Original Article

Pharmaceutical Development Of Methotrexate Loaded Transferosomal Gel For Skin Cancer By Doe Approach

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Abstract

Objective: The goal of this study is to use Design Expert Software to evaluate a transfersomal gel formulation for transdermal administration of Methotrexate utilising the Design of Experiments (DOE) Approach (Version 12, Stat- Ease Inc., Minneapolis, MN).

Method: Transferosomes are ultra-flexible supra-molecular aggregates with a great ability to permeate intact mammalian skin. The formulation were designed by Box-Behnkenn Design by using Design Expert Software (Version 12, Stat- Ease Inc., Minneapolis, MN). For usage as a transferosomal gel, drug encapsulation in various transfersomal formulations having various ratios of different drug concentrations (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 g) and Carbopol-940(0.5, 1.2 g) is being studied.

Result: Optimized best formulation containing phosphatydylcholine and sodium deoxycholate (60:40:2)) was identified by Design Of Experiments® 12 software using Box-Behnkenn design exhibited particle size(125.7±0.05nm), entrapment efficiency(85.2±0.23%) and polydispersitivity index(0.245±0.31) and characterized the vesicles by Scanning electron microscopy and transmission electron microscopy shows stable vesicles. *Ex-vivo* permeation studies were performed by using goat skin showed good permeation. The transdermal flux of the Methotrexate loaded transferosomal gel is found to be 7.02±0.16 μg/cm^{-2}/h at the end of 12th h.

Conclusion: Transferosomes, according to this study, are a promising long-term delivery route for Methotrexate and are relatively stable. This research reveals that transferosomes containing Methotrexate could be used to treat Squamous Cell Carcinoma via transdermal drug delivery.

Key words: Transferosome, Edge Activator, Felixibility, Penetration, Methotrexate, Skin cancer

INTRODUCTION

Over two decades ago, it was predicted that skin cancer would account for more than one-third of all cancers. This prophecy has already begun to take shape. Skin cancers are classified into two categories based on the cells that are involved: keratinocytes and melanocytes. The two sorts of classifications are non-melanoma skin cancer (NMSC), which is more common, and melanoma skin cancer (MSC), which is more dangerous. The most frequent type of skin cancer is melanoma. There are two types of melanoma: benign and malignant [1]. A benign tumour is non-cancerous and does not spread quickly. The vast majority of the time, it is seen as a precancerous sign. Melanoma in its malignant stage is very lethal because there are no visible symptoms. It is caused by abnormal cell production that spreads throughout the body. Dermatologists have significant challenges in recognizing malignant tumors at an early stage [2].The most frequent type of skin cancer in the first category is basal cell carcinoma (BCC). BCC seldom spreads beyond the primary tumour site and is rarely deadly. However, if not treated promptly, it can be disfiguring. The majority of severe or malignant melanomas are dark-colored pigmented lesions. Although the majority of instances are treatable, they can result in mortality. MSC skin cancer is the worst form of skin cancer. Damaged DNA generates mutations in melanoma, which are genetic faults that allow tumoral skin cells to reproduce rapidly [3].

Colloidal particles, often known as vesicles, are aqueous sacs wrapped by a bilayer of concentric polymers. They are appropriate for vesicular drug administration because hydrophilic pharmaceuticals are contained in the inner aqueous compartment while hydrophobic drugs are trapped in the lipid bilayer. Transferosomes are highly deformable (ultra-flexible) and self-optimizing unconventional drug carrier vesicles, with membrane flexibility, hydrophilicity, and the ability to sustain vesicle integrity being the most critical variables in their transit over the skin [4]. Furthermore, Transfersomes can successfully protect a drug from undesirable rapid clearance from cutaneous blood vessels, extending the drug's circulation time and increasing its bioavailability [5]. Transfersomes have been utilised to transport a variety of chemicals, including macromolecules such as steroids, proteins [6], insulin [7], corticosteroids [8], ketoprofen [9], and anticancer medicines [10]

Many tests must be carried out after the formulation approach has been developed in order to develop a final formulation. The application of a systematic methodology and DOE has proven to be a very effective technique for formulation development. DOE enables the formulation scientist to analyze various elements and their interactions while maintaining complete control over the number of experiments[11].Here we have used 3 level 3 factorial design Box-Behnken design by using Design-Expert (Version 12, Stat- Ease Inc., Minneapolis, MN).

MATERIALS AND METHODS

Materials:

The pure drug Methotrexate was obtained as a gift sample from Pfizer Inc. soya lecithin, methanol, and chloroform were procured from Delpha Drugs And Pharmaceuticals India. S.D. Fine Chemicals Ltd., India, given soya phosphatidyl choline, Carbopol-940, isopropyl alcohol, and potassium dihydrogen orthophosphate. All of the chemicals used in the experiments were of analytical grade. Purified water that had been freshly prepared was used.

Experimental design for optimization of drug loaded transferosomes using factorial design

The formulations were done by using Box-Behnken design by using Design Expert Software (Version 12, Stat- Ease Inc., Minneapolis, MN).

A design matrix consisting of 17 experimental runs is constructed, for which the non-linear computer generated quadratic model is defined as: Y 14 b0 b1X1 b2X2 b3X3 b12 1 2 b13 1 3 b11X1 2 b22X2 2 b33X3,

where Y is the measured response associated with each factor level combination; b0 is constant; b0 is constant; b1, b2, b3 are linear coefficients; b12, b13, b33 are interaction coefficients between the three factors; b11, b22, b33 are quadratic coefficients computed from the observed experimental values of Y from experimental runs; and X1, X2 and Xi 2 I 14 1, 2 or 3) represent the interaction and quadratic terms, respectively. As independent variables, phosphatydylcholine (X1), Sodium deoxycholate (X2), and solvent mixture (X3) were chosen. The dependent variables in the formulation of transfersomes were particle size (Y1), entrapment efficiency (Y2), and poly dispersive index (PDI) (Y3). The concentration ranges of the independent variables under research, as well as their low and high levels, are listed in the Table 1.

