International Journal of Early Childhood Special Education (INT-JECSE)
DOI: 10.48047/intjecse/V12I1.201057 ISSN: 1308-5581 Vol 12, Issue 01 2020

PERFORMANCE OF OPTIMIZED FORMULATION IN VITRO AND IN VIVO FOR EFFECTIVE DELIVERY

DR. PANKAJ NAINWAL
Department of Pharmacy, Graphic Era Hill University, Dehradun, Uttarakhand, India 248002

Abstract

Simvastatin is a cholesterol-lowering medication that may be used to treat hypercholesterolemia, coronary heart disease, and dyslipidemia. Simvastatin (SV), on the other hand, has been proven to have poor oral absorption in the GI tract. Developing proliposomal formulations was undertaken with the sole intent of increasing SV's oral bioavailability. The proliposomes were made using a film deposition on carrier technique. Morphology, entrapment efficiency, drug-polymer compatibility, and in vitro and in vivo tests were all evaluated for the proliposomes. There was no evidence of a drug-polymer interaction in the FTIR or DSC analyses. The maximum medication release time observed with the optimized formulation (PL6) was 12 hours (99.78 0.067%). Pure SV had a maximum plasma concentration of 10.4 2.921 g/mL and an elimination half-life of 67.124 0.23 hours, whereas SV proliposomes (SVP) had an AUC0- of 179.75 1.541 hours. The adjusted SVP significantly improves SV rate and uptake.

Key words: Simvastatin; proliposome; cholesterol; phospholipid; in vivo studies

INTRODUCTION

The fungus Aspergillus terreus is fermented to produce the lipid-lowering chemical known as synthetic simvastatin (SV). The enzyme 3-hydroxy-3-methylglutaryl COA reductase, which catalyzes the last step in cholesterol biosynthesis, may have a role in suppressing steroid hormone production. By stimulating hepatic LDL receptors, LDL cholesterol is degraded at a faster rate. One kind of statin medication is called SV. Long-term SV use reduces the risk of mortality from atherosclerosis-related disorders such myocardial infarction, ischemic attack, and coronary heart disease. Multiple tissues may be affected by SV due to its pleiotropic nature, notwithstanding its key advantages. Solubility is a key rate-limiting element in achieving the necessary concentration in systemic circulation for therapeutic action. As a result, researchers in dosage forms have a significant difficulty in addressing the solubility issue associated with SV (3). Just 2.24 g of SV may be dissolved in 1 milliliter of water. Dissolution-controlled absorption is the conventional view for substances with extremely low water solubility. It has a biological half-life of 3 hours, a bioavailability of 5%, and is well absorbed in the GIT, all of which hint to considerable first pass metabolism in the liver. Therefore, it is crucial to consider the oral solid formulation's dissolving rate, water solubility, and bioavailability. This article discusses a novel liposome approach for preparing solid SV proliposomes powder, which not only increases SV bioavailability but also improves solubility at pH 7.4, with drug release lasting for more than 8 hours. It was said that liposomes were employed to provide drugs orally. Literature analysis led us to the conclusion that some poorly absorbed medications have been enclosed in liposomes to improve their absorption and bioavailability in the gastrointestinal tract. Peptide medicines' stability and rate of absorption may be improved as well.

LITERATURE REVIEW

Khan, M. I., Yaqoob, S., Madni, A., Akhtar, M. F., Sohail, M. F., Saleem, A., Tahir, N., Khan, K. U., & Qureshi, O. S. (2016), Because of their inherent ability to self-regulate and self-optimize, transfersomes

(TFS) hold great promise as transdermal carriers for a wide range of low- and high-molecular-weight medicines. For simultaneous transdermal administration, we demonstrate the synthesis and characterization of TFS loaded with the nonsteroidal anti-inflammatory drug meloxicam (MLX) and the steroid dexamethasone (DEX). Thin-film hydration was effectively used to create TFS formulations (TFS-1 through TFS-6) with varied concentrations of lecithin, Span 80, and Tween 80. Studies on drug release found that Tween 80-based TFS was more effective than Span 80-based TFS at a pH of 7.4. Vesicles were found to have a spherical shape when seen using a scanning electron microscope (SEM) for the chosen formulas -1 and TFS-3. In addition, carbopol-940 gels were made using three different transfersomal formulas depending on entrapment efficiency. Based on the kinetic modeling of the release and permeation data, it seems that the drug diffusion-based moment follows the Korsmeyer-Peppas model. In contrast, we discovered that the inflow of MLX and DEX into ordinary gel was only 26.18% and 22.94%, respectively. Our results provide credence to the concept of using TF-G containing MLX and DEX as an alternate drug carrier to improve transdermal flow and, by extension, therapeutic efficacy.

