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Original Article

# Development of liposomal topical gel of bexarotene for effective management of cutaneous t-cell lymphoma: Formulation to preclinical assessment

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**ABSTRACT**

**Background and Aims:** The objective of this work was to formulate liposomal gel formulation of bexarotene for Cutaneous T-cell Lymphoma (CTCL). Low solubility and high log P value make the drug a poor candidate for its penetrability and absorption through the transdermal route. When bexarotene is incorporated into liposomal formulation, its solubility and permeability can enhance.

**Methods:** In the present investigation, the liposomes of bexarotene were prepared by thin film-hydration method and optimized for different critical processing parameters such as the amount of lipid and the time of stirring. Checkpoint batches were prepared to validate the mathematical model.

**Results:** The final optimized liposome formulation, which has more than 85% entrapment efficiency and vesicle size of 625 nm, was prepared. The optimized liposomes were loaded (equivalent to 1% w/w bexarotene) into the carbopol gel (1.5% w/w) and, evaluated for physico-chemical parameters. In vitro drug permeation and deposition of promised liposomal gel were performed through rat skin. The skin irritation studies of the liposomal gel were examined on rats, in vivo. MTT assay was performed to determine the cytotoxicity and cell apoptosis on CTCL specified cell line (Hut-78) by bexarotene liposomal gel. The optimized liposomes of bexarotene (FL1) were found to be spherical having a vesicle size of 639 nm with PDI 0.115 and a zeta potential value of -19.3 mV. The promised liposomal gel (LG5) evaluations were found in the limit. The LG5 was shown 31% bexarotene deposition in the skin. The experiment revealed a significant decrease ($p<0.005$) in the number of viable cells following MTT assay.

**Conclusion:** The liposomal gel formulation of bexarotene improved the treatment and management of CTCL.

**Keywords:** Bexarotene, Liposome, Topical Gel, Lymphoma, MTT assay, UV skin irritation studies

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## INTRODUCTION

The cutaneous T-cell lymphoma (CTCL) is the most common form of non-Hodgkin's lymphoma that involves skin, blood, lymph nodes, and other internal organs (Olsen, 2015). In the early and advanced stages of the disease, the symptoms are observed only in the skin, including itching, dryness, rashes, and enlargement of lymph nodes. Various epidemiological studies have substantiated that CTCL disease is also promoted or triggered by environmental and external exposures, viz., air pollution and chemical exposure, including pesticides, detergents, and UV rays (Zinzani et al., 2016).

Bexarotene is a highly effective anticancer agent that has been approved for the treatment of CTCL. Bexarotene, a third-generation selective retinoid X receptor agonist, triggers the receptor and initiates prompting cell variation, which has been shown to demonstrate promising effects in the management and treatment of CTCL. The mechanism of action of bexarotene is not completely understood. Recent studies reported that the induction of apoptosis in CTCL cell lines (MJ, Hut78, and HH; $IC_{50}$ ~160-171mM) in association with activation of caspase-3 and cleavage of poly–adenosine diphosphate-ribose polymerase and interleukin-4 production, which plays a major role in the systemic immunosuppression that is characterized by advancing Sézary syndrome, may be inhibited by bexarotene (Chen et al., 2014). However, the topical delivery of bexarotene is diminished due to its high log P (6.9) value and poor aqueous solubility, therefore, it is difficult to penetrate the different layers of skin (Agarwal, Katare, & Vyas, 2001). Liposomal delivery of bexarotene can improve the transdermal delivery and increase cell membrane penetration as well as the treatment of cutaneous T-cell lymphoma.

Liposomes are spherically-shaped and nano-structured vesicles composed of an outer bilayer envelope made up of one or more non-toxic, biodegradable, and biocompatible phospholipids with an aqueous core that easily entraps both hydrophobic and hydrophilic therapeutic agents. They are proven for the effective delivery of the therapeutic agents topically for various disorders of the skin as (Sharma & Verma 2017; Minh et al., 2015). The present research aims to develop a liposomal topical gel of bexarotene and characterize it for various physicochemical parameters followed by skin irritation studies and *in vitro* cytotoxicity activity (Hashemi, Karami-Tehrani, & Ghavami, 2004).

## MATERIALS AND METHODS

### Materials

Bexarotene was received as a gift sample from Apicure Pharmaceuticals Pvt. Ltd. (Vadodara, India); Phospholipon 90H (PL-90H), Phospholipon 100S (PL-100S), and Soya phosphatidylcholine (SPC) were purchased from Himedia (Mumbai, India), Cholesterol from Lobachem (India), and ethanol and ethyl acetate from SD Fine Chemicals Ltd. (India). Carbopol 934P was acquired as gift sample from Lubrizol Limited (India). Ethanol was purchased from Finar Pvt. Limited (India). Cellophane membrane (MW 12000–14000) was purchased from Himedia, (India); methanol and other chemicals used were of analytical grades.

