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Formulation and evaluation of transdermal ultradeformable vesicles of aspirin

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ABSTRACT

Transfersomes are incredibly elastic and deformable vesicles composed of phospholipids and edge activators. This study proposed aspirin-loaded transfersomes for transdermal administration to prevent gastrointestinal side effects, boost drug permeation rate, and extend drug action. The formulations were prepared via the thin-film hydration method using soy lecithin as a vesicle forming agent and tween 80 as an edge activator. The formulation trials were optimized by 'Custom design' JMP SW13. The optimum formulation yielded a vesicle size of 74.4 nm, a zeta potential of -27.4 mV, and a % entrapment efficiency of 90.5%±0.25 with a drug release of 88.65 %±0.34. A 1% carbopol gel incorporated the optimum formula. The homogeneous gel had a drug content of 95.8±1.5 %, a viscosity of 1762cP, a pH of 5.74±0.78, and % a drug release of 85.5%±0.85. The study concluded that transdermal transfersomes would be a promising approach to treating angina.

Keywords: Transfersomes, Ultra-deformable, Vesicles, Phospholipids, Optimization

INTRODUCTION

Classically, angina (or angina pectoris) refers to a pressure-like substernal chest discomfort brought on by physical or emotional stress. It is the most acute symptom of ischemic heart disease. Angina can be acute or chronic. A restriction in coronary blood flow due to coronary artery spasm, obstructive

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atherosclerotic plaque, a non-coronary problem such as acute anemia, coronary microvascular dysfunction, or hypotension might limit myocardial oxygen supply. The cause may be a significant rise in myocardial oxygen demand. A rise in heart rate (HR), supraventricular tachycardia (atrial fibrillation or flutter), hyperthyroidism, or other factors are some of the incidences that lead to oxygen demand[1].

Medications used to treat angina are calcium channel blockers, nitrates, and beta-blockers. These agents decrease myocardial ischemia through heart rate regulation and vasodilatory processes[2]. Ranolazine and ivabradine are unconventional antianginal drugs used to treat symptomatic angina in chronic, stable ischemic heart disease patients[3]. Most -adrenoceptor antagonists are effectively absorbed after oral treatment. However, many are subjected to first-pass hepatic degradation, limiting their oral bioavailability to different degrees[4].

Aspirin's mechanism of action involves irreversible inhibition of platelet-dependent enzyme cyclooxygenase, [COX], identified as isoenzymes as COX -1 and Cox-2. Platelet aggregation happens from the production of thromboxane A2, a powerful promotor produced by COX -1, thus preventing prostaglandin synthesis. This irreversible inactivation of COX -1blocks thromboxane A2 and produces the antiplatelet effect. Platelet activation and aggregation with subsequent activation of the clotting cascade play critical roles in the onset of acute occlusive vascular events, such as MI and occlusive cerebrovascular accident (CVA). Because platelets do not have a nucleus and thus cannot regenerate COX, they become an excellent target for antithrombotic therapy, while aspirin shows both immediate and long-term effects on platelets. Aspirin belongs to the biopharmaceutics classification system (BCS) II drug with high solubility and permeability profile[5]. The reported water solubility of aspirin is 2-4 mg/ml.[5]

The transdermal route has several advantages over other traditional drug delivery routes. The benefits include reducing unfavorable side effects, preventing hepatocytes' metabolism, and extending predictable drug action. Also, it avoids fluctuation in drug blood levels, improves the physiological and pharmacological response, and, last but not least, the ability to deliver drugs with a short half-life. Specific features such as higher encapsulation capacity for hydrophobic and hydrophilic drugs, biodegradability, non-toxicity, and drug encapsulation in the vesicular structure make them preferential to other vesicular carriers. The prolonged drug presence in the circulation, the potential to target different organs and tissues, and enhanced bioavailability are also additional advantages[6]. Transferosomes are lipid-based-vesicular carriers comprised of four elements: phosphatidylcholine., dipalmitoyl phosphatidyl-

choline, edge activator such as a surfactant or a bile salt, low concentration of alcohol, and water. Transferosomes mimic the behaviour of a cell engaged in exocytosis, making them ideal for regulated and targeted drug administration. When added to aqueous systems, the carrier aggregate comprises at least one amphipathic molecule (such as phospholipids), which self-assembles into a bilayer of lipid that finally shuts into a lipid vesicle in the presence of a bilayer softening agent (a biocompatible surfactant).[7,8]

Transdermal anti-anginal drugs are helpful for the treatment of moderate to severe chronic angina. The advantages of transdermal transferosomes of the anti-anginal drug include ease of administration and therapeutic efficacy. These factors, coupled with preventing the drugs from hepatic metabolism, and lowering the untoward side effects, are likely to contribute to targeted delivery and better therapeutic efficacy. Therefore, the current work concentrated on developing transferosomes via a thin-film hydration technique to optimize the formulation via a custom approach and evaluate the formulation for its percentage of drug loading, vesicle size, stability, and *in vitro* drug release profile to substantiate its ability to pass via skin.

METHODOLOGY

Materials

Aspirin from Central Drug House (P) Ltd, soya lecithin, and tween 80 from Sigma Aldrich Ltd. All of the other chemicals and reagents utilized in this experiment were of an analytical grade.

Methods

Preparation of Transfersomes

Screening of surfactants and preparation of blank formulation

Two surfactants were screened (tween 80 and span 80) with different concentrations (10-25%). The blank formulation was dissolving the lipid (Soya lecithin) in a volatile organic solvent (chloroform: methanol) (1:1) mixture, and the organic solvent was allowed to evaporate in a rota evaporator; once the organic solvent evaporates, a thin film formed. (Phase transition temperature of soy lecithin is 570C, and cholesterol is 370C) This thin film was kept for 12-24 hr, ensuring the complete evaporation of the organic solvent, thin-film hydrated with the phosphate buffer solution 7.4 pH with gentle shaking above its phase transition temperature[9].

Preparation of drug loaded transfersomes

Based on the blank formulations, tween 80 as the surfactant (10-25% w/w of phospholipids) and soya lecithin as the phospholipid/vesicle forming agent (300-600mg) was selected (Figure1.)

