing

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greater absorption from epithelial cells. To progress bioactive compounds bioavailability, it has been showed that nanocarriers are a virtuous candidate.[9-12] To increase bioavailability and therapeutic activities, nanomedicine is an auspicious tool. Usually, a large surface area is provided by nanocarriers, which have their own characteristics, merits, and demerits; in addition, it will be helpful in overcoming anatomic barriers.[13,14] In clinical use or being developed, many drugs are insoluble in water and have low bioavailability. Liposomal delivery in the digestive system is considered a promising method for improving drug dissolution and absorption. This is because liposomes are biocompatible and can retain water-repelling molecules inside their fatty layers. However, there are some issues, such as liposomes not passing easily through the cells in the intestines and becoming unstable due to their larger size. Despite the development of parenteral liposomes, no liposomal formulations have been approved for oral use. In the last decade, with the rapid growth in available studies, liposomal oral delivery has resurged.[15-18] Owing to their ability to solubilize water-insoluble medications into nanoscale structures and modify their in *in vivo* behavior to decrease toxicity, liposomes and other particulate delivery systems have gained much attention. One of the most comprehensively tried systems among the nanoparticles is liposomes, since they are biocompatible enough to be approved for parenteral administration.[19-21] Water-insoluble drugs can be dissolved in the liposomal membrane lipid domain by liposomes, which are vesicles bound by phospholipid bilayers.[22-25] The structural and compositional similarity of liposomes to bio-membranes, along with their solubilizing capacity and biocompatibility, has promoted their application in non-invasive oral delivery of poorly permeable medications.[26] Liposomes can help to dissolve drugs that do not mix well with water. In the digestive system, they protect these drugs from breaking down and help them pass through cell walls in the lining of the stomach and intestines. All of these factors make it easier for the body to absorb drugs when taken orally. Thus, liposomes are a better option for delivering drugs that are not water soluble. However, even though liposomes have these benefits, they have not been very effective in improving the absorption of drugs, such as peptides and proteins. To improve drug absorption through the mouth, biocompatible carriers such as liposomes are used. Along with their many benefits, these nanocarriers are useful because their structures are similar to those of the cell membranes. They adhere well to biological membranes, making them suitable for uptake by the lymph system. Moreover, when mixed with bile salts, they can form complexes that help dissolve drugs that do not mix well with water. Improving the oral bioavailability of a variety of compounds, including hydrophilic and lipophilic drugs, peptides, and proteins they have been popular. The main apprehension for oral liposomes is their stability under GI conditions; however, to increase the stability of oral liposomes, promising approaches have been suggested. These include polymer coating, appropriate use of lipid compositions, double liposome preparation and proliposomes, addition of stabilizing lipids to liposomal structures, and some other innovative approaches.[27] ATV a lipid-regulating drug, is widely used to decrease the cholesterol and triglyceride levels. Drug delivery approaches that can increase solubility and avoid hepatic effects must be developed owing to their poor water solubility and hepatic metabolism. For this purpose, atorvastatin (ATV) loaded liposomes were prepared and characterized.[28] A novel approach was developed to improve ATV bioavailability and stability of ATV by constructing liposome with ATV. However, statins have some limitations such as poor oral absorption and low aqueous solubility, and when administered orally, they have limited bioavailability. Currently, nanotechnology is a well-developed field, and new methods provide systems with enhanced statin aqueous solubility, bioavailability, higher absorption, and controlled release of statin at the site of administration controlled release of the statin.[29] Due to their considerable molecular weight and limited aqueous solubility, statins have low oral bioavailability. Furthermore, drug-related adverse effects can develop in a significant number of patients. The bioavailability of statins can be increased by effective delivery methods, which could decrease the toxicity and side effects associated with increased statin plasma concentrations.[30]

MATERIALS AND METHODS

Materials

Atorvastatin (ATV) was received as gift from Dr. Reddy's Lab. (Hyderabad, India). Soya-L-α-lecithin (SPC), cholesterol (CHL), chloroform, Tween 80, Acetonitrile were purchased from Merck (Mumbai, India). butylated hydroxy toluene (BHT) was procured from Qualigens Fine Chemicals (Mumbai, India). Marketed tablet of ATV (Lipitor) was purchased from local market.

Methods

Prepartion of Liposomes

Using the thin film hydration method, ATV-containing nanoiposomes were prepared. ATV, CHL, and SPC (among the ingredients' specific amounts). were mixed together and dissolved in 10 mL of chloroform in a 250 mL round-bottom flask. After thoroughly mixing the content, the solvent was removed at 40°C using an aspirator and a rotary vacuum evaporator (Rotavap, model: PBU-6). The resulting thin film was placed overnight in a vacuum desiccator. It was subsequently soaked in PBS (pH 7.4) at 60°C with stirring at 160 rpm for one hour, followed by an additional hour at a frequency of 30 ± 3 KHz with an ultrasonicator (Trans-o-Sonic, Mumbai, India). At 15,000 rpm the mixture was centrifuged for one hour at 4°C in a refrigerated centrifuge (3K30 Sigma Lab Centrifuge). To assess the optimal dry mass, the liposome mass was collected in a Petri dish and freeze-dried using a laboratory freeze-dryer (Laboratory-Freeze Dryer, Instrumentation India Ltd., Kolkata).

