PHARMACEUTICAL DEVELOPMENT OF TAMOXIFEN CITRATE LOADED TRANSFEROSOMAL GEL FOR SKIN CANCER BY DOE APPROACH

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Abstract

Objective: The purpose of the present study is to evaluate the transfersomal gel formulation for transdermal delivery of Tamoxifen citrate by DOE Approach.

Materials and Methods: Transferosomes are ultra-flexible supra-molecular aggregates with a great ability to permeate intact mammalian skin. The formulation were designed by Box-Behnkenn Design. 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-940-934 (0.5, 1.2 g) is being studied.

Results: Entrapment efficiency (EE percent), drug content, in-vitro skin penetration testing, and stability studies were all found in the produced formulations. Transmission Electron Microscopy confirmed that the vesicles were spherical in shape. According to the findings, tamoxifen citrate was effectively pinned with a standardised drug concentration in all formulations. The 0.1 g Tamoxifen citrate optimised transferosome formulation MG2 exhibited encouraging results, with maximum drug release (94.32%) and maximum drug release (94.32%), respectively (94.32%).

Conclusion: Transferosomes, according to this study, are a promising long-term delivery route for Tamoxifen citrate and are relatively stable. This research reveals that transferosomes containing Tamoxifen citrate could be used to treat squamous cell carcinoma via transdermal drug delivery.

Keywords: Transferosome, Edge Activator, Felixibility, Penetration, Tamoxifen citrate

INTRODUCTION

Skin cancer was projected to account for more than a third of all cancers over two decades ago. 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. Non-melanoma skin cancer (NMSC), which is more common, and melanoma skin cancer (MSC), which is more deadly,are the two types of classifications. The most frequent type of skin cancer is melanoma. There are two types of melanoma: benign and malignant.1

(1) A benign tumor is non-cancerous and does not spread quickly. The vast majority of the time, it is seen as a precancerous sign.

2) 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 stage2. Basal cell carcinoma is the most common type of skin cancer in the first category (BCC). BCC seldom spreads beyond the primary tumor location and rarely becomes fatal. It can, however, be disfiguring if not treated promptly.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 rapidly3.

Colloidal particles, often known as vesicles, are aqueous sacs surrounded by a concentric Because polymer bilayer. hydrophilic medications are contained in the inner aqueous compartment and hydrophobic drugs are trapped in the lipid bilayer, they are ideal for vesicular drug administration.Transferosomes are highly deformable (ultra-flexible) and selfoptimizing 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 skin4. Furthermore, Transfersomes can successfully protect a drug undesirable rapid clearance from from cutaneous blood vessels, extending the drug's increasing circulation time and its bioavailability5. Transfersomes have been utilised to transport a variety of chemicals, including macromolecules such as steroids, proteins6, insulin 7, corticosteroids8, ketoprofen9, and anticancer medicines10

After the formulation strategy has been defined, many experiments need to be conducted in order to develop a final formulation. The use of systematic approach and DOE has proven to be a very effective tool for formulation development. DOE allows for the formulation scientist to evaluate multiple factors and their interions while fully controlling the number of experiments11.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

The pure drug tamoxifen was obtained as a gist 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. The formulations were done by using Box-Behnkenn design design expert software version 12.

Experimental design for optimization of drug loaded transferosomes using factorial design

A 3 factor, 3-level factorial design was utilised to explore the quadratic response surfaces and for developing second for second order polynomial models using Design-Expert (Version 12, Stat- Ease Inc., Minneapolis, MN).A design matrix comprising of 17 experimental runs constructed, for which the non-liner computer generated quadratic model is defined as: Y ¹/₄ b0 b b1X1 b b2X2b b3X3b b12 1 2 b b13 1 3b b11X1 2 b b22X2 2 b b33X3, where Y is the measured response associated with each factor level combination; 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 1/4 1, 2 or 3) represent the interaction and quadratic terms, respectively. Lipoid S 100 (X1), Tween 80 (X2), and ethanol were chosen as independent variables (X3). With limits on the formulation of transfersomes, the dependent variables were particle size (Y1), entrapment efficiency (Y2), and poly dispersive index (PDI) (Y3). The Table lists the concentration ranges of the independent variables under investigation, as well as their low and high levels12

Compatibility studies through FT-IR

FTIR spectra obtained through the compatibility of the pure drug and excipient was observed using Bruker FTIR13. The spectra were reported at wave numbers ranging from 3500 to 500cm-1.

Standard calibration curve of tamoxifen citrate

A UV visible spectrophotometer was used to conduct the calibration curve which was measured at 234.5nm.The absorbance of the solution was measured at 234.5 nm and 275 nm using UV Vis spectrophotometry14.