Preformulation studies

Compatibilty studies through FT-IR

FTIR spectra obtained through the compatibility of the pure drug and excipient was observed using Bruker FTIR [12, 13]. The spectra were reported at wave numbers ranging from 3500 to 500cm-1.

Standard calibration curve of Methotrexate

A UV visible spectrophotometer was used to conduct the calibration curve which was measured at 240 nm. The absorbance of the solution was UV Vis spectrophotometry [14].

Formulation of Methotrexate loaded transferosomes

Formulations were done by using Design expert software version 12.Phosphatydylcholine, sodium deoxycholate , and the Methotrexate are dissolved in 10 ml of a mixture of two organic solvents (chloroform: methanol) at suitable ratio as shown in the formulation table 1 in a clean, dry bottom flask. A magnetic stirrer carefully evaporated the organic solvent to create a lipid film on the flask wall, and a phosphate buffer solution (pH 7.4) was hydrated by rotation at room temperature at 60 rpm for 1 hour and kept at room temperature for 2 hours for swelling. The multilaminar lipid vesicles (MLV) are then sonicated for 10 minutes with a probe sonicator (Heldolph vcx750) [15].

Table 1: Optimization Formulation table by DOE approach using design expert software

Factors	**Levels**	
Independent variable	Low	High
X_1=Phosphatydylcholine(mg)	30	90
X_2= Sodium deoxycholate(mg)	20	60
X_3=Solvent mixture(Choloform: methanol) ml	1	3

Characterization of Methotrexate loaded transferosomes

Vesicular size determination

The diameter of the vesicle can be determined using photon correlation spectroscopy. A sample is made with distilled water. The samples are diluted with filtered saline after passing through the 0.2 mm membrane filter [16].

Zeta Potential Analysis

The zeta potential, size distribution, and vesicle size of the optimised formulation were measured using Zetasizer (DTS Version 5.03, Malvern) and the light scattering process, also known as Photon Correlation Spectroscopy (PCS). The zeta sizer is set at 25°C at a 90° angle in this system. For Zeta potential estimation and size determination, water is used as a dispersant [17].

Entrapment Efficiency:

Entrapment efficiency is represented as a percentage of what is added in terms of the amount of drug present. Mini-column centrifugation was used to isolate the trapped medication [18].

$$ENTRAPMENT\ EFFICIENCY - \frac{Amount\ entrapped}{Total\ amount\ added} X\ 100$$

Percentage Drug Content

For the determination of percentage drug content transferosome formulation of about 1gm was taken. Sonication was carried out with ethanol to lyses the vesicles for 15 min. For half an hour, centrifugation at a speed of 14000 rpm was carried out by placing the solution in a centrifugation tube. Methanol of 100 ml was used to dilute the clear solution obtained. 100 ml phosphate buffer of pH 7.4 was made by diluting 10 ml of the prepared solution Aliquots were withdrawn after regular time intervals and by using UV spectrophotometer at 234.5 nm the drug content was calculated for Methotrexate [19].

In-Vitro Drug Release Studies

To investigate different transfersomal formulation drug release trends, a cellophane membrane (Molecular weight cut off 12000-14000, HI Media Ltd, Mumbai, India) was utilized. An exact amount of formulation was spread out on a membrane positioned between the donor and receptor chambers (Franz-diffusion cell apparatus) with an accessible diffusion zone. The receptor compartment is filled with a repeatedly stirred phosphate buffer pH 7.4 at a rate of 50 rpm with a small magnetic bar at a temperature of 37 0.5 °C. At different time intervals, 5 ml aliquots were withdrawn and restored with the same amount of phosphate buffer solution. The samples were analyzed in a spectrophotometer, and a graph depicting the amount of drug penetrating through the membrane over time was created to produce the Invitro drug release [20].

Preparation of Methotrexate Loaded Transferosomal Gel Using Carbopol-940

Carbopol-940 of three different concentrations

Table 2 was used to determine the appropriate percentage of carbopal-940. The mixture was swirled until it thickened. Poly ethylene glycol- 400 (PEG-400) (5ml) was progressively added into the aqueous dispersion of Carbopol-940 after complete dispersion. Then, 5ml of isopropyl alcohol (IPA), 5ml of propylene glycol (PG), and 1ml of triethanolamine (TEA) were added. To achieve a homogenous gel dispersion, 100g of distilled water (q.s.) was also added. To obtain the best batch of Carbopol-940 gel, these three distinct gel formulations were tested for various assessment metrics [21, 22]. To the prepared carbopal gel transferosomes of Methotrexate which is equivalent to 100 mg drug was incorporated as shown in the table 3.

Table 2: Formulation table of Methotrexate loaded Transferosomal Gel

S.NO	Transfersome (mg)	Carbopol-940 (mg)	Triethanolamine (ml)	Propylene Glycol (ml)	Isopropyl Alcohol (ml)	Water
TamG1	100	250	10	5	5	Q.S
TamG2	100	500	10	5	5	Q.S
TamG3	100	1000	10	5	5	Q.S

Characterization of Transferosomal Gel Loaded with Methotrexate

Homogeneity

Three different formulations of Carbopol-940 gel were developed and tested for physical appearance through visual observation [23].

pH Value of Topical Transferosome Gel

For determining the pH, each of the gel formulations was taken to measure pH using digital pH meter. The measurement of the pH of each system was replicated thrice [24].

Grittiness

The light microscope was used to determine microscopically the presence of particles in all the formulations prepared. The gel formulation shows satisfying results of freedom requirement from grittiness and the particular matter as it is a desired characteristic for any topical formulation [25].

Spreadability Test

The gel formulation of 350 mg which was taken on one glass slide and another glass slide was containing about 5.8 ± 1g of gel which was allowed to drop from a 5 cm distance. After 1 min, the spread gel was examined to determine the diameter of the circle.

Extrudability Test

After applying the weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds, the gel quantity (g/cm^2) extruded from the lacquered aluminum collapsible tube was determined. The extrudability can be calculated using the formula provided.

$$Extrudability = \frac{Weight\ applied\ to\ extrude\ gel\ from\ the\ tube\ (g)}{Area\ in\ sq\ cm}$$

Viscosity

The viscosity of the Methotrexate loaded transferosomal gel was determined by Brookfield viscometer.