Nicolai Tirumalesh, Krishna Prasad Chowdary (2019), In order to obtain Approximately 85% dissolving in 10 min, the current research aims to improve a tablet formulation of Valsartan using CD, Starch 1500, and Soluplus using a 23-factorial design and then evaluates the optimized formulations using In Vitro and In Vivo (Preclinical) methodologies. In a 23 factorial design, eight different tablet formulations of valsartan were created by varying only three variables. The effectiveness of direct compression in making valsartan tablets was assessed. There is a very significant (P 0.01) relationship between the dissolving rate of Valsartan tablets and the three parameters CD, Starch 1500, and Soluplus. Optimal tablet formulation of valsartan achieved the desired dissolving rate of 85.75 percent in 10 minutes. When compared to the current Valsartan product on the market, the improved Valsartan IR tablet formulation showed faster absorption, higher plasma concentrations, and greater bioavailability (123.5%). In conclusion, Valsartan IR tablets with a dissolution rate of around 85% after 10 minutes might be improved with the adoption of a 23-factorial design.

Basant A. et al. (2018), The limited oral bioavailability (49%) of the effective anti-gout medication febuxostat (FXS) is due to its low water solubility and relatively strong first-pass impact. The purpose of this research was to improve the solubility and bioavailability of FXS by using a chosen FXS self-nanoemulsifying system (s-SNES) in sublingual fast-dissolving films (SFs). Solvent casting was used to transform the s-SNES into SFs. In a fully factorial study, the effects of polymer and plasticizer type on the SFs' mechanical properties and FXS dissolution profile were analyzed. The most desirable SF was identified through numerical optimization based on a set of criteria. Polyvinylpyrrolidone K30 (at 6% w/v), polyethylene glycol 300 (at 20% w/w), and s-SNES (1 g) were combined, and 0.5% w/v Avicel PH101 to create the optimized SF (O-SF). O-SF was shown to be effective in delivering FXS via the sublingual tissue of sheep. The disintegration profile of FXS did not alter much when O-SF was stored for three months. The effectiveness of O-SF in vivo in rabbits was compared to that of commercially available oral tablets (StaturicVR 80 mg). Pharmacokinetic parameters were determined using a cross-over design with no sequence effect. Compared to commercially available tablets, O-SF statistically performed better, with greater Cmax, AUC0-24, AUC0-1, apparent t1/2 and lower tmax and apparent kel. O-SF was shown to have a relative bioavailability of 240.6% compared to the commercially available tablet. This demonstrates that the study's goals of increasing the dissolution rate and bioavailability of FXS via the use of a patient-friendly formulation were successfully accomplished.

Brito Raj et al. (2019), Polydispersity index, millivolt zeta potential, scanning electron microscopy, entrapment efficiency, and nanoparticle size were all used to assess the Simvastatin NLC preparation that was made using an improved hot homogenization approach. The PI for the improved NLC F7 formulation is 0.480 0.24. NLC loaded transdermal patches have been shown in in-vivo

pharmacokinetic experiments to boost the bioavailability of simvastatin relative to the commercially available oral dose version. The results of the research demonstrated that a transdermal NLC patch containing the medication is an effective method of providing drugs with limited bioavailability.

Hypertension is a cardiovascular illness that requires lifelong treatment, according to research published in 2018 by Zafar et al. Oral administration is a common method of delivering medication to patients. The release from the S-PE-SNEDDS formulation was much higher (p 0.05; 97.874.89% in 1 h vs. 27.872.65% from pure PE) in the first hour. It outperformed PE dispersion in terms of antioxidant activity and antibacterial efficacy against Staphylococcus aureus and Pseudomonas aeruginosa. Better antihypertensive effect (p 0.05) and 4.92-fold greater relative bioavailability were seen in the in vivo activity in rats compared to pure PE dispersion. Overall, the findings suggest that S-PE-SNEDDS may be a more effective strategy for oral administration in terms of absorption and therapeutic action.

METHODOLOGY

Materials

Biocon Ltd of Bangalore, India, donated simvastatin. VAV Life Sciences private limited in Mumbai, India, generously provided the soybean phosphatidylcholine. We got our cholesterol from the Factory in Mumbai, India. All of the chemicals and materials used were of the highest possible quality.

Methods

Preparation of Simvastatin Proliposomes

The vacuum rotary evaporator film deposition on carrier technique was used to create SV-containing proliposomes. The proliposomes were made by combining lecithin, mannitol, and cholesterol in varying concentrations. Tables 1 and 2 show the breakdown of the ingredients. To ensure full mannitol drying, the flask containing the appropriate formulations was spun under vacuum at 115 rpm for 30 minutes. Chloroform and methanol (8:2, v/v) were used to dissolve the necessary quantities of SV, cholesterol, and lecithin. A 5 mL aliquot of the organic solvent was poured into the round bottom flask and the temperature was set to 37 degrees Celsius. After thoroughly drying, the last 5 mL of each aliquot of solution was discarded. Before being sieved into # 100 meshes, Overnight, we dried the flask holding the proliposomes in a vacuum desiccator. After collecting the powder, it was placed in a tightly closed container for analysis.

Fourier Transformed Infrared

Fourier transformed infrared (FT-IR) analysis verified the existence of possible drug-excipient interactions. A drug-polymer or formulation combination was pressed together with powdered potassium bromide to create pellets. The FT-IR spectra of powder diffuse reflection was obtained using an FT-IR spectrophotometer (Shimadzu- 8400S, Japan).

