DESIGN AND *IN VIVO* EVALUATION OF NAPROXEN-LOADED TRANSFEROSOMAL GEL FOR TRANSDERMAL DELIVERY

HIMA BINDU E., RADHA G. V.*

Department of Pharmaceutics, GITAM School of Pharmacy, GITAM Deemed to be University, Visakhapatnam-530045, Andhra Pradesh, India
*Corresponding author: Radha G. V.; *Email: radhagadela@gmail.com

ABSTRACT

Objective: The main objective of the present study was to formulate, optimize, and evaluate naproxen transfersomal gels.

Methods: Reverse phase evaporation was used to create thirteen different formulations of naproxen-loaded transfersomes. Using Response Surface Methodology (RSM) and Central Composite Designs (CCD), the influence of independent process variables, such as soy lecithin, cholesterol content and surfactant concentration, on dependent variables, such as entrapment effectiveness and vesicle size of naproxen transfersomes, was assessed. *In vitro*, *ex-vivo*, and *in vivo* drug release of formulations were also studied.

Results: It was discovered that the NTG7 formulation of transfersomes had the maximum entrapment effectiveness and ideal vesicle diameter. The optimized NTG7 formulation displayed a maximum drug content of 97.4% and a maximum drug release of 88.03%. The release of naproxen from the final gel adhered to the Korsmeyer-Peppas release model. The *ex-vivo* drug release of the optimized formulation was found to be 85.91% for 24 h. The maximum drug concentration after oral administration was 843.54±7.67ng/ml, and T_{max} was 3.0±0.08h. The improved formulation's $AUC_{0-\infty}$ was greater than the commercial formulation's. A higher drug concentration in the blood compared to the marketed formulation suggested better systemic absorption of naproxen from the gel formulation. After three months, at a temperature range of 2 to 8 ℃, the formulation demonstrated correct semisolid consistency and good stability and there was also no appreciable change in the initial values of appearance, pH, and % drug content.

Conclusion: The above findings imply that the gel created using naproxen-loaded transfersomes may be a potentially valuable new formulation.

Keywords: Naproxen, Transfersomal gel, Reverse phase evaporation, Central composite design, Independent process variables, Dependent variables

INTRODUCTION

Rheumatoid arthritis (RH) is one of the leading causes of disability, especially in older people [1]. A non-steroidal anti-inflammatory drug (NSAID) called naproxen is regarded as the first-line treatment for the symptoms of ankylosing spondylitis and rheumatoid arthritis [2]. The anti-inflammatory effect of NSAIDs is brought on by the inhibition of cyclooxygenase, and the consequent decrease in prostaglandin synthesis causes undesirable side effects. Because the majority of NSAIDs must be taken in numerous daily doses to maintain therapeutic blood levels, patient noncompliance is another prevalent therapeutic issue. The half-life of naproxen is between two and five hours. Following oral treatment, naproxen absorbs efficiently. Within two to three hours of oral treatment, the plasma concentration reaches its peak. Adopting new drug delivery strategies is necessary to prevent naproxen's systemic side effects and improve its therapeutic effectiveness and patient compliance [3, 4]. These drawbacks of oral administration can be avoided by topical administration of these drugs [5-7].

Drug delivery has long been made possible by transdermal drug delivery systems (TDDS) [8, 9]. Most importantly, TDDS can be successfully used when medication therapy is required for long-term or chronic use. Therefore, the creation of TDDS is a possibility for treating several pathological illnesses, including rheumatoid arthritis [10]. The use of nano-formulations has become a practical way to get around the drawbacks of transdermal therapy. The field of transdermal drug administration makes substantial use of transfersomes and ultra-flexible liposomes. They can effectively transfer the medicine into or through the skin depending on the application, thanks to their ultra-flexible membrane characteristics. Transfersomes are more suited for skin penetration because they are more elastic than other vesicular delivery techniques [11-14].

Despite the present advancements, several issues need to be tackled. To fully comprehend the mechanism of penetration, fundamental and thorough molecular-level investigations are needed. The safety issues from the formulation aspect should be thoroughly assessed to hasten the transition from the clinic to the market [15]. New and potential therapeutic modalities against a variety of diseases may result from further scientific research on transfersomes [16]. The formulation of transferosomes was attempted in the current investigation, and CCD has been used to optimize formulation variables that may have an impact on the formulation characteristics of naproxen transferosomes [17].

MATERIALS AND METHODS

Materials

Naproxen was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad, India. Soyalecithin, Cholesterol, Span 80, and carbopol-934 were purchased from Sigma Aldrich Ltd, India. All of the other chemicals and reagents utilized in this experiment were of an analytical grade.

Methods

Preparation of naproxen-loaded transfersomes

According to the literature, transfersomes were formed using the reverse phase evaporation method with a few changes. At first, lipids such as soya lecithin and cholesterol were taken in a beaker. In the same beaker add Span 80 as an edge activator, which was then dissolved in a solvent solution of diethyl ether and chloroform. The thin coating formed after 24 h of keeping the beaker at ambient temperature. A probe sonicator was used to sonicate the drug solution, which contained 1000 mg of the drug per ml of water, for two minutes. After that, the film was hydrated with phosphate buffer saline (pH 7.4), and it was then subjected to a further 2-minute sonication to produce transferosomal suspensions. To improve chemical penetration, 2% v/v of dimethyl sulfoxide (DMSO) was added to each solution. All the prepared transferosomal suspensions were filtered through Whatman® filter paper No. 40. Finally, the suspensions were put in 1% w/v carbopol and kept in a cool, dark environment to prevent oxidation [18, 19].

Experimental design

In the current investigation, Design expert software Version 12 was employed to enhance two key transferosomal parameters, specifically

vesicle size and percentage entrapment efficiency. Component A is cholesterol (soy lecithin), and component B is the edge activator were chosen as the independent variables. The size of the vesicle and percentage entrapment efficiency was chosen as dependent variables. Components A and B were given values of 10 mg for the lower value and 90 mg for the higher value. Response surface plots were created

utilizing the CCD based on the mentioned independent and dependent variables. Based on preliminary experiment results and the feasibility of creating transfersomes at extreme values, the experimental ranges for each element were selected. All variable values and batch codes are listed in table 1. To choose which optimum formulation to utilize, the software's solution was used [20-22].