Transmission Electron Microscopy Studies

Transmission electron microscopy was used for determining the formulated gel [26].

Scanning Electron Microscopic Studies

Scanning electron microscopic studies are utilized to gain knowledge about the morphology of surface.

Drug Content

1gm of a transferosome gel formulation was used, and the vesicles were lysed by sonication in 25 ml of ethanol for 15 minutes. This solution was then placed in a centrifuge tube and spun at 14000 rpm for 30 minutes. Methanol was used to dilute the clear solution to 100 ml. The solution was then diluted to 100 ml with phosphate buffer pH 7.4. Aliquots were taken, and the drug concentration of Methotrexate was determined using a UV spectrophotometer set to 227 nm.

In-vitro Release Study

The *Invitro* drug release study was carried out as mentioned in the transferosome evaluation.

Ex-Vivo permeation study

Using a Franz Diffusion cell with an efficient diffusion surface area and receiver chamber capacity, in-vitro permeation was performed on excised, defatted goat skin tissues [26]. The tissue was kept at 21ºC in a deep freezer. It was brought to room temperature and inserted between the donor and receiver compartments of the Franz diffusion cell during the experiment. The donor compartment faced the superficial layer of the goat's skin, while the receiver compartment faced the other side. Before the experiment, the skin tissue was stabilised with stimulated skin fluid (SSF) (pH-4.2). SSF was fed into the incubator shaker's reception chamber and agitated at a speed of 100 rpm with a magnetic rotor to keep the temperature at 37ºC. To preserve stability, the entire media was replaced every 30 minutes with new buffer. After six cycles of stabilisation, 1 ml of the sample (0.5 % w/v Methotrexate transfersomal gel) was placed in the donor compartment with 0.75 ml of SSF to replicate the skin milieu. Phosphate buffer (20 ml) was added to the receptor compartment (pH 4.5). The samples were taken at regular intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 24 hours) and filtered through a 0.45 mm membrane filter. The samples were then tested for drug content using UV spectrophotometry, and the cumulative percentage drug release was calculated [27]. The flow (mg/cm2/h) and permeability coefficient (Kp) were determined using the following formulae:

$$Flux(mcg/cm2/h) = Cumulaive\ amount\ of\ drug\ permeated\ vs\ time$$
$$Permeability\ coefficient(Kp) = Flux/Drug\ concentration\ in\ donor\ compartment$$

Stability Study

For the stability study evaluation, the formulation was maintained at room temperature (25 ± 2ºC) for two months. To evaluate the formulation, pH, spreadability and extrudability were checked after the 1st and 2nd month.

RESULTS AND DISCUSSION

Preformulation Studies

Drug Excipient Compatibility Study by FT-IR

The IR spectra indicate there were no interactions between the drug and excipients.

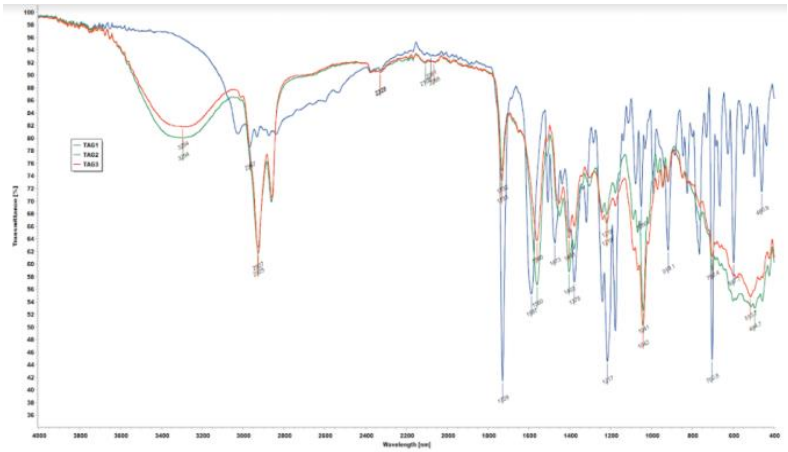


Fig.1: FT-IR graph of Methotrexate +phosphatydylcholine+ sodium deoxycholate and Carbopol-940 gel. FTIR graph shows no compatibility with drug and excipients.

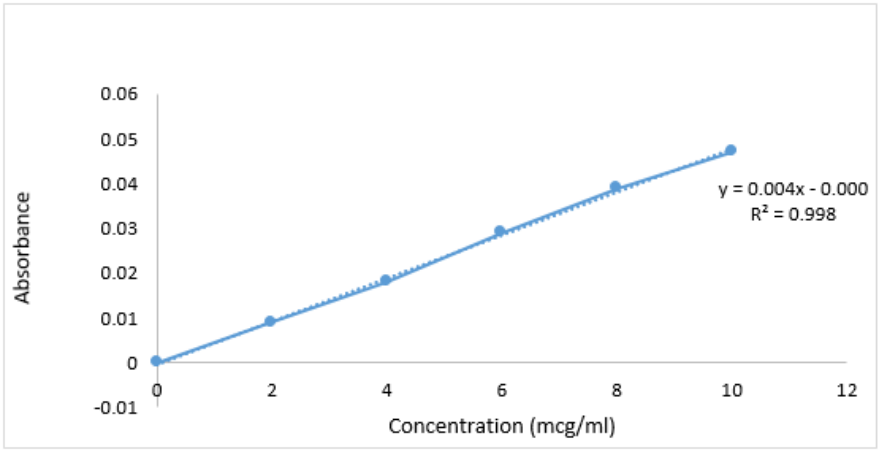


Fig. 2: Calibration curve of Methotrexate

Calibration curve shows linearity

Optimization of a characterization of Methotrexate loaded transferosomal formulation

The optimum Methotrexate-loaded tranferosomal formulation systems were chosen based on particle size, percent entrapment efficiency, and polydispersivity index, while minimising vesicle size utilising the Design Expert Software's point prediction approach as shown in table 3. (Version 12, Stat- Ease Inc., Minneapolis, MN).