Differential Scanning Calorimetry

The DSC-60 equipment was used to conduct the analysis on both individual pharmaceuticals and combined formulations. High-purity alpha-alumina discs were employed to represent an empty cell. Nitrogen gas was used as the atmosphere and a heat rate of 10°C min1 was used for the complicated scans. A value of J/Kcal has been assigned to the energy.

DOI: 10.48047/intjecse/V12I1.201057 ISSN: 1308-5581 Vol 12, Issue 01 2020

Table 1: Formulation Chart of Simvastatin Proliposomes with Different Carriers

Sr. No.	Simvastatin	LECIVA-S70	Cholesterol	D-mannitol	D-sorbitol	MCCP
1	5 mg	100 mg	50 mg	1 g	–	–
2	5 mg	100 mg	50 mg	–	1 g	–
3	5 mg	100 mg	50 mg	–	–	1 g

Table 2: Formulation Chart of Simvastatin Proliposomes

Formulation	Drug (mg)	LECIVA-S70 (mg)	Cholesterol (mg)	D-mannitol (mg)
PL 1	5	50	100	1000
PL 2	5	100	100	1000
PL 3	5	100	50	500
PL 4	5	100	100	500
PL 5	5	50	100	500
PL 6	5	100	50	1000
PL 7	5	50	50	500
PL 8	5	50	50	1000

Drug Content

In order to extract the medication from the proliposomes, In order to extract the sample formulation, 1000 mg was put in a 50 mL volumetric flask and dissolved in 100% methanol. (1st dilution). In a 25-milliliter volumetric flask, 5 milliliter aliquots of the original solution were diluted with pure methanol. The absorbance at 237.7 nm was then measured using a UV-Vis spectrophotometer to determine the medication concentration. (Shimadzu- UV 1800, Japan).

Percentage Yield (% Yield)

The proliposome yield was calculated by weighing the processed product together with the phospholipid, carrier, and cholesterol that went into making the proliposomes. The formula was used to calculate the interest rates in percentage terms.

$$\text{Percentage yield} = \frac{\text{Mass of proliposomes}}{\text{Mass of drug} + \text{Mass of the excipients}} \times 100$$

DOI: 10.48047/intjecse/V12I1.201057 ISSN: 1308-5581 Vol 12, Issue 01 2020

Percentage Encapsulation Efficiency

As soon as the proliposomes were ready, they were placed in a centrifuge and spun for 30 minutes at 15,000 RPM. The % efficiency of SV-loaded proliposomes was estimated using the centrifugation technique. Centrifuged at 15,000 rpm for 30 minutes, the prepared proliposomes were left in the centrifuge tube. One milliliter of the supernatant was taken out and diluted with methanol. Samples were appropriately diluted before UV absorption measurements were taken. The free SV was measured at 237.7 nm using a UV spectrophotometer. The supernatant contains the unbound medication in its entirety as free SV. Encapsulation efficiency is reported as a percentage of the medicine that was successfully encapsulated.

$$\%\text{Encapsulation efficiency} = \frac{\text{Total mass of the drug} - \text{free dissolved drug}}{\text{Total mass of the drug}} \times 100$$

In Vitro Diffusion Studies

At 37 0.5°C, in phosphate buffer pH 7.4, the drug, physical mixes, and proliposome formulations were introduced to 900 mL of paddle type and stirred at 50 rpm speed. Five-milliliter aliquots were obtained and replaced with new medium every hour from 1 to 12. Using a UV-visible spectrophotometer calibrated with a blank reading of 237.7 nm, the collected samples were analyzed for drug content.

In Vivo Studies

These tests were conducted to determine how well the produced formulation functioned in plasma relative to the pure medication. After receiving clearance from the JSS Faculty of Pharmacy's animal ethics committee, the study was conducted (Institutional Animal Ethics Committee). Wistar rats that were 4 months old and weighed between 150 and 200 g were used in the experiments. Rats of both sexes were employed in the experiment. Before receiving the test formulations, the rats fasted for 12 hours prior to the experiment. Blood samples were taken at certain intervals after the formulations were applied. All of the rats used in the experiment were kept in a controlled environment before being randomly assigned to one of three groups: group I received the proliposome formulation; group II received pure SV; and group III served as a control. Each group was given 10 milligrams of SV per kilogram of body weight. At 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours after receiving the optimized formulation, the rats' jugular vein blood was taken, and the plasma was separated (without additional storage at 20°C) and the medication was extracted. Simvastatin peak area ratio calibration curves were used to calculate the concentration of untested plasma samples.

Extraction Procedure

In order to get protein samples out of rat plasma for chromatographic analysis, A ratio of 2.5 mL of acetonitrile to 0.5 mL of plasma was employed. After centrifuging at 5000-6000 rpm for 10 minutes, the precipitate was resuspended in 1 mL of acetonitrile using a 1-minute vortexing procedure. After being centrifuged, the samples were analyzed using HPLC injection of 20 L of material.