Table 1: Naproxen transferosomal gel formulation

Std	Run	Factor 1A: Lipid	Factor 2B: Edge activator	Drug (Naproxen mg)	Carbopol 934 (%w/v)
8	1	50	50	25 mg/10 ml	1%
9	2	50	50	25 mg/10 ml	1%
13	3	106.569	50	25 mg/10 ml	1%
6	4	50	50	25 mg/10 ml	1%
3	5	50	50	25 mg/10 ml	1%
4	6	-6.56854	50	25 mg/10 ml	1%
7	7	50	-6.56854	25 mg/10 ml	1%
2	8	10	10	25 mg/10 ml	1%
11	9	90	10	25 mg/10 ml	1%
1	10	50	106.569	25 mg/10 ml	1%
5	11	90	90	25 mg/10 ml	1%
12	12	50	50	25 mg/10 ml	1%
10	13	10	90	25 mg/10 ml	1%

Characterization of transfersomes

Fourier transform-infrared spectroscopy (FT-IR) studies

For infrared analysis of samples utilizing the KBr pellet technique, the FTIR spectrum of drug sample and drug combined with excipients was used. Dry potassium bromide was combined with around 1-3 mg of the sample, and the mixture was then used to analyze the samples in transmission mode over a 4000-400 cm^{-1} wave number range [23, 24]. The peaks correspond to those in fig. 5 depict the distinctive drug absorption for various functional groups.

Vesicle size, entrapment efficiency, and drug loading

Using a Malvern Zetasizer 3000, dynamic light scattering (DLS) was used to measure the vesicle's size. The polydispersity index (PI), which is a gauge of the width of the size distribution, is produced by DLS together with the mean diameter. Ultra-purified water was used to dilute the sample before the experiment. At 20,000 rpm and 10 °C for 30 min, the produced transferosomal suspensions were ultra-centrifuged. Following centrifugation, 1 ml of the supernatant was diluted by adding 9 ml of 7.4 phosphate saline buffer, and the absorbance was then determined using a UV-Vis spectrophotometer by measuring absorbance at 330 nm for naproxen [25, 26]. The formula below was used to determine the drug entrapment efficiency and drug loading,

$$\% \, EE = \frac{Qt - Qs}{Qt} * 100$$

$$\% \, DL = \frac{Qt - Qs}{Qu} * 100$$

In vitro drug release studies

With the aid of a modified Franz diffusion cell, research on the *in vitro* release of several transfersome formulations was conducted. The cellophane membrane was fixed to a 2.5 cm diffusion area diffusion cell assembly. The receptor fluid was stirred at 100 rpm in a 22.5 ml phosphate buffer with a pH of 5.5 that was kept at 37±0.5 °C for the duration of the tests. The produced formulation was inserted into the donor compartment's membrane. At predetermined intervals, aliquots of the 2 ml sample were removed and promptly replaced with an equivalent volume of fresh diffusion medium [27, 28]. By that time, a graph of the drug release was calculated and plotted and shown in fig. 6 and 7.

Ex-vivo study

For the *ex-vivo* permeation experiment employing a modified manufactured Franz diffusion cell, the complete thickness of the skin of hairless Swiss albino mice was utilized. A 2.5 cm² effective diffusion area of the skin clamped between the donor and receptor

chambers. Phosphate buffer pH 5.5, freshly prepared, was placed into the receptor chamber. The solution in the receptor chamber was continually stirred at 300 rpm using a magnetic stirrer and a hot plate while the diffusion cell was kept at 37 °C. The donor chamber was gently inserted with 2 ml of transferosome formulation containing naproxen. At 1, 2, 3, 4, 5, 6, 12, 18, and 24 h, 5.0 ml of the solution in the receptor compartment was taken out, analyzed with a UV-Vis spectrophotometer, and then immediately replaced with an equal volume of fresh PBS with a pH of 5.5 [29].

pH measurement

A digital pH meter was used to determine the pH of the gel composition.0.25 g of transferosome-based gel was precisely weighed and dissolved in 25 ml of distilled water. Before each usage, the pH meter was calibrated using buffer solutions with pH values of 4.0, 7.0, and 9.0.

Spreadability index

To have a successful therapeutic response, the formulation must have sufficient dosage availability to absorb from the skin. Two to five grams of gel were placed between two slides and gradually raised in weight by adding it to a weight pan. The amount of time it took for the top plate to face a distance of 10 cm after 80 grams of weight were added was observed. Less spread time is indicated by good spreadability. The below formula determines it.

$$S = m * l/t$$

Viscosity

The Brookfield viscometer was used to measure the viscosity of the manufactured topical transferosomal gel of naproxen, with an ideal speed of 10 rpm.

Zeta potential

An instrument called a Zetasizer was used to calculate the gel's zeta potential. A transparent, reusable zeta cell was used to operate the device at a constant temperature of 25 °C. The particles' mean diameter and PI were determined by using the cumulant analysis and the results are shown in fig. 10 [30].

Stability

Transferosomal gel preparations underwent stability testing for approximately 3 mo at room temperature, and characteristics like pH, preparation appearance, and drug content of optimized formulations were assessed and the results are shown in table 12 [31, 32].

Differential scanning calorimetry (DSC)

To determine the physical compatibility, a DSC was used to conduct a thermal examination of a few different drugs and polymer mixes.

Amounts of the sample ranging from 1 to 2 mg were placed in sealed aluminium sample pans during each scan at a temperature of 10 °C/min in a nitrogen environment between 25 and 350 °C. As a guide, an empty aluminum pan was used [33].

Transmission electron microscopy (TEM)

TEM was used to examine the formulation's morphology. On a film-coated copper grid, one drop of diluted transferosomal suspension was placed, stained with one drop of 2% w/v aqueous phosphotungstic acid solution, and then allowed to dry for contrast enhancement. The material was analyzed using TEM at a 72000X magnification [34].

Confocal laser scanning microscopy (CLSM)

Transfersomes made with Rhodamine B dye were applied evenly and non-occlusively to the excised abdomen skin of Wistar rats for 24 h. The skin was washed with distilled water and alcohol before being placed on the slide with the SC facing up. The slide was made with a thickness of 5 to 10 m. After that, the slide was examined for the z-axis using a CLSM that was outfitted with a 540 nm stimulating Argon laser beam and a 625 nm emission. The Rhodamine B dye was applied in the same way as described above, using a simple solution for comparison [35].

