APPLICATION OF RESPONSE SURFACE METHODOLOGY IN OPTIMIZATION OF PACLITAXEL LIPOSOMES PREPARED BY THIN FILM HYDRATION TECHNIQUE

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INTRODUCTION

Paclitaxel (PT) is a chemical compound isolated from the bark of Taxus brevifolia (northwest Pacific Yew Tree), empirical formula (C47H51NO13) and on the basis of characterization named it as Taxol [1]. It has the potential anticancer drug based on previous reports, PT shows antitumor activities towards breast cancer [2], ovarian cancer [3], lung cancer [4] and pancreatic cancer [5]. However, PT exhibits poor aqueous solubility and permeability owing to its low water solubility and bioavailability. Therefore, by consideration of these problems, there is a need to develop a novel formulation of such effective and efficient anticancer drug.

For improving poor aqueous solubility and permeability of PT, a number of formulation strategies have been developed and used. Some of them were modified, due to some excipient–drug interactions. For improving the solubility, PT dissolved in a mixture of polyoxyethylated castor oil (Cremophore EL): dehydrated ethanol (1:1) ratio as a delivery vehicle. The formulation produced hypersensitivity and non-linear pharmacokinetic behavior after intravenous administration. The hypersensitivity reaction at the site of administration could be due to an inclusion of Cremophore EL [6-7]. After that, the delivery vehicle was replaced with the addition of tween 80 alone or combination of tween 80: dehydrated alcohol, and diluted with aqueous media. The diluted formulation showed the precipitation of PT from solution due to low solubility [8]. These attempts, techniques, with persistent low solubility problem, has been overcome by creating novel formulation with the aim of improving aqueous solubility, permeability, and bioavailability of PT. It includes novel oral formulation [9], novel PT self-emulsifying drug delivery system (SEDDS) [10], novel ligands based PT targeting formulation [11], micellar formulation [12], liposomal formulation [13], bioconjugates [14], dendrimers [15] and nanocarrier systems [16]. In all these formulation techniques the problem associated with PT was shown to be improved significantly.

The liposome is emerging techniques for specialized drug delivery [17] and best suitable for lipophilic drug due to its biocompatibility and reducing drug toxicity, with maintaining efficacy of the anticancer drug for a maximum period of time. Some previous studies include asulacrine [18], docetaxel [19] and tamoxifen [20] with these approaches, their poor aqueous solubility and bioavailability were found to be improved. So, need to develop and optimize the paclitaxel -loaded liposomes (PTL) for effective anticancer treatment. In pharmaceutical technology, in the development and optimization of different pharmaceutical dosage forms, there are a high number of factors which influence the product characteristics. Therefore, complex, expensive and time-consuming formulation studies are often necessary for the development of a product with required and desired properties. The experimental design methodology is a strategy to use a smaller number of experiments and to avoid unnecessary experiments [21-22].

Experiments were designed to determine the effect of the independent variables (factor) on the dependent variable (parameter/response) of a process or formulation. RSM, one of the designs of experiments, is a powerful tool for determining the relationship between a response and a set of quantitative involved factors. RSM is a technique used to find the optimum response by using the quadratic polynomial model [23]. The advantage of RSM is the reduced amount of experiments required, thereby reducing the cost of expensive analysis methods. The application of RSM is useful for understanding or mapping a region of the response surface, finding the variable level of optimum response, and selecting the process condition or formula to meet the specifications [24]. This research was carried out to optimize PTL formula with independent variables such as phospholipid (phospholipon 90G) concentration and cholesterol (CH) concentration. The optimum formula was obtained from RSM using 3 factorial design. The optimization approach was applied to obtain desired % EE and % DR for PTL.
**MATERIALS AND METHODS**

**Materials**

Paclitaxel (PT), (purity=90%) was received as a gift sample from MAC-CHEM Products (India) Pvt. Ltd. Bhopal, Thane, India. The phospholipids samples viz., Phospholipon 90G® (PL90G), Phospholipon 80H® (PL80H) and Phospholipon 90H® (PL90H) with purity >90%, was obtained as a free gift sample from Lipoid GmbH, Ludwigshafen, Germany. The solvents namely chloroform and methanol were purchased from Merck Ltd. Mumbai, India. Cholesterol (CH), potassium dihydrogen phosphate and sodium hydroxide pellets were obtained from Sigma Chemicals, Sigma-Aldrich Corporation, St. Louis, MO. Chemical used in this work were of analytical grade (AR).