Experimental Animals

Swiss albino mice (9 males, 9 females; average weights: 25-30 g. A total of 18 mice, divided into three groups) were used for the bioavailability assay under the approval of NCPT/IAEC-028/2025 by the IAEC of NSHM Knowledge Campus (Animal House) in Kolkata. A single dose of 10 mg/kg was administered orally to mice for 24 hr. Male Albino mice were kept in a controlled environment at the NSHM Knowledge Campus for one week before the experiment started. The conditions were temperature 22 ± 3°C and relative humidity 50 ± 5%. Before the experiment, the mice were kept under a regular dark and light cycle and fasted overnight. Table 1 describes the optimization of the liposome preparation.

Preparation of Calibration curve of ATV

Using a UV/vis spectrophotometer, drug absorption maxima were found in various working solutions, including PBS with 0.5% (w/v) TWEEN and in ACN. Respective absorbance against concentrations was determined in PBS at pH 7.4 and ACN. The data were plotted to develop the respective calibration curves shown in respectively.

Drug-excipients interaction study

The preliminary drug-excipients interaction was assessed by Fourier transform infrared (FTIR) spectroscopy. The individual components of the experimental formulations viz, ATV, CHL and SPC, a mixture of ATV, CHL & SPC; and experimental formulation were run in FTIR spectrophotometer within 4000 cm-1 to 400 cm-1. The spectrums were recorded and compared with the principle peaks from each other to know any changes in the structure.

Vesicle size and zeta potential

The size of nanoparticles was measured using a Nano ZS90 Zetasizer. The effective electric charge on the nanoparticles was checked at a temperature of 25°C with a fixed angle of 173° to reduce multiple scattering.

Surface Topography By Field-Emission Scanning Electron Microscope (Fe-Sem) And Cryogenic-Transmission Electron Microscopy (Cryo-Tem) Study

High-resolution images of the experimental formulation was captured using a field-emission scanning electron microscope (Thermofisher Apreo 2S, India). The small quantity of proliposomes were placed on double-sided sticky carbon tapes, were coated with platinum for 2 min to ensure an even layer on the surface of about 5 nm. and then attached to FE-SEM sample holders and images were taken at different magnification. The surface morphology was further substantiated by Cryo-TEM (Titan Krios ,Thermo Fisher, India). The sample under testing was placed on a carbon coated copper grid, followed by removal of extra solvent by blotting and then the grid was subjected to vitrification (Treated the grid with liquid nitrogen. The vitrified grid was placed in Cryo TEM sample holder and images were recorded and analyzed.

In vitro drug release

For *in vitro* drug release experiments, Franz diffusion cells with dialysis membranes were used for in vitro drug release experiments. The optimized formulation was administered to the donor compartment, whereas a phosphate buffer solution (pH 6.8) was administered to the receptor compartment. Using a circulating water bath, both compartments were maintained at 37°C with a 0.5% tolerance. A 2 mL aliquot was taken and 2 mL of freshly prepared buffer was added to maintain sink conditions at different time intervals (0, 15, 30, 60, 360, and 720 min), filtered, and analyzed using UV Spectroscopy.

Further to establish the mechanism of drug release, the release data were fitted in the different kinetics of drug release and the

Table 1: 3^2 factorial design with the experimental response values for the various formulation of ATV loaded lipid based nano carriers.

Formulation code	Factors (levels)		Responses		
	Phosphatidylcholine (mg) (A)	Rotation per minute (rad/s)	Drug Loading (%)	Particle Size (nm)	Zeta Potential (mV)
NL1	50 (-1)	12000 (-1)	41	140	-32.31
NL2	100 (0)	15000 (0)	51	123	32.09
NL3	100 (0)	18000 (+)	63	110	-58.7
NL4	50 (-1)	15000 (0)	57	125	-20.33
NL5	100 (0)	12000 (-1)	49	135	62.11
NL6	50 (-1)	18000 (+1)	59	111	-29.33
NL7	150 (+1)	15000 (0)	54	120	31.87
NL8	150 (+1)	18000 (+1)	68	110	54.22
NL9	150 (+1)	12000 (-1)	45	133	67.11

release mechanism was established by observing the correlation coefficient (R2) value.

Statistical Optimization

Optimization of ATV loaded lipid based nano carriers to improve bioavailability by Response Surface Methodology (RSM).

Analyzing data and verifying the optimization model

In the existing optimization study, numerous Response Surface Methodology (RSM) calculations were performed using State-Ease Inc. Design Expert Software (Model 13). Multiple Linear Regression Analysis (MLRA) was used to create polynomial fashions that blanketed interplay and quadratic phrases for each reaction variable. The following equation illustrates the overall shape of the MLRA model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_1^2 X_2$$

Within the over condition, β_0 speaks to the captured and demonstrates that the scientific cruel of all quantitative comes about obtained from the nine tests. The coefficients β_1 to β_7 are inferred from the test reaction values of Y, whereas X_1 and X_2 demonstrate the coded levels of the impartial variable. The terms X_1, X_2, and Xi^2 (i = 1 to 2) were compared to the interaction and quadratic conditions, respectively. The factual legitimacy of the polynomial conditions was based on an ANOVA investigation within the Plan Master Computer program. These plots are exceptionally valuable for observing the interaction effects between the factors on the reaction.