Naproxen *in vivo* study in animals

Twelve Male white New Zealand rabbits were procured from Vab bioscience, 1-6-197/45/D, Bapuji Nagar, Musheerabad, bearing the Reg No: 282/PO/RcBt/s/2000/CPCSEA of weight 2–2.5 kg were checked for the absence of any diseases and selected for the *in vivo* study by randomized cross-over design. The animals were kept in 25 °C, ventilated animal rooms with a 12/12 h light/dark cycle. Water and food were available whenever needed. Before beginning the study, the animals were given a week to become acclimated. All animal experiment protocols have been approved by the CMR College of Pharmacy's institutional animal ethics committee (CPCSEA/1657/1AEC/CMRCP/COL-20/97). Before the day of administration, rabbits were fasted overnight but were allowed access to water. The weight of the animals was measured and recorded before the start of each experiment. Thereafter, six rabbits were given oral doses of Naproxen Marketed drug. The hair of the other six rabbits at the abdominal site of the gel application was clipped before the experiment. Before applying the gel, the skin was gently wiped with warm water followed by an alcohol swab, and patted dry. Gel with an area of about 20 cm containing Naproxen was then applied to the shaved skin. Blood samples of approximately 0.5 ml were collected in dried heparinized tubes at regular intervals after oral administration and administration from the jugular vein and, as soon as possible, 0.1 ml of the whole blood was transferred accurately to another tube which was then frozen at −20 °C and stored until analysis. A blood sample of 1 ml was collected periodically to prevent clotting 1 ml of heparin solution was added previously to the sample container. These samples were then centrifuged for 30 min at 2500 rpm. 1 ml of clear supernatant was taken after suitable dilution. The supernatant layer was separated and reconstituted with 500 ml of mobile phase and vortexed and filtered through 0.45 mm syringe filters and 20 ml of the sample solution was injected for HPLC analysis [36]. The validated analytical method was found satisfactory and proved to be adequate for the determination of Naproxen in plasma. The corresponding pharmacokinetic (PK) parameters of rabbit plasma are summarized in table 13.

PK analysis

The PK parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max} i.e., T_{max} and $t_{1/2}$ values, the area under the plasma concentration-time curve (AUC) from zero to the last sampling time (AUC_{0-t}), the AUC from zero to infinity ($AUC_{0-\infty}$). AUC_{0-t} was calculated by the linear trapezoidal rule [37].

RESULTS AND DISCUSSION

Statistical formulation optimization based on the design of experiments has typically been used in the development of numerous pharmaceutical dosage forms by designing a set of experiments that measure the responses by fitting a mathematical model to the data and also conducting suitable statistical tests to ensure that the best possible model is chosen and determining the values of factors investigated. In the development of diverse drug delivery systems, CCD, one of many statistical designs for formulation optimization, is frequently used. A response surface design, which is a component of the CCD, provides details on the individual effects, pair-wise interactions, and curvilinear variable impacts of the many elements explored in the design.

Design-Expert Software Version 12 suggested a CCD for the current study that took into account the concentration of the surfactant and the amount of soy lecithin in a total of 13 experimental formulations of transferosomal gels containing naproxen. As a result, it was investigated how these factors affected the % entrapment efficiency and vesicle size of prepared transferosomal gels containing naproxen. Table 1 provides a summary of the matrix of the design, which also includes the experimental strategy and observed response values. To obtain model equations involving distinct primary components and interaction factors for each response under study, the values of observed responses measured for all trial formulations were fitted in the central composite design. One-way ANOVA was used to statistically test these models ($p<0.05$). The models seemed to be significant ($p<0.05$) when the model p values for both measured responses were less than 0.05.

Response 1 (Vesicle size)

Table 2: Fit summary

Source	Sequential P-value	Lack of Fit P-value	Adjusted R²	Predicted R²	
Linear	<0.0001		0.8623	0.7604	
2FI	0.0111		0.9280	0.8531	Suggested
Quadratic	0.1692		0.9443	0.7690	
Cubic	0.0298		0.9809	0.4902	Aliased

Table 3: Sequential model sum of squares [Type I]

Source	Sum of squares	df	Mean square	F-value	P-value	
Mean vs Total	2.253E+06	1	2.253E+06			
Linear vs Mean	1.004E+05	2	50182.02	38.58	<0.0001	
2 FI vs Linear	6889.00	1	6889.00	10.13	0.0111	Suggested
Quadratic vs 2FI	2436.14	2	1218.07	2.31	0.1692	
Cubicvs Quadratic	2780.47	2	1390.23	7.70	0.0298	Aliased
Residual	903.12	5	180.62			
Total	2.366E+06	13	1820E+05			

Table 4: ANOVA for 2FI model

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	1.073E+05	3	35751.01	52.58	<0.0001	significant
A-Cholesterol: soyalecithin	97106.93	1	97106.93	142.81	<0.0001	
B-Surfactant	3257.11	1	3257.11	4.79	0.0564	
AB	6889.00	1	6889.00	10.13	0.0111	
Residual	6119.74	9	679.97			
Lack of Fit	6119.74	5	1223.95			
Pure Error	0.0000	4	0.0000			
Cor total	1.134E+05	12				

The model is noteworthy because of its Model F-value of 52.58. An F-value this large could only happen owing to noise with a 0.01% chance. Significant model terms are those with P-values less than 0.0500. In this instance, the model terms A and AB are significant. Values over 0.1000 shows that the model terms are insignificant. Model reduction may enhance our model if there are numerous insignificant model terms (except those needed to maintain hierarchy).

Table 5: Fit statistics

Std. Dev.	26.08	R^2		0.9460
Mean	416.31	Adjusted R^2		0.9280
C. V. %	6.26	Predicted R^2		0.8531
		Adequate (Adeq) Precision		22.6528

The Adjusted R^2 of 0.9280 and the Predicted R^2 of 0.8531 are reasonably in agreement; the difference is therefore less than 0.2. The signal-to-noise ratio is measured by Adeq Precision. The ideal ratio is greater than 4. Our ratio of 22.653 denotes a sufficient signal. The design space can be explored using this model.
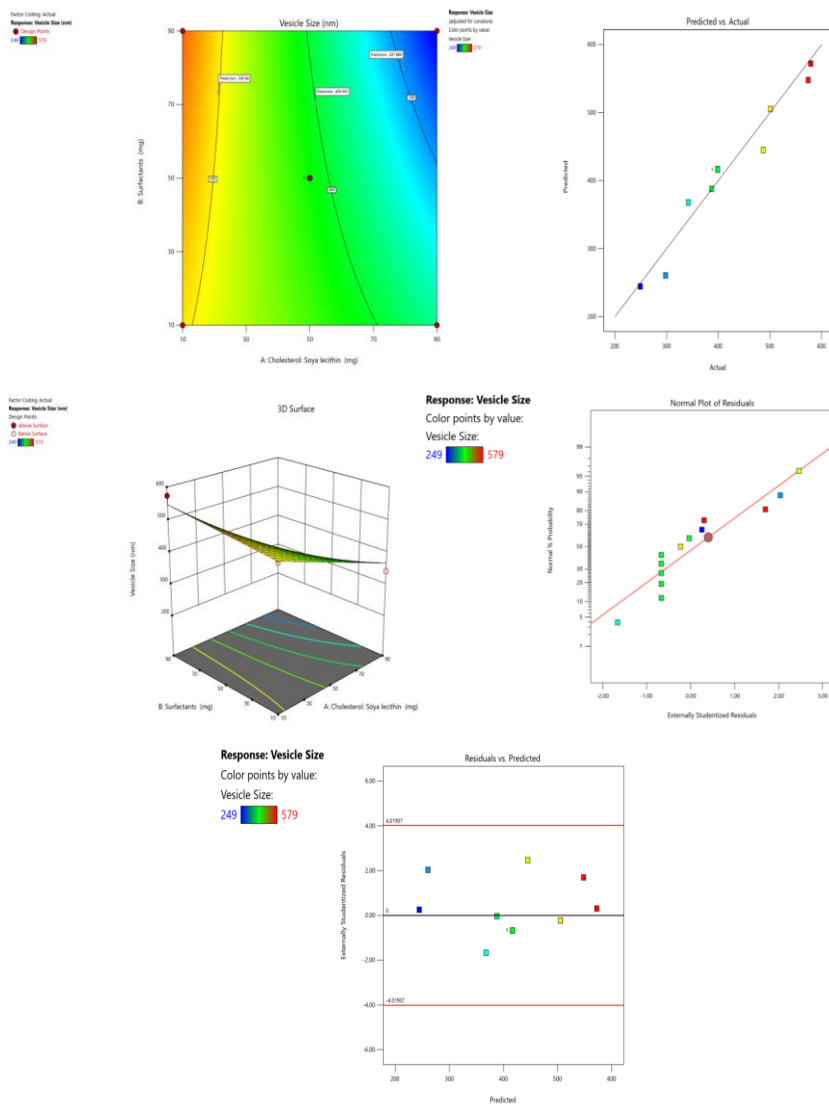