**Experimental design (3² full factorial design)**

To reduce the number of trials and attain the highest amount of information on product properties, the screening was done by applying full factorial design (3²), systematically study the joint influence of the independent variables on the dependent variables. So, in this study two factors were evaluated, each at three levels and experimental trials was performed at all nine possible combinations. Amount of phospholipid (PL90G-1, 2 and 3 moles) and amount of cholesterol (CH-1, 2 and 3 moles) was selected as the second independent variables (X₂, w) for liposomes. These variables varied at three levels, low level (-1), medium level (0), and high level (+1).

All the calculations were done at milligram level. Amount of PT (10 μM) and final formulation volume 15 ml was kept constant. Percent entrapment efficiency (% EE) (Y₁) and percent in vitro drug release at 12 h (% DR) (Y₂) were selected as dependent variables. Values of variables and batch codes are shown in table 1 and 2. Design Expert® DX 10.0.7.0 (Stat-Ease Inc., MN) license version software was used for the generation and evaluation of statistical experimental design [25-26].

**Preparation of paclitaxel-loaded liposomes (PTL)**

Liposomes were prepared by the thin film hydration method (TFH). The 10 μM PT (mol wt., 853.9) constant for all batches and the required quantities of phospholipid (PL90G) (mol. wt., 758.07) and cholesterol (CH) (mol. wt., 386.67) were taken in a 100 ml round bottom flask and dissolved in 10 ml chloroform. All the batches were prepared according to the experimental design in table 1. Chlороform was evaporated using rotary vacuum evaporator (Model: PBV-7D, Vertical condenser, rotavap, superfil™® continental Pvt. Ltd., Mumbai, India) and kept overnight under vacuum. Then it was hydrated by 15 ml of phosphate buffer pH 7.4 for 1 h with 10 min of extensive vortexing. The suspension of liposomes was sonicated in the water bath at 60 °C to reduce the size of liposomes. Non-incorporated PT was separated by ultracentrifugation at 10,000 rpm for 30 min at 4 °C. The supernatant was then discarded and PT loaded liposomes in the precipitate were redispersed in required volume of phosphate buffer pH 7.4. This was transferred to vials and stored at 4 °C [27].

**Statistical analysis and optimization of formulation using RSM**

Response surface modeling and evaluation of the quality of fit of the model for the current study were performed employing Design Expert® DX 10.0.7.0 license version software [23-26, 30]. Polynomial models including linear, interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis (MLRA). A second-order polynomial equation that describes the effect of independent factors on the response is expressed in the following forms:

Linear model: \[ Y = \beta_0 + \beta_1X_1 + \beta_2X_2 \]  
2FI (interaction model): \[ Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_1\beta_2X_1X_2 \]  
Quadratic model: \[ Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_1\beta_2X_1X_2 + \beta_1^2X_1^2 + \beta_2^2X_2^2 \]  
Where \( Y \) is the dependent variable; \( \beta_0 \) is the arithmetic mean response of the nine runs and \( \beta_1 \); \( \beta_2 \); \( \beta_1\beta_2 \) and \( \beta_1^2 \) is the estimated coefficient for the corresponding factor \( X_1 \); \( X_2 \); \( X_1X_2 \); and \( X_1^2 \) and \( X_2^2 \). The main effects \( X_1 \) and \( X_2 \) represent the average influence of each factor on the response. Analysis of variance (ANOVA) for the responses was performed to identify a significant effect of factors on responses and the model parameters were obtained. The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. Two-dimensional contour plots and three-dimensional response surface plots resulting from equations were obtained by the Design Expert software. These plots are very useful in a study of the effects of two factors on the response at one time and predict the responses of dependent variables at the intermediate levels of independent variables. Subsequently, a numerical optimization technique by the desirability and graphical optimization technique by the overlay plot approach were used to generate the new formulation with the desired responses. An optimized formulation was developed by setting constraints (goals) on the dependent and independent variables. To validate the chosen experimental design, the resultant experimental values of the responses were quantitatively compared with those of the predicted values and calculated the percent relative error (PRE) by the following equation 5.

\[ \% \text{Relative error} = \frac{| \text{Predicted value} - \text{Experiment value} |}{\text{Predicted value}} \times 100 \]  

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Determination of percent entrapment efficiency (% EE)

