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Development, Optimization, and In-Vitro Evaluation of Transfersomes Rectal Gel of Meclizine as a Model Drug Using Box–Behnken Design

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Rectal drug delivery provides significant advantages over oral and intravenous routes, particularly for patients unable to take medications orally and in emergencies. This route bypasses hepatic first-pass metabolism, potentially enhancing bioavailability, and benefits from the neutral pH of the rectal environment that maintains drug stability. The study focuses on developing a meclizine-loaded transfersomes-based gel for rectal delivery to improve bioavailability and patient compliance. Meclizine, a poorly water-soluble drug with low oral bioavailability, is used to treat motion sickness and vertigo. Transfersomes, elastic lipid vesicles, enhance drug delivery through biological barriers. A Box–Behnken design was employed to optimize and evaluate the effects of phospholipid: surfactant ratio and sonication time on entrapment efficiency, vesicle size, and drug flux. It was found that the optimized meclizine transfersomes gel formulation, composed of (180 mg) phospholipid: surfactant (75:25) (w/w) ratio, (10 mg) cholesterol, and at 2 min as sonication time, fits well with prerequisites of an optimum formulation. The optimized formulation showed (78.5%) entrapment efficiency, (222 nm) mean vesicle size, and (-44.3 mV) zeta potential as shown in Figure 6 and (36 ug/cm^2.hr) flux. The study highlights the potential of transfersomes-based rectal gels as effective delivery systems for enhancing therapeutic outcomes, particularly for drugs with low oral bioavailability. The findings suggest that rectal gels containing transfersomes can offer a promising alternative for systemic drug delivery, ensuring better patient compliance and therapeutic efficacy. Future research should focus on clinical evaluations to confirm the effectiveness and safety of these formulations.

Keywords: Meclizine, Transfersomes, Box Behnken Design, Surface response, gel.

1. Introduction

Rectal drug delivery offers unique benefits compared to oral or intravenous administration, particularly for specific patient populations and medications [1, 2]. It bypasses the liver's first-pass metabolism, allowing drugs to enter the systemic circulation directly via lower rectal

veins, potentially increasing bioavailability [3, 4]. This method is ideal for emergencies and patients who cannot take medications orally. The neutral pH of the rectal environment helps maintain medication integrity [5, 6]. Rectal drug delivery offers various forms, including suppositories, enemas, rectal gels, and foams, to meet different clinical needs [7, 8]. However, it presents challenges such as patient compliance issues and potential irritation [9]. Rectal formulations, including suppositories, microspheres, nanoparticles, enemas, and gels, enhance drug absorption and stability, with studies showing improved bioavailability in drugs like ibuprofen and theophylline [10, 11]. The rectal route offers a useful, noninvasive alternative route of administration when the local or systemic effect is intended. The rectum provides a relatively constant environment for drug delivery that allows effective concentration of drugs in blood and avoids gastrointestinal (GI) absorption difficulties and hepatic first-pass metabolism [12, 13]. However, conventional solid suppositories are usually accompanied by discomfort that may lead to poor compliance and patient refusal. Moreover, if they lack mucoadhesion, conventional suppositories might reach the end of the colon. Therefore, incorporated drugs may be exposed to first-pass metabolism. Therefore, another rectal dosage form that is easy to administer and has mucoadhesive properties has to be used [14]. Rectal formulations of analgesics, antiepileptic drugs, and hormones like progesterone have improved bioavailability and patient compliance [15]. Rectal drug administration provides a viable alternative with enhanced bioavailability and rapid onset of action, particularly in pediatric patients [16]. Transfersomes are a novel drug delivery system that enhances therapeutic agents through the skin and other biological barriers. These ultra-deformable, elastic lipid vesicles can squeeze through narrow skin constrictions, making them highly efficient for transdermal delivery [17]. Transfersomes encapsulate hydrophilic and lipophilic moieties, offering versatility in drug delivery. Transfersomes are composed of a bilayer formed as a phospholipid and edge activator. Transfersomes are more elastic than the conventional liposomes. Elasticity in these vesicles is attributed to an edge activator, which can weaken the lipid bilayer of the vesicles and increase their deformability and flexibility. Edge activators are single-chain surfactants with a high radius of curvature. Spans, Tweens, sodium cholate, and sodium deoxycholate were employed as edge activators. Transfersomes, being deformable, can squeeze themselves through pores in the biological membranes, which are too much smaller than their diameters [18]. Transfersomes are primarily used for transdermal drug delivery, vaccine delivery, cancer therapy, hormone delivery, and cosmetic applications [19]. However, their efficacy can affect stability, manufacturing complexity, and skin variability [20]. Transfersomes face regulatory challenges regarding approval and standardization, requiring extensive clinical evaluation. They are also used in the cosmetic industry to enhance active ingredient delivery into the skin [21]. Transfersome-based gels for rectal delivery offer improved drug absorption enhanced stability, and patient compliance [22]. Meclizine is a white to slightly yellowish crystalline powder with a chemical structure of 1-[(4-chlorophenyl) (phenyl)methyl]-4-[(3-methylphenyl) methyl] piperazine. It is insoluble in water, alcohol, chloroform, and dilute acids, with a melting point of approximately 214°C [23]. It is well absorbed from the gastrointestinal tract and has an onset of action within 1 hour, lasting between 8 and 24 hours [24]. However, meclizine shows low oral bioavailability (22–32%) [25]. Meclizine is widely distributed throughout the body, including the central nervous system, and is highly protein-bound. It is primarily excreted through urine with an elimination half-life ranging from 6 to 12 hours [26]. Meclizine treats nausea, vomiting, and dizziness associated with motion sickness and vertigo [27]. A rectal gel formulation allows better control

over drug release and absorption kinetics. Different excipients can be added to modulate drug release rates and optimize absorption profiles based on individual patient needs. This flexibility in formulation design could potentially enhance therapeutic outcomes by tailoring treatment regimens [28]. Response surface methodology (RSM) investigates the impact of several explanatory factors on one or more response variables. Generally, an experimental design entails selecting the proper combination of independent factors and the level of each factor to be investigated. Nevertheless, because experimental runs are costly in terms of both time and money, it is important to keep the number of runs to a minimum while still achieving the required results. Some techniques, such as Box–Behnken, are widely employed. Optimization with response surface analysis effectively minimizes the time required to develop pharmaceutical dosage forms and improve research output [29]. Response surface methodology builds upon factorial design by developing mathematical models that define the association between factors and responses. This technique uses statistical regression analysis to fit curves or surfaces that represent these relationships. It helps identify optimal factor combinations that result in desirable characteristics. Response surface methodology could generate contour plots showing how changes in composition affect specific responses [30]. The use of statistical approaches in optimizing drug formulations offers several advantages. Firstly, these techniques provide a systematic and efficient way to evaluate multiple factors simultaneously, saving time and resources compared to traditional one-factor-at-a-time approaches. Secondly, they enable the identification of critical factors and interactions that may not be evident through trial-and-error methods alone. Finally, by generating mathematical models and predictive simulations, statistical approaches facilitate informed decision-making during formulation development [31]. The study aims to assess the possibility of developing the transfersomes-loaded gelling system as a carrier for the rectal delivery of meclizine as an effective treatment for nausea and vomiting, overcoming the drawbacks of oral delivery. The study involved a comprehensive *in-vitro* evaluation of the developed system to attain an optimized, effective, and promising delivery system.