### Development of liposomes

Liposomes of bexarotene were prepared by the thin-film hydration method using a rotary evaporator (Heidolph Hei VAP Advantage AQ/G3, Germany). The drug was solubilized in a blend of ethyl acetate and ethanol in the ratio of 1.5:1.0. The thin film formed by using a mechanical dispersion of bexarotene, different phospholipids like phospholipon 90H, phospholipon 100S, soya phosphatidylcholine (1-4% w/v), and cholesterol (1% w/v) was appropriately mixed in ethyl acetate in a 250 mL round bottom flask (RBF) as given in Table 1. By using the rotary evaporator in reduced pressure, organic solvents were condensed and recovered, which created a thin film around the inner surface of the flask at 50ºC and 90 rpm. The obtained film was subjected to vacuum for an hour for the complete removal of the remaining solvent. Then, the dried film was hydrated with a phosphate buffer (pH 7.4) for 30 min. The ready mixture was vortexed and sonicated in a bath sonicator (Ultra Sonic, India) for 15 min. and the solution of liposome was prepared and stored in the refrigerator for further evaluations (Akbari et al., 2020).

### Characterization of liposomes

*Vesicle size determination*

The dynamic light scattering method was employed to determine the vesicle size of bexarotene liposomes. The liposomal suspension was diluted with water (10 times, MilliQ®) and transferred in the zeta sizer cell (Malvern Zetasizer NanoZS90, UK), and the vesicle size was determined according to the standard operating procedure. The temperature of the cell was kept constant at 25°C and three values were obtained at 175°scattering angle (Manosroi, Kongkaneramit, & Manosroi, 2004).

*Zeta potential determination*

Polydispersity index was determined by zeta potential, which could be a measure of uniformity. The sample for determining the zeta potential was prepared similarly as that for the determination of vesicle size. It was then examined thrice from -200 mV to +200mV as per the method of Dicko *et al.,* 2010.

*Morphological evaluation*

The surface morphology of liposomes was evaluated using transmission electron microscopy (TEM) by JEM 2100 microscope (Hitachi, H-7650, Japan). The diluted liposomal dispersion was fixed on the copper grids (Formvar®) for morphological studies. The diluted liposomal suspension was stained with the solution of uranyl acetate (2%, w/v) for proper imaging of samples (Fathalla Youssef, & Soliman., 2020).

*Entrapment efficiency and drug loading*

For determination of entrapment efficiency (%, EE) the ultrafiltration centrifugation method was applied, wherein 2 mL of the formulation was centrifuged at the rate of 10.000 rpm for 30 minutes utilizing a cooling centrifuge AmiconUltra-4 (Ultracel-10) centrifugal filter (Merck Millipore Ltd., Germany) (Fathalla et al., 2020). Both pellet and supernatants were collected and the amount of free drug within the supernatant liquid was determined utilizing the RP-HPLC assay method. The entrapment efficiency in liposomes was calculated as

$$\%\ Entrapment\ efficiency = \frac{Ct - Cr}{Ct} \times 100$$

Ct = Total drug concentration, Cr = Free drug concentration.

*In vitro drug release studies*

The dialysis method was employed to study the in vitro drug release from liposomes. Ten milliliters of the bexarotene liposomes were placed in a dialysis bag (Spectra Por S/P 2 dialysis membrane, 12K–14K molecular weight) and dipped in 40 mL dialysis media (phosphate buffer, pH 7.4 with Tween 80 (0.02%, v/v) to maintain sink condition at 37±0.5°C, and stirring rate was kept constant at 50 rpm. Samples of two milliliters of release medium were withdrawn for analysis at one-hour intervals and replaced with a fresh medium. Drug release studies were continued for 12 hours, and the obtained samples were analyzed using the RP-HPLC method (Singh et al., 2005).

**Optimization of liposomes**

In the preliminary studies, various lipids were screened out for the preparation of bexarotene liposomes viz., PL-100S, PL-90H, and SPC, respectively, and sonication time, cholesterol-lipid ratio, and drug loading during the experiment were studied. Bexarotene liposomes were optimized using Response Surface Methodology by $3^2$ full factorial design methods. The two factors at three-level design were employed for constructing second-order polynomial models (quadratic) using Design Expert 11 (Version Trial; Stat-Ease Inc, Minneapolis, Minnesota) software. A total of nine formulations, as shown in Table 2, were prepared according to experimental design. The selected independent variables were the amount of phospholipon-100S (X1) and time for rotation (X2). The entrapment efficiency (%) and vesicle size (nm) were selected as dependent variables (Garg et al., 2017).