Fig. 1: Three-dimensional surface plots showing the effect of variables on vesicle size

Table 6: Response 2: entrapment efficiency

Source	Sequential P-value	Lack of Fit P-value	Adjusted R^2	Predicted R^2	
Linear	<0.0001		0.8456	0.7246	
2FI	0.4228		0.8409	0.6730	
Quadratic	0.0137		0.9400	0.7510	Suggested
Cubic	<0.0001		0.9993	0.9813	Aliased

Table 7: Sequential model sum of squares [Type I]

Source	Sum of squares	Df	Mean square	F-value	P-value	
Mean vs Total	93585.31	1	93585.31			
Linear vs Mean	372.65	2	186.32	33.85	<0.0001	
2FI vs Linear	4.00	1	4.00	0.7053	0.4228	
Quadratic vs 2FI	36.07	2	18.03	8.43	0.0137	Suggested
Cubic vs quadratic	14.85	2	7.43	297.04	<0.0001	Aliased
Residual	0.1250	5	0.0250			
Total	94013.00	13	7231.77			
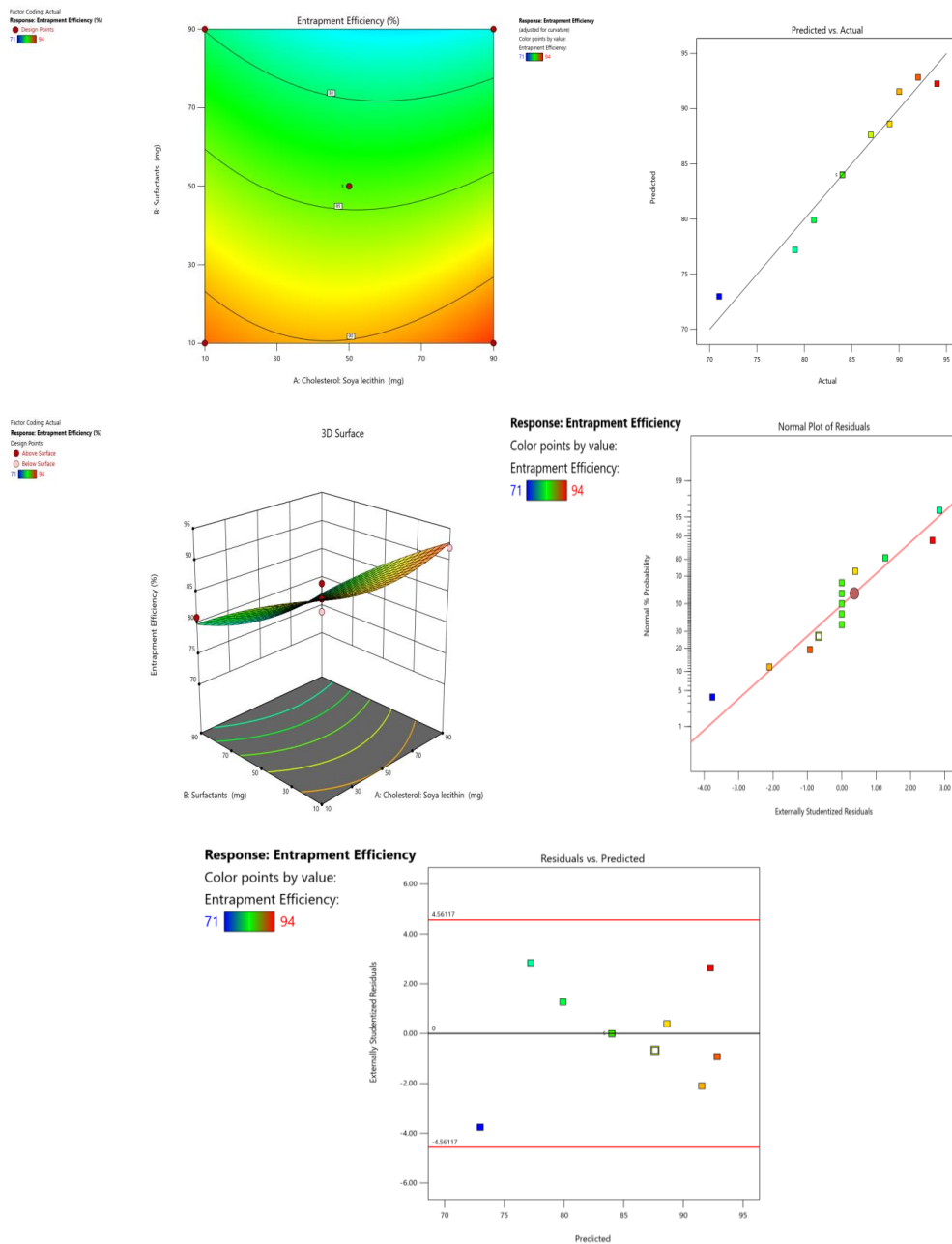
Fig. 2: Three-dimensional surface plots showing the effect of variables on entrapment efficiency

Table 8: ANOVA for quadratic model

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	412.72	5	82.54	38.58	<0.0001	Significant
A-Cholesterol: Soyalecithin	1.00	1	1.00	0.4674	0.5162	
B-Surfactant	371.65	1	371.65	173.70	<0.0001	
AB	4.00	1	4.00	1.87	0.2138	
A^2	29.59	1	29.59	13.83	0.0075	
B^2	3.29	1	3.29	1.54	0.2550	
Residual	14.98	7	2.14			
Lack of Fit	14.98	3	4.99			
Pure Error	0.0000	4	0.0000			
Cor total	427.69	12				

The model is significant because of the Model F-value of 38.58. An F-value this large could only happen owing to noise with a 0.01% chance. Significant model terms are those with P-values less than 0.0500. In this instance, B and A2 are important model terms. If the value is higher than 0.1000, the model terms are not considered relevant. Model reduction may enhance our model if there are numerous insignificant model terms.