The following diagram depicts the composition of the main components as well as a full review. Particles with a wide range of properties were created using 27 different transferosomal formulas created using the design expert programme version 12. The average size ranged from 125 to 390 nanometers. Phosphatydylcholine (60ml), sodium deoxycholate (40mg), a solvent mixture of chloroform and methanol (3:1), and 100 mg of Methotrexate were determined to meet the requirements of an ideal formulation F2 (60,40,2 concentration) Design expert software version 12 was used to create response 3D graphs. These plots were used to investigate the effects of three independent factors on answers while keeping the fourth variable constant.

Table 3: Optimization table of 3 level 3 factorial design of transferosomes

	Factor 1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**	**Response 3**
Factors and responses	**X1:Phosphatydyl choline**	**X2:Sodium Deoxycholate**	**X3:Solvent Mixture**	**Y1:Particle Size**	**Y2:Entrapment Efficiency**	**Y3:Polydispersivity Index**
Units	mg	Mg	ml	Nm	%	no unit
F1	60	60	1	345	43.2	0.859
F2	60	40	2	125.7	85.2	0.245
F3	30	40	1	386	67.9	0.765
F4	60	40	2	127.3	88.1	0.253
F5	60	40	2	128.1	86.7	0.258
F6	60	40	2	126.2	86.2	0.255
F7	90	20	2	255	45.9	0.546
F8	60	20	3	289	55.9	0.873
F9	60	60	3	276	47	0.957
F10	90	40	1	378	53	0.864
F11	90	40	3	332	33.9	0.765
F12	60	40	2	128.3	85.6	0.843
F13	60	40	2	127	87.9	0.255
F14	60	40	2	128.7	88.3	0.26
F15	90	60	2	376	44.7	0.764

F16	60	40	2	127.1	85.4	0.243
F17	60	40	2	128.3	85.9	0.254
F18	60	40	2	129	86	0.234
F19	30	20	2	289	65	0.923
F20	60	40	2	126.4	85.9	0.253
F21	60	40	2	112.8	76	0.774
F22	60	40	2	118.8	65	0.454
F23	30	40	3	324	49	0.435
F24	60	40	2	125.5	86.3	0.258
F25	30	60	2	234	52	0.667
F26	60	40	2	127.3	87.3	0.255
F27	60	20	1	378	55	0.764

The above optimization table is derived from Design expert software® 12 software with the appropriate concentration (F1 TO F27). After optimizing the formulation 60,40,2 concentrations (F2) seems to be optimum batch for the preparation of transferosomal gel

Effect of phospholipid: surfactant ratio on Entrapment Efficiency and Drug Loading

The % entrapment efficiency of deformable vesicle formulations ranged from 43.2% to 88.34 (Table 3). With increasing surfactant concentration from 5 to 10% (w/w) in transfersomes produced with sodium deoxycholate, the percent EE increased significantly ($P<0.05$). When surfactant concentrations above 15%, mixed micelles coexisted with transfersomes, resulting in lower drug entrapment due to the stiffness and smaller size of mixed micelles. According to Patel et al., the influence of phospholipids and surfactant ratio in lipid components of vesicles on the entrapment efficiency of the lipophilic pharmaceutical, Methotrexate, reduced with increasing surfactant ratio. The phospholipid and surfactant ratios also have an impact on drug loading. The current study discovered that phospholipid and surfactant concentrations improved drug loading only in a few batches, and overall dependency is modest, but surfactant has a considerable positive effect. The F-value of 15.76 for the model indicates that it is significant. An F-value this large might arise owing to noise only 0.07 percent of the time. Model terms are significant when "Prob > F" is less than 0.0500.

Effect of vesicle composition

The percentages of drug entrapment in various transfersomal compositions are compared to a blank gel and a marketed product in Table 3. The EE of transfersome formulations was significantly higher than that of pure drug suspension and marketed formulation ($P>0.05$). This result may be due to interactions between the surfactants and Methotrexate, which created a complex that was injected into the transfersomes bilayer. In comparison to liposomes, Fang et al. discovered that adding the surfactant sodium deoxycholate to phosphatidylethanolamine vesicles significantly increased the entrapment efficacy of 5-aminolevulinic acid. Gupta et al. discovered that transfersomes were substantially more successful in trapping than liposomes and niosomes[16]..

Effect of phospholipid & surfactant ratio on the Particle Size and PDI

The use of phospholipid and surfactant ratios in the preparation of the formulation resulted in a significant change in the average particle size and stability of the transfer group formulation. The phospholipid/surfactant ratio varies widely, between 85.5 and 95.15%. As the phospholipid/surfactant ratio increases, the particle size increases sharply.

Vesicles Size and PDI

Malvern Mastersizer determined the vesicle size analysis values for transfersomes. The particle size of the formulations F1 to F27 appears to be 118.3 nm to 378 nm when the lipid ratio of phospholipids and surfactant concentration is reduced. Following formulation optimization, the F2 formulation was revealed to have the ideal concentration. As a result, at this concentration, the transferosomal gel must be prepared using the following approach.

Zeta Potential Analysis:

The size of the vesicles and its distribution is confirmed by the obtained size distribution curve.

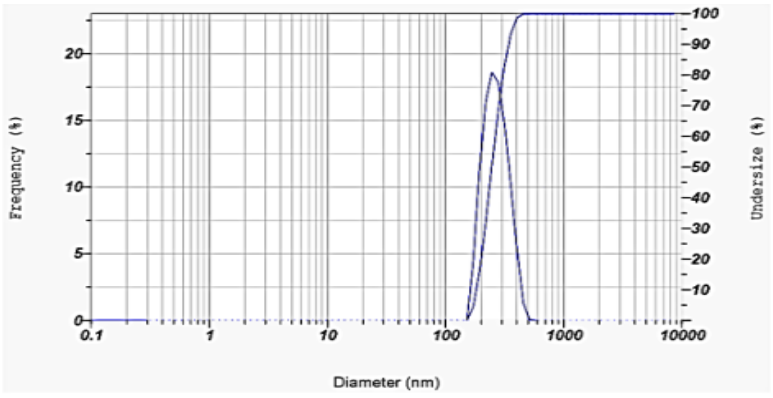


Fig. 3: Zeta Potential of the optimized formulation

The table 4 shows the average vesicle size and PDI of Methotrexate loaded transferosomes. The transferosomes were measured to be between 120 and 380 nanometers in size. All of the vesicles' PDI values were in the range of 0.250 to 0.957 nm, indicating that the dispersion is homogeneous.