The responses were calculated using interactive and polynomial terms of the mathematical model.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{12}X_1X_2$$

In the equation, Y denotes the dependent variable, $b_0$ is the arithmetic mean $b_1$, and $b_2$ are the coefficients for the factors $X_1$ and $X_2$. Optimized formulation was validated, and analysis of variance (ANOVA) was used to analyze the results. ANOVA was applied to find out the significance of factors (Jangde & Singh, 2016).

*Checkpoint batches*

The checkpoint batches were prepared and compared with values obtained from the software utilizing the equations of the model validity. Checkpoint batches were prepared and evaluated for percent entrapment efficiency and vesicle size. The predicted value and observed values were compared and residual values were determined (Table 3).

*Optimized batch*

Final optimized Bexarotene liposome batch FL1 was prepared as per the suggested quantity of X1 and X2 factors, which was set targeted for entrapment efficiency (85%) and vesicle size (625 nm), as shown in Table 3. The method of preparation of the liposome was the same as that mentioned previously.

**Preparation of liposomal topical gel**

For the preparation of the liposomal topical gel, firstly the Carbopol 934P (0.5 to 1.75%, w/w) was splashed into distilled water (Table 4). The topical gel was formed by swelling and hydration carbomer for 3-4 h. The acidic nature of Carbopol suspension was neutralized using triethanolamine for crosslinking and gel formation. Then isopropyl alcohol, methyl paraben, and propyl paraben were added and blended with a gel base. The liposomal suspension (FL1) was at that point blended with this Carbopol gel base by mechanical blending at a speed of 1000-1500 rpm for 1 h to make the liposomal topical gel dispersion (Yu et al., 2019).

**Characterization of liposomal topical gel**

The developed liposomal topical gels were characterized physically for their color, transparency, odor, morphology, and

**Table 1. Development of bexarotene liposomes.**

| Form. Code | Lipid (%, w/w) | | | Cholesterol (%, w/w) | Drug (%, w/w) | Visual Appearance | Vesicle Size (nm) | EE (%) | PDI |
|---|---|---|---|---|---|---|---|---|---|
| | PL-90H | SPC | PL-100S | | | | | | |
| L1 | 1 | - | - | 1 | 1 | Rough layer | 835.5±5.4 | 61.1±2.1 | 0.519 |
| L2 | 2 | - | - | 1 | 1 | Rough layer | 901.3±6.8 | 72.3±2.8 | 0.573 |
| L3 | 3 | - | - | 1 | 1 | Rough layer | 983.5±8.2 | 82.1±3.8 | 0.641 |
| L4 | 4 | - | - | 1 | 1 | Rough layer | 995.4±9.3 | 73.3±6.5 | 0.654 |
| L5 | - | 1 | - | 1 | 1 | Rough layer | 779.3±3.7 | 61.1±1.9 | 0.522 |
| L6 | - | 2 | - | 1 | 1 | Rough layer | 895.4±4.8 | 69.3±2.8 | 0.571 |
| L7 | - | 3 | - | 1 | 1 | Rough layer | 885.4±8.3 | 71.2±2.5 | 0.481 |
| L8 | - | 4 | - | 1 | 1 | Rough layer | 991.4±7.4 | 83.5±2.6 | 0.594 |
| L9 | - | - | 1 | 1 | 1 | Smooth layer | 182.4±2.4 | 65.1±1.5 | 0.123 |
| L10 | - | - | 2 | 1 | 1 | Smooth layer | 213.2±2.9 | 81.2±1.8 | 0.271 |
| L11 | - | - | 3 | 1 | 1 | Smooth layer | 639.2±3.6 | 89.9±1.9 | 0.116 |
| L12 | - | - | 4 | 1 | 1 | Smooth layer | 715.2±3.7 | 74.2±2.4 | 0.317 |

**Table 2. Design matrix $3^2$ full factorial design, ANOVA responses, and the observed coefficient for optimization of bexarotene liposomes.**