Stability Studies

The research' end objective is to predict how long a product will stay fresh by hastening its decomposition, most ideally by improving temperature (T) and relative humidity (RH) conditions (RH). Studies on stability have been conducted using the optimized formulations. Experimental conditions

were maintained at 25 2°C, 60 5% RH, and 40 2°C, 75 5% RH for a total of 30 days, during which time the optimal formulation was placed in a container with a screw lid. Physical appearance and spectrometric analysis of the drug ingredient at 237.7 nm have been performed on samples taken on days 0, 5, and 30.

RESULTS AND DISCUSSION

FT-IR Study of Drug-Loaded Proliposomes

The FTIR spectra of the pure medication exhibited no discernible differences from those of the physical combination and the excipients. The large SV sums at 3549.62 cm1, 2924 cm1, 1268.24 cm1, and 1165 cm1 were all preserved (Fig. 1), suggesting that the pure medication and the polymer do not interact. As can be observed in Fig. 1, the FT-IR spectra of SV-loaded proliposomes are quite similar to those of the pure drug.

The expected SV peaks could be seen in proliposome spectra. Hence, the pharmaceuticals and excipients are suitable for one another. This demonstrated that there had been little alteration to the drug's molecular structure. The free O-H vibration frequency of pure SV samples was 3549 cm1, the CH vibration frequency was 2924 cm1, and the LV frequency was 1268.24 cm1. All of these defining peaks appear at about the same wave number in the SV spectra of proliposomes and physical mixtures.

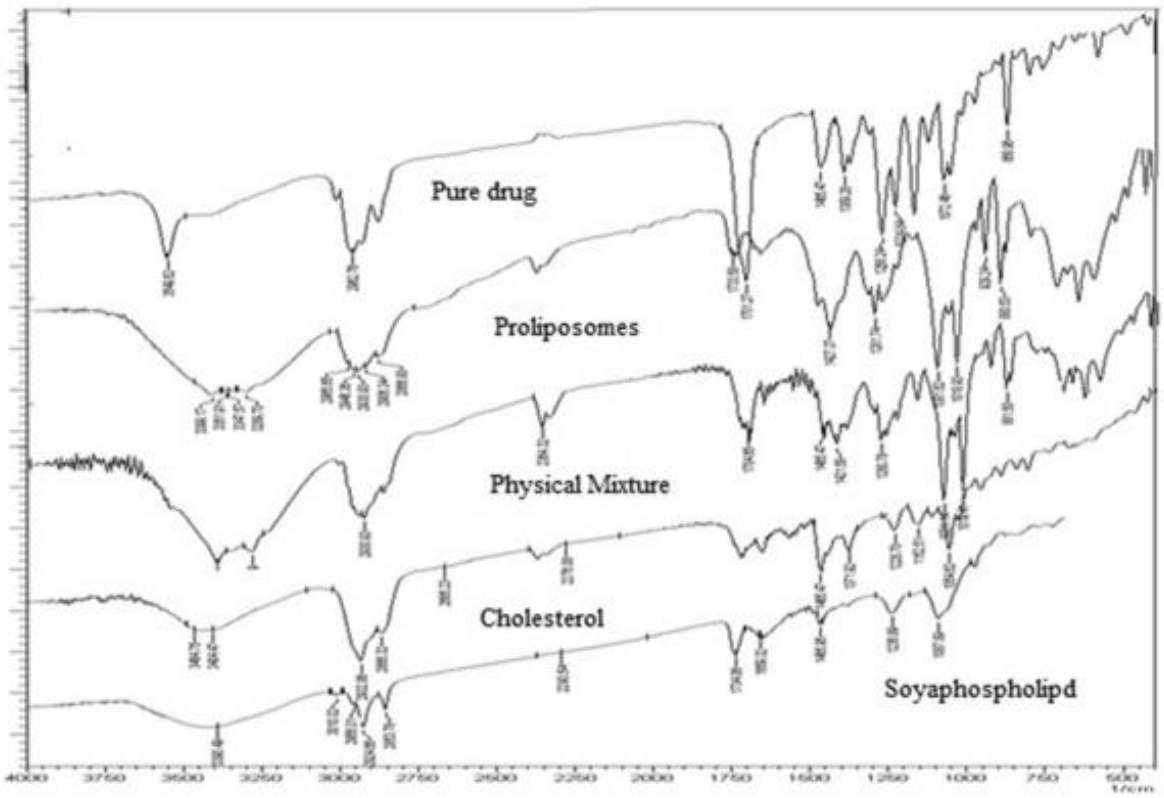


Fig. 1: Comparative FT-IR spectra

Differential Scanning Calorimetry

In the DSC thermogram of SV, an endothermic peak was clearly visible at 138.41 °C. There was no drug-to-drug interaction seen since the endothermic peak of the physical combination matched that of the medicine. The melting endothermic peak in the DSC thermogram of pure drug occurs at 138.41

DOI: 10.48047/intjecse/V12I1.201057 ISSN: 1308-5581 Vol 12, Issue 01 2020

degrees Celsius, with a fusion enthalpy (Hf) of -31.72 J/g. The melting point of SV-loaded proliposomes, as shown by their thermal curve, is 138.44 degrees Celsius, with a fusion enthalpy of 18.31 J/g. The thermograms of the physical combination, pure simvastatin, and the optimized formulation are shown side-by-side in Figure 2. (PL6).