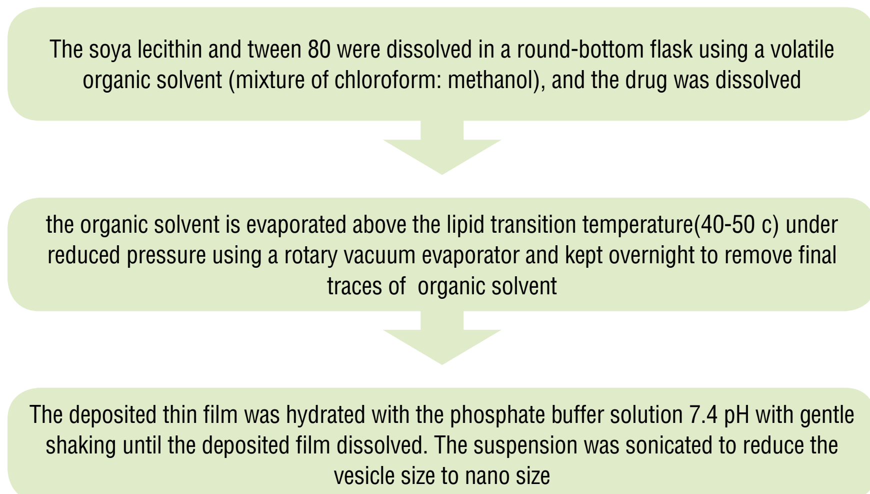

Figure 1. Schematic representation of preparation of transfersomes

Design of experiment

Using custom design via JMP version 13, the effect of formulation variables on quality attributes was studied. The formulation variables such as the number of phospholipids (300-600 mg), the concentration of surfactant (10-25%), and hydration volume (10-15ml) as factors on responses entrapment efficiency (%), particle size (nm), zeta potential (mV) were studied is given in Table 1. The design generated 12 runs given in Table 2[10]

Table 1. Experimental design

Factors	Low		High
X1= Weight of phospholipids	300mg		600mg
X2= Concentration of surfactants	10% w/w of phospholipid		25% w/w of phospholipid
X3= Hydration volume	10ml		40ml
Responses	Goal	Low limit	High limit
Y1=%Entrapment efficiency	Maximise	80%	95%
Y2= Vesicle size	Minimise	50nm	200nm
Y3= Zeta potential	Minimise	-20mV	-35mV

Table 2. Formulation table

Formulation code	Drug (mg)	Weight of phospholipids (mg)	Concentration of surfactant (%)	Hydration volume (ml)
F1.	100	600	25	10
F2.	100	600	15	40
F3.	100	300	15	40
F4.	100	300	25	40
F5.	100	600	15	40
F6.	100	300	15	10
F7.	100	300	25	10
F8.	100	600	25	10
F9.	100	300	25	40
F10.	100	300	15	10
F11.	100	450	20	25
F12.	100	450	20	25

Characterization of transfersomal suspension

Entrapment efficiency (%EE)

To separate the entrapped drug from the unentrapped drug, 2ml of transfersomal suspension was centrifuged at 11000 rpm for one h. The supernatant diluted in methanol and drug content was quantified spectrophotometrically as the free drug. The percentage of drug entrapment is calculated by the below-mentioned formula[10].

$$\% \, Entrapment \ efficiency = \frac{Total \ drug - free \ drug}{Total \ drug} * 100$$

Vesicle size analysis (Horiba SZ-100 particle size analyser)

The dynamic light scattering (DLS) technique measured the vesicle size of all drug-loaded transfersomes. Transfersomal suspension was diluted with double distilled water before subjecting for measurements, and each sample was measured in triplicate at a scattering angle of 90° at 25.2°C[11].

Zeta potential

The zeta potential measurement was carried out by SZ-100 HORIBA scientific using the principle of electrostatic light scattering. Zeta potential was determined after suitable dilution of the trasfersomes samples with distilled water. The diluted samples were placed into were placed in the zeta measurement cell. The electrophoretic mobility of the nanodispersion was measured by tracking the movement of trasfersomes in an electrical field, and the electrical charges were determined.7 Electrophoretic mobility (μ) measurements were used for determining Z. The Smoluchowski equation converted the mobility to ZZ = μη/ε Where η is the viscosity and ε is the permittivity of the solution[11].

In vitro drug release of formulations

Using Franz diffusion cell assembly, in vitro drug release studies were performed on trasfersomes suspension. The study used a dialysis membrane (70; Hi media) with a diffusional area of 70 cm2. The donor compartment contained (50 mg equivalent) formulation, and the receiver compartment had a volume of 50 ml of phosphate buffer solution (PBS) pH 7.4. The dissolution medium in the receptor compartment was magnetically stirred (at 100 rpm) and kept at 37 ± 0.5 ^{0}C for 24 h. The sample aliquots (1 ml) collected at predetermined intervals were replaced with the same buffer volume. The aliquots were filtered through a 0.45 mm membrane filter. After appropriate dilution, the samples were analyzed using UV–a visible spectrophotometer at a λmax 266.4nm against PBS as blank. The measurements were done in triplicate[12].

Evaluation of experimental design

The results of the responses from the experimental study were substituted into the experimental design and evaluated for model fit. The model identified the design space and determined the desirability function and surface response curves. The transfersome gel was prepared from the optimized formulation.

Fourier transform infrared spectroscopy (FTIR)

Spectra of optimized formulation were recorded using the Perkin Elmer FTIR spectrophotometer (RXIFT-IR system, USA). Samples were mixed with dry potassium bromide in a 1:1 ratio and then compressed into a transparent disc by applying 10kg/cm2 pressure in a hydraulic press at a scanning range from 4000-400 cm-1. The spectra obtained were compared and interpreted for the functional group peaks[13].

Differential scanning calorimetry (DSC)

A differential scanning calorimeter (Perkin Elmer) was used to measure the thermal nature of drugs and additives. The pure drug and a physical mixture of aspirin, soya lecithin, and tween 80 were used in the study. A sealed aluminum pan was availed to place the sample, which was flushed with nitrogen (50 ml/min). The sample was scanned at a ten °C/min rate from 20 °C to 250 °C. Thermograms were recorded. An empty aluminum pan was used as a reference[13].