| Batch | X1: Amount of Phospholipon-100S (%w/w) | X2: Time of Rotation (Minutes) | Y1 Entrapment Efficiency (%) | Y1 Vesicle Size (nm) |
|---|---|---|---|---|
| OL1 | -1 | -1 | 60.34±1.33 | 170.92±10.32 |
| OL2 | -1 | 0 | 65.13±1.24 | 182.45±10.56 |
| OL3 | -1 | +1 | 63.16±2.23 | 176.84±15.52 |
| OL4 | 0 | -1 | 78.34±2.34 | 242.56±16.25 |
| OL5 | 0 | 0 | 81.25±2.48 | 213.24±13.33 |
| OL6 | 0 | +1 | 79.46±2.89 | 225.28±25.49 |
| OL7 | +1 | -1 | 86.52±3.79 | 620.46±16.49 |
| OL8 | +1 | 0 | 89.96±2.32 | 639.27±18.33 |
| OL9 | +1 | +1 | 90.56±3.49 | 656.59±25.33 |
| **Coded Value** | | **-1 (Low)** | **0 (Intermediate)** | **+1 (High)** |
| X1 | Amount of Phospholipon-100S (%, w/w) | 1 | 2 | 3 |
| X2 | Time of Rotation (Minutes) | 10 | 15 | 20 |
| **Independent Variables** | | **Y1** | **Y2** | |
| | | **Entrapment Efficiency (%)** | **Vesicle Size (mm)** | |
| | | **ANOVA Response** | | |
| F Value | | 20.71 | 188.24 | |
| P Value | | 0.0156 | 0.0006 | |
| $R^2$ | | 0.9718 | 0.9968 | |
| Adjusted $R^2$ | | 0.9249 | 0.9915 | |
| Predicted $R^2$ | | 0.6687 | 0.9663 | |
| Adeq Precision | | 14.13 | 39.2413 | |
| | | **Coefficients** | | |
| $b_0$ | | 73.222 | 376.333 | |
| $b_1$ | | 9.833 | 196 | |
| $b_2$ | | -10.833 | 76.5 | |
| $b_{12}$ | | -0.25 | 20 | |
| $b_{11}$ | | 2.166 | 49 | |
| $b_{22}$ | | -0.833 | -12.5 | |

separation of phases. The pH of the prepared liposomal topical gels was determined using a digital pH meter (Benchtop pH meter, Mettler Toledo, Mumbai). The topical gel was dispersed in milliQ water, and the electrode of pH meter was plunged inside the topical gel container and the pH was recorded until a constant reading was achieved. The parallel plate method was used to determine the spreadability of the liposomal topical gel. The liposomal topical gel (100 mg) was put on a transparent glass slide and another transparent slide of glass was put upon the slide containing the topical gel. Initially, the spread topical gel diameter was recorded by the weight of the glass slide only. After stress given by weight (ranging from 5 g to 100 g for a 30-second interval) on the liposomal topical gel, the spread diameter was calculated after the addition of each weight (Bavarsad et al., 2016). The spreadability of the topical gel was determined by the following equation:

$$S = m\,X\,\frac{l}{t}$$

where, S = spreadability, m = weight on upper slides, l = glass slide length (7.5 cm), t = time (s).

The viscosity of the liposomal topical gel was determined with the help of a Brookfield viscometer (S-62, LVDV-E) at 25 ºC with a fixed speed of 12 rpm. Drug content of the liposomal topical

**Table 3. Checkpoint batches and optimized batch.**

| Form. Code | Lipid (% w/w) Phospholipon 100S | Rotation Time (minutes) | Entrapment Efficiency (%) | | | Vesicle Size (nm) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Predicted Valve | Observed Value | % Residual Value | Predicted Valve | Observed Value | % Residual Value |
| LC1 | 1.5 | 12.5 | 73.99 | 74.23±12.35 | 0.32 | 254.20 | 248.12±12.39 | 2.45 |
| LC2 | 1.5 | 17.5 | 63.28 | 61.26±6.31 | 3.29 | 320.78 | 333.10±18.21 | 3.69 |
| LC3 | 2.5 | 12.5 | 83.95 | 86.91±11.25 | 3.40 | 440.20 | 448.31±15.28 | 1.80 |
| LC4 | 2.5 | 17.5 | 72.99 | 75.84±10.56 | 3.75 | 526.70 | 551.45±19.84 | 4.48 |
| FL1 | 3 | 15.5 | 84.99 | 89.9±1.9 | 4.67 | 623.26 | 639.2±4.9 | 2.49 |

**Table 4. Characterization of liposomal topical gel formulations.**

| Code | Polymer (%, w/w) | Formulation Incorporated/Drug | Appearance | Viscosity (cPs) | Drug Content (mg) | Spreadability (cm) | pH |
|---|---|---|---|---|---|---|---|
| G1 | 1.5 | Pure Drug | Loose, Non-greasy, Homogenous | 4332.2 | 99.1±2.6 | 4.5±0.55 | 6.9±0.2 |
| LG1 | 0.5 | FL1 | Loose, Non-greasy, Homogenous | 1291.6 | 95.3±1.9 | 5.4±0.22 | 6.7±0.1 |
| LG2 | 0.75 | FL1 | Non-greasy, Homogenous | 1869.5 | 97.3±1.3 | 5.3±0.35 | 6.9±0.3 |
| LG3 | 1.0 | FL1 | Non-greasy, Homogenous | 2494.7 | 94.6±2.4 | 5.0±0.51 | 6.9±0.1 |
| LG4 | 1.25 | FL1 | Non-greasy, Homogenous | 3196.8 | 96.5±1.9 | 4.7±0.52 | 6.7±0.3 |
| LG5 | 1.5 | FL1 | Non-greasy, Homogenous | 4286.9 | 93.7±1.4 | 4.6±0.24 | 6.8±0.2 |
| LG6 | 1.75 | FL1 | Hard, Non-greasy, Heterogeneous | 5619.7 | 97.2±1.9 | 3.8±0.68 | 6.9±0.4 |