In vivo bioavailability study

For this study, a High Performance Liquid Chromatography (HPLC) system (Shimadzu Corp) was used. The mobile phase was made with a mixture of 30% phosphate buffer and 70% methanol, and it flowed at a speed of 1 mL per minute. The retro-orbital route was used to collect blood (0.5 mL) before dosing at 15, 30, 60, 360, and 720 min. Blood samples were centrifuged for 10 min at 5500 rpm. Blood plasma was stored and kept at 20°C. We mixed 0.1 mL of blood was mixed with acetonitrile (0.5 mL) to extract the drug and allowed to stand for 10 min. Then, 150 µL of plasma and 50 mg of Internal Standard (IS) were added to each 1.5 mL centrifuge tube. After adding 300 µL of acetonitrile, the samples were shaken for five minutes and spun again at 6600 rpm for 5 min to complete deproteinization. We used micropipettes to collect clear liquid from each sample. The liquid was then filtered through a 0.45 micron filter. Finally, 20 µL of pure top layer was injected into the HPLC system. For the bioavailability study, three groups of six animals each were used. One group was the control group, one received the marketed formulation, and the third received ATV-loaded liposomes as nano-carriers. Pharmacokinetic studies were conducted by measuring the drug concentration over time to determine parameters such as C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, and K_{el}. These pharmacokinetic data helped estimate the ATV, and the results are presented as the mean ± standard deviation.

Table 2: Formulation composition, loading efficiency and drug entrapment of different formulations.

Code for creation	ATV: SPC: CHL (weight-based)	Loading Efficiency %*	Drug entrapment
NL1	1:5:5	44.61±2.05	42.2±0.21
NL2	1:10:5	81.26±5.01	77.4±0.24
NL3	1:15:5	87.81±3.41	79.1±0.21

Table 3: Comparison of pharmacokinetic data of ATV loaded liposome (formulation) and marketed ATV tablet. Optimal conditions were selected based on the drug release requirements. The optimization resulted in 100 solutions from which 9 were selected. After preparing and evaluating these 9 solutions, the desired drug release pattern was determined by comparing the predicted and observed responses.

Pharmacokinetic Parameter	Marketed Tablet	Formulation
C_{max} (µg/mL)	9.9±1.10	4.5±0.2
t_{max} (hr)	1.7 ±0.2	2.20 ±0.25
AUC_{0-t} (µg hr/mL)	123.6 ±2.5	392.4 ±5.5
$AUC_{0-\infty}$ (µg hr/mL)	172.1 ±23.1	610 ±12.3
k_{el} (hr^{-1})	0.040±0.3	0.050±0.6
$t_{1/2}$ (hr)	4.5±0.2	5.4±0.3

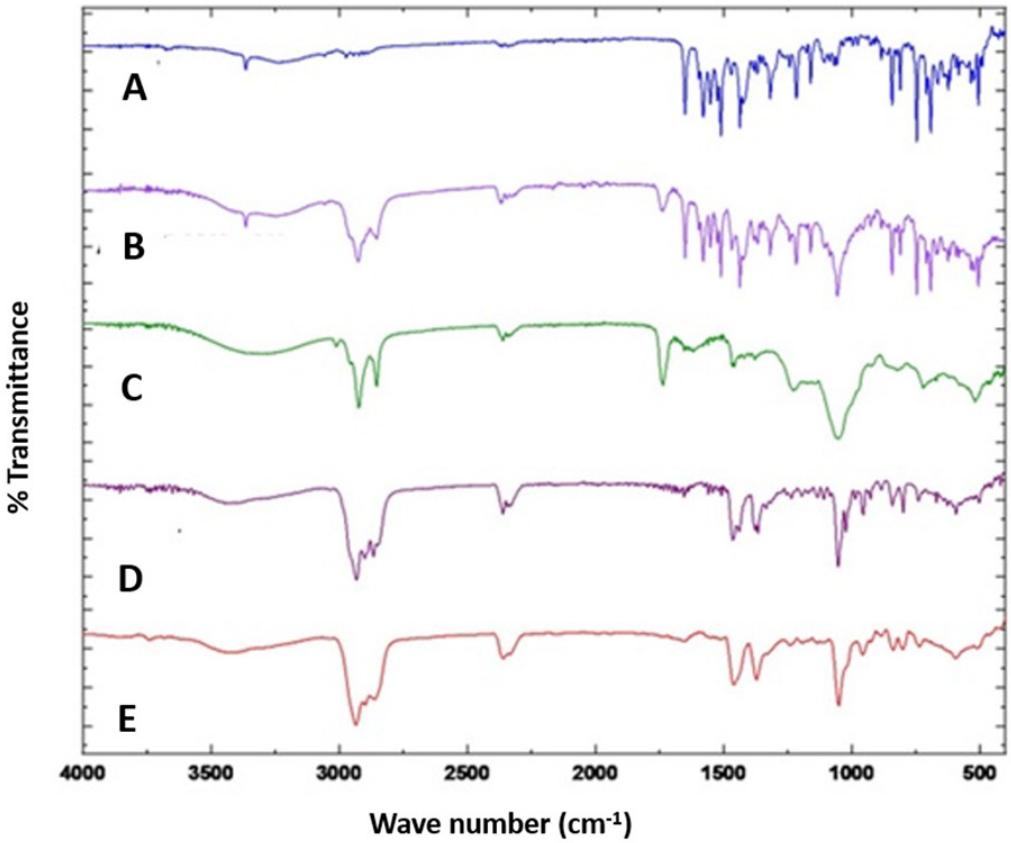


Figure 1: FTIR spectrums of Atorvastatin (A), mixture of ATV, SPC and CHL(B), SPC (C), CHL(D), and experimental formulation (E)

RESULTS

Determination of lambda max and Calibration Curve of ATV

At 245 nm, the maximum absorption of ATV in a PBS containing 0.5% (w/v) Tween at pH 7.4 was noted. The absorption maximum of ATV in ACN was detected at 246 nm.