Morphology using Transmission electron microscopy (TEM)

The morphology of transferosomes containing the drug was examined by the 200-transmission electron microscope (TEM) (FEI type FP5018/40 Tecnai G2 Spirit Bio TWIN). The transferosomal suspensions containing aspirin in Milli-Q water were dropped on a standard carbon-coated copper grid (mesh) and air-dried for five h, and the surface pictures were observed[13].

Preparation of aspirin loaded transfersomal gel

Preparation of blank gel

The gel was prepared with different concentrations (0.1, 0.5, 0.75, and 1%) of the gelling agent carbopol 934. The accurate weight of carbopol 934 was sprinkled into a beaker containing 100 mL boiling distilled water and soaked overnight. A homogenous gel prepared under magnetic stirring, followed by the neutralizing agent triethanolamine, was added. The gels were examined for consistency, pH, and viscosity[14].

Preparation of drug loaded transfersomal gel

Drug-free gel (1%w/w) was prepared using the procedure mentioned in the blank gel formulations. The optimized aspirin transfersome was introduced into the gel base with continuous stirring on a magnetic stirrer. Preservatives such as methylparaben and propylparaben were added, followed by triethanolamine dropwise, and adjusted the prepared gel's pH to pH 5.5[14].

Preparation of drug solution gel

A gelling agent of 1% carbopol 934 was used to form a drug solution gel. Carbopol 934 was accurately weighed and dusted into a beaker containing 100 mL boiling distilled water, where it soaked overnight. The drug solution was introduced with continuous stirring on a magnetic stirrer to ensure homogenous dispersion inside the gel base. Preservatives such as methylparaben and propylparaben were used. The solution was neutralized by adding triethanolamine as a neutralizing agent drop by drop, constantly mixing until a homogeneous gel was produced. Then the amount of added neutralizing agent was controlled to adjust the pH of the prepared gel to pH 5.5 using a pH meter[14].

Evaluation of the transfersomal gel

Rheology of the gel

The prepared gels were evaluated for the viscosity using Brook-field Viscometer (Brookfield Engineering Laboratories, Inc. Middleboro, MA, USA) with an S94 spindle; at speeds of 10, 12, 20, 30, 50, 60, and 100 rpm at 37⁰C. After a predetermined time of 5 minutes, constant viscosity readings were obtained and recorded in centipoises[15].

pH measurement, Homogeneity and spreadability

A digital instrument16 measured the pH of the prepared gel.

A small amount of gel was pressed between the thumb and index finger and checked for the presence of a lumpy feeling[16].

Spreadability was checked by pressing 0.5 g of transfersomal gel between two transparent circular glass slides by measuring the diameter of the produced circle[13].

Drug content

The drug content of the transfersomal gel was determined by diluting 0.5g of gel (10 mg equivalent trasfersomal suspension) with methanol and stirred for 2hr. The solution was filtered and was analyzed spectrophotometrically at 276 nm[15,16].

In vitro release study

Using Franz diffusion cell assembly, in vitro drug release studies were performed on trasfersomes suspension. The study used a dialysis membrane (70; Hi media) with a diffusional area of 70 cm 2. The donor compartment contained (50 mg equivalent) formulation, and the receiver compartment had a volume of 50 ml of phosphate buffer solution (PBS) pH 7.4. The dissolution

medium in the receptor compartment was magnetically stirred (at 100 rpm) and kept at 37 ± 0.5 ⁰C for 24 h. The sample aliquots (1 ml) collected at pre-determined intervals were replaced with the same buffer volume. The aliquots were filtered through a 0.45 mm membrane filter. After appropriate dilution, the samples were analyzed using UV–a visible spectrophotometer at a λmax 266.4nm against PBS as blank. The measurements were done in triplicate[17-20].

Stability testing

The stability of transfersomes and transfersomal gel was studied at 250±20 C/60 % RH ±5% and 5°±3°C. In this study, the samples were stored for three months and evaluated for % EE, vesicle size of transfersomes in transfersomal suspension, and drug content in transfersomal gel[18].

RESULTS and DISCUSSION

Preparation of transfersomes

Screening of surfactants and preparation of blank formulation

Surfactants were evaluated for their vesicle size, physical observation, and microscopic structure. The microscopic image showed that the vesicles were distributed widely, and the lamellae were visible, whereas they were not much prominent in the formulation with a span of 80, as shown in Figure 2.

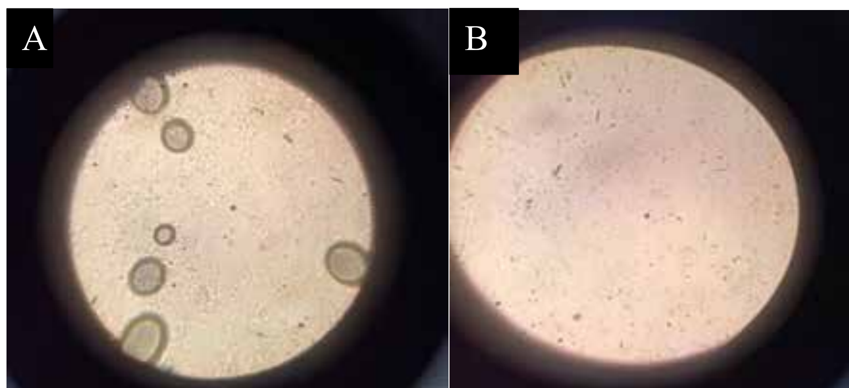

Figure 2. Microscopic view of transfersomes formulated with (A) tween 80(15%) (B) Span 80 (15%)

Formulation with Tween 80 and span 80 resulted in a transparent film, which on subsequent hydration and sonication, resulted in a vesicle size of 1025.5 nm and 1436 nm, respectively, as given in Figure 3. With this evaluation, tween 80 was used further in formulation trials.