**Table 5. Skin irritation data.**

| Group | Treatment | Dose (mg/cm$^2$) | No. of Animals | Wrinkle Score |
|---|---|---|---|---|
| Group I | Control | - | 06 | 0 |
| Group II | UV treated + Drug free gel topically | 50 | 06 | 2.83±0.408$^{NS}$ |
| Group III | UV treated + Drug free Liposomal gel topically | 50 | 06 | 2.33±0.516$^{NS}$ |
| Group IV | UV treated + Drug Liposomal gel topically | 50 | 06 | 2.28±0.753$^{NS}$ |

Values are expressed as Mean±SD One-way Anova followed by Bonferroni test,
*P<0.050 significant and NSP<0.050 non-significant compared to the UV treated group.

gel was estimated by shaken 100 mg bexarotene equivalent liposomal topical gel with an adequate quantity of methanol to extract the bexarotene and then estimated by utilizing the RP-HPLC method. The *in vitro* diffusion of the drug from the liposomal topical gel and plain drug-loaded topical gel was examined utilizing a Franz diffusion cell apparatus kept at 37±1$^0$C. The actual diffusion area diameter of the diffusion cell apparatus was 2.30 cm$^2$. The receptor compartment consisted of 6.5 mL phosphate buffer (pH 7.4) and was stirred at 100 rpm. Cellophane membrane (MW; 12K–14K Dalton) was fixed between the compartments of the donor and the receptor. The formulation was placed in the donor compartment. The

aliquots were taken from the Franz diffusion cell at different time intervals and after each sampling, the diffusion media already kept at 37±1$^0$C was exchanged into the receptor compartment (Priprem et al., 2018).

*Skin permeation studies*

The shaved dorsal skin of sacrificed albino rats was used for diffusion studies. The fat was separated from the near dermis side using a blade and alcohol. Collected skin was cautiously washed with phosphate buffered saline solution (PBS, pH 7.4) and stored in the deep freezer (-20°C). Before initiating diffusion studies, the diffusion medium (PBS pH 7.4 containing Tween® 80 (0.02%, v/v) to maintain sink condition) was added to the Franz diffusion cell and kept at 37±1$^0$C for one hour. The optimized liposomal topical gel was applied on dorsal skin. Aliquots were withdrawn at different time intervals. The amount of the receptor fluid was kept constant by introducing a new receptor fluid sample. The RP-HPLC method was used to analyze the samples (Surini, Leonyza & Suh, 2020).

*Drug deposition studies*

The rodent skin which was used in the skin permeation studies was carefully expelled. The skin was washed with the mixture of methanol and distilled water (1:1) to eliminate traces of the drug formulation on the outer part of the skin portion. The washed skin was divided into small portions by cutting and dipped in a 10 mL volume of 0.05% trypsin solution. Sample tests were put on a mechanical stirrer at 100 rpm and at 37±1$^0$C for 24 h. Samples were analyzed after filtration using the RP-HPLC method.

*Skin-irritation studies*

The designed protocol for the animal studies was approved by IAEC (PBRI/IAEC/PN-19016). The animals were subdivided into 4 groups of 24 animals. Animals of Group I served as the control group, while Animals of Group II received five-minute ultraviolet light exposure two times a day and served as the irradiated control group; drug-free topical gel was applied. The test groups III and IV received both ultraviolet radiation and drug-free liposomal topical gel and bexarotene liposomal topical gel treatment, respectively. The treatment was provided to animals four hours before ultraviolet radiation exposure as per the protocol guidelines. All the animals were kept in a locally prepared wooden ultraviolet simulator or chamber. The ultraviolet lamp (300W Waton® bulb, Germany) was fixed inside on the top of the chamber so the animals received radiation from a distance of 40 cm. The bulb produces the full spectrum of ultraviolet radiation from 260 - 400 nm. Ultraviolet radiation exposure was controlled by exposure time. The animals were treated for a month to study the changes obtained on dorsal skin (Jewett *et al.* 2020; Raimondi, Suppa & Gandini, 2020).