2. Materials and Methods

Materials

Meclizine (purity >99%), Soybean phosphatidylcholine, cholesterol, and HPMC K4M were obtained from Hangzhou Hyper Chemicals Limited, Zhejiang (China). Span 80 was purchased from Alpha Chemika (India). All other solvents and chemicals were of analytical grade.

Methods

Statistical Modelling for Optimizing Meclizine-Loaded Transfersomes Formulation

The formulated meclizine-loaded transfersomes were optimized using Box–Behnken Design (BBD) as a response surface methodologies (RSM) tool. Three factors, three levels (33) Box–Behnken Design was constructed using three independent variables representing phospholipid/surfactant ratio (X1), surfactant concentration (Span 80) (X2) and sonication time (X3), with three levels high (+1), medium (0) and low (-1), as shown in **(Table 1)**. The dependent variables examined were entrapment efficiency, EE% (Y1), vesicle size (Y2), and flux (Y3). The Design-Expert version 12.0 software (Stat-Ease, Minneapolis, MN, USA) was used to evaluate the effects of formulation variables on the investigated dependent variables.

Thirteen runs were prepared according to the experimental design to obtain the optimized formula with the desired responses. The analysis of variance (ANOVA) test was adopted to analyze the received data, assess the model's significance, and prove the statistical analysis. A, B, and C represent the main factors, AB, BC, and AC indicate the interactions between main characteristics, and A2, B2, and C2 represent the polynomial terms. The p-values related to the regression coefficients stated the significance of the independent factors on the dependent responses.

Table 1. Variables of Box–Behnken Design for Meclizine Transfersomes Formulations Showing Independent Variables and Their Level of Variation.

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Soybean PC/Span 80	mg	Numeric	100	400	-1 ↔ 100	+1 ↔ 400	223.53	109.14
B	Cholesterol	mg	Numeric	10	60	-1 ↔ 10	+1 ↔ 60	32.35	17.86
C	Sonication Time	min	Numeric	2	8	-1 ↔ 2	+1 ↔ 8	4.47	2.18

Preparation of meclizine-loaded transfersomes

Transfersome dispersions were developed using the mechanical dispersion sonication technique [32]. They were using the Box–Behnken model. Precise amounts of phospholipids: Span 80 (75:25 w/w), cholesterol, and meclizine were mixed in a test tube. A vortex mixer (Heidolph, Germany) was used to mix until a clear solution was formed. The solution was kept in a bath sonicator (Power Sonic 410, Korea) at 70±5°C for 15±2 minutes, then vortexed along with the injection of phosphate-buffered saline (PBS) (pH 7.4) at 70 ±5°C to the solution drop by drop from a needle syringe at a constant rate until the total volume of the dispersion reached 5 ml. The dispersions were left at room temperature for an additional two hours for swelling. The prepared transfersomes dispersions were sonicated via probe sonicator (QSONICA, USA) for five min., with an interval of 2 sec., pulse cycle, and amplitude of (20±2%). The prepared dispersions were stored in the refrigerator at 4±1°C and used the next day for evaluation studies. Thirteen formulations were prepared according to the experimental design; the entrapment efficiency (EE%), vesicle size, and flux of meclizine transfersomes are presented in **(Table 2)**. Meclizine gels 5% w/w were formulated using HPMC (1%) hydrogel previously prepared as gelling agents. A blank hydrogel of HPMC K_4M was prepared by dissolving a specified weight of the polymer in a specified volume of deionized water, mixing well in a beaker using a magnetic stirrer with continuing heating at (60 ± 5 °C). The final volume was completed by deionized water to obtain (1 %, w /v) HPMC gel, and it was left aside for 24 h. to eliminate any bubbles. Methylparaben was used as a preservative at (0.1 % w /v) and added during the gel preparation. Meclizine-loaded transfersomes gels were prepared by incorporating and mixing a specific volume of transfersomes dispersion with a gelling agent until a homogenous, clear gel formed. The final meclizine transfersomes-loaded gel concentration was (5%, w /w). formulas were stored in a refrigerator at (8 ± 2 °C) during the study [33]. The plain gel was prepared by dissolving (50 mg) of meclizine in (1 ml) of ethanol and mixed well using a bath sonicator at (25 ± 2 °C) until a clear solution was obtained. The gelling agent was added to the solution with stirring until a homogeneous gel formed, in which the final concentration was equal to (5%, w /w) of meclizine transfersomes gel.

Table 2. The Independent Variables Used for Optimizing Different Transfersomes Formulations and The Detected Results of Dependent Variables

Formula	Factor 1 A: Soy PC/Span 80 (75:25) (w/w) (mg)	Factor 2 B: Chol. (mg)	Factor 3 C: Son. time (min)	Res 1 EE %	Res 2 V. size (nm)	Res 3 Flux (ug/cm^2.hr)
F1	100	10	4	65±1.3	164±3	26±1.5
F2	100	30	2	77±1	190±7	34±1.8
F3	100	30	8	58±1.8	158±5	36±2.2
F4	100	60	4	78±0.6	108±2	24±1.4
F5	200	10	2	82±1.5	210±1	36±2.4
F6	200	30	4	71±0.7	185±4	32±1.9
F7	200	10	8	62±1.9	131±8	43±1.1
F8	200	60	2	82±0.2	226±2	42±1.7
F9	200	60	8	64±1.1	189±3	37±2.7
F10	400	10	4	72±0.4	202±1	42±2.2
F11	400	30	2	88±1.8	253±6	34±1.2
F12	400	30	8	62±0.7	157±3	44±2.7
F13	400	60	4	75±0.8	187±3	35±2.5

Res: Response, n=3

Optimization of the prepared meclizine loaded transfersomes formulas

Entrapment Efficiency Determination (EE%)

Meclizine entrapment efficiency (EE%) was measured by the direct method using (1 ml) of the prepared dispersions, which theoretically contained (10 mg) of meclizine. It involves separation of the aqueous phase (PBS layer) from the phospholipid layer using refrigerated centrifugation (Eppendorf, Germany) at 4 ±0.5 oC with 12000 ±1000 rpm rotation speed for 30 ±5 min., repeated twice and then at 8000 ±1000 rpm rotation speed for 15± 2 min once. The separated lipid layer was dissolved in (25 ml) absolute ethanol. Samples from this volume were drawn by syringe, filtered by microfilter, and suitably diluted for measurement of UV absorbance at 232 nm [34].

$$EE\% = \left(\frac{(Melizine)total-(Meclizine)aqueous}{(Melizine)total}\right) \times 100\% \qquad (1)$$

Determination of Vesicles Size

The optimized transfersomes vesicles' size was measured using a zetasizer (Malvern, UK). The dynamic light scattering technique assessed the formulations' vesicle size. Samples were adequately diluted with distilled water and measured in the instrument using a quartz cuvette via dynamic light scattering at 25oC with a scattered back angle of 175^o [35]. All measurements were done in triplicate.