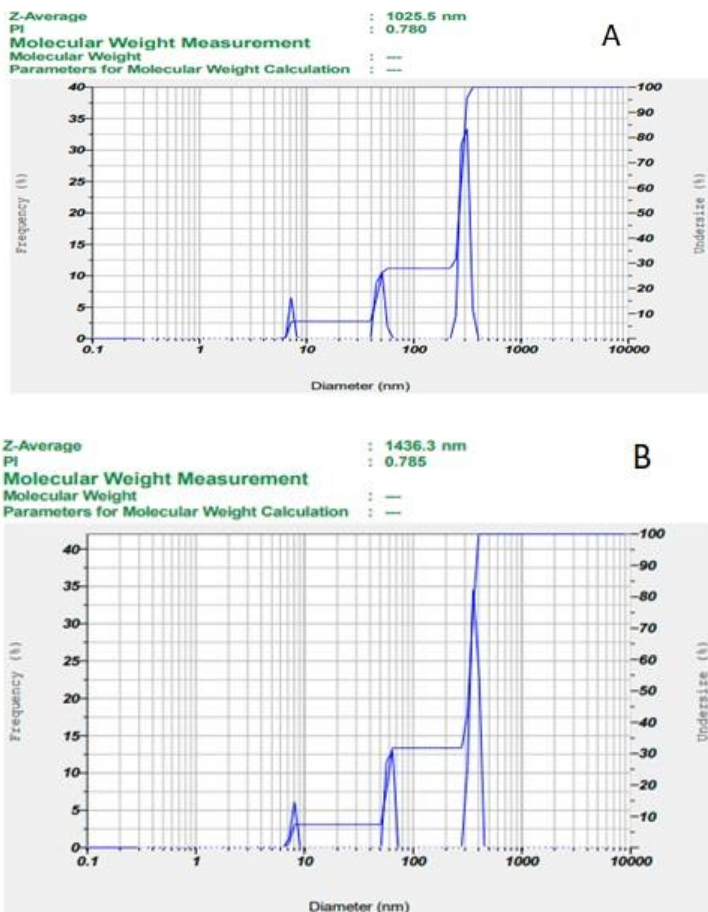

Figure 3. Vesicle size of formulation A) with tween 80 surfactant, B) span 80 surfactant

Vesicle size, zeta potential and entrapment efficiency

Formulations were optimized via a custom design approach using JMP 13 SW. Weight of phospholipid, the concentration of surfactant, and hydration volume was selected as factors for the responses % EE, vesicle size, and zeta potential. The experimental design generated 12 runs with two center points. A vesicle size of 50.4- 100.5 nm, the zeta potential of -32.6 to -21.5mV, and % EE in the 82.3-92.5% range were observed. The results are reported in Table 3.

Table 3. Physical observation of blank formulation

Surfactant	Vesicle size (nm)	% EE	Physical observation
Tween 80	1025.5	89.4%	Clear thin film
Span 80	1436.3	81.5%	Clear Thin film

In vitro drug release of drug loaded transfersomal suspension

In vitro drug release of drug-loaded transfersomal suspension of all formulations was carried out for 24 h and the percentage release was in the range of 49.7-89.4 %, as given in Figure 4. The full release was found in the F6 formulation; this data can support the most miniature particle size and highest entrapment of the drug in the vesicle.

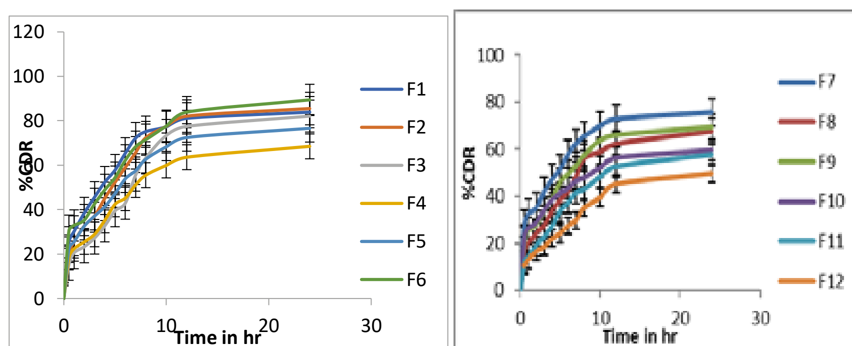

Figure 4. *In vitro* drug release profile of transfersomal suspension (F1-F6) and (F7-F12)

Optimization of experimental design

The actual vs. predicted plot for the responses % EE, vesicle size, and zeta potential showed the R2 value of 0.80, 0.85, 0.86, and the P-value of 0.0039, 0.0012, and 0.001 as given in Figure 5. This data indicated that the factors and selected factors' levels were statistically significant ($p \leq 0.05$) for all the responses. The optimized formula was obtained from the desirability plot with desirability of 0.703 and found maximum desirability of 0.80, as shown in Figure 6. The optimized formulation contains 381mg of a phospholipid, 15% w/w concentration of surfactant, and 10ml hydration volume, all factors in lower levels. The surface profiler indicates that the variables selected had a linear influence on the responses and no curvature effect, as seen in Figure 7. The prediction variance profile demonstrates that about 50 % of the fraction of de-

sign space had a variance less than 0.175, a desirable parameter in optimizing the formulations. Both prediction variance profiler and the fraction of design space are given in Figure 8 and figure 9, respectively.