*In-vitro cell proliferation studies*

A well-developed MTT assay was used to assess the proliferative ability of cells treated with bexarotene-loaded topical gel and bexarotene-loaded liposomal topical gel. Around 5×10$^3$ Hut78 cells *per* well were inoculated in 96-well formats. Serum medium RPMI was changed to serum-free RPMI medium after a one-day incubation period (Madan et al., 2012). After completion of a day, cells were treated with bexarotene-loaded topical gel and bexarotene-loaded liposomal topical gel at gradient concentrations ranging from 0-150µM/mL in PBS (pH 7.4) for three days. At the end of treatment, 5 mg/mL MTT was added to each well, and the plate was incubated at 37°C in the dark for 4 h. The formazan product was then dissolved in 100 µL of DMSO after removing the medium or content of each well (Bhatia et al., 2009).

**Ethical approval**

Ethics committee approval was obtained for the study from Pinnacle Biomedical Research Institute (PBRI) Bhopal Ethics Committee with the date 24.08.2019 and decision number PBRI/IAEC/PN-19016.

## RESULT AND DISCUSSION

**Development of liposomes**

Liposomes prepared with three lipids Phospholipon 90H, Phospholipon 100S, and soya phosphatidylcholine exhibited vesicle size from 182 to 995nm. Liposomes prepared using Phospholipon 100S (182.4±2.4 nm, 0.123) appeared to have a smaller size, size distribution, and PDI when compared with Phospholipon 90H (835.5±5.4nm, 0.519) and soya phosphatidylcholine (779.3±3.7 nm, 0.579), as shown in Table 1.

Liposomes with Phospholipon 100S showed lower PDI values, indicating smaller size distribution compared to others. The small vesicle size of the liposome formulation made up of Phospholipon 100S was shown to have a higher entrapment efficiency value (L11, 89.9% ±1.9).

The liposomes stabilized by using the cholesterol in their structure. Rigidity improved the leakage of drug from bi-lipid layers prevented by the use of cholesterol. It also reduced the flexibility of the liposomes, which increases the stability. The entrapment efficiency improved due to prevention of leakage and flexibility.

The cholesterol-lipid ratio was optimized for 1:1, 1:2, 1:3, and 1:4. It was witnessed that by increasing the amount of cholesterol and lipid the percent entrapment efficiency of the drug was increased gradually. The vesicle size and PDI also increased as the ratio of cholesterol-lipid was increased. In the preliminary studies, it was found that if EE is >85% then there is no effect of sonication time. Hence sonication time was optimized to 15 minutes based on the results of vesicle size, PDI, and EE (%).

A $3^2$ full factorial design was employed in the optimization of liposome development. In the used design two factors are evaluated, each at three levels. All nine possible combinations were performed in an experimental trial. The amount of Phospholipon-100S ($X_1$) and the time for rotation ($X_2$) were selected as independent variables. The entrapment efficiency (%) and vesicle size (nm) were selected as dependent variables. ANOVA was applied to detect insignificant factors. The fit of the model was dependent upon the lower p-value, high F-value, high level of adjusted $R^2$, and predicted $R^2$ (Table 2). From the data of entrapment efficiency (%) of the factorial formulations OL1 to OL9, polynomial equations for entrapment efficiency (%) had been derived using Design Expert 11 software. The co-

efficients for entrapment efficiency (%) (Y1) and Vesicle Size (nm) (Y2) of the factorial formulations are shown in Table 2. In the case of entrapment efficiency (%) (Y1), the positive sign for coefficients of X1 indicate that as the amount of Phospholipon-100S increases, entrapment efficiency (%) increases. The negative sign for coefficients of the time for rotation (X2) indicates that as the time increases, entrapment efficiency (%) decreases. In the case of vesicle size (nm) (Y2), the positive sign for coefficients X1 and X2 indicated that as the concentration of Phospholipon-100S and the time for rotation (X2) increases, Vesicle Size (nm) increases.

The validity of the optimization model was verified using four extra design checkpoint formulations (LC1 to LC4) and determining their entrapment efficiency (%) and vesicle size (nm). The nearness of the predicted and observed values of LC1 to LC4 in the method indicates by their low value of percent residual value. So results also proved the derived equations for the dependent variable for entrapment efficiency (%) and vesicle size (nm) (Table 3) predicted by software. The software-generated contour plots of response surface optimization method for the dependent variable are shown in Figures 1A and 1B, respectively.

The optimized batch (FL1) was prepared for the entrapment efficiency (85%) and vesicle size (625 nm). The software suggested the amount of Phospholipon-100S (3%, w/w) and time of rotation (15.5 minutes) to get optimized results. Here, a very low deviation was observed in predicted value and observed value. The average vesicle size of the optimized batch (FL1) was found to be below 639 nm and PDI was found to be below the desired value of 0.15 (Figure 2A). The zeta potential of the optimized formulation was observed to be -19.3 mV (Figure 2B). TEM results showed the lamellarity and morphology of optimized bexarotene liposomes. (Figure 2A, insert).