The FTIR spectra of ATV (A), physical mixture (B), SPC (C), CHL(D), and experimental formulation (E) were analyzed to investigate the compatibility of the drug with the selected liposomal excipients. The FTIR spectrum of A) exhibited its characteristic absorption bands corresponding to O–H stretching (~3400 cm^{-1}), aliphatic C–H stretching (~2920–2850 cm^{-1}), carbonyl (C=O) stretching (~1700 cm^{-1}), aromatic C=C vibrations (~1600–1500 cm^{-1}), and C–O/C–F stretching vibrations in the fingerprint region (1200–1000 cm^{-1}), confirming the identity and structural integrity of the drug. Soya lecithin (C) showed characteristic C–H stretching bands at 2920–2850 cm^{-1}, ester carbonyl absorption near 1735 cm^{-1}, phosphate (P=O) stretching around 1240 cm^{-1}, and P–O–C stretching near 1060 cm^{-1}. Cholesterol (D) displayed characteristic O–H stretching around 3400 cm^{-1}, aliphatic C–H stretching at 2930–2860 cm^{-1}, and C–O stretching in the region of 1050–1100 cm^{-1}. The experimental batch (E) retained all the characteristic peaks of both components without the appearance of any additional bands or significant peak shifts, Similarly, the physical mixture of atorvastatin, cholesterol, and soya lecithin (B) showed the characteristic absorption peaks of atorvastatin, cholesterol, and soya lecithin with only slight variations in peak intensity and broadening. No new absorption bands, disappearance of characteristic peaks, or significant shifts in wavenumber were observed. These findings indicate the absence of any chemical interaction or incompatibility between atorvastatin and the lipid components.

The minor changes in peak intensity and slight broadening observed in the physical mixture are attributed to weak physical interactions, such as hydrogen bonding, van der Waals f orces, and hydrophobic interactions between atorvastatin and the phospholipid bilayer constituents. Such non-covalent interactions are expected during liposome preparation and facilitate the incorporation of the drug within the lipid bilayer without altering its chemical structure.

Overall, the FTIR results confirm that ATV is compatible with cholesterol and soya lecithin, demonstrating no evidence of chemical interaction among the formulation components. The observed physical interactions are desirable, as they promote drug entrapment and stabilization within the liposomal vesicles, thereby supporting the successful development of a stable ATV-loaded liposomal formulation.

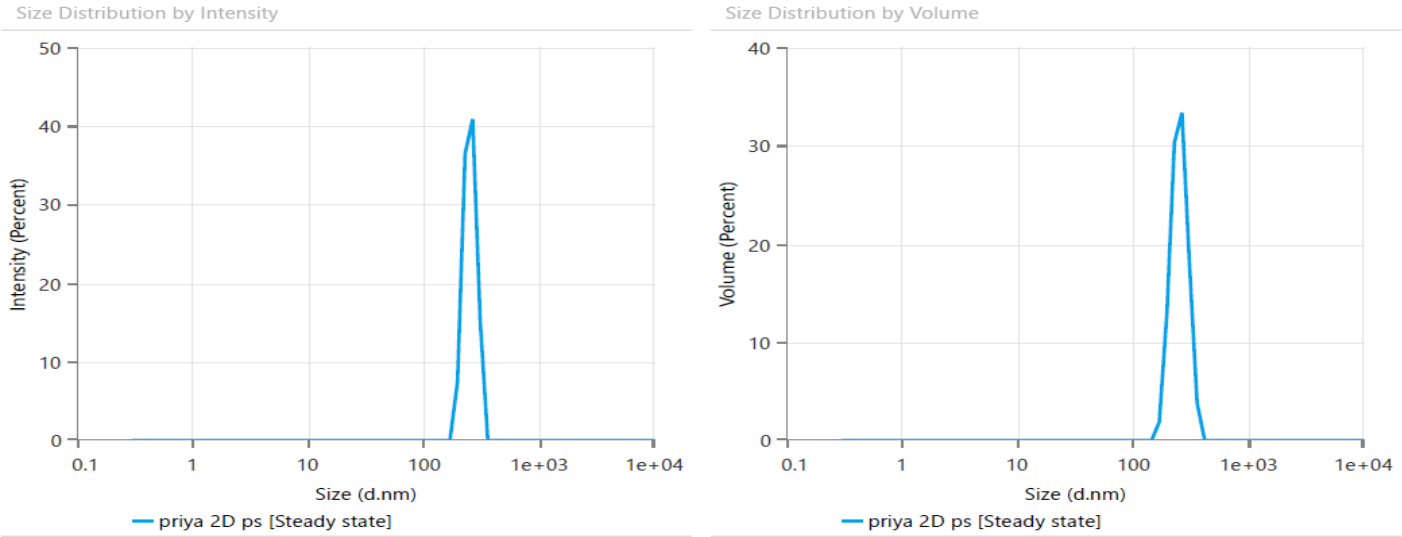


Figure 2: Average vesicle size Distribution (DLS) of the optimized nanoliposomes (NL3).