Table 9: Fit statistics

Std. Dev.	1.46	R^2	0.9650
Mean	84.85	Adjusted R^2	0.9400
C. V. %	1.72	Predicted R^2	0.7510
		Adeq Precision	19.9767

The Predicted R^2 of 0.7510 and the Adjusted R^2 of 0.9400 are reasonably in agreement; that is, the difference is less than 0.2. The ratio of signal to noise is measured by Adeq Precision. A ratio of at least 4 is preferred. Our ratio of 19.977 suggests that the signal is sufficient. To move around the design space, utilize this model.

Solubility studies

All of the excipients, including the medication, should completely dissolve in the solvent and yield a clear, transparent solution to improve film-forming ability and good stability following hydration. The solvents in the formulation may potentially promote drug flux through the membrane by increasing penetration. Since naproxen is more soluble in ethanol than in water in this investigation, ethanol improves the formulation's ability to permeate the skin.

Chemical compatibility studies (FTIR)

It was found that all of the naproxen characteristic peaks were present in the combination FT-IR spectra of the drug and the drug with the polymers, suggesting the compatibility of the medicine with the utilized polymer.
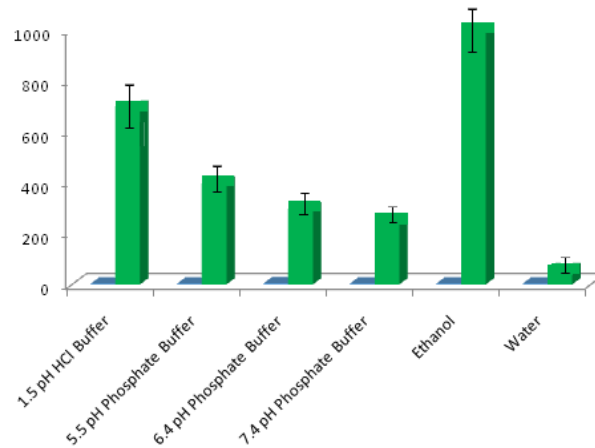

Fig. 3: Solubility chart of naproxen, (All determinations were performed in triplicate and values were expressed as mean±SD, n=3)
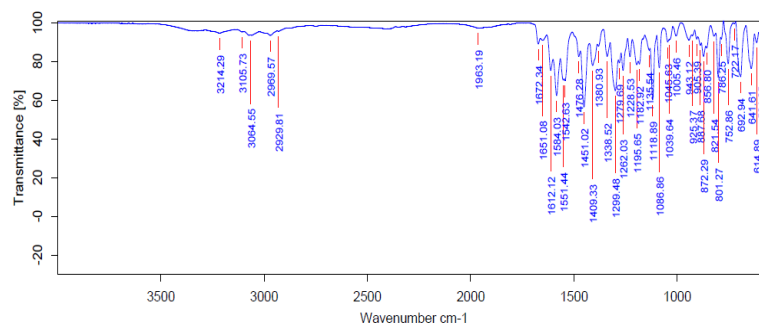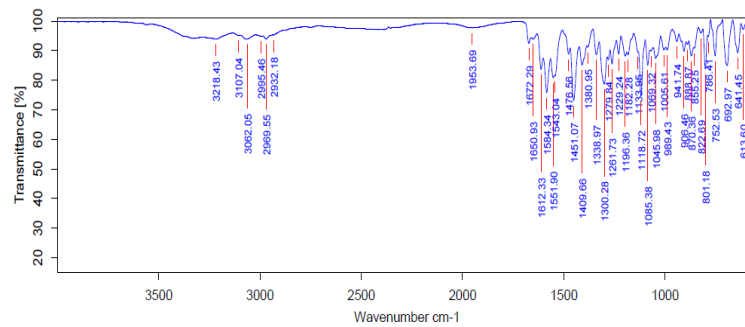

Fig. 4: FTIR spectrum of naproxen

Fig. 5: FTIR spectrum of naproxen+soyalecithin+Cholesterol+Span 80

Table 10: FTIR interpretation of naproxenandexcipients

S. No	Functional group	Wave number	Pure naproxen	Naproxen+Soyalecithin+Tween 20+Tween 80
1	$(C_6H_6)_2$	1500-400	1391.57-511.48	1457.62-521.41
2	C=0	1660-1690	1584.27	1636.60s
3	COOH	1760-1690	-	1733.67

Vesicle size, % entrapment efficiency and drug content

Different transferosomes (n=13) were created in this study using RSM, CCD, and characterization before use to ensure uniform batches. Table 11 displays the % yield, drug content and vesicle size of transferosomes. The prepared transferosomes of naproxen were found to have vesicle sizes between 249 and 579 nm, which is said to be an ideal nano size for increasing the permeability and bioavailability of the drug. This enhanced decrease in the obtained VS values might be attributed to the decrease in interfacial tension during vesicle formation and due to the steric repulsion between the charged edge activator molecules arranged on the surface of the transferosomes, causing an increase in the curvature of the vesicles membrane [23]. The surfactant concentration on any vesicle or carrier preparation plays a very important role, as it was stated in the literature that increasing surfactant concentration decreases the permeability and bioavailability of the vesicle. The concentration of surfactants was retained at its maximum because the current research's surfactant concentration was performed by Design Expert software. As a result, all formulations were found to have an entrapment efficiency of between 71 and 94%. This indicates that there is no leakage and all of the formulations have good entrapment efficiency. Edge activator with higher HLB yielded vesicles with the larger aqueous spaces needed for drug entrapment within the formed lamellae and provided greater protection for drug leakage from the formed transferosomes, resulting in higher encapsulation efficiency [24]. When compared to other formulations, the NTG7 formulation demonstrated higher entrapment efficiency. All formulations had drug content ranging from 88.4% to 97.4%. When compared to other formulations, the F7 formulation demonstrated a high of 97.4%.