Table 4: Particle size and zeta potential analysis of optimized formulation

Particle Size & Z-Potential Analysis		
Formulation	**Z-Average size (d.nm)**	**PDI**
F2	254.9 ± 60.2	0.783

Mean ± SD (n=3)

Table 5: Predicted Vs Observed

Formulation Code	Appearance	Grittiness	Spreadability (gm.cm/sec.)	Extrudability	Viscosity	%Drug Content	pH
TG1	White and opaque	No	3.76 ± 0.5	5.5±0.25	4.1±0.54	87.38 ± 0.85	7.0
TG2	Highly viscous	No	2.06± 0.1	7.7±0.20	3.9±0.14	83.06±0.05	6.9
TG3	Clear and soft	No	1.70 ± 1.9	6.2±0.20	4.2±0.32	94.12±0.91	6.8

Mean ± SD (n=3)

Table 6: Results for the optimized Methotrexate loaded transferosomal gel formulation

Composition	Optimized level	Response	Experimental value	Predicted value
Phosphatylcholine	60	Particle size nm	125.7±0.05nm	125.7nm
Sodium deoxycholate	40	% EE	85.2±0.23%	86%
Solvent mixture	2	PDI	0.245±0.31	0.245

Mean ± SD (n=3)

All of the developed transferosomal gel formulations had optimal values for ph, viscosity, drug content, extrudability, and spreadability. Because the formulation is topical, the ph values of all the formulations were found to be appropriate and similar to skin ph.. In the case of gel preparation, viscosity is an important metric to consider when defining the gel because it affects extrudability and drug release. The three formulas had the same viscosity of 4.2 Pa.s the spreadability and extrudability of each formulation were judged to be satisfactory. The spreadability rating reflects how easily the gel can be spread with a small amount of shear. The tube's extrusion is critical during its application and in patient acceptability. The homogeneity of the various formulations was evaluated visually and by applying pressure between the thumb and index finger, and it was found to be excellent

Transmission Electron Microscopy and Scanning electron microscopy

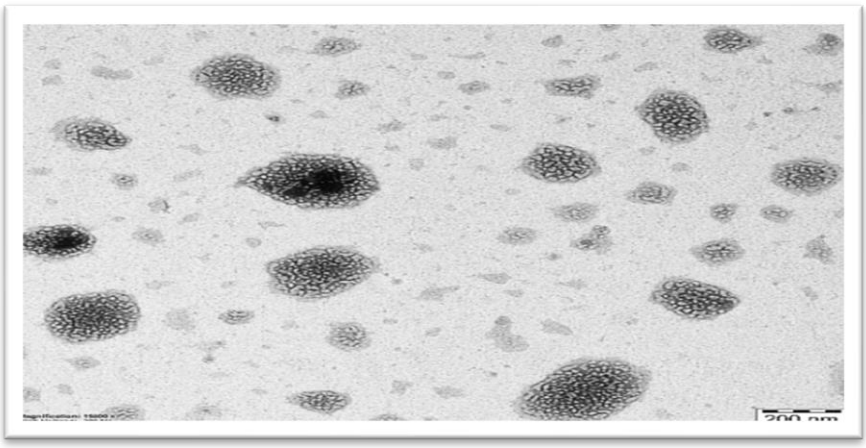


Fig. 4: TEM of optimized TG3 Formulation

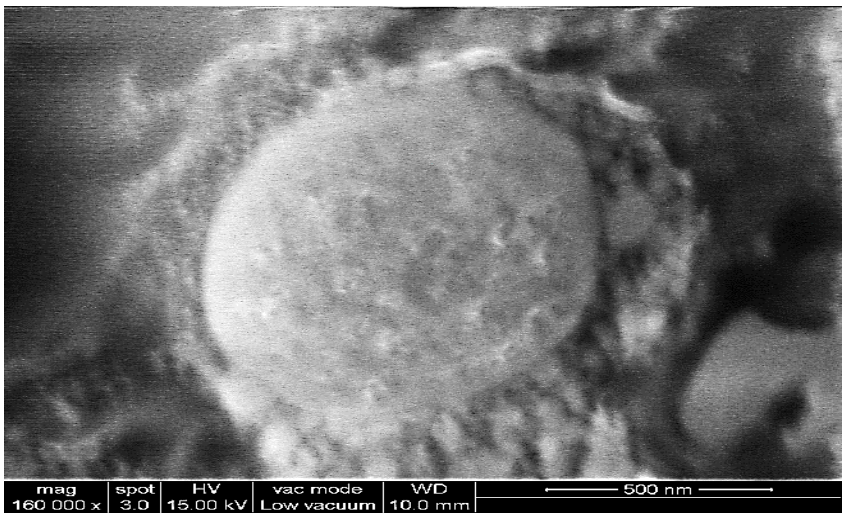


Fig. 5: SEM of optimized TG3 Formulation.

The optimised formulation had a slightly smooth, spherical shape, according to SEM analysis. Particle size analyzers were used to estimate the sizes shown in the experiments

Invitro drug release of Methotrexate loaded transferosomes

The *Invitro* drug release study was performed for the Methotrexate loaded transferosomes to compare the transferosomes and transferosomal gel.