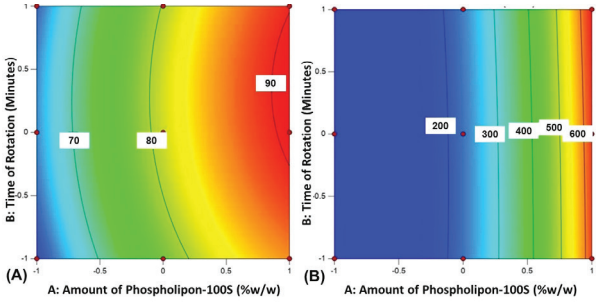


**Figure 1.** Contour plots indicating the relationship between the factors A and B on the response variables, (A) Entrapment Efficiency (%) (B) Vesicle Size (nm).

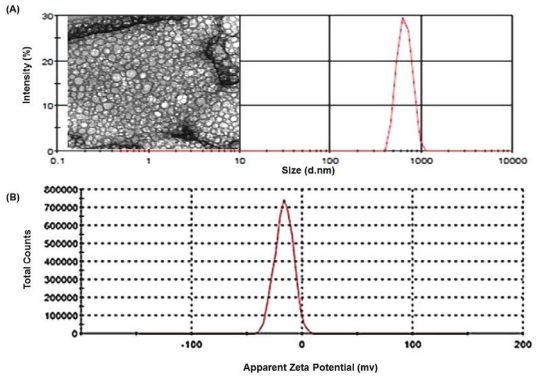


**Figure 2.** (A) Vesicle size, insert TEM photograph and (B) zeta potential distribution of optimized bexarotene liposome formulation (FL1).

## Development of bexarotene liposomal topical gel

Several carbopol concentrations (0.5 to 1.75%, w/w) were used and the best was selected based on viscosity, consistency, gel-forming capacity, and spreadability. Except for G1, all other formulations gave sufficient viscosity, consistency, and spreadability to enable topical application that was homogenous and non-greasy in nature and appearance. It was observed that the topical gel dried and made a hard film a while after application. To elude the drying nature of the topical gel, isopropyl alcohol, methyl paraben, and propyl paraben were added to the liposomal topical gel. The values of various evaluation parameters were found for the drug content (93 to 97%), viscosity (1292 to 5620 cps), spreadability (3.8 to 4.4 cm), and pH value (6.7 to 6.9). The high spreadability value shows that the liposomal topical gel was spread easily with a small application of stress. The high value of spreadability was useful in CTCL for even distribution and spreading of liposome on the dorsal skin surface. The rheological behavior of the liposomal topical gel was also responsible for the spreadability and residence time of topical gel on the skin surface. If viscosity values are between 4000 to 5000 cps at 25 ºC with a fixed speed of 12 RPM with spindle S-62, LVDV-E is ideal for topical gel. Rheological behavior was interpreted by the viscosity values optimized against shear rates and from the results it was observed that the viscosity reduced with an increase in shear rate. So, the formulated topical gel was having pseudo-plastic behavior, which was ideal for the topical formulation and application (Saka et al., 2020).

## *In vitro* drug release and *ex vivo* permeation studies

For release studies of bexarotene drug solution and bexarotene liposome formulation, the withdrawn samples from the dialysis apparatus were diluted and analyzed. For the drug solution dialysis, the complete release was perceived within 10 h (Figure 3), whereas from the liposome formulation, drug release was found in a controlled manner and 60% of the drug was released within 12 h. In the study of drug diffusion from liposomal topical gel from Franz

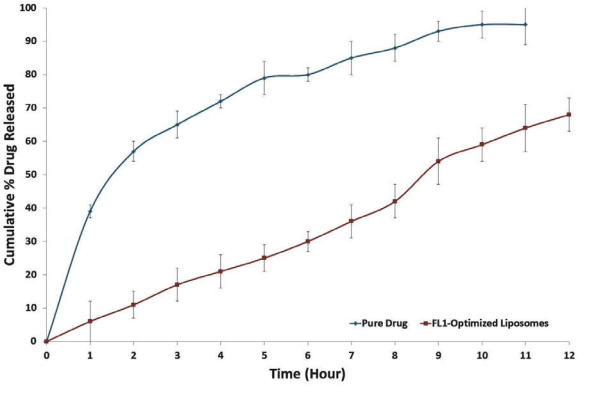


**Figure 3.** Comparative in-vitro dissolution profile of pure bexarotene drug and optimized liposome formulation (FL1) for each time interval in phosphate buffer (pH 7.4).

diffusion cell, the results depicted that more than 50% of drug was diffused through the cellophane membrane in 8 h. Likewise, in the ex-vivo diffusion model the bexarotene liposomal topical gel gave similar results as in the in-vitro diffusion studies. These outcomes demonstrate that the drug is being released in a sustained and controlled manner over a prolonged period of time, which is important for skin disorders like CTCL where once or twice a day application is sufficient, thus improving patient compliance.