Table 11: Vesicle size, % entrapment efficiency, and drug content of Naproxen

S. No.	Formulation number	Vesicle size (nm)	Entrapment efficiency (%)	Drug content
1	NTG1	399	84±0.02	92.8±0.032
2	NTG2	399	84±0.123	92.8±0.043
3	NTG3	298	87±0.042	93.5±0.642
4	NTG4	399	84±0.056	92.8±0.853
5	NTG5	399	84±0.132	92.8±0.214
6	NTG6	579	89±0.032	94.5±0.123
7	NTG7	487	94±0.342	97.4±0.062
8	NTG8	501	90±0.845	95.2±0.742
9	NTG9	342	92±0.123	96.1±0.942
10	NTG10	387	71±0.153	88.4±0.213
11	NTG11	249	79±0.064	90.2±0.842
12	NTG12	399	84±0.321	92.8±0.731
13	NTG13	574	81±0.871	92.2±0.021

(All determinations were performed in triplicate and values were expressed as mean±SD, n=3)

*In vitro d*iffusion studies for naproxen transferosomes and release kinetics

Fig. 6 and 7 depict the dissolving profiles of 13 various naproxen-loaded transferosomal formulations. The *in vitro* release tests revealed that naproxen from transferosomes was released quickly and completely. This initial flush might be due to the release of the drug entrapped near the surface layers of the formed transferosomes, while the rest of the drug entrapped in the deep layers was released in a slower manner later [26]. After 24 h, all formulations showed a drug release of at least 80%. At 24 h, F7 had a maximum drug release of 88.03%. The extended-release might be due to the enhanced viscosity of the formed gels, which in turn reduced the drug release. Surfactant molecules make transferosomes more organized and less leaky, preventing drug release. High surfactant ratios may generate mixed micelles that are less responsive to concentration gradients [33].

The *in vitro* drug release data were fitted to the Korsmeyer-Peppas release model to understand the process of drug release from the transferosomes, and the interpretation of release exponent values sheds insight on the mechanism of drug release from the dosage form. The optimized formulation demonstrated zero-order kinetics with an anomalous diffusion mechanism (super case II transport), according to these values.
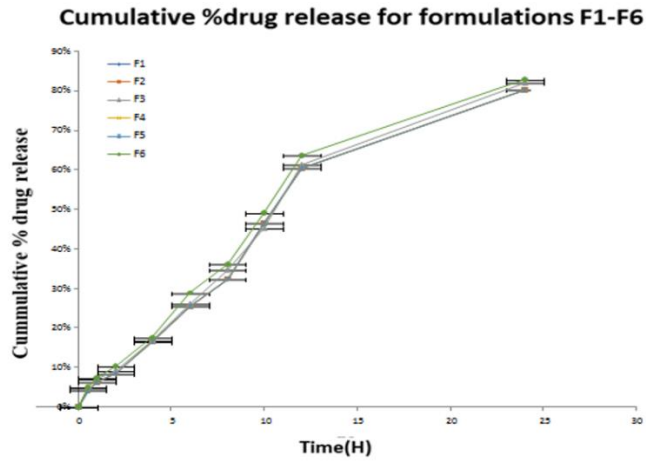
Fig. 6: %Cumulative drug release for formulations F1-F6, (All determinations were performed in triplicate and values were expressed as mean±SD., n=3)
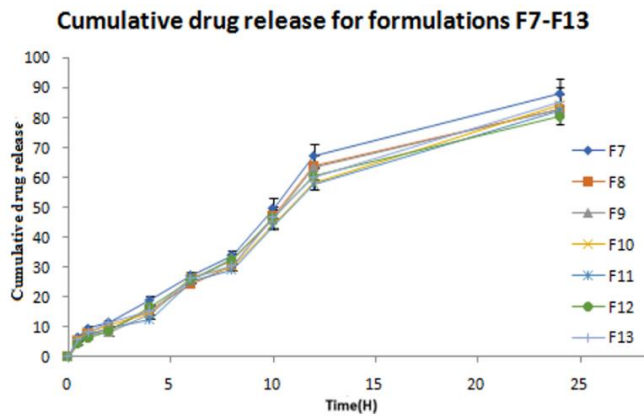

Fig. 7: %Cumulative drug release for formulations F7-F13, (All determinations were performed in triplicate and values were expressed as mean±SD., n=3)
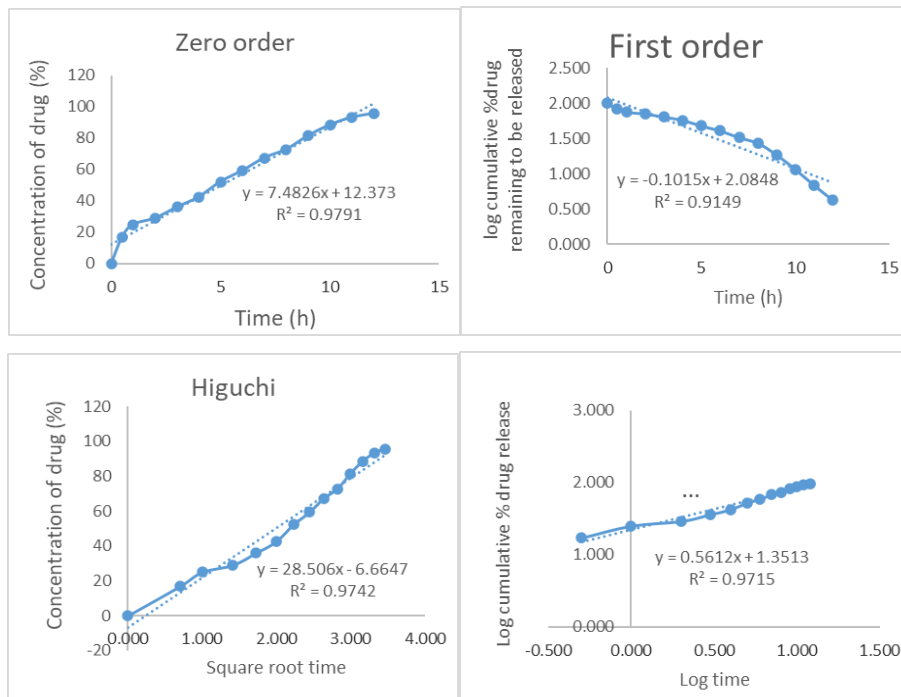

Fig. 8: Drug release kinetics for F7 formulation of naproxen transferosomes

Ex-vivo permeation study for F7 formulation of naproxen

Formulation, NTG7 of naproxen, was determined to generate better entrapment efficiency (94%) and optimal vesicle size based on the results of 2 primary answers (dependent variables) selected for design expert software or optimization. Therefore, these two formulations were taken into account for the subsequent assessments. The *ex-vivo* drug release of the improved formulation (F7) was determined to be 85.91% for 24 h in the fig. 9 below. Transfersomes have better permeation ability than other liposomal formulations because transferosomal vesicles are ultra deformable elastic and flexible vesicles [30].