Drug Content

According to Table 3, more than 95.26 percent of the DC was detected in the manufactured proliposomes. All dosage forms of the medicine have received the same distribution. Drug concentration ranges from 95.26 0.025 to 99.12 0.014 percent.

Percentage Yield

Throughout the process of formulating proliposomes, the final return on investment was less than 100%. As a result, the drug's yield percentage has changed. When film deposits have been finished on the carrier system, the percentage of the product that will be recovered has been calculated. The percentage yields vary from 74.130.02 to 76.880.94 for PL6, the most frequent and lowest of the PL3 formulations. The results are shown in Table 3.

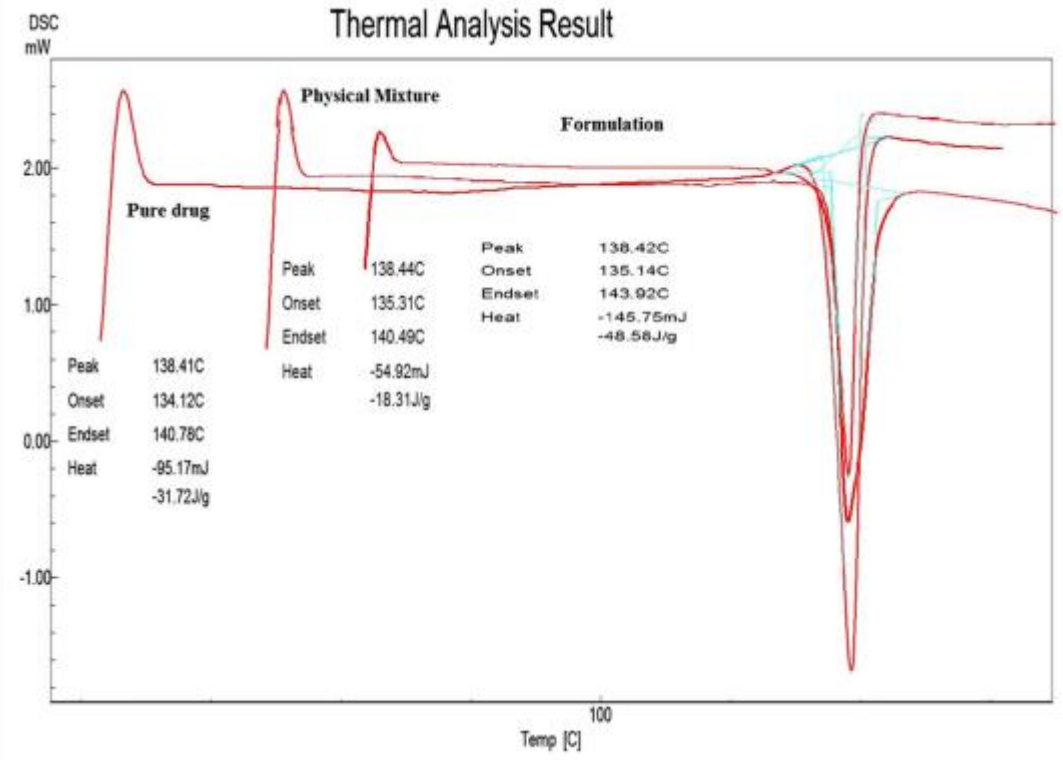


Fig. 2: Comparative DSC thermogram

Determination of % Entrapment Efficiency

The quantity of medication enclosed by the lipid bilayer is proportional to its encapsulation efficiency. The formulation's phospholipids, transporters, and cholesterol determine how well the encapsulation process works. Table 3 shows the EE ranging from 45.14 0.079 to 53.23 0.084% for the various formulations. Since they raise the stiffness of the liposomal membrane, cholesterol and phospholipids improve entrapment efficiency. Due to increased drug competition in the bilayer for cholesterol packing, an increase in cholesterol content decreased the trapping efficiency. Over a specific quantity of

DOI: 10.48047/intjecse/V12I1.201057 ISSN: 1308-5581 Vol 12, Issue 01 2020

cholesterol, the regular linear shape of vesicular membranes may be altered, contributing to the observed drop in trapping effectiveness. The drug concentration, yield, and encapsulation efficiency of proliposomes are shown graphically in Figure 3.

Table 3: Drug content, % Yield, % Encapsulation efficiency data of proliposomes

Formulation	% Drug content	% Yield	% Encapsulation efficiency
PL 1	95.26 ± 0.025	74.13 ± 0.02	45.64 ± 0.192
PL 2	98.28 ± 0.043	76.01 ± 0.08	46.16 ± 0.161
PL 3	96.53 ± 0.012	73.13 ± 1.84	45.14 ± 0.079
PL 4	97.34 ± 0.015	75.88 ± 0.84	52.23 ± 0.084
PL 5	95.62 ± 0.017	74.83 ± 1.03	47.74 ± 0.078
PL 6	99.12 ± 0.014	76.88 ± 0.94	53.23 ± 0.084
PL 7	98.47 ± 0.017	76.22 ± 0.97	47.87 ± 0.081
PL 8	97.07 ± 0.014	75.23 ± 0.98	48.23 ± 0.049