Study the permeation of the prepared meclizine transfersomes

Gel formulations via *in-vitro* diffusion (Transdermal flux) A vertical diffusion cell, HDT 1000 (Copley, UK), was applied for the *in-vitro* diffusion study in which the receptor compartment used was 12 ml volume and 15 mm opening diameter with a screw-type sample holder. The study was done at 37 ±1^{o}C, in which the cell was filled carefully in the receptor compartment

with a solution of sodium lauryl sulfate (SLS) in PBS (0.8% w/v) to achieve sink condition. A magnetic stirrer bar inside the cell is used to rotate the solution during the study, which is set to rotate at 600 rpm [36]. Strat M® membranes (70 nm pore size) were taken and carefully fixed between the donor and receptor compartments. The effective surface area of the used cell for diffusion was (1.76 cm^2) so the Strat-M® membrane was cut optimally to suit that area [37]. Formulations were evaluated for a permeation study in which 200 mg of gel equivalent to (10 mg) of meclizine was taken and added to the donor compartment. All measurements were done in triplicate. Sampling was done every half hour by taking (100 µL) of the receptor solution and compensating it with fresh buffer to maintain the sink condition. During the test, the diffusion cell was examined to see if bubbles were present on both sides of the membrane. A modified HPLC analysis method was used to determine meclizine in samples. The process was previously validated in terms of (linearity, precision, and accuracy). Formulations were evaluated for a permeation study in which 200 mg of gel equivalent to (10 mg) of meclizine was taken and added to the donor compartment. All measurements were done in triplicate. Sampling was done every half hour by taking (100 µL) of the receptor solution and compensating it with fresh buffer to maintain the sink condition. During the test, the diffusion cell was examined to see if bubbles were present on both sides of the membrane. A modified HPLC analysis method was used to determine meclizine in samples. The process was previously validated in terms of (linearity, precision, and accuracy). High-performance liquid chromatography (HPLC) method was used to determine the concentration of meclizine in the formulations [27]. Separation was performed on the C18 column (octylsilane chemically bonded to totally porous silica particles of 5 µm particle size). Detection was achieved using UV–A visible detector at a wavelength of 232 nm. The mobile phase was composed of a mixture of methanol: water (65:35) containing 0.7 gm of monobasic sodium phosphate in each 100ml, which was adjusted to the pH of 4 with phosphoric acid. The analysis was performed in isocratic mode with a mobile phase at a 1.5 ml/min flow rate. A volume of 100 µl of each sample was injected into the analytical column. All measurements were done in triplicate. The target permeation flux in humans was based on the human clearance of meclizine (Cl= 2450 ml/h) and the mean steady-state concentration of ONDS (Css= 23.5 ng/ml) [26]. The *in-vitro* diffusion area (1.76 cm^2) was used to calculate the desired permeation flux of meclizine via equation 2 [38].

$$Desired\ flux\ (J) = \frac{(Css\ x\ Cl)}{(Membrane\ area)} \qquad (2)$$

Accordingly, humans' target flux was (32.5 µg/cm^2.h). Steady-state flux (Jss) and enhancement ratio (ER) were calculated using equations 3 and 4, respectively.

$$JSS = \frac{Slope\ of\ the\ permeation\ curve}{Surface\ area\ of\ the\ membrane} \qquad (3)$$

$$Enhancement\ ratio\ (ER) = \frac{(Jss\ test)}{(Jss\ plain)} \qquad (4)$$

Where (Jss) is for the transfersomes gel formula and (Jss) plain is for the plain gel.

Evaluation of the optimized meclizine transfersomes loaded gel

The highly desirable meclizine-loaded transfersomes gel formula was selected to be evaluated as follows:

Physical inspection

The selected formulation was inspected visually to assess the homogeneity of the formulations [39].

Estimation of pH value

The pH measurement was investigated at room temperature using a calibrated digital pH meter (HANNA, Germany). It was triplicated, and the average reading was taken [40].

Spreadability test

This experiment investigated the selected gel's spreadability and measured the spreading diameter when applied to the applied area. Briefly, the gel was retained between two slides, and a definite weight was fastened for 1 minute over the upper slide. The spreading area diameter was measured to indicate the spreadability [41].

Rheological studies

A rotational viscometer (Myr, Spain) was used to measure the viscosity of the developed transfersomes gels at 25 °C using Spindle R6 (radius 0.75 cm) at 200 rpm. The viscosity was determined in triplicate, and the mean reading was taken [42].

Drug content determination

Accurately, 0.5 g of the selected gel preparations (equivalent to 25 mg of meclizine) was diluted to ten milliliters using ethanol. The sample was sonicated at (25 ± 2 °C) until a clear solution was obtained. Solutions were placed in test tubes, centrifuged at 4000 rpm for (5 ±1 min), filtered and suitably diluted with ethanol, and measured spectrophotometrically at 232 nm [43]. All measurements were done in triplicate.

Texture properties

Texture analysis was performed using a TAXTPlus texture analyzer with a 5 kg load cell. A rapid, straightforward analytical technique, texture profile analysis (TPA), has been used for the mechanical characterization of pharmaceutical gels and semi-solid systems [44]. A cylinder probe was twice moved into the sample to a defined depth and speed, allowing a delay period between successive penetrations. Firmness and consistency were evaluated using a stainless-steel cylinder probe (P/10) validated to the company probe specifications. The probe was attached to the device arm. The texture analyzer and probe height were calibrated before the test initiation. The probe moved into a container (30 mm height and 20 mm diameter) filled about (75 ±5%) of its capacity with the gel sample being assessed. Cohesiveness was calculated by dividing work to penetrate for the second time by work to penetrate during the first cycle. The test was done at room temperature. Data analysis for the studied factors was performed using exponent® 32 software (Ver. 6) supplied by Stable Micro Systems®.

Thermal analysis

Thermal analysis was used as a compatibility study protocol in which the meclizine pure sample, HPMC, and the selected formula were tested using differential scanning calorimetry (DSC). Two aluminum pans were used; the first was for the reference (empty pan), and the second was for the test sample. Both were crimped by compression before the test. The instrument's temperature was raised from 25 - 300 °C using a heating rate of 10 °C/min and nitrogen gas at a 50 mL/min flow rate. Thermograms were obtained and analyzed using (TA-60WS) software (Ver. 2.2) for data acquisition and analysis [25].