Ex-vivo Permeation Study for F-2 formulation of Naproxen
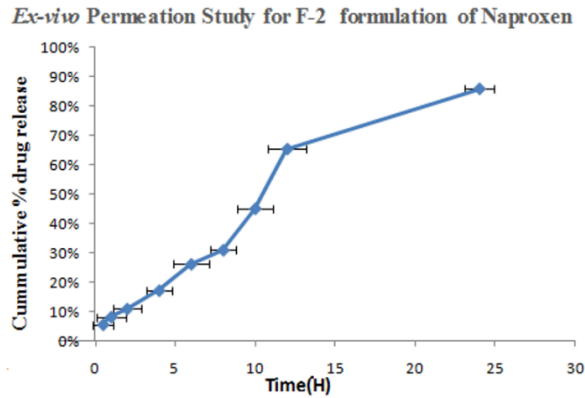
Fig. 9: %Cumulative drug release for F7 formulation of naproxen, (All determinations were performed in triplicate and values were expressed as mean±SD., n=3)

pH results

To check irritation, the pH must be determined. Since the transferosomal gel must be given topically, the pH ranges from 5.5 to 6. This appears to be the best way to apply anything transdermally. NTG7 formulation pH was found to be 5.8. The stated pH was acidic therefore, skin discomfort was possible. The negative charge of transferosomal vesicles enhances transdermal drug absorption [38].

Spreadability

The spreadability of transferosomal gels should be as stable as possible because extremes in either direction suggest ineffective spreading. The transferosomal gels should spread effectively and uniformly since they display pseudoplastic flow. The outcomes of NTG7 (spreadability index 14.87 gcm/sec) have demonstrated that the formulation's spreadability is at its best.
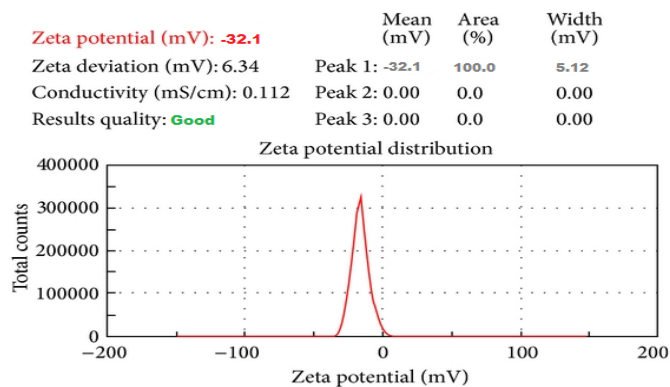
Viscosity

NTG7 was discovered to have a viscosity of less than 3,500 centipoises which is 3100 cps, which appears to be gel-like rather than cream-or oil-like.

Zeta potential

According to fig. 10's depiction of the NTG7 formulation's zeta potential, which is suitably high (-32.1 mV), the complexes would be stable and their tendency to aggregate would be minimal. The colloidal suspensions are homogeneous because they have a low PI. The results confirmed colloidal stability of all transferosomal vesicles. Due to significant electrostatic repulsion, vesicles do not aggregate, ensuring stability [39].

Stability studies

For roughly 90 d, stability investigations were carried out at room temperature, and appearance, pH, and % drug content were assessed. With NTG7, there was also no appreciable change in the initial values of appearance, pH, and% drug content. Thus, it was discovered that the formulations were stable at room temperature and they have the capacity for protection of drug molecules. The physical stability of the prepared transferosomal formulation and the corresponding in situ gel could be confirmed, with no drug leakage from the formed vesicles [33].

	Mean (mV)	Area (%)	Width (mV)
Zeta potential (mV): **-32.1**			
Zeta deviation (mV): 6.34	Peak 1: -32.1	100.0	5.12
Conductivity (mS/cm): 0.112	Peak 2: 0.00	0.0	0.00
Results quality: Good	Peak 3: 0.00	0.0	0.00

Fig. 10: Zeta potential of NTG7 formulation

Table 12: Stability studies of naproxen transferosomal gel

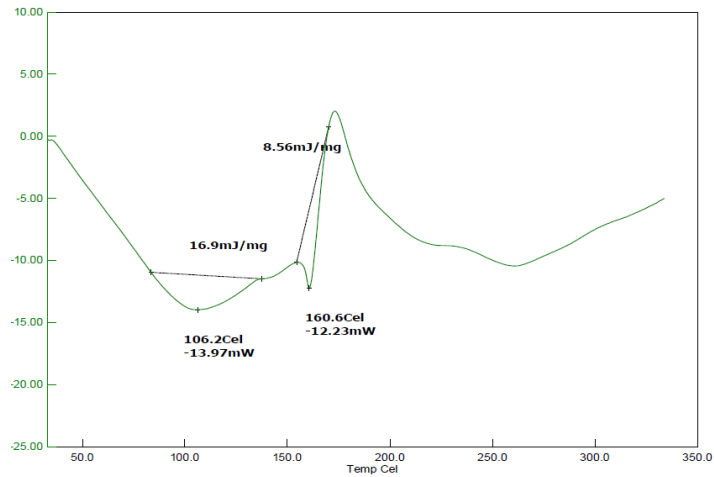| S. No.	No. of days	Evaluation parameter	Initial	NTG7
1	30 d	Appearance	Light Yellow color	No Change
2	60 d	pH	5.6	No Change
3	90 d	% Drug content	96.7	No Change

Fig. 11: DSC thermograms of NTG7 and naproxen with excipients

DSC

The thermal stability of the formulation is demonstrated by a DSC thermogram. The drug was present in the amorphous phase and may have been uniformly distributed throughout the transferosomes, according to DSC measurements.

TEM

The TEM indicates the morphology of vesicles, and the TEM images for NTG7 were found to be spherical with a uniform surface. The transferosomal vesicles were clearly spherical with very flawless borders. The measured vesicle size matched ZetaSizer readings within ≤200 nm. The uniform surface and shape of vesicles have proved that there won't be any drug leakage happening from the formulations [40].