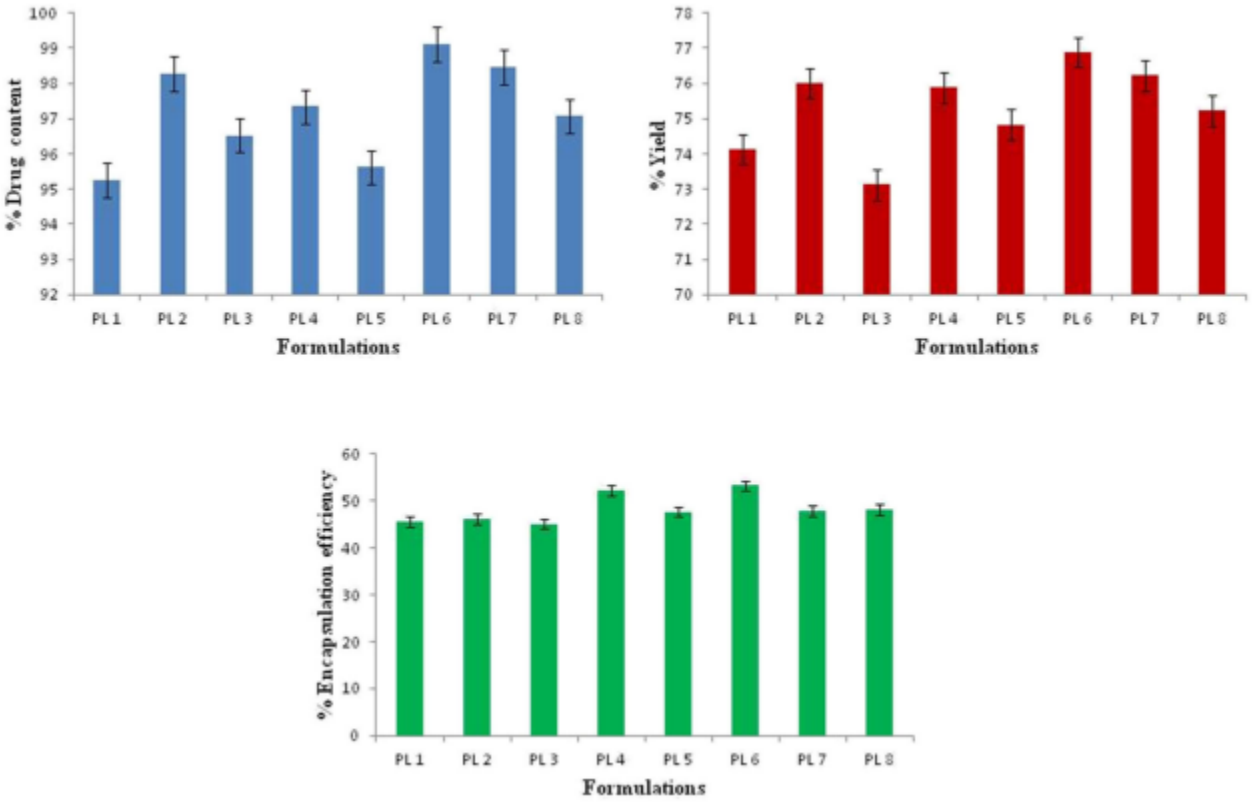


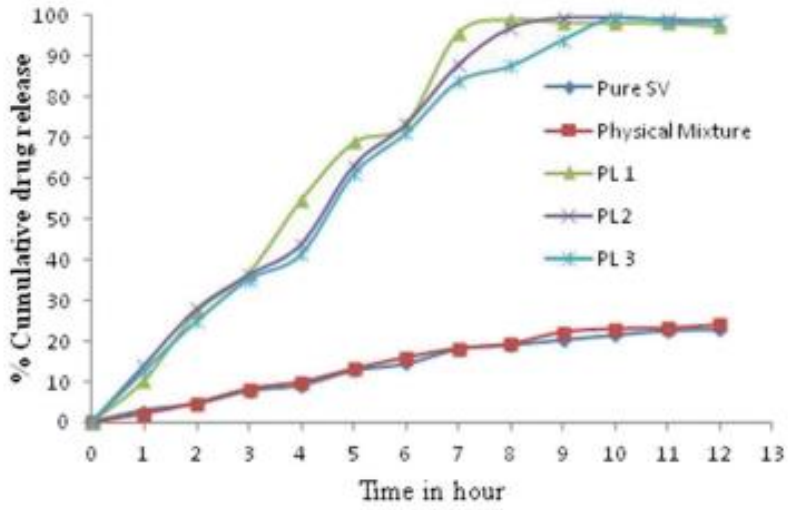
Fig. 3. Bar graph showing encapsulation efficiency of proliposomes