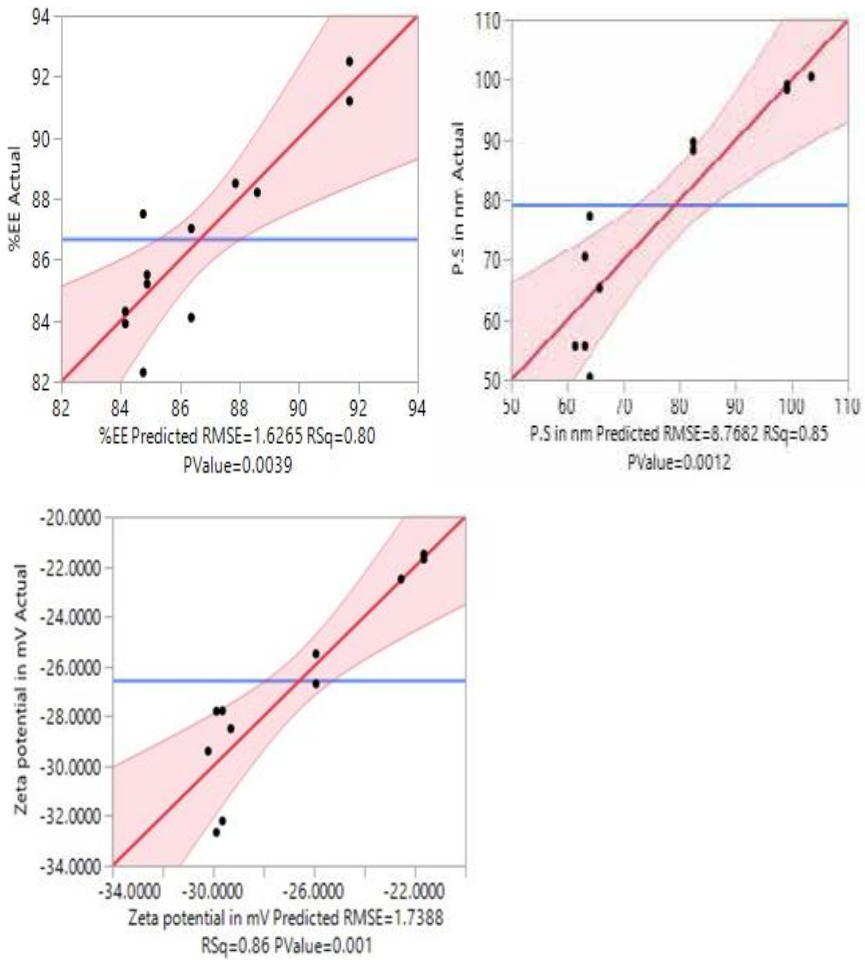

Figure 5. The graph of % EE, Vesicle size and Zeta potential

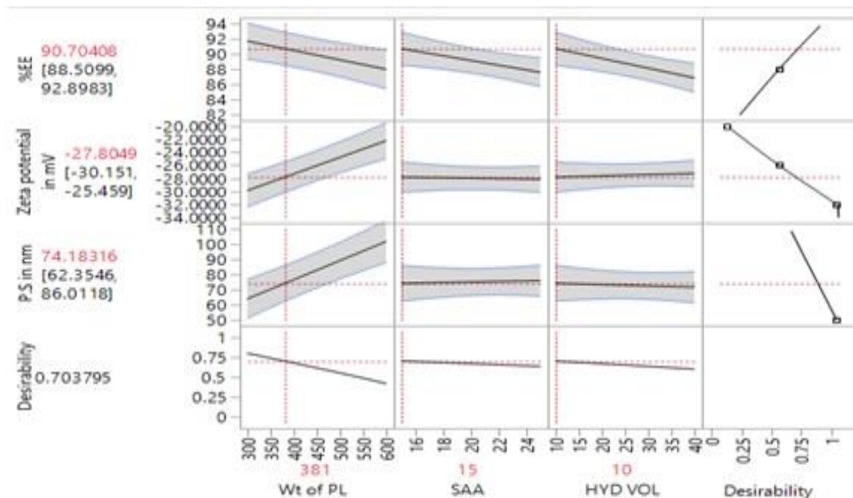

Figure 6. Prediction profiler and desirability for DOE

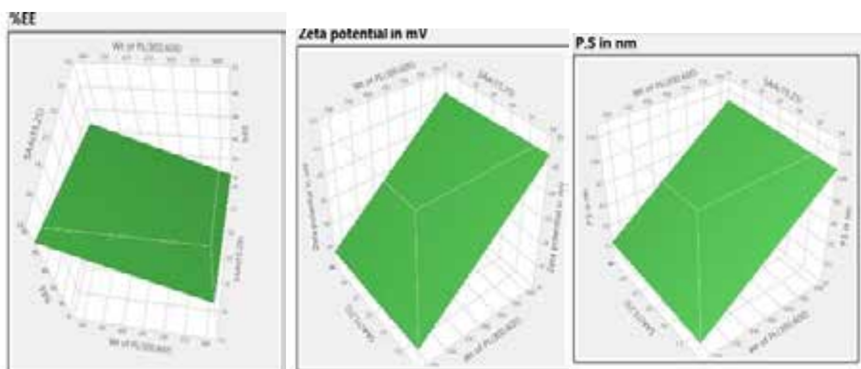

Figure 7. Surface profiler for % EE, zeta potential, vesicle size

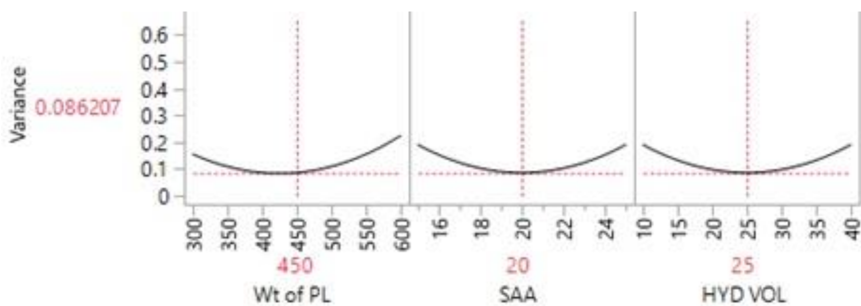

Figure 8. Prediction variance profile

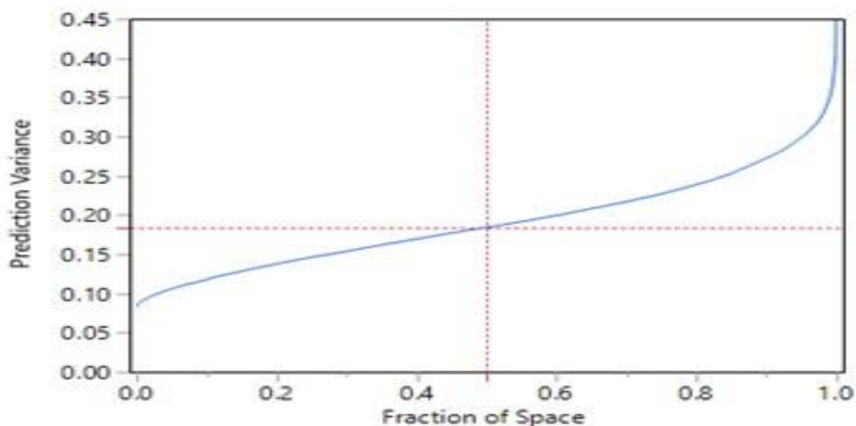

Figure 9. Fraction of design space plot

Evaluation of optimised formulation

The optimized formulation was prepared per the predicted formula based on the desirability approach. The formulations evaluated for the vesicle size, zeta potential, % EE and % CDR were found through diffusion studies. The vesicle size was 74.4 nm, zeta potential to be -27.4mV as shown in Figures 10 and 11, and % EE to be 90.5%±0.25. As reported in the literature, the optimum ratio of phospholipid: surfactants can affect the size, zeta potential, and drug entrapment efficiency. It is seen that the surfactant at higher concentration has, on the contrary, an effect on entrapment efficiency as the changes in membrane permeability can expel the drug from the core. However, higher surfactant concertation results in smaller vesicle size up to an optimum level. After that, it promotes micellar formation rather than vesicles.