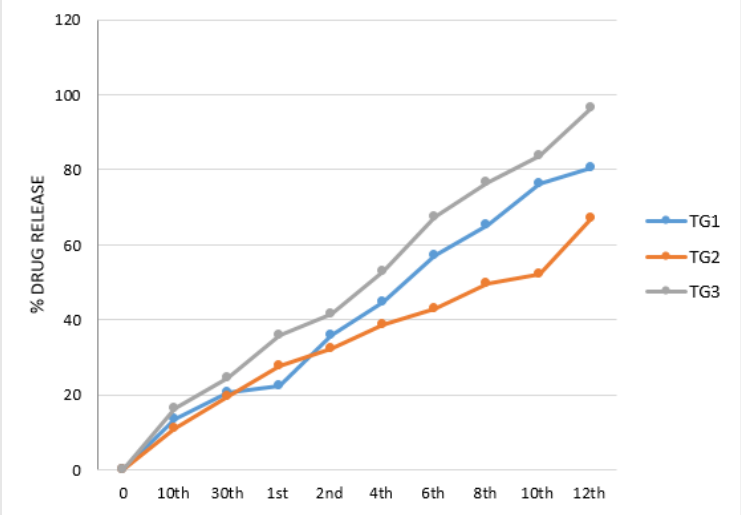


Fig. 6: *Invitro* drug release of Methotrexate loaded transferosomal gel formulation TG1, TG2 and TG3

Fig. 6 explains the *invitro* drug release of Methotrexate loaded transferosomal gel. TG3 shows maximum drug release as compared to TG1 and TG2

TABLE 7: Cumulative drug release of different formulations

S.NO	TIME INTERVAL	CUMULATIVE DRUG RELEASE OF DIFFERENT FORMULATIONS		
		PURE DRUG SUSPENSION	MARKETED FORMULATION	OPTIMIZED FORMULATION
1	0	0	0	0
2	2	15.3±0.45	8.24±1.23	13.2±0.87
3	4	23.3±0.34	14.3±2.45	31.3±0.34
4	6	29.3±0.65	20.4±0.35	41.5±1.12
5	8	30.2±0.23	29.0±1.43	56.7±2.67
6	10	49.3±0.54	35.5±1.46	63.3±1.32
7	12	59.2±0.33	47.4±0.87	73.8±0.23
8	16	73.3±0.78	63.7±0.38	83.4±0.45
9	24	86.3±0.21	84.2±0.66	92.3±0.34

Values are average of triplicate values ± standard deviation

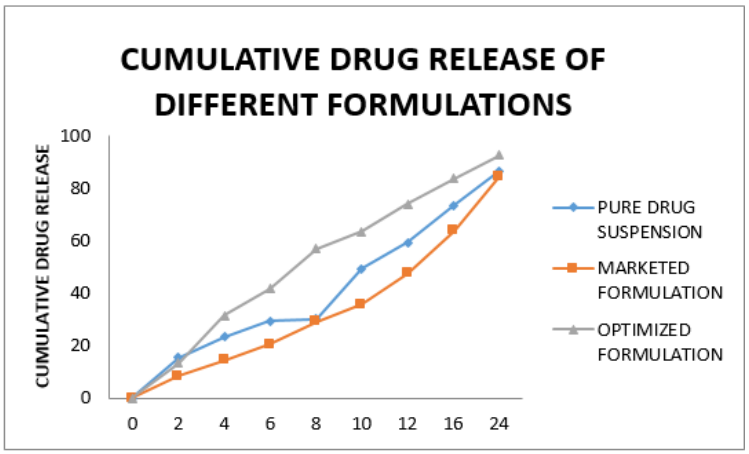


Figure 7: Cumulative drug release of different formulations

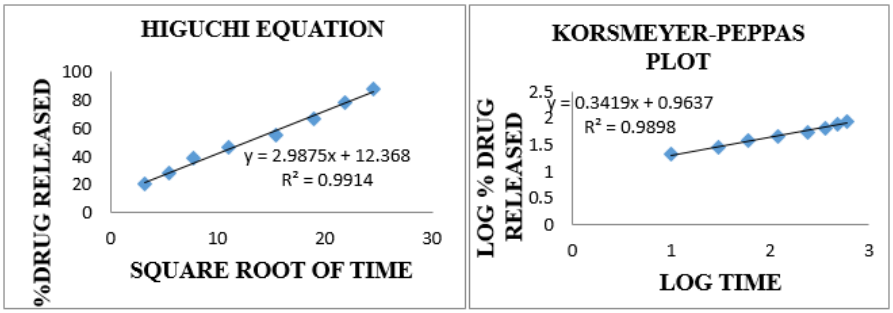


Fig. 8: Graphical representation of Drug release kinetics of Methotrexate loaded transferosomal gel TG3

Release Kinetics of Methotrexate Loaded Transferosomes

Release kinetics from the optimized formulation of Methotrexate Loaded Transfersomes was compared to different kinetic models. Results showed that the model was best fitted with data in the Higuchian equation (R^2 = 0.991). This model explains the drug release from an insoluble matrix time-dependently based on Fickian diffusion. The release constant was computed from the slope of the suitable plots and the regression coefficient was determined. Also, the obtained values proved that after Higuchian model, the best linearity was followed by the first-order kinetics model (R^2 =0.991).A kinetic model was used to calculate the correlation coefficient for transferosomal gel. The results of in vitro drug release of the transferosomal gel formulation TG3 were fitted to release models, but the results showed Higuchi's and Korsmeyer's models. The models were created to show the release of semisolid dosage forms containing low-soluble drugs.

Ex-vivo permeation study

Table: 8 Ex-vivo permeation study

Formulation code	Transdermal flux **(µg/cm^{-2}/h)**	Permeability coefficient **(Cm/h)**	Lag time **(hr)**	Diffusion coefficient **(Cm^2/h)**
Transferosomal gel TamG3	7.02±0.16	0.249±0.42	1.6	8.3

Mean ± SD (n=3)

Transgel has a significantly greater flux value than drug suspension gel. This is due to the existence of polimer, which are important in drug dispersion and penetration. Another explanation is the nano-sized, ultra-transformable character of the transvesicles, as well as the increase in interfacial area, which affects drug delivery.

The slope of the liner portion of the graph was used to determine the mean + S.D. Kp was determined by dividing flux by the drug concentration in the donor cell.