To determine the penetrative property of topical gel formulation, dermal uptake studies were carried out using drug disposition studies. The amount of drug deposited in the skin was also calculated. It was concluded that formulation LG5 showed the highest (31.29%) restoration of the drug in the skin. It showed that the high concentration of drug was available in the skin for a long period of time for local application of the drug in the effective management of CTCL (Figure 4).

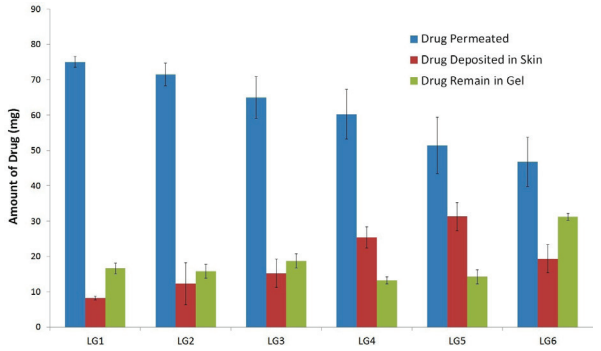


**Figure 4.** Ex vivo skin permeation study and drug deposition (retention) study of optimized liposomal topical gel formulations following transdermal application.

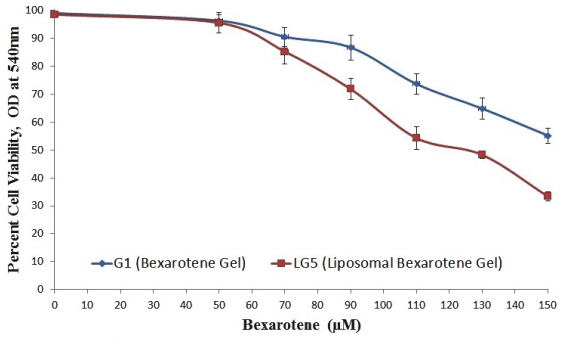


**Figure 5.** Cytotoxicity activity of pure bexarotene (G1, $IC_{50}$ >150 µM) and optimized liposomal topical gel formulations (LG5, $IC_{50}$ ~128 µM*) in Hut 78 cell line (n=6, StatTM Software, P<0.05;*implies Significant Changes Observed).

### Skin-irritation study

The liposomal topical gel (LG5) is topically applied and used with UV radiation for more effective treatment and management of CTCL. The skin irritation studies were observed under UV light exposure. It was found that the liposomal topical gel reduced the wrinkle score of the skin. The results clearly show that the topical gel does not produce any irritation to the skin while in the presence of UV light (Table 5).

### *In-vitro* cell proliferation studies

The cytotoxic activity was performed by determining the minimum inhibition concentration of optimized pure drug-loaded gel (G1) and liposomal topical gel of bexarotene (LG5) formulations using the standard MTT cell viability assay. The results of the MTT cell viability method depicted IC50 and value of > 150 µM and 128 µM by G1 and LG5 formulation against the Hut 78 cell line associated with CTCL, respectively. The result revealed that the amount of drug needed for preventing cell line proliferation is maximum by bexarotene-loaded topical gel than other nano-vesicular formulations (Liposomes) as shown in Figure 5, and the absorbance was measured at 263 nm using a plate reader (BioRad). An ANOVA test was employed to find the statistical significance of the observed value.

## CONCLUSION

The liposomal topical gel of bexarotene was designed, formulated, and evaluated for various parameters. The liposomes were prepared by the optimization technique for desired vesicle size, high drug loading, and stable dosage form. The promised liposomes were incorporated into Carbopol topical gel and characterized for different parameters. The prepared topical gel has good spreadability and rheological properties. The promised liposomal topical gel had shown controlled diffusion of drug and sufficient deposition of drug in the skin. This study indicates that the liposome topical gel of bexarotene can be considered in the management of CTCL.

**Peer-review:** Externally peer-reviewed.

**Informed Consent:** Written consent was obtained from the participants.

**Author Contributions:** Conception/Design of Study- S.S.,N.S., S.P.V., D.P.B.; Data Acquisition- S.S.,N.S., S.P.V.; Data Analysis/Interpretation- S.S.,N.S., S.P.V., D.P.B.; Drafting Manuscript- S.S.,N.S., S.P.V., D.P.B.; Critical Revision of Manuscript- S.S., D.P.B.; Final Approval and Accountability- S.S.,N.S., S.P.V., D.P.B.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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