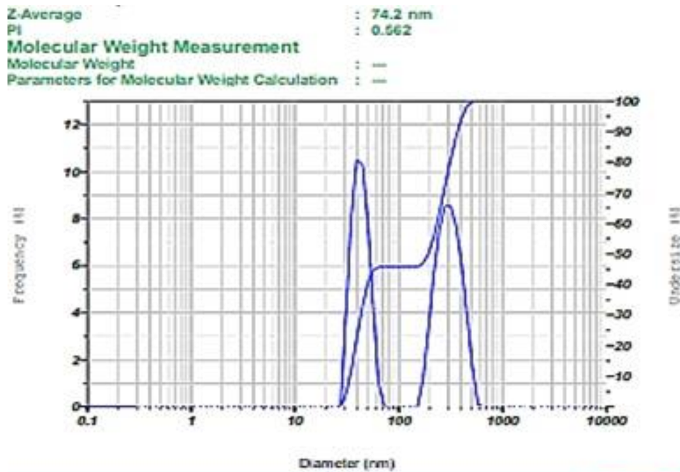

Figure 10. Particle size analysis of optimised formulation

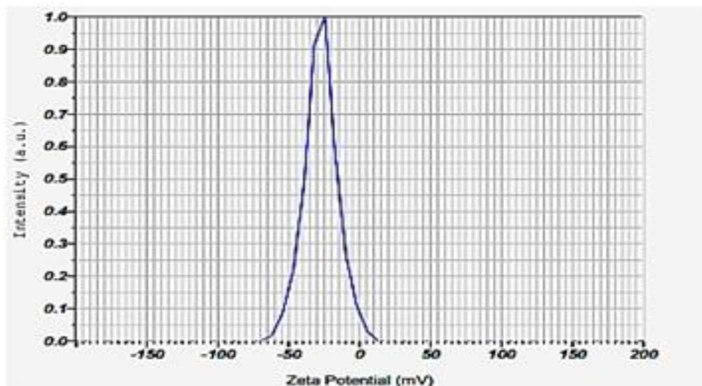

Figure 11. Zeta potential of Optimised formulation

Moreover, the poydispesibility index is found to be low, which may be due to reduced interfacial tension. The results showed that the phospholipid has a prominent effect on the responses; a low amount of phospholipid resulted in low zeta potential value, vesicle size, and higher % EE. The hydration volume had an almost negligible effect on the responses. The lesser the hydration volume is, the more secondary is zeta potential, and with more hydration volume, a slight increase in the vesicle size and % EE was observed18-20. The actual values are as per the practical observation, as shown in Table 4, and the observation of optimized formulation is given in Table 5.

Table 4. Results showing %Entrapment efficiency, Zeta potential and Vesicle size

Formulation code	% Entrapment Efficiency	Zeta potential (mV)	Vesicle size (nm)
F1	85.5	-22.5	100.5
F2.	84.3	-21.5	98.5
F3.	88.5	-28.5	55.6
F4.	82.3	-32.2	70.5
F5.	83.9	-21.7	99.2
F6.	92.5	-32.6	50.4
F7.	88.2	-29.40	65.3
F8.	85.2	-22.5	100.5
F9.	87.5	-27.78	55.6
F10.	91.2	-27.8	77.4
F11.	87.02	-26.7	88.4
F12.	84.1	-25.50	89.65

Table 5. Evaluation of optimised formulation

	Vesicle size predicted	Vesicle size actual	Zeta potential predicted	Zeta potential actual	% EE predicted	% EE actual	% CDR
Optimised formulation	74.1nm	74.4nm	-27.9mV	-27.4mV	90.70%	90.5%±0.25	88.65%±0.34

Morphology of transfersomes

The TEM image shows the transfersomes are present in a spherical shape, and a unilamellar structure was observed, which is given in Figure 12.

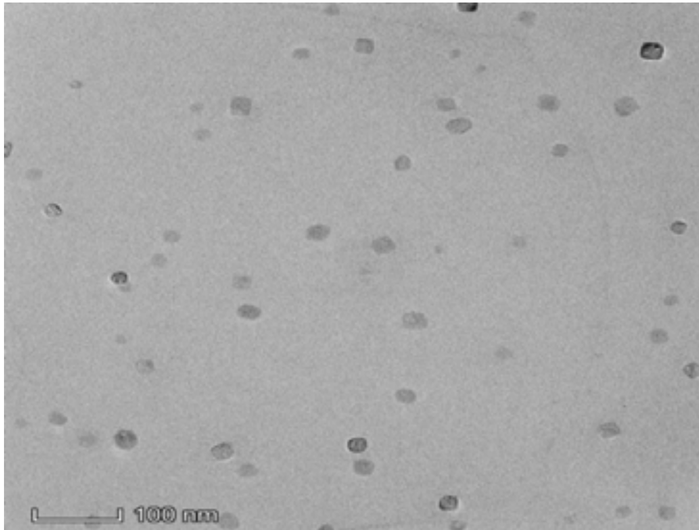

Figure 12. TEM image depicting the structure and morphology of transfersomes

FTIR Study of optimised formulation.