In Vitro Drug Release

There was a rapid onset to the liposomes' behavior, followed by a slower, more drawn-out phase that lasted for 12 hours. The first, sharp increase in release might be due to an explosion in the drug's liposomes' outer area caused by a non-trapped molecule. There may be a negative correlation between how well a trap works and how quickly medications are released. Drug release from PL6 is clearly slower than that of other formulations. The entrapment efficiency data complements the in vitro release information. Initial SV release lasted for the first 2 hours, with concentrations ranging from 9.44 to 19.45%, followed by a gradual slowing of the release rate. As can be shown in Fig. 4, the in vitro drug release profile for all eight formulations (PL1-PL8) was between 99.07 and 99.78% after 12 hours. As the lipid bilayers are cholesterol stabilized, the findings demonstrated that it takes some time for SV to be released from the liposomes (Fig. 5). As a result, liposomes may be used to create a depot effect. The insoluble SV medication was dramatically enhanced in its dissolving efficiency when encapsulated in proliposomes. This may be because phospholipid molecules improve SV's solubility, or because the medication has changed from a crystalline to an amorphous form. Since the drug is stable and released gradually at the site of action, the findings imply that the formulation of proliposomes meets the demand for an effective approach for the sustained the medicine in delivery.

In Vivo Release Studies

PL6 formulations were selected for in vivo research because they met the criteria for optimal particle size, entrapment efficiency, and in vitro release. Figure 5 displays the chromatographic peak of pure simvastatin after standardizing the chromatographic conditions for the determination of retention time and wavelenght. Optimized formulation was subjected to a pharmacokinetic investigation in Wistar rats (per mandated CPCSEA criteria) (PL6). All of the rats used in the experiment were healthy ones; there were three groups total, with the first receiving the formulation, the second receiving pure SV, and the third serving as a control. Both groups were given an SV dosage of 10 mg/kg.



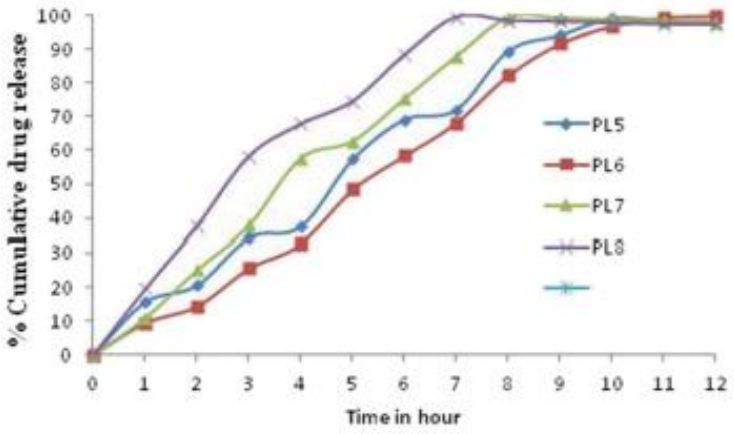


Fig. 4: In vitro dissolution profile of formulations (PL1–PL8), pure simvastatin, physical mixture

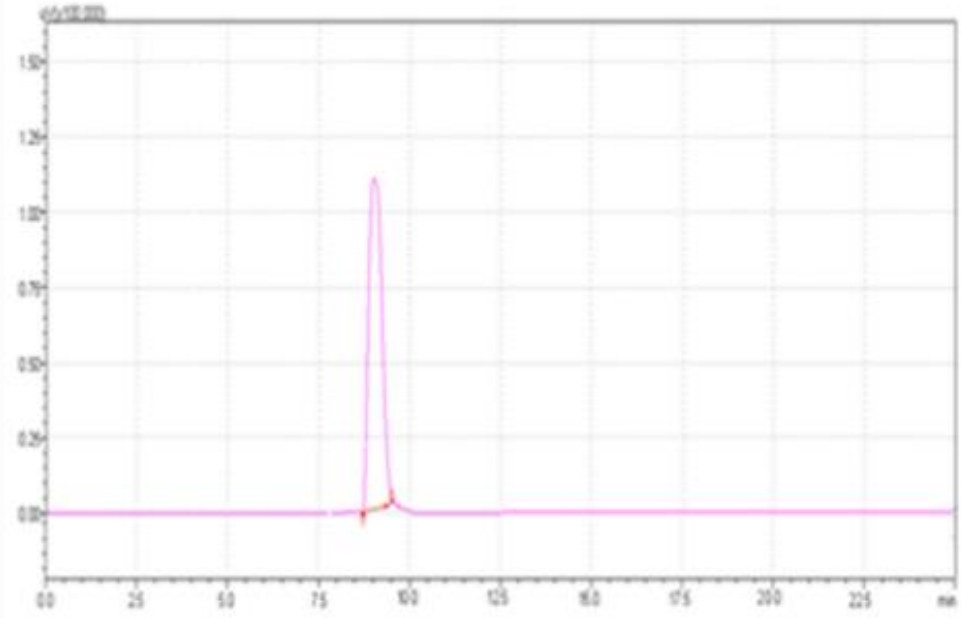


Fig. 5: Simvastatin peak obtained by HPLC at the end 12 h

CONCLUSION

In this research, proliposomes' potential as an improved oral delivery mechanism for SV was investigated. The SV proliposoms were created using a film deposition technique, with mannitol serving as the carrier and LECIVA-S70 and cholesterol serving as the active ingredients in different ratios. Physicochemical characterisation, dissolution tests, and in vitro drug release were used to fine-tune the PL6 formulation. Studies of the pharmacokinetics of SV in living organisms have shown its superior absorption and oral bioavailability.