Statistical analysis

Results obtained from the experimental work were demonstrated as mean ± standard deviation (SD) of three measurements. A one-way analysis of variance (ANOVA) was used to examine the significance of different formulas. The level of significance was defined as ($P<0.05$).

3. Results and Discussion

Preliminary Studies for Preparation of meclizine-Loaded Transfersomes

Transfersomes loaded with meclizine were prepared using the mechanical dispersion method. This process was chosen because forming full vesicles during hydration enhanced the encapsulation efficiency percentage [45]. Preliminary trials were performed to select the surfactant that produces transfersomes with the highest entrapment efficiency percentage. It was noticed that the transfersomes formulation prepared using Span 80 as an edge activator exhibited an acceptable EE% compared to that prepared from the hydrophilic Tween 80. These findings are correlated to the edge activators' HLB values. Edge activators with a low HLB value (HLB value of Span 80 = 4.3) produce transfersomes with a high EE%, resulting from the increased lipid volume ratio in the transfersomes vesicles to the encapsulated aqueous volume [46]. Consequently, in this study, span 80 was adopted to give flexibility to the transfersomes' membrane. Next, for the formulation and optimization of meclizine-loaded transfersomes, a three-factor, 3-level Box–Behnken experimental design was adopted. A total of 13 formulations with different amounts of phospholipid: surfactant ratio and sonication times were prepared, as shown in **(Table 2)**. The amount of phospholipid: the amount of surfactant (A), cholesterol amount (B), and the sonication time (C) were set as independent factors.

Analysis of Box–Behnken Design

The relationships between independent variables, at three levels (-1, 0, +1), with dependent responses, such as the entrapment efficiency (Y1), vesicle size (Y2), and flux (Y3), were assessed by the Box–Behnken design, using the design Expert® software. The quadratic model was the optimum model for all the dependent responses. According to the 3-level Box–Behnken design investigations, the amounts of lipid-forming vesicles (phospholipid), edge activator concentration (Span 80), and sonication time significantly impacted the entrapment efficiency and flux. These observations ensure the selection of the independent variables. The significance of the model was estimated by ANOVA, where, at P-value < 0.05, the model is considered significant. The p-value < 0.05 clarifies that the quadratic model is statistically significant in describing the interrelationship between the independent factors and the dependent responses. Soya phosphatidylcholine was chosen because it has a more excellent membrane deformability index than egg phosphatidylcholine, so the interaction ability of soy phosphatidylcholine with surfactants will be higher [47]. The influences of the independent factors on (EE%) of meclizine-loaded transfersomes are represented by surface response graphs. Increasing the Span 80 concentration from 25 mg to 50 mg resulted in a proportional decrease in the entrapment efficiency of meclizine. However, a further increment of Span 80 to 100 mg resulted in a marked increase ($P<0.05$) in the EE%. As shown in **(Figure 1)**, increasing the Span 80 concentration from 25 mg to 100 mg resulted in a proportional increase

in the entrapment efficiency of meclizine. As illustrated in **(Table 2)**, the maximum entrapment efficiency was 88 ±1.8% for F11, while the minimum value was 58±1.8 for F3.

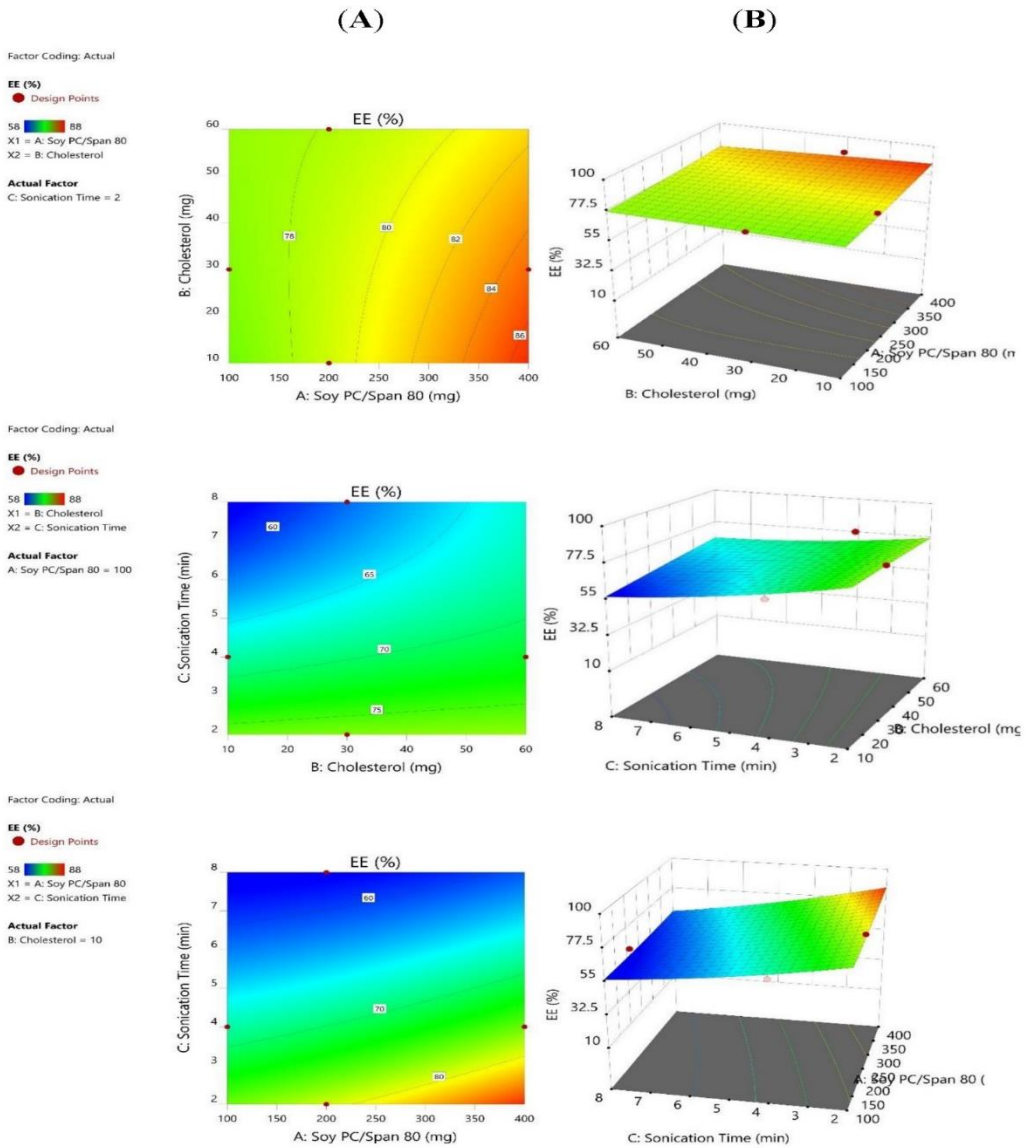

Figure 1**.** Contour plots (A) and corresponding response surface plots (B) show the effects of the independent variables on entrapment efficiency (Y1). Two independent variables are considered simultaneously, while the third remains constant

High concentrations of soya phosphatidylcholine will increase the number of vesicles formed along with the increasing number of adsorbed drugs so that the value of the entrapment efficiency will also increase [48]. The entrapment efficiency of lipophilic drugs like meclizine decreases insignificantly (P >0.05) if the percentage of cholesterol increases since they occupy the same space in the lipid bilayers [49]. Sonication time showed a significant difference (P

<0.05) in EE%. It caused a slight decrease in EE% after eight min., which agreed with previous studies that reported the cause was probably vehicle destruction leading to drug leakage [50]. As meclizine loading affects the vesicle size, the reduced EE% after eight min. of sonication could result from the smaller vesicles formed [51].