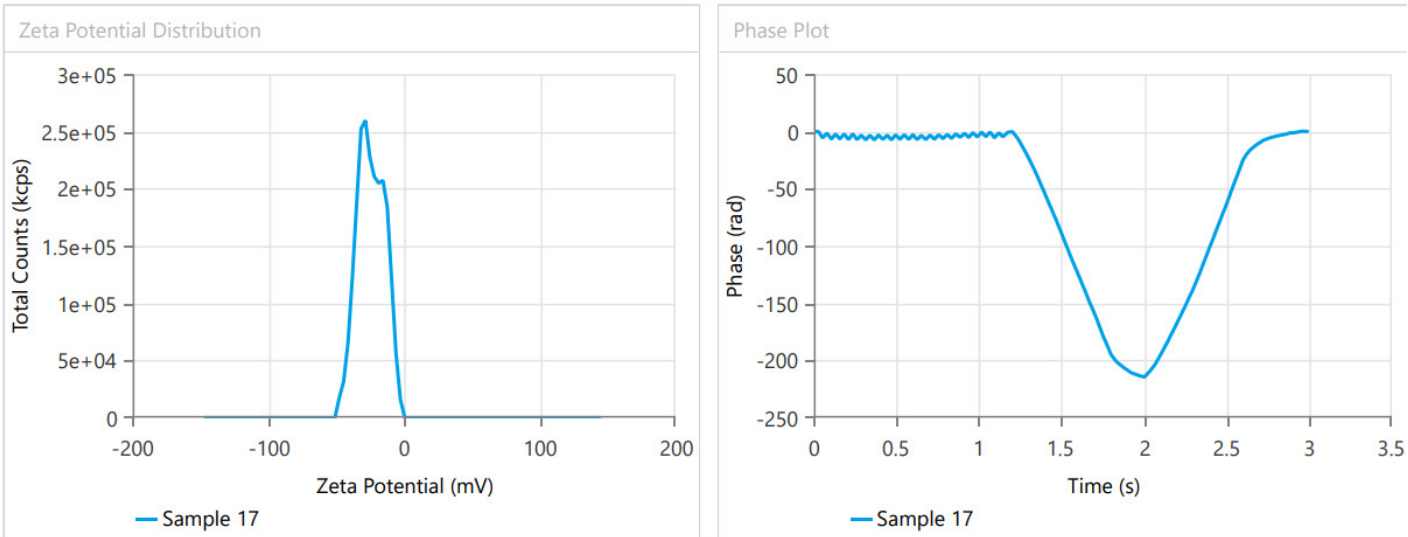


Figure 3: Zeta potential distribution of the optimized nanoliposomes (NL3).

Vesicle Size & zeta potential

The particle size of NL3 (optimized best formulation) is shown in Figures 2 and 3, which represent particle sizes of 110.3 ± 14.6 nm, PDI values of 0.28 ±0.03, and zeta potentials of -58.7 ± 7.1 mV (Figures 2 and 3).

FE-SEM replace please with FE-SEM AND CRYO-TEM STUDY

This image shows the surface morphology and topography of the samples at the nanometer scale with spherical shape and uniform distribution throughout the matrix of the experimental sample of proliposomes batch with size less than 100 nm.

Further, the particle morphology for shape, size was confirmed by by Cryo-TEM study of the optimized formulation as shown in Figure 4 and 5. The size is less than 100 nm with smooth surface and intact unilamellar vesicle were observed.

In vitro drug release

In vitro dissolution data of liposomes were suitable for various kinetic equations, the correlation coefficient (R^2) value was calculated from different kinetics models, and the value of Korsmeyer-Peppas release was 0.999. Table 2 describes two different in *in vitro* studies of the three best formulations with different ratios of drug and excipients.

Statistical Optimization

Experimental Design by RSM

Formulation trials were carried out for the formulation, physicochemical characterization, and assessment of atorvastatin loaded lipid based nano carriers to improve bioavailability With SPC and RPM (independent variables) and loading, particle size, and zeta potential (dependent variables) at different ratio levels used as per the experimental design (Figures 6 and 7).

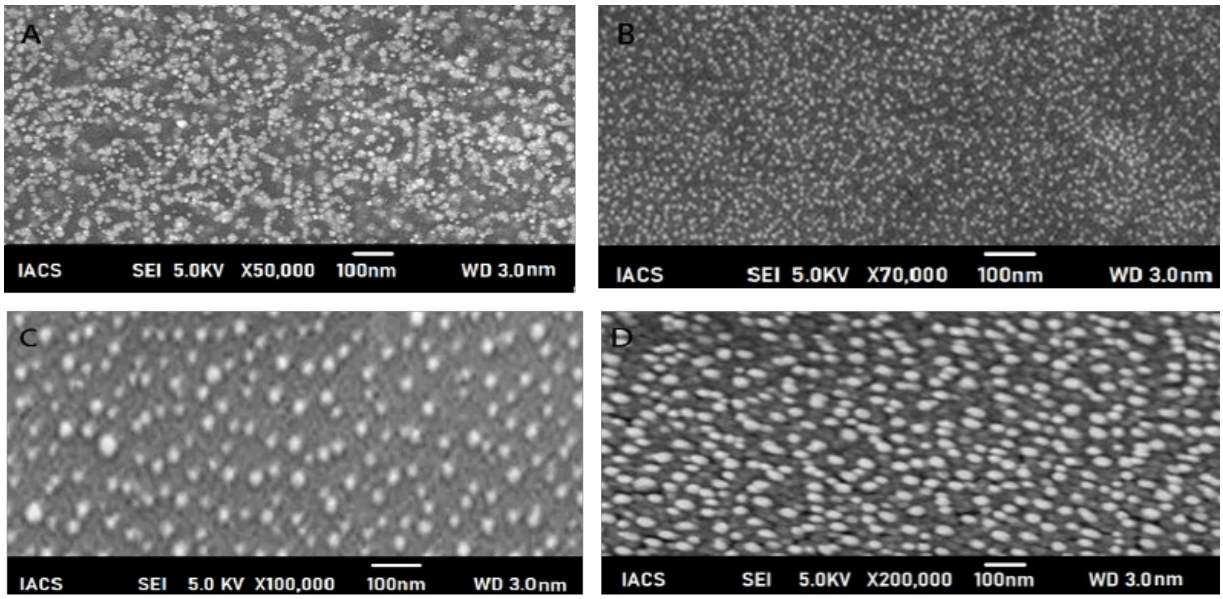


Figure 4: The FESEM images of optimized pro-liposomes (NL3) at different magnification at 50000×, 70000×, 100000×, and 200000×