Optimization of Formulation of tamoxifen loaded transferosomes

Formulations were done by using Design expert software version 12.Phosphatydylcholine, sodium deoxycholate, and the tamoxifen citrate 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 byDOE 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

EVALUATION STUDIES

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 Formula:

Amount entrapped

_____X 100

Total amount added Percentage Drug Content

For the determination of percentage drug content transferosome formulation of about 1gm was taken. Sonication was carried out with ethanol to lyse 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 Tamoxifen citrate20

In-Vitro Drug Release Studies:

The cellophane membrane (Molecular weight cut off 12000-14000, HI Media Ltd, Mumbai, India) was used to test different transfersomal formulation drug release trends. On a membrane placed between the donor and receptor chambers (Franz-diffusion cell apparatus) with an accessible diffusion region, an exact amount of formulation was spreaded out. The receptor compartment is filled with a continuously stirred phosphate buffer pH 7.4 with a small magnetic bar at a rate of 50 rpm held at a temperature of 37 ± 0.5 °C. The 5 ml

aliquots were removed at different time intervals and replaced with the same amount of phosphate buffer solution. The samples were analysed in a spectrophotometer after which a graph is shown with the accumulated quantity of drug permeating through the membrane across time to build the invitro drug release 21.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
S.No	A:Phosphatydylcholine	B:Sodium Deoxycholate	C:Solvent Mixture	Particle Size	Entrapment Efficiency	Polydispersivity Index
	mg	mg	ml	nm	%	no unit
1	60	60	1	345	43.2	0.859
2	60	40	2	125.7	85.2	0.245
3	30	40	1	386	67.9	0.765
4	60	40	2	127.3	88.1	0.253
5	60	40	2	128.1	86.7	0.258
6	60	40	2	126.2	86.2	0.255
7	90	20	2	255	45.9	0.546
8	60	20	3	289	55.9	0.873
9	60	60	3	276	47	0.957
10	90	40	1	378	53	0.864
11	90	40	3	332	33.9	0.765
12	60	40	2	128.3	85.6	0.843
13	60	40	2	127	87.9	0.255
14	60	40	2	128.7	88.3	0.26
15	90	60	2	376	44.7	0.764
16	60	40	2	127.1	85.4	0.243
17	60	40	2	128.3	85.9	0.254
18	60	40	2	129	86	0.234
19	30	20	2	289	65	0.923
20	60	40	2	126.4	85.9	0.253
21	60	40	2	112.8	76	0.774
22	60	40	2	118.8	65	0.454
23	30	40	3	324	49	0.435
24	60	40	2	125.5	86.3	0.258
25	30	60	2	234	52	0.667
26	60	40	2	127.3	87.3	0.255
27	60	20	1	378	55	0.764

Table 2: Optimization table 3 level 3 factorial design of transferosomes

After optimizing the formulation 60,40,2 concentrations seems to be optimum batch for the preparation of transferosomal gel

PREPARATION OF TAMOXIFEN CITRATE LOADED TRANSFEROSOMAL GEL USING CARBOPOL-940- 940: Carbopol-940 of three different concentrations

Suitable percentage of carbopal-940 was taken with respect to the table 2. The mixture was stirred until thickening occurred. After complete dispersion, PEG-400 (5ml) was added slowly into the aqueous dispersion of Carbopol-940-940. Then, other ingredients such as isopropyl alcohol (IPA) 5ml, propylene glycol (PG) 5ml and triethanolamine (TEA) 1ml were added. Distilled water (q.s.) for 100g of gel was also added to get homogeneous dispersion of gel. These three different formulations of gel were tested for various evaluation parameters to obtain the best batch of Carbopol-940 gel 22

To the prepared carbopal gel transferosomes of Tamoxifen citrate which is equivalent to 100 mg drug was incorporated as shown in the table 3

 Table 3: Formulation table of Tamoxifen loaded Transferosomal gel

S.NO	Transfersome (mg)	Carbopol- 940 (mg)	Triethanolamine (ml)	PropyleneGlycol (ml)	IsopropylAlcohol (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

TRANSFEROSOMAL GEL LOADED WITH TAMOXIFEN CITRATE.

Homogeneity:

Three different formulations of Carbopol-940 gel were developed and tested for physical appearance through visual observation23.

p H 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 thrice24.

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 formulation25.