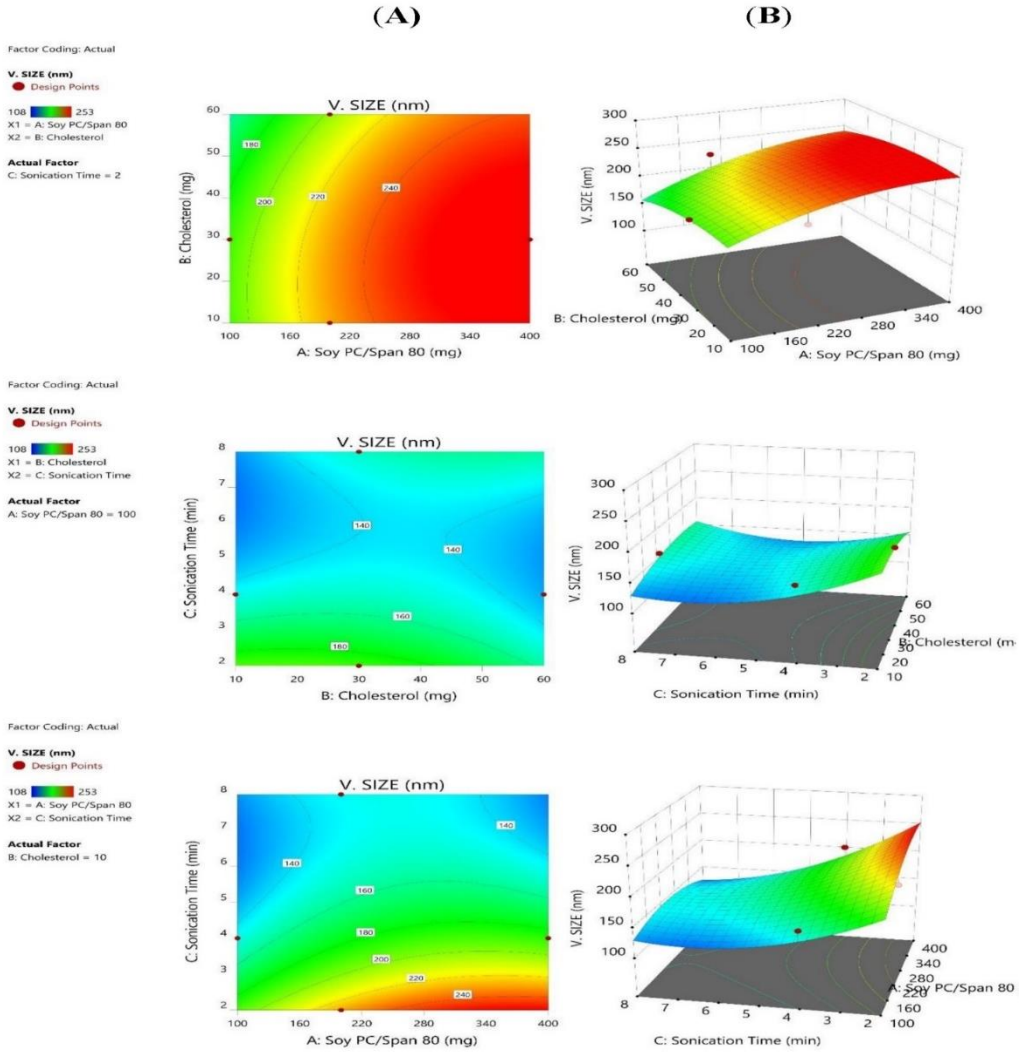

Figure 2. Contour plots (A) and corresponding response surface plots (B) show the effects of the independent variables on vesicle size (Y2). Two independent variables are considered simultaneously, while the third one remains constant

It was observed that soybean PC displayed the most potent positive effect on vesicle size. As shown in **(Figure 2)**, The mean vesicle size (P <0.05) increased with an increase in soybean PC content. It was due to the long saturated and unsaturated hydrocarbon chains of soybean PC molecules, which increases the bilayer thickness of transfersomes vesicles. As illustrated in **(Table 2)**, the smaller mean vesicle size was seen in F4 (108±2 nm), and the larger mean

vesicle size was seen in F11(253±6 nm). Furthermore, the increased mean vesicle size with an increase in span 80 could have been due to forming a sizeable micellar structure. It is expected that at low concentrations, the cholesterol reduces the mean vesicle size due to the close packing of surfactant monomers. While, at high concentrations with a constant soybean: surfactant ratio, cholesterol enters the bilayer vesicle structure, increasing their chain order with the hydroxyl group heading towards the aqueous layer and the aliphatic chain arranging itself to the hydrophobic chains, which results in increased hydrophobicity of vesicles, which imparts a strain in the bilayer structure. Thus, to establish the thermodynamic stability of vesicles, the mean vesicle size is increased significantly (P <0.05) with high cholesterol content [52]. The analysis of meclizine using HPLC for the *in-vitro* permeation study showed that meclizine elutes with a characteristic sharp peak at a retention time of 12.45 minutes, as shown in **(Figure 3)**, which was agreed with previous studies [27]. At constant sonication time (2 min.), data showed that as the amount of soybean PC: Span 80 ratio increased, the flux increased significantly (P<0.05).