Table 9: Permeation Parameters Of Methotrexate Loaded Transferosomes Across Goat skin

Formulation code	Transdermal flux **(µg/cm-2/m)**	Permeability coefficient **(Cm/h)**	Lag time **(hr)**	Diffusion coefficient **(Cm^2/h)**
Pure drug gel	5.13±0.34	0.210±0.53	2.5	6.4
Marketed gel formulation	5.82±0.21	0.234±0.27	2.1	7.1
Transferosomal gel TamG3	7.02±0.16	0.249±0.42	1.6	8.3

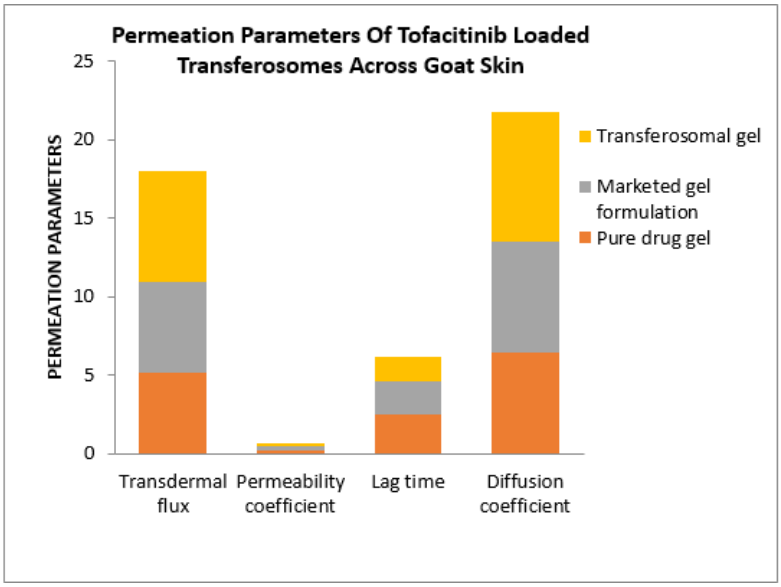


Fig.9: Permeation Parameters Of Methotrexate Loaded Transferosomes Across Goat Skin

3D response surface plot showing effect of independent variable on PDI Methotrexate Loaded Transferosome Formulation

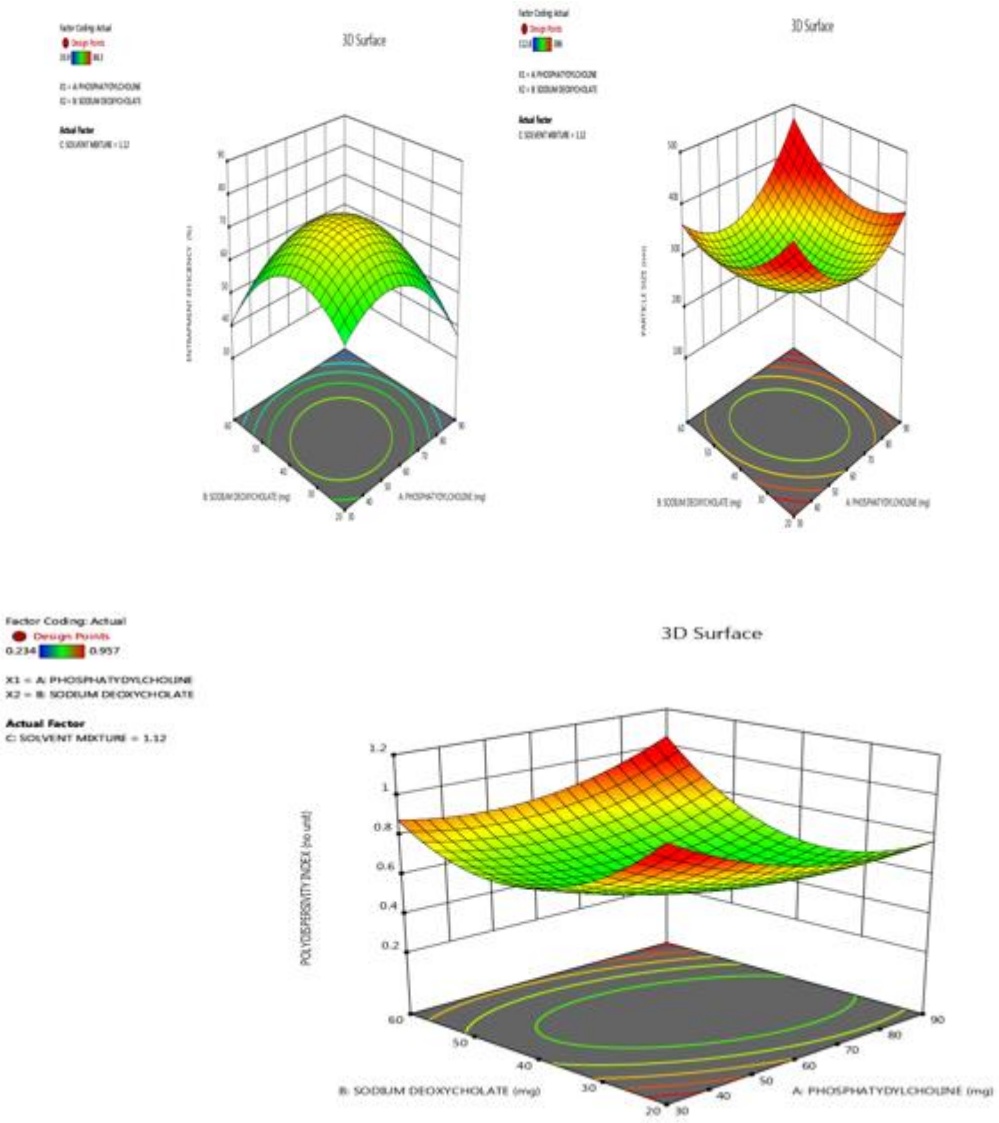


Fig.10: 3D Response surface plot showing effect of independent variable on PDI Methotrexate Loaded Transferosome Formulation

The experimental results were analyzed by 3D Response surface plot and pareto chart to determine the significant effects. The higher t-value observed in the response curve endorse the selection of predominant effects affecting particle size.