Spreadability Test:

The gel formulation of 350 mg which was taken on one glass slide and another glass slide was containing about 5.8 ± 1 g 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:

The gel quantity (g/cm2) extruded from the lacquered aluminum collapsible tube after the application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 s was determined. The extrudability can be measured by using the formula given.

Extrudability =

Weight applied to extrude gel from the tube (g)

Area in $\rm cm^2$

Viscosity

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

Transmission Electron Microscopy Studies:

Transmission electron microscopy was used for determining the formulated gel.

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 taken and the vesicles were lysed with 25 ml of ethanol by sonication for 15 min. Later this solution was placed in a centrifugation tube and centrifuged at 14000 rpm for 30 minutes. The clear solution was diluted to 100 ml with methanol. Then 10 ml of the solution was diluted to 100 ml with phosphate buffer pH 7.4. Aliquots were withdrawn and drug content was calculated for Tamoxifen citrate by using UV spectrophotometer at 227 nm.

In-vitro Release Study :

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

Ex-Vivo permeation study

Excised, defatted goat skin tissues were used to perform in-vitro permeation utilising a Franz Diffusion cell with an effective diffusion surface area and receiver chamber capacity. The tissue was kept at _21 _C in the deep freezer. It was brought to room temperature and installed between the donor and receiver compartments of the Franz diffusion cell during the experiment. The donor compartment was facing the superfacial layer of goat skin, while the receiver compartment was facing the opposite side. Before beginning the experiment, the vaginal tissue was stabilised with stimulated skin fluid (SVF) (pH-4.2). In the incubator shaker, SVF was fed into the receiver chamber and agitated with a magnetic rotor at a speed of 100 rpm to keep the temperature at 37 1 C. Every 30 minutes, the entire media was changed with fresh buffer to maintain stability. After six cycles of stabilisation, 1 mL of the sample (tamoxifen transfersomal gel containing 0.5 percent w/v) was deposited in the donor compartment with 0.75 mL of SVF to simulate the vaginal milieu condition. The receptor compartment was filled with 20 mL of phosphate buffer (pH 4.5). The samples were withdrawn at regular interval

(0.5,1,2,3,4,6,8,10, 12, 14,16 & 24 h) and filtered through 0.45 mm membrane filter the samples were then analysed for drug content by UV spectrophotometry and cumulative percentage drug release was determined . Using the following formulae, the flux (mg/cm2/h) and permeability coefficient (Kp) were calculated:

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 ($25 \pm 20C$) for two months. To evaluate the formulation, pH, spreadability and extrudability were checked after the 1st and 2nd month.

RESULTS FOR TAMOXIFEN CITRATE LOADED TRANSFEROSOMES

PREFORMULATION STUDIES:

Drug Excipient Compatibility Study by FT-IR:

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



Figure 1: FT-IR graph of tamoxifen citrate +phosphatydylcholine+ sodium deoxycholate and Carbopol-940 gel



Figure 2: Calibration curve of Tamoxifen citrate

Optimization of a characterization of tamoxifen loaded transferosomal formulation.

The optimum tamoxifen loaded tranferosomal formulation systems was selected based on particle size , % entrapment efficiency and polydispersivity index, minimising the vesicle size by applying point prediction method of the Design Expert Software Version 12.

The composition of the various factors and comprehensive evaluation were shown below.

Based on 27 different transferozomal formulations designed by the design expert software version 12, particles with a wide. Average size range from 125 to 390 nm were obtained. Among these formulations (f2) phosphatydylcholine (60ml) , sodium deoxycholate (40mg) and solvent mixture of chloroform and methanol(3:1) and 100 mg of tamoxifen was found to

fulfil requisites of an optimum formulation F2 (60,40,2 concentration)Response 3D plots were drawn by using design expert software version 12. These plots were utilized to study about the impacts of 3 different independent variables in the responses while holding the fourth variable at constant level.

Zeta Potential Analysis :

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



Figure 3: Zeta Potential of the optimized formulation

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

PARTICLE SIZE & Z-POTENTIAL ANALYSIS

Formulation	Z-Average size (d.nm)	PDI
M2	254.9 ± 60.2	0.783

Table 5: Results for the optimized tamoxifen loaded transferosomal gel formulation

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

Invitro drug release of Tamoxifen citrate loaded transferosomes.

The invitro drug release study was performed for the tamoxifen citrate loaded transferosomes to compare the transferosomes and transferosomal gel.