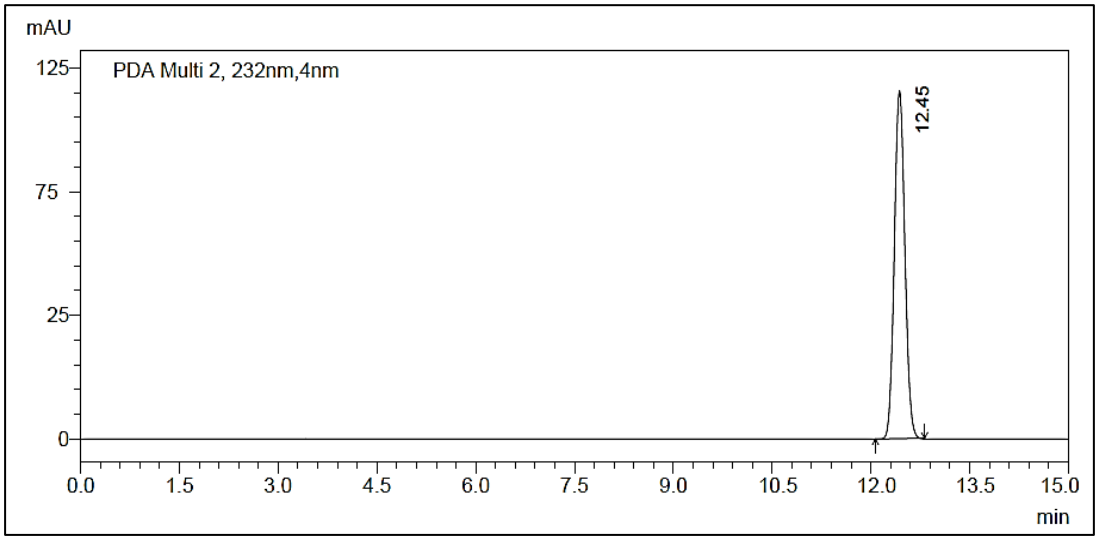

Figure 3. Chromatogram of meclizine using mobile phase (methanol: water 65:35)

The phospholipid fluidizes the vesicle membrane components, and surfactant, i.e., span 80, makes the vesicles more flexible. As illustrated in **(Table 2)**, the lowest flux was seen in F4 (24±1.4 ug/cm^2.hr), while the highest flux was seen in F12 (44±2.7 ug/cm^2.hr). Phospholipid disturbs the structure of the strat M® membrane and increases the fluidity. Transfersomes are entered into disturbed membranes through channels. Thus, the drug is released from transfersomes vesicles in the membrane layers due to the fusion of vesicles within the membrane components [53]. The cholesterol amount showed an insignificant effect (P>0.05) in the flux values as cholesterol was used as a stabilizer and enhancer for the rigidity of the vesicles. Still, it might act synergistically with the soybean PC and surfactants as permeation enhancer substances. As shown in **(Figure 4)**, The sonication time showed a significant effect (P<0.05) on the flux of the transfersomes vesicles as the sonication time prolonged from 2 to 8 min. The time of sonication for the formation of vesicles was found to have a significant effect on the formation of stable vesicles. The less time given for swelling of the vesicles, the larger the size. The vesicle size is also related to incorporating a significant molecular weight

hydrophobic drug.The bilayer's vital property, membrane thickness, is strongly associated with the permeability and bending modulus of the membrane [54].

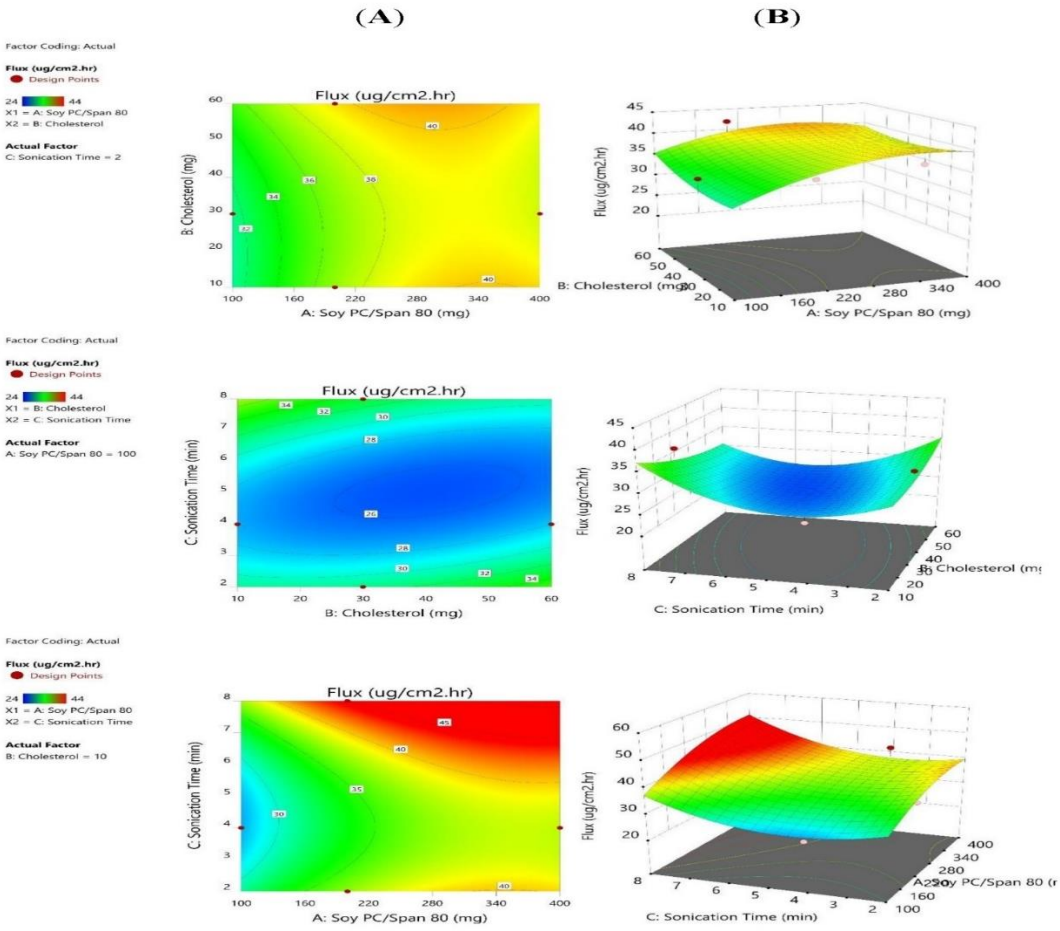

Figure 4. Contour plots (A) and corresponding response surface plots (B) show the effects of the independent variables on flux (Y3). Two independent variables are considered simultaneously, while the third remains constant

Selection of the optimized formulation of meclizine-loaded transfersomes

After constructing the Box–Behnken experimental design, the optimized formulation with the desired properties was specified utilizing the design expert® software (the point prediction method). As shown in **(Table 3)**, the optimized formula was selected from 13 experiments by shifting the criteria towards maximum values of (Y1), the entrapment efficiency percentage, lower sonication time (Y2), and maximum flux (Y3) after nine hours.