Stability studies

The stability studies show that there was a negligible increase in the particle size from 35.41± 0.52 to 36.55±3.34 nm during the storage conditions (4oC and 25oC). The initial % Entrapment efficiency of the optimized transfersomes was found to be 84.24 ± 0.38 %. After 6 months storage at 4ºC and 25ºC it was found to be 81.05±0.62% and 79.24±0.45% respectively. In contrast, there were no significant changes in the % EE during storage of formulation for 6 month 4ºC and 25ºC. Thus optimized formulation was found to be stable at 4ºC and 25ºC temperatures for six months. The data are represented in table 8

Table 10: Stability studies of different formulations

Parameters	Initial values 0 month	1 month		3 months		6 months	
		25±2ºC, 60±5%RH	**(40±2ºC,75±5 %RH)**	**(25±2ºC, 60±5%RH)**	**(40±2ºC,75±5 %RH)**	**(25±2ºC, 60±5%RH)**	**(40±2ºC,75±5 %RH)**
Mean particle size	125.7 ±0.87 nm	235.41± 0.52 nm	235.41± 0.52 nm	235.81± 0.38 nm	235.81± 0.38 nm	236.55±3.34 nm	236.55±3.34 nm
% entrapment efficiency	85.2±0.57 %	81.05±0.62%	81.05±0.62%	80.75±0.35%	80.75±0.35%	79.24±0.45%	79.24±0.45%
Drug content	97.35%	97.20%	97.20%	96.52%	97.02%	95.64%	96.87%

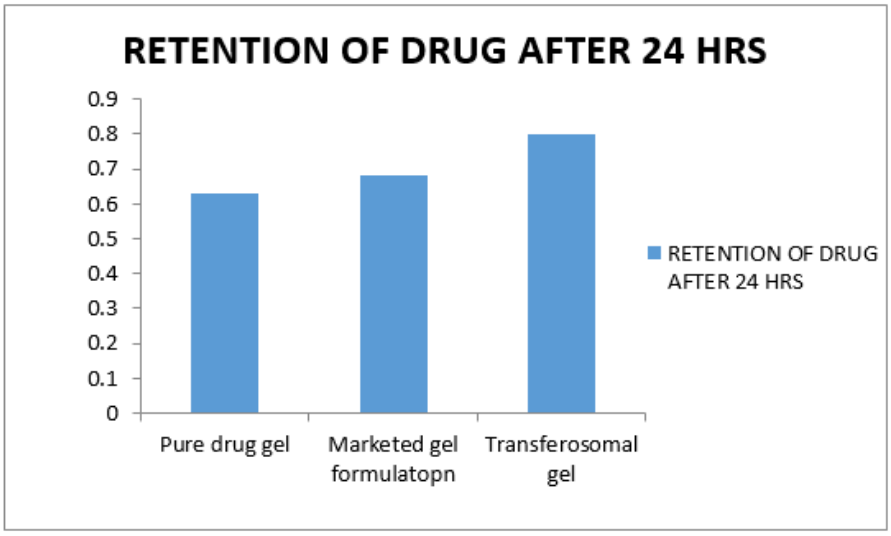
Values are average of triplicate values ± standard deviation

Skin Retention Study:

The skin retention studies of different formulations were performed in order to analyze the content of Methotrexate in the skin after 24 h of diffusion. The study showed that percentage drug retention of formulations was found higher for TG3 as compared to pure drug (PDT). The % retention was near about similar for Marketed formulation, 0.68±0.32 and that of plain drug loaded gel was 0.63±0.280and Transfersomal gel was 0.80±0.05. Skin penetrating effect of the carbopol based novel gel formulations (TG) were studied. The result showed that the nanovesicular moieties embedded in the gel has the ability to penetrate deeper in to the dermal layer and exert their depot effect. The polymeric shell also facilitates its entry into the cells. The percent drug retention of the TG formulation after 24 hours was satisfactory in comparison to the marketed formulation.

FORMULATION CODE	RETENTION OF DRUG AFTER 24 HRS
Pure drug gel	0.63±0.28
Marketed gel formulatopn	0.68±0.32
Transferosomal gel	0.80±0.05

Values represent as mean + S D (n=3)



CONCLUSION

In conclusion, a three-level three-factorial Box-Behnken design was used to optimise a Methotrexate-loaded transferosomal gel. Methotrexate is a cancer-fighting drug. Transfersomes, a type of drug carrier, aid in the non-invasive delivery of medications across the skin. These extremely deformable vesicles' drug-loaded transferosomes can penetrate deeper into the soft tissues. As a result, the Methotrexate transferosomal gel combines safety, effectiveness, and penetrating activity. The findings of using different concentrations of phosphatydylcholine and sodium deoxycholate revealed that transferosomal formulations comprising 60,40,2 concentrations of phosphatydylcholine, sodium deoxycholate, and solvent mixture correspondingly were the most effective. The thin film hydration process was used to create the optimal transferosome. For skin penetration, the formulation possessed the right vesicle shape, size, and % entrapment efficiency. The produced transferosome was then integrated into a Carbopol-940 gel matrix and homogeneity, spreadability, ph and viscosity, as well as an *Invitro* drug release study, an *ex-vivo* permeation research, cumulative drug release of pure drug, marketed formulation and prepared transferosomal gel and skin retention study were all performed. As a result, the researchers came to the conclusion that the proposed formulation benefits from its nano size and promises improved therapeutic efficacy. As a result, the proposed study suggests that the ultra-deformable transferosomal system could be used to treat skin cancer.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

ABBREVIATIONS

MTH: Methotrexate, SCC- Squamous cell carcinoma, BCC-Basal cell carcinoma, EE-Entrapment Efficiency, PDI-Polydispersivity Index, MLV-Multilaminar Lipid Vesicle, MSC- Melanoma Skin Carcinoma, NMSC-Non-Melanoma Skin Carcinoma. TEM-Transmission Electron Microscopy, SEM-Scanning Electron Microscopy, SSF-Stimulated Skin Fluid, TEA- triethanolamine, PCS- Photon Correlation Spectroscopy, PG- Propylene Glycol, IPA- Isopropyl Alcohol, DOE-Design Of Experiments, FTIR-Fourier Transit Infrared Spectroscopy

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