The FTIR graph of the optimized formulation showed a peak at 1634.5 cm-1, establishing the presence of the carboxylic acid group, which was found to agree with the standard range 1500-1700cm-1. C-O stretching for carboxylic acid and ester can be observed at 1239.46cm-1 and 1197.8cm-1, which follow the standard range 1200-1250cm-1 and 1100-1200cm-1, respectively represented in Figures 13 and 14. And the peaks of O-H stretching meta substitution and C-H bending signify the drug's presence in the formulation, and no interaction was observed.

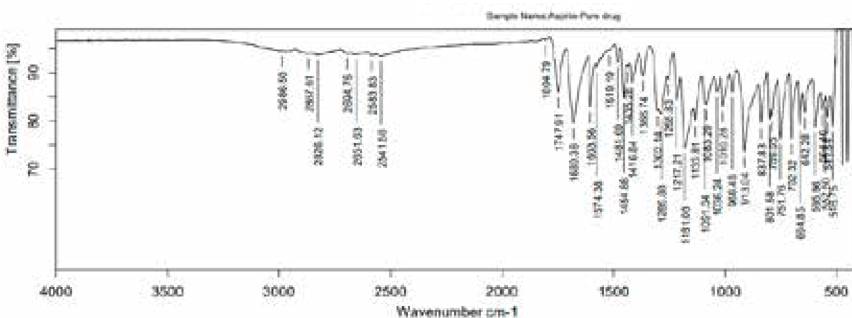

Figure 13. FTIR spectrum of pure drug aspirin

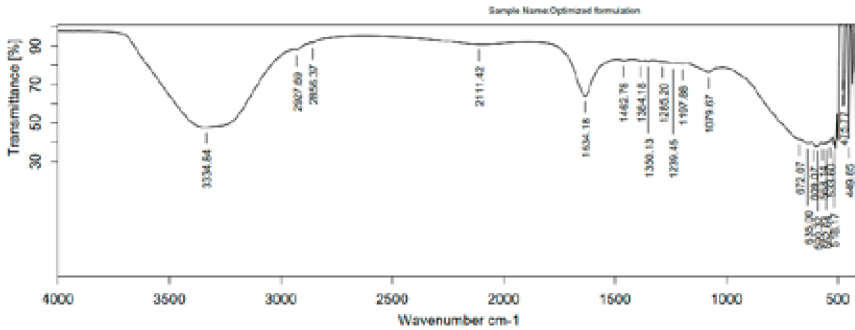

Figure 14. FTIR spectrum of optimised formulation

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is one of the most extensively used calorimetry techniques for characterizing the solubility and physical state of drugs in lipid vesicles is differential scanning calorimetry (DSC). Figure 15 shows the DSC analysis of the pure drug and the optimized formulation. The DSC study of the pure drug shows a significant endothermic peak at 144.9°C, which corresponds to aspirin's melting point. This peak intensity was reduced in the DSC thermogram of optimized transfersomes produced with tween 80. The absence of aspirin's melting endotherm suggested that the drug was in a more soluble amorphous state. (Figure 16)

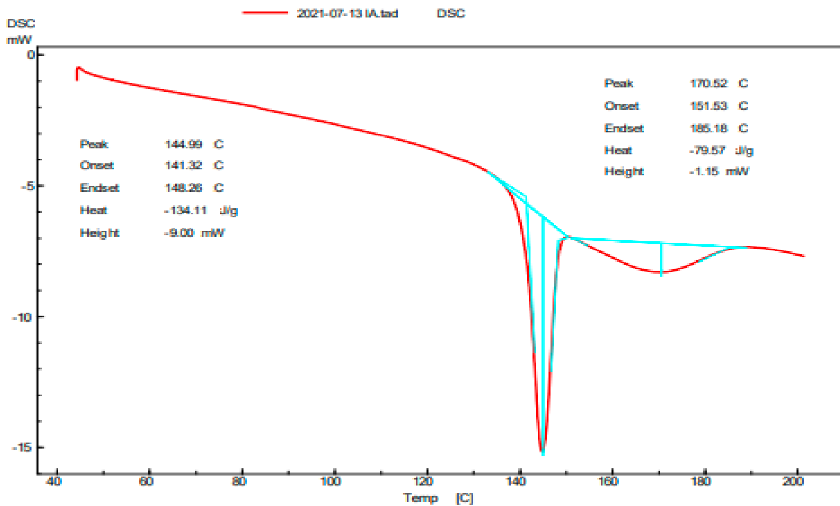

Figure 15. DSC thermogram of pure drug

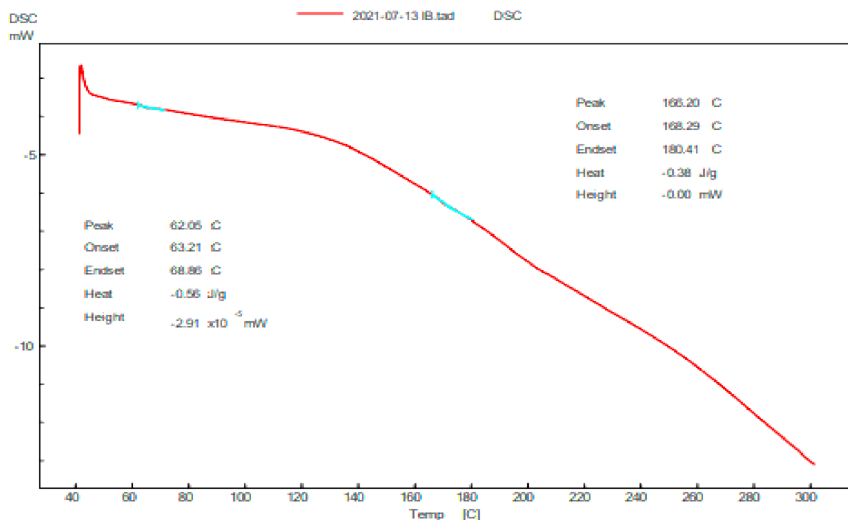

Figure 16. DSC thermogram of optimised formulation

The inhibition of aspirin crystallization and solubilization in transferosomes could explain the shift in melting behavior. This shows that the aspirin in the prepared transfersomes was amorphous. The conversion of a drug's physical state to an amorphous or partially amorphous state results in a high-energy state with the high disorder, resulting in increased solubility.