Table 3. Constraints For the Optimization of Criteria

Name	Goal	Lower Limit	Upper Limit
A: Soy PC/Span 80 (mg)	minimize	100	400
B: Cholesterol (mg)	minimize	10	60
C: Sonication time (min.)	minimize	2	8

EE%	maximize	58	88
Vesicle size (nm)	minimize	108	253
Flux (ug/cm^2.hr)	maximize	32.5	44

It was found that the meclizine transfersomes gel formulation **(Figure 5)**, composed of (180 mg) phospholipid: surfactant (75:25) (w/w) ratio, (10 mg) cholesterol, and at 2 min as sonication time, fits well with the prerequisites of an optimum formulation.

Figure 5. Optimized Formula

The optimized formulation showed (78.5%) entrapment efficiency, (222 nm) mean vesicle size, and (-44.3 mV) zeta potential as shown in **(Figure 6)** and (36 ug/cm^2.hr) flux.

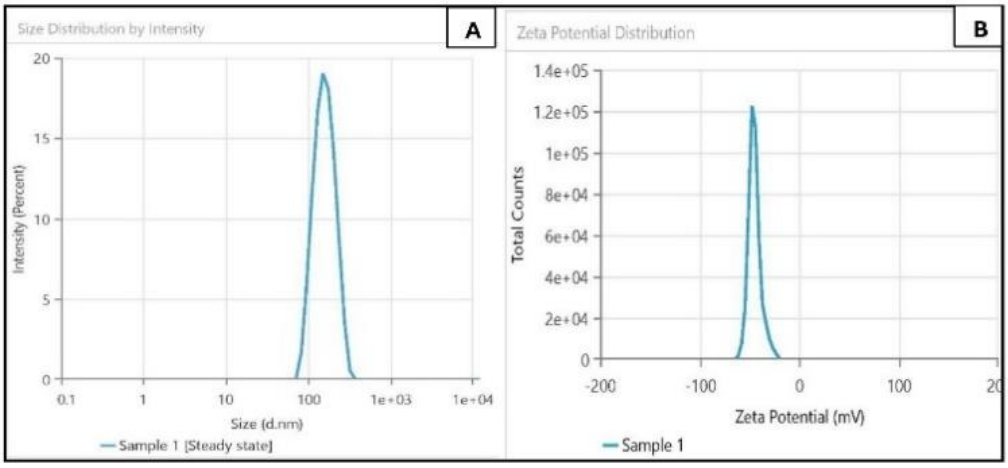

Figure 6. Graphical representation for optimized transfersomes dispersion, A) Size distribution, B) Zeta potential (ζ) distribution. (n=3)

Validation of the Developed Response Surface Methodology (RSM) Model

The theoretical values of both dependent responses, Y1, Y2, and Y3, for all developed meclizine-loaded transfersomes, were determined by plugging their corresponding A, B, and C values into the appropriate mathematical equations generated by the software. The obtained actual and predicted values of the responses are depicted in **(Table 4)**.

Table 4. Constraints For the Optimization of Criteria

Res.	**Pred. Mean**	**Pred. Median**	**Obs.**	**SD**	**SE**	**95% CI low for the Mean**	**95% CI high for the Mean**
EE	78.5	78.5	80.2	4.6	3.7	69.6	87.3
V. Size	222.8	222.8	209.7	18.9	15.2	186.6	258.9
Flux	36.0	36.0	35.8	3.4	2.7	29.5	42.5

It was obvious that the expected and actual values were in reasonably good agreement, as illustrated in **(Table 4)**. These findings confirmed the validity of the developed response surface methodology model. Therefore, the generated polynomial equations using BBD could be utilized to predict the dependent response values. (**Figure 7)**, demonstrates the linear correlation plot of the predicted versus the actual responses, indicating that the predicted R^2 for the three responses reasonably agrees with the adjusted R^2. Additionally, a lack of fit for the dependent's responses clarified significant values (F-value), that is, 4, 4.75, and 4, respectively, and p-values less than 0.05 for all reactions ($P < 0.05$), concluding the validity of the model.

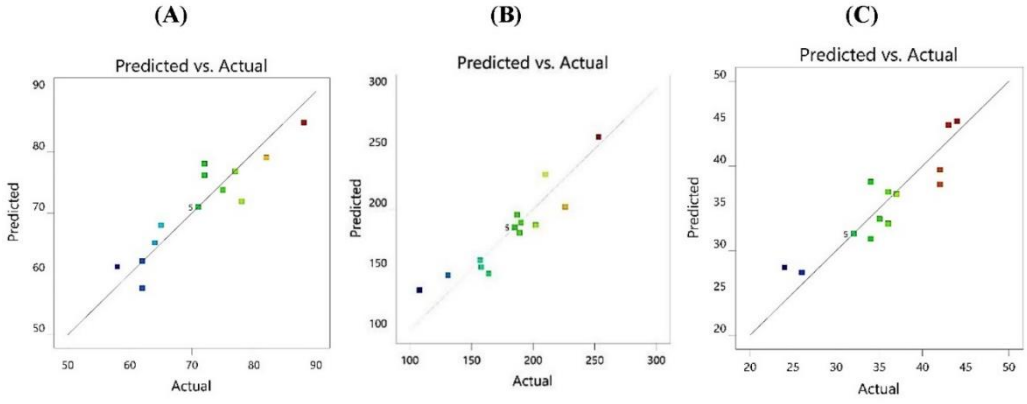

Figure 7. Linear correlation between actual and predicted values for all dependent variables: (A) for Y1, (B) for Y2 and Y3, (C) for Y3

Evaluation of the optimized meclizine-loaded transfersomes gel

Transfersome gel loaded with meclizine was evaluated for consistency, homogeneity, pH, and spreadability. The gel was smooth, clear, homogeneous, and spreadable. When compressed between slides, the distance traveled by transfersomes gel was used to estimate spreadability. The gel traveled a total distance of (37.35 ± 1.58 mm). The pH of the gel was reported to be (7.2 ± 0.25), which was deemed suitable for rectal application [55]. The gel's viscosity was measured as 5433 ± 297 mPa.s, indicating enough consistency to be applied to the skin. The drug content of the selected formula was (99.2 ± 1.9%) which is acceptable according to USP, which stipulates that the uniformity of the first ten dosage units should fall within the range of (100 ±15%) for accepted content uniformity [56]. A texture analysis profile for the selected formula was performed, and results showed a force–time plot in which the firmness of the

sample was equal to the Stickiness and work of adhesion were also calculated from the peak of the force in the negative axis and the area in the force-time plot, respectively. All data are shown in **(Table 5)** and **(Figure 8)**.

peak positive force (g), and the consistency of the transfersomes gel formula was also measured.