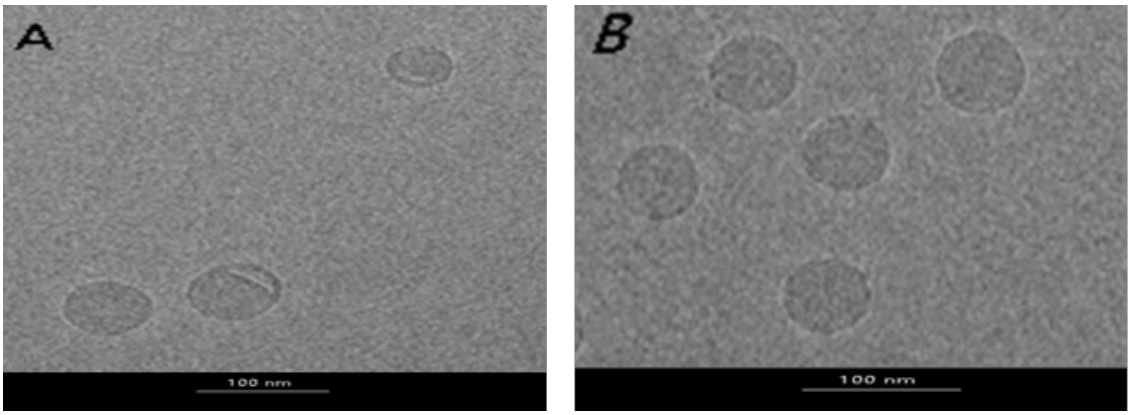


Figure 5: Cryo-TEM image of the optimized Nanoliposomes (NL3).

(a) Drug Loading (%) = 1028.28 + 970.45+ 77.83B + 165.38AB + 1.28A2 + 0.5408B2

Where F value = 85.45, R^2 = 0.984, $p < 0.05$.

(b) Particle Size (nm)= 479.78 + 451.83 + 27.95B + 1021.44AB + 2.52A2 + 0.9339B2

Where F value = 113.47, R^2 = 0.974, $p < 0.05$.

(c) Zeta Potential (mV) = 1047.12+ 991.20+ 101.92B +121.77AB + 0.2812A2 + 0.0952B2

Where F value = 94.29, R^2 = 0.964, $p < 0.05$.

Where;

(a) Significance results for % of Drug Loading (b) Particle Size (c) Zeta potential. The given equations show how the process variables interact to produce the response and its quantifiable effect. To assess the significance of the model, an analysis of variance (model: ANOVA 13) was performed using Design Expert software as described below. At a significance level of 5%, a model is considered significant if the *p*-value is less than 0.05.

In vivo bioavailability study

A C_{max} of ATV following orally administered atorvastatin with liposome was 9.9±1.10 µg/mL and then it took few hours to reach t_{max} and atorvastatin liposome had C_{max}, and the t_{max} was 26.12.±1.5 µg hr/mL respectively. The $AUC_{0-\infty}$ as significantly elevated in atorvastatin-loaded liposome, compared to marketed ATV ranging from 172.1±23.1 in µg hr/mL up to 610.2±12.3µg hr/mL. Table 3 describes the different evaluation parameters of *in vivo* studies with the optimized formulation.

DISCUSSION

Statistical optimization of the selected formula was performed to obtain the best ratio and the most optimized formula. Nine formulation variables, including the SPC-to-CHL ratio, were optimized using a factorial design. Statistical analysis of the data

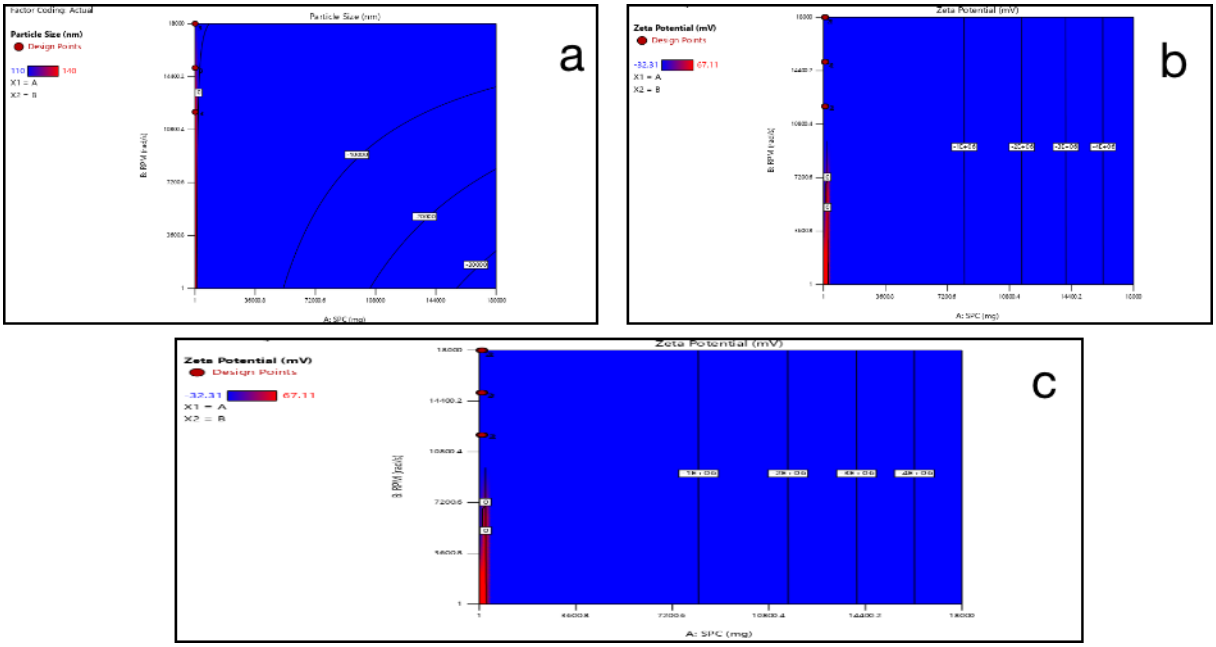
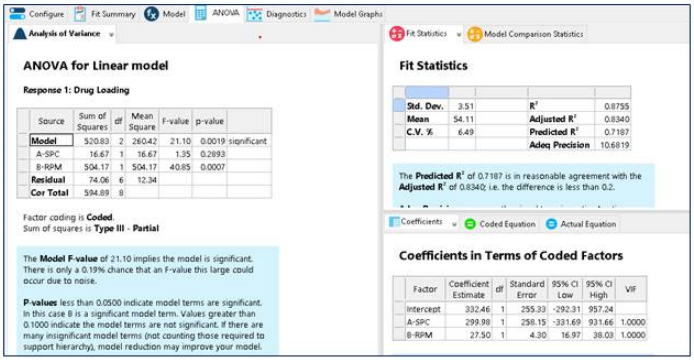