Evaluation of transfersomal gel

The viscosity of drug solution gel and transfersomal gel was evaluated at 100 pm. The gel formed with the drug solution was thick compared to the transfersomal gel, represented in Figure 17.

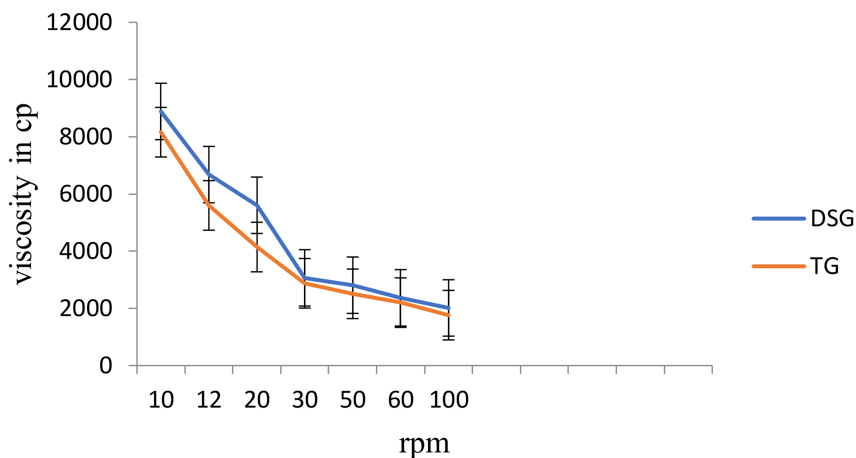

Figure 17. Viscosity in cP of drug solution Gel (DSG) and transfersomal Gel (TG)

The viscosity and consistency of the plane gel are represented in Table 6, and the pH, homogeneity, spreadability, and drug content are given in Table 7.

Table 6. Evaluation of drug solution gel and transfersomal gel

Evaluation parameters	Drug solution gel	Transfersomal gel
Viscosity(cP) at 100 rpm	2014.5	1762
pH	5.04±0.38	5.74±0.78
Homogeneity	Good	Good
Spreadability (cm)	10.6 ± 0.73	7.2 ± 0.85
Drug content (%)	85.54±2.2	95.8±1.5

Table 7. Effect of carbopol concentration on viscosity and consistency

Carbopol 934 concentration	Viscosity(cP) at 100 rpm	Consistency
0.1%	57	**Liquid**
0.5%	455	**Slightly gel consistency**
0.75%	501	**Gel consistency**
1%	600	**Gel consistency**

In vitro drug release study

Drug release from the drug solution, drug solution gel, and transfersomal gel was compared to see the effectiveness of the drug release when presented as the solution and solution gel. The drug solution showed a % CDR of 45.6 ±0.48% after 24 hrs; release is less due to the partial solubility of the drug in water and permeation of the drug through the membrane. Drug solution gel and transfersomal gel % CDR were 70.9%±0.45 and 85.5±0.52%, respectively. The graph of % CDR v/s time was plotted and shown in Figure 18.

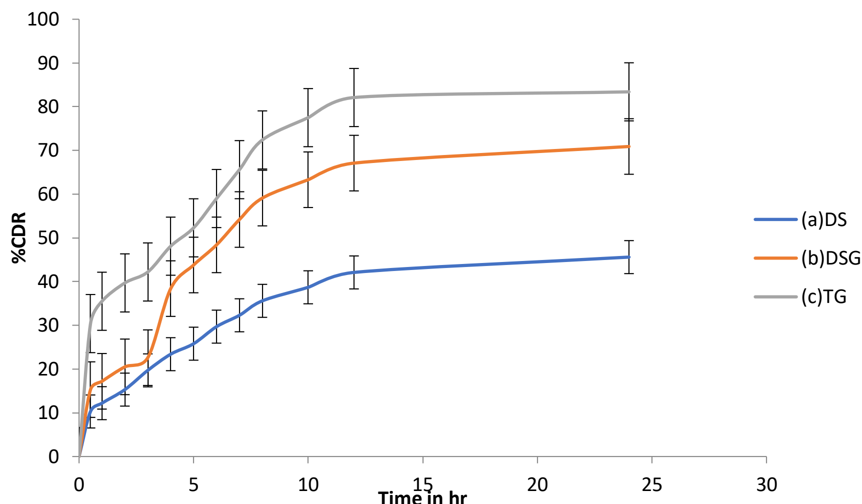

Figure18. *In vitro* drug release profile of (a) (DS) Drug solution (b) (DSG) Drug solution gel (c)(TG) Transfersomal gel

Stability studies

The optimal transfersomal suspension and gel were studied at 250±20 C/60 % RH ±5% and 50±30C. The samples were evaluated for % EE, vesicle size, and drug content. After 90 days of the study period, the % EE and drug content remain the same without any changes indicating a stable formulation, as shown in Table 8.

Table 8. Accelerated stability testing

Sl no	Days	% Entrapment efficiency Transfersomal suspension		Vesicle size transfersomes in suspension		Drug content of gel	
		Optimal formulation	25°±2° C/60 % RH ±5%	Optimal formulation	25°±2° C/60 % RH ±5%	Optimal formulation gel	25°±2° C/60 % RH ±5%
1	0	90.5%±0.25	90.5%±0.25	74.4nm	74 nm	95.8±0.38	95.8±0.38
2	90	90.5%±0.25	89.15%±0.15	74.4nm	73.4nm	94.1±0.08	95.12±0.09

Transferosomes are flexible and biocompatible non-invasive carriers utilized in drug delivery systems to achieve adequate drug concentration. The present work was an attempt to formulate ultra-deformable vesicles of aspirin to improve the therapeutic efficacy of the drug via a non-invasive drug delivery system. The vesicles prepared by the thin-film hydration method preserved their properties, such as zeta potential and particle size. The formulation trials were optimized via a custom design approach. The optimized vesicle incorporated gel formulation exhibited appreciable viscosity. The drug release from trasferosomal gel formulation exhibited a 3-fold increment in drug release compared to the solution and a two-fold to solution gel. The study can be extrapolated to *ex vivo* permeation using biological tissue samples, and also pharmacokinetic studies can be carried out to support *in vitro* data. Hence, the study proved the feasibility of transferosomes as a stable carrier to aspirin in treating angina pectoris.

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