EVALUATION STUDIES OF TRANSFERSOMAL TAMOXIFEN CITRATE FORMULATION IN CARBOPOL-940-940 GEL:

The evaluation studies of the three differently formulated tamoxifen loaded transferosomal gel was tabulated in table

S. NO	FORMULATION CODE	APPEARANCE	GRITTINESS	SPREADABILITY (GM.CM/SEC.)	EXTRUDABILITY	VISCOSITY	% DRUG CONTENT	РН
1	TG1	White and opaque	No	3.76 ± 0.5	5.5±0.25	4.1±0.54	87.38 ± 0.85	7.0
2	TG2	Highly viscous	No	2.06± 0.1	7.7±0.20	3.9±0.14	83.06±0.05	6.9
3	TG3	Clear and soft	No	1.70 ± 1.9	6.2±0.20	4.2±0.32	94.12±0.91	6.8

Table 6: Results of transferosomal gel formulation

Transmission Electron Microscopy and Scanning electron microscopy



Figure 5: TEM of TG3



Figure 6: SEM of TG3

INVITRO DRUG RELEASE OF TAMOXIFEN LOADED TRANSFEROSOMAL GEL



Figure 7: Invitro drug release of Tamoxifen loaded tranferosomal gel Drug release kinetics



Figure 8: Graphical representation of Drug release kinetics of tamoxifen loaded transferosomal gel TG3



Figure 9: 3D Response surface plot showing effect of independent variable on PDI Tamoxifen Loaded Transferosome Formulation

Discussion

Tamoxifen citrate is an anticancer drug. The special drug carrier transfersomes helps in non invasive delivery of drugs accross the skin. The drug loaded transferosomes from these ultra deformable vesicles can penetrate more deep into the soft tissues. Thus the transferosomal gel of tamoxifen citrate combines the safety, efficacy and penetration activity. From different concentration of the phosphatydylcholine and sodium deoxycholate used,the results have concluded that transferosomal formulation containing 60,40,2 concentration of phosphatydylcholine,sodium deoxycholate and solvent mixture respectively.

The mean vesicle size and PDI of Tamoxifen citrate loaded transferosomes as shown in the table. The size of the transferosomes was measured in the range of 120 nm to 380 nm. The PDI values of all the vesicles was measured in the range of 0.250 to 0.957 nm indicates the dispersion homogeneous

The results for ph, viscosity, drug content, extrudability and spreadability of all the prepared transferosomal gel formulations shows optimum values. The ph values of all the formulations were found to be acceptable and similar to skin ph, as the formulation is topical.

Viscosity is the important parameter in case of gel preparation for characterizing gel as it affects the extrudability and drug release. The viscosity of the three formulations were found to be 4.2 Pa.s

Each formulations spreadability and extrudability were found be good.

The value of spreadability indicates that the gel is easily spreadable by small amount of shear.

The extrusion from the tube is very important during its application and in patient acceptance.

Homogeneity of the various formulations were tested visual observation and also by applying pressure between the thumb and index finger and was found to be excellent.

SEM studies of the optimized formulation showed slightly smooth, spherical structure.

The size shown in the studies are approximately measured by particle size analyzer.

The correlation coefficient was determined for transferosomal gel by kinetic model. The results of invitro drug release of transferosomal gel formulation TG3 was fitted to the release models, the results however showed Higuchi's and Korsmeyer peppas model .The models were developed to depict the release of low soluble drugs incorporated in semisolid dosage forms.

Flux value for transgel was significicantly higher than drugs suspension gel. This can be attributed to the presence of polimer which play a major role in drug diffusion and permeation. Another reason is nano sized ultra transformable nature of that transvesicles and increase in interfacial area which influences transportation of drug.

Mean + S.D was calculated from the slope of liner portion of graph. Kp was calculated by dividing flux with the concentration of the drug in donor cell.

Hence, methods adopted for gel formulations were found to be suitable.

Thus, we can utilize the combined effect of tamoxifen citrate and transferosome for the topical delivery for the treatment of skin cancer.

Conclusion

In summary, tamoxifen loaded transferosomal gel were optimized using 3 level 3 factorial Box-Behnken design. The optimized transferosome was formulated by thin film hydration technique. The formulation had a suitable vesicle shape, size and maximum percentage entrapment efficiency for the penetration to the skin. The prepared transferosome was finally incorporated into Carbopol-940 gel matrix and suitably characterized by homogeneity, spreadability and ph and viscosity, invitro drug release study and ex-vivo permeation study.

Hence, it was concluded that the developed formulation benefits from its nano size and promises better therapeutic efficacy. Thus, the proposed study indicates that the potential use of ultra deformable transferosomal system in the treatment of skin cancer

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AUTHORS CONTRIBUTIONS

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CONSENT FOR PUBLICATION

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CONFLICT OF INTERESTS

Declared none

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