Figure 6: Impact of SPC and RPM on % drug loading, particle size and zeta potential by ANOVA.

A.



ANOVA for Linear model

Response 1: Drug Loading

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	520.83	2	260.42	21.10	0.0019	significant
A-SPC	16.67	1	16.67	1.35	0.2693	
B-RPM	504.17	1	504.17	40.85	0.0007	
Residual	74.06	6	12.34			
Cor Total	594.89	8				

Factor coding is Coded.
Sum of squares is Type III - Partial

The Model F-value of 21.10 implies the model is significant. There is only a 0.19% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	3.51	R^2	0.8755
Mean	54.11	Adjusted R^2	0.8340
C.V. %	6.49	Predicted R^2	0.7187
		Adeq Precision	10.6819

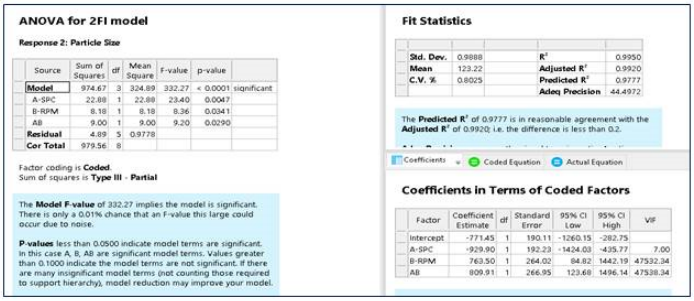
The Predicted R^2 of 0.7187 is in reasonable agreement with the Adjusted R^2 of 0.8340; i.e. the difference is less than 0.2.

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	332.46	1	255.33	-292.31	957.24	
A-SPC	299.99	1	258.15	-331.69	931.66	1.0000
B-RPM	27.50	1	4.30	16.97	38.03	1.0000

(A) Significance result for % of Drug Loading

B.



ANOVA for 2FI model

Response 2: Particle Size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	974.67	3	324.89	332.27	< 0.0001	significant
A-SPC	22.88	1	22.88	23.40	0.0047	
B-RPM	8.18	1	8.18	8.36	0.0341	
AB	9.00	1	9.00	9.20	0.0290	
Residual	4.89	5	0.9778			
Cor Total	979.56	8				

Factor coding is Coded.
Sum of squares is Type III - Partial

The Model F-value of 332.27 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	0.9888	R^2	0.9950
Mean	123.22	Adjusted R^2	0.9920
C.V. %	0.8025	Predicted R^2	0.9777
		Adeq Precision	44.4972

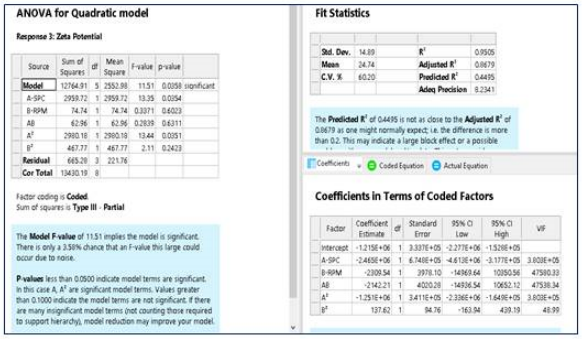
The Predicted R^2 of 0.9777 is in reasonable agreement with the Adjusted R^2 of 0.9920; i.e. the difference is less than 0.2.

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	-771.45	1	190.11	-1260.15	-282.75	
A-SPC	-929.90	1	192.23	-1424.03	-435.77	7.00
B-RPM	763.50	1	264.02	84.82	1442.19	47532.34
AB	809.91	1	266.95	123.68	1496.14	47538.34

(B) Significance result for Particle Size

C.