Confocal laser scanning microscopy (CSLM) studies

The drug's vesicle penetration depth was investigated using CLSM. The created transfersomal formulation was evenly dispersed throughout the skin's strata, as demonstrated by the CLSM's high fluorescence intensity. Rhodamine B dye was put into an improved transfersomal formulation, and a confocal laser scanning microscopic image of the formulation revealed that it penetrated much deeper and fluoresced much more intensely than the Rhodamine B dye solution. This effective transferosome transport of Rhodamine B shows that it has increased penetration and subsequently fused with the membrane lipids in the deeper layers of the skin, supporting the findings of previous researchers [41]. As a result, the plain dye solution was not able to penetrate to the same degree as the transfersomal formulation.
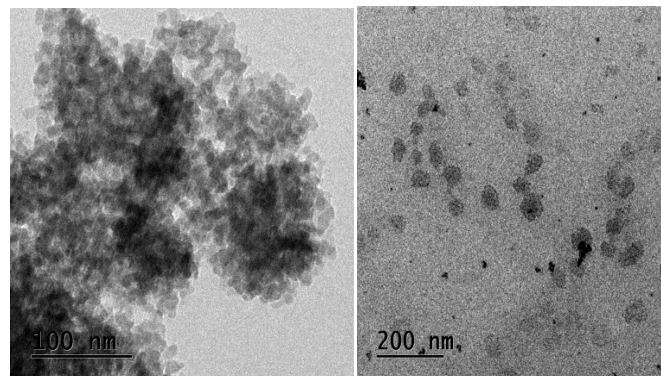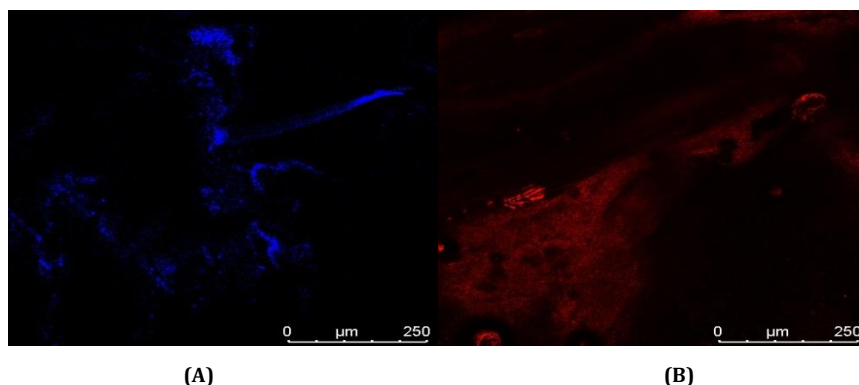

Fig. 12: TEM image of NTG7

| (A) | (B) |

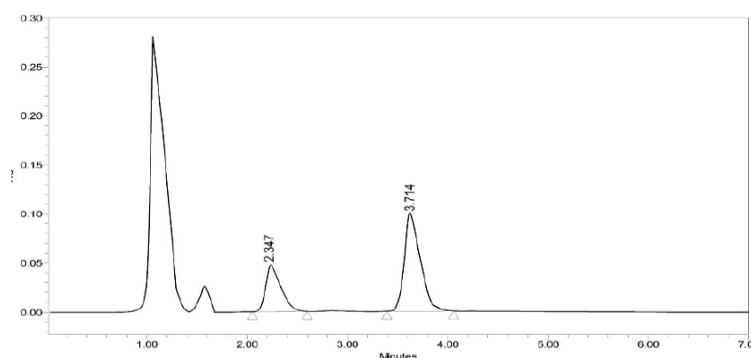**Fig. 13: CLSM images of naproxen and transferosomal naproxen gel**

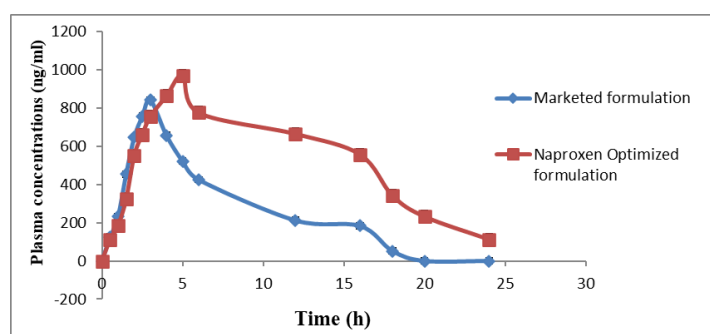Fig. 14: Standard HPLC chromatogram of naproxenin rabbit plasma

Fig. 15: Comparison of plasma drug concentrations for marketed formulation and naproxen-optimized formulation, (All determinations were performed in triplicate and error bars were omitted)

Table 13: Comparison of PK parameters of marketed formulation and naproxen-optimized formulation

PK parameters	Marketed formulation	Naproxen optimized gel
C_{max} (ng/ml)	843.54±7.67	971.32±3.87
AUC_{0-t} (ng. h/ml)	5157.76±10.34	6166.84±13.83
$AUC_{0-\infty}$(ng. h/ml)	6454.22±16.13	7767.15±19.29
T_{max}(h)	3.0±0.08	5.0±0.4
$t_{1/2}$ (h)	10.4±0.28	14.02±0.95

(All determinations were performed in triplicate and values were expressed as mean±SD, n=3)

PK analysis

The *in vivo* bioavailability studies in rabbits were conducted to examine the ability of the laboratory-developed gel of Naproxen Plasma concentration profiles of Naproxen marketed formulation with gel-optimized formulation in rabbits are shown in fig. 15. The results of PK parameters, including C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$ are given in table 13. The maximum drug concentration, C_{max}, after oral administration was 843.54±7.67ng/ml, and T_{max} was 3.0±0.08h. For the Naproxen gel, C_{max} and T_{max} were 971.32±3.87ng/ml and 5.0±0.4h, which was significantly different compared to the marketed Drug. The T_{max} values in all rabbits were higher for gel than oral administration. AUC is an important parameter in evaluating the bioavailability of a drug from the dosage form, as it represents the total integrated AUC and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for the optimized formulation was higher (7767.15±19.29ng. h/ml) than the marketed formulation (6454.22±16.13ng. h/ml). Statistically, the AUC_{0-t} of the gel formulation was significantly higher (P<0.05) as compared to the marketed formulation. A higher amount of drug concentration in blood indicated better systemic absorption of Naproxen from gel formulation as compared to the marketed formulation.

As we go deeper into this cutting-edge subject of nanotechnology, it becomes imperative to comprehend that it provides a significant component of their employment in a variety of compositions to maximize the permeability of many therapeutic chemicals. Many transferosome products are currently being used in cutting-edge clinical trials, which serve as evidence of this. The success of various transferosome treatments for cutaneous and transdermal distribution may be soon witnessed on the global market, which is interesting.

CONCLUSION

The effectiveness of the Naproxen-loaded transferosomes was created, optimized, and tested in the current work. The produced vesicles were in the nano-size range and demonstrated an acceptable level of entrapment effectiveness. When compared to the marketed formulation, the prepared naproxen gel formulation showed a higher amount of drug concentration in blood, indicating better systemic absorption of Naproxen from the gel. The C_{max} and T_{max} values in all rabbits were higher for gel than for oral administration. $AUC_{0-\infty}$ infinity for the optimized formulation was higher than the marketed formulation. In conclusion, Naproxen effectively increased skin permeability by being entrapped within transfersomal vesicles, which increased the drug's efficacy. Therefore, transfersomal drug delivery systems may be potential carriers for overcoming the skin's natural barrier function and enhancing drug absorption via the skin.

carry out the research work. They also extend their gratitude towards the head of the department and principal of pharmaceutical sciences, GITAM University, Visakhapatnam, Andhra Pradesh, India for their encouragement and support.

AUTHORS CONTRIBUTIONS

Contributions from each author are equal.

CONFLICT OF INTERESTS

The authors state that this paper has no actual, potential, or perceived conflicts of interest.

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