REFERENCES

1) Basant A. Habib, Amina S. Abd El-Samiae, Boushra M. El-Houssieny & Randa Tag (2018), Formulation, characterization, optimization, and in-vivo performance of febuxostat self-nano-emulsifying system loaded sublingual films, Drug Delivery, 28:1, 1321-1333, DOI: 10.1080/10717544.2021.1927247

2) Khan, M. I., Yaqoob, S., Madni, A., Akhtar, M. F., Sohail, M. F., Saleem, A., Tahir, N., Khan, K. U., & Qureshi, O. S. (2016). Development and In Vitro/Ex Vivo Evaluation of Lecithin-Based Deformable Transfersomes and Transfersome-Based Gels for Combined Dermal Delivery of Meloxicam and Dexamethasone. BioMed research international, 2022, 8170318. https://doi.org/10.1155/2022/8170318

3) N. Tirumalesh and K. P. R. Chowdary, (2019) "Formulation Optimization and In Vitro and In Vivo Preclinical Evaluation of Valsartan IR Tablets," International Research Journal of Pharmacy and Medical Sciences (IRJPMS), Volume 2, Issue 2, pp. 66-70.

4) Brito Raj, S., Chandrasekhar, K.B. & Reddy, K.B. (2019), Formulation, in-vitro and in-vivo pharmacokinetic evaluation of simvastatin nanostructured lipid carrier loaded transdermal drug delivery system. Futur J Pharm Sci 5, 9. https://doi.org/10.1186/s43094-019-0008-7

5) Zafar, A., Imam, S.S., Alruwaili, N.K., Alsaidan, O.A., Elkomy, M.H., Ghoneim, M.M., Alshehri, S.M., Ali, A.M., Alharbi, K.S., Yasir, M., Noorulla, K.M., Alzarea, S.I., & Alanazi, A.S. (2018). Development of Piperine-Loaded Solid Self-Nanoemulsifying Drug Delivery System: Optimization, In-Vitro, Ex-Vivo, and In-Vivo Evaluation. *Nanomaterials, 11*.

6) Garg, V.; Singh, H.; Bhatia, A.; Raza, K.; Singh, S.K.; Singh, B.; Beg, S. Systematic Development of Transethosomal Gel System of Piroxicam: Formulation Optimization, In Vitro Evaluation, and Ex Vivo Assessment. AAPS PharmSciTech 2017, 18, 58–71.

7) Ahmed, T.A. Preparation of Transfersomes Encapsulating Sildenafil Aimed for Transdermal Drug Delivery: Plackett–Burman Design and Characterization. J. Liposome Res. 2015, 25, 1–10.

8) Ahmed, A.; Ghorab, M.; Gad, S.; Qushawy, M. The Application of Plackett-Burman Design and Response Surface Methodology for Optimization of Formulation Variables to Produce Piroxicam Niosomes. Int. J. Drug Dev. Res. 2013, 5, 121–130.

9) Gilani, S.; Mir, S.; Masood, M.; Khan, A.K.; Rashid, R.; Azhar, S.; Rasul, A.; Ashraf, M.N.; Waqas, M.K.; Murtaza, G. TripleComponent Nanocomposite Films Prepared Using a Casting Method: Its Potential in Drug Delivery. J. Food Drug Anal. 2018, 26, 887–902.

10) Verma, P.; Pathak, K. Nanosized Ethanolic Vesicles Loaded with Econazole Nitrate for the Treatment of Deep Fungal Infections through Topical Gel Formulation. Nanomed. Nanotechnol. Biol. Med. 2012, 8, 489–496.

11) Mbah, C.C.; Builders, P.F.; Agubata, C.O.; Attama, A.A. Development of Ethosomal Vesicular Carrier for Topical Application of Griseofulvin: Effect of Ethanol Concentration. J. Pharm. Investig. 2019, 49, 27–36.

12) Ghanbarzadeh, S.; Hariri, R.; Kouhsoltani, M.; Shokri, J.; Javadzadeh, Y.; Hamishehkar, H. Enhanced Stability and Dermal Delivery of Hydroquinone Using Solid Lipid Nanoparticles. Colloids Surf. B Biointerfaces 2015, 136, 1004–1010.

13) Garg, B.J.; Garg, N.K.; Beg, S.; Singh, B.; Katare, O.P. Nanosized Ethosomes-Based Hydrogel Formulations of Methoxsalen for Enhanced Topical Delivery against Vitiligo: Formulation Optimization, In Vitro Evaluation and Preclinical Assessment. J. Drug Target. 2016, 24, 233–246.

14) Ahmed, A.; Ghorab, M.; Gad, S.; Qushawy, M. Design, Formulation, and Evaluation of Piroxicam Niosomal Gel. Int. J. PharmTech Res. 2014, 6, 185–195.

15) Ramakrishna, G.A.; Manohar, S.D.; Bhanudas, S.R. Ethosomes: Carrier for Enhanced Transdermal Drug Delivery System. J. Adv. Pharm. Educ. Res. 2014, 4, 380–387.