ANOVA for Quadratic model

Response 3: Zeta Potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	12764.91	5	2552.98	11.51	0.0358	significant
A-SPC	2959.72	1	2959.72	13.35	0.0354	
B-RPM	74.74	1	74.74	0.3371	0.6023	
AB	62.96	1	62.96	0.2839	0.6311	
A^2	2980.18	1	2980.19	13.44	0.0351	
B^2	467.77	1	467.77	2.11	0.2423	
Residual	665.28	3	221.76			
Cor Total	13430.19	8				

Factor coding is Coded.
Sum of squares is Type III - Partial

The Model F-value of 11.51 implies the model is significant. There is only a 3.58% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	14.89	R^2	0.9505
Mean	24.74	Adjusted R^2	0.8679
C.V. %	60.20	Predicted R^2	0.4495
		Adeq Precision	8.2341

The Predicted R^2 of 0.4495 is not as close to the Adjusted R^2 of 0.8679 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	-1.215E+06	1	3.337E+05	-2.277E+06	-1.526E+05	
A-SPC	-2.465E+06	1	6.748E+05	-4.612E+06	-3.177E+05	3.803E+05
B-RPM	-2309.54	1	2978.10	-14969.64	10350.56	47580.33
AB	-2142.21	1	4020.28	-14936.54	10652.12	47538.34
A^2	-1.251E+06	1	3.411E+05	-2.336E+06	-1.649E+05	3.803E+05
B^2	137.62	1	94.76	-163.94	439.19	48.99

(C) Significance result for Zeta potential

A. The model is significant, with RPM strongly influencing drug loading, while SPC is insignificant, showing good fit and predictive ability.

B. The 2FI model is highly significant, with SPC, RPM, and their interaction significantly influencing particle size, showing excellent model fit

C. The quadratic model is significant, SPC and quadratic terms influence zeta potential, but low predicted R^2 suggests limited predictability

Figure 7: ANOVA and Regression Analysis of Drug Loading, Particle Size, and Zeta Potential.

on drug loading (%), particle size, and zeta potential showed that two variables, X1 and X2, were highly important for the formulation. As shown in Figure 6, formulation NL3 was selected as an atorvastatin-loaded lipid-based nanocarrier to improve its bioavailability by SPC and CHL. The medicine ATV calcium is hydrophobic and artificial with low bioavailability. Liposomes are tiny round vesicles with one or more lipid bilayers that can be crafted from herbal or artificial lipids. To avoid first-pass metabolism, liposomes can protect hydrophobic medicines from metabolism in physiological fluids and increase their solubility and bioavailability. The goal was to create, characterize physicochemically, and evaluate atorvastatin-loaded liposomes to increase ATV's bioavailability of ATV. The current study aimed to enhance the solubility, dissolution rate, and bioavailability

of poorly soluble ATV. The procedure entails creating an ATV calibration curve in Acetonitrile (ACN) and PBS with 0.5% Tween 80, conducting an FTIR analysis, and creating ATV-loaded NLs using the thin-film hydration process and DLS analysis. Using PBS containing Tween and ACN, the drug absorption maxima were observed at 246 and 245 nm, respectively. All excipients were compatible according to the FTIR results. Based on numerous observations, the NL3 formulation was found to be the optimal formulation for the final product yield, drug abuse, and loading efficiency rate in the process. This formulation was approved for additional in research *in vitro* and *in vivo*. The zeta potential was -58.7 ± 7.1 mV, PDI was 0.28 ± 0.03, and particle size was 110.3 ± 14.6 for NL3, Cryo-TEM Showed unilamiller that produce unform distribution and AUC of animal studies increased two-fold rather than marketed formulation.

CONCLUSION

Nanocarrier technology represents a breakthrough for the delivery of pharmaceutical drugs. Tests show that these carriers boost drug bioavailability fold, which solves a basic problem in medicine: getting drugs exactly where they need to go. Traditional delivery methods are nowhere nearly as effective, with less than half the drug reaching its intended target. These systems excel through precise targeting and smart release. They work exceptionally well across different applications, from better cellular uptake to stronger antitumor effects. Market numbers validate these clinical results and indicate a promising future for nano-based delivery systems. Patients can expect better outcomes with higher absorption rates, fewer side effects, and easier treatment plans. Our ongoing research builds foundations for better therapeutic solutions that can change patient care worldwide.

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ABBREVIATIONS

ATV: Atorvastatin; **CHL:** Cholesterol; **SPC:** Soybean Phosphatidylcholine; **RSM:** Response Surface Methodology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

Original draft writing, methodology & data collection: MM, Concept & data interpretation: TKS, Writing, reviewing and validation: BD.

ETHICAL CONSIDERATION

Approved by the Ethical committee (NCPT/IAEC-028/2025).

SUMMARY

Administration of drugs via Nano liposomes could be an effective method for enhancing the bioavailability of poorly water-soluble drugs. This study showed a two-fold higher bioavailability than that reported for atorvastatin. According to *in vivo* tests, the C_{max} of atorvastatin increased dramatically when utilizing liposomes loaded with atorvastatin (formulation) compared to marketed atorvastatin 10 mg tablets. The AUC determination from experimental rat blood plasma revealed that liposome-encapsulated atorvastatin had greater bioavailability than the marketed formulation and test sample.

- Atorvastatin-loaded nano-liposomes have been successfully developed to overcome poor solubility and extensive first-pass metabolism.
- The liposomal formulation resulted in nanosized vesicles (~110 nm) with a narrow size distribution and high colloidal stability.
- Optimized formulation showed high drug loading efficiency (>60%).
- Nano-liposomes enhanced the oral bioavailability of atorvastatin by approximately few-fold compared with that of conventional dosage forms.
- Liposomal delivery demonstrated potential for dose reduction and sustained therapeutic action in hyperlipidaemia management.

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