Table 5. Texture Analysis for the Selected Formulation*

Formula	Mean Max. +ve Force 'Firmness' (+/- SD) (g)	Mean Max. -ve Force 'Stickiness' (+/- SD) (g)	Mean +ve Area 'Consistency' (+/- SD) (g.s)	Mean -ve Area 'Work of adhesion' (+/- SD) (g.s)
SF**	32.035 ±2.8	-18.699 ±4.2	41.436 ±5.2	-29.939 ±6.9

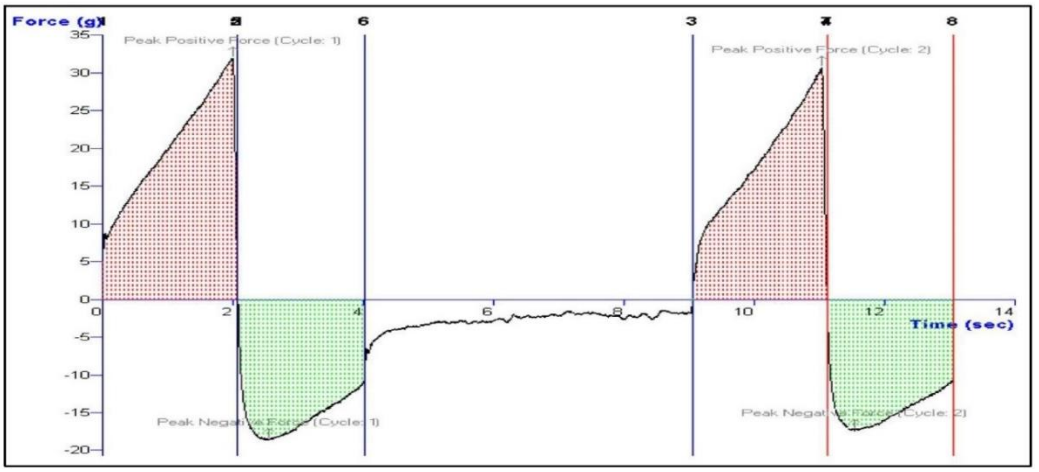

Figure 8. Force–time plot for TPA analysis as obtained from TAXTPlus for the selected transfersomes gel formula, (n=6)

Data in **(Figure 9)** shows a thermogram of pure meclizine and optimized meclizine transfersomes gel. Meclizine showed a sharp endothermic peak at 214 ◦C, corresponding to its melting point and indicating the crystalline properties of the pure powder. A significant reduction ($P < 0.05$) in the peak intensity of meclizine was observed in the formula, indicating the conversion of a crystalline form of free meclizine to an amorphous state in transfersomes vesicles [25].

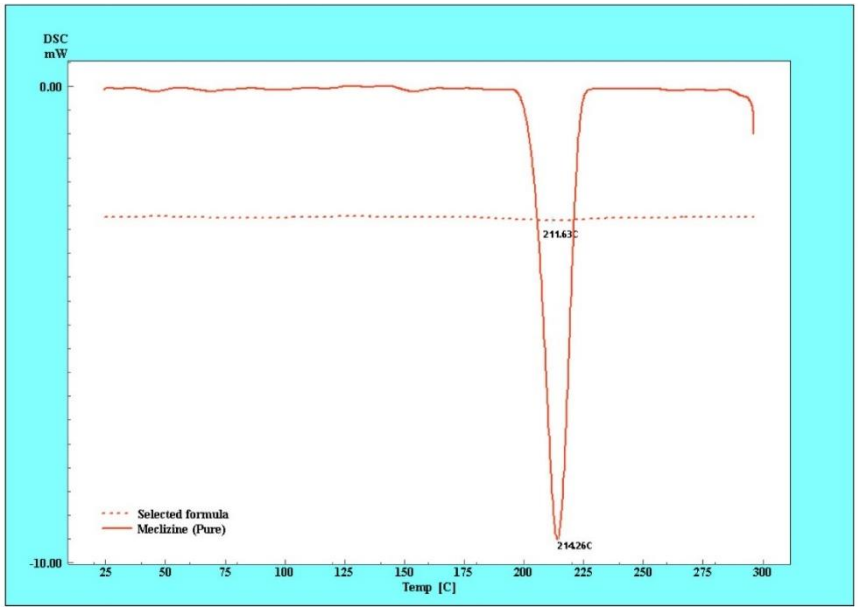

Figure 9. Thermograms of meclizine and the selected (optimized) formula

The *in-vitro* permeation studies for the optimized formula showed a flux (Jss) of 36 ug/cm^2.hr; the simple plain meclizine gel was 4.2 ug/cm^2.hr. This gel showed an enhancement in permeation about eight times that of a cross Strat-M® membrane, as shown in **(Figure 10)**.

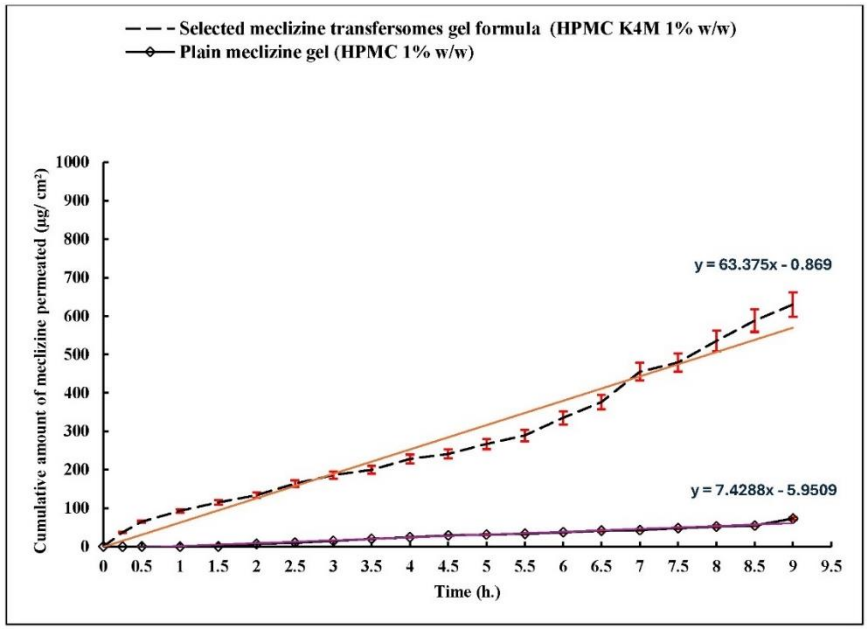

Figure 10. Permeation plot for the optimized formula and the plain (simple) meclizine gel

4. Conclusion

In the current study, gel loaded with meclizine-loaded transfersomes was prepared and optimized. The prepared vesicles were in the nano-size range and showed reasonable entrapment efficiency. The *in-vitro* permeation studies through strat-M® showed an 8-fold increase in drug flux from transfersomes gel formulation compared to the meclizine-loaded gel. Most importantly, the formulated meclizine-loaded. Encapsulation of meclizine within transfersomes vesicles efficiently improved drug permeation and enhanced the entrapped drug's antiemetic activity. Consequently, transfersomes drug delivery systems represent potential carriers for circumventing the barrier function of biological membranes and improving drug delivery.

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Ethics statement: None

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