Purification of PTL formulation was done by the ultracentrifugation method [26]. To quantify the amount of entrapped PT, 2 ml of the vesicular dispersion was centrifuged at 10,000 rpm for 1 h at the...
controlled temperature of 4 °C (Remi cooling centrifuge, Remi Elektrotechnik limited, India). Supernatant contains unentrapped drug was withdrawn and measured UV spectrophotometrically (at λmax 230 nm) (Model: SPECTRO 2060 PLUS, Analytical Technologies Ltd., Gujarat, India) against 30:70 ratio of methanol: phosphate buffer solution (PBS) (pH 7.4). All the determinations were made in triplicate. A calibration plot was produced by diluting stock solutions of PT with 30:70 ratio of methanol and PBS (pH 7.4).

% EE was calculated and expressed as a percent of the available dissolved solute actually encapsulated. The amount of drug entrapped in liposomes was determined by equation 1.

\[
\% \text{ entrapment efficiency (EE)} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug}} \times 100
\]  

Percent in vitro drug release study (% DR) at 12 h.

The in vitro drug release study for PT from different PTL formulation was carried out as per the procedure described by Utreja [29] with little modifications. In brief, the Franz diffusion cell apparatus was employed for this study. The apparatus is consisted of donor and receptor compartment, with an effective surface area for dissolution was (2.303 cm²). The dialysis membrane (LA395, Dialys Membrane−110 AV, flat width ~31.12 mm, Average diameter ~21.5 mm, and approximate capacity = 3.63 ml/cm²; HI media laboratories, Mumbai, India) was employed and pretested as per the directions were given by the manufacturer. After proper pretreatment, the membrane was cut into desired size and shape, then mounted between the effective surface area of donor and receptor compartment. The PTL dispersion (2 ml) was placed over the membrane, accompanied by an addition of PBS (20 ml, pH 7.4) contain 0.1% tween 80 as dissolution media in the receptor compartment. The contents of receptor compartment were stirred at 100 rpm using magnetic stirrer at 37±1 °C. At specified time intervals, 2 ml aliquots were withdrawn from sampling port of apparatus, diluted suitably with fresh media and the absorbance of the resulting solution was read at 230 nm using UV-visible spectrophotometer (Model: SPECTRO 2060 PLUS, Analytical Technologies Ltd., Gujarat, India).

Vesicle morphology study of liposomes

The liposomes were mounted on glass slides and viewed under a Motic Digital Microscope (Type DM-1802) for morphological observation after suitable dilution. The size analysis of PTL was examined at the magnification of ×40 using calibrated eyepiece micrometer. The images were recorded using Motic image plus 2.0 ML software, accompanying with the instrument.

Determination of percent drug content

One milliliter of dispersion was pipette from the PTL formulation and lyed with methanol. It was further diluted with 30:70 ratio of methanol: phosphate buffer solution (PBS) (pH 7.4) and sample were analyzed spectrophotometrically at λmax 230 nm for PT [26].

Determination of particle size, poly-dispersion index (PDI) and zeta potential (ζ)

The size of liposomes was measured by dynamic light scattering with a Malvern zetasizer. Dihuted (1:100) PTL dispersion was added to the sample cuvette and then cuvette is placed in zetasizer. The sample is stabilized for two minutes and reading was measured. The average particle size was measured after performing the experiment in triplicate. The zeta potential of developed PTL formulation was determined using Malvern zetasizer (Malvern zetasizer ver. 6.20, UK). The zeta potential was calculated by Helmholz-Smoluchowski’s equation from the electrophoretic mobility of liposomes at 25 °C [26].

RESULTS AND DISCUSSION

Experimental design and data acquiring (3^full factorial design)

Full factorial design (3^) was applied to optimize the PTL formulation. All nine batches of PTL were prepared according to the formulation variables as shown in table 2. Liposomes were obtained by the TFH method. RSM was exploited to estimate the influence of the molar ratio of PL90G and CH as independent variables and their interactions on the investigated responses (dependent variables: % EE and % DR). This experiment aimed to identify considerable factor effect influencing the formulation performance and to set up to their excellent levels for the desirability of responses shown in table 2.

Statistical analysis and optimization of formulation using RSM

To evaluate the quantitative effects of factors (X1 and X2) and their levels low (-1), middle (0) and high (+1) on the preferred responses, the experimental values of the flux were analyzed by Design Expert® DX 10.0.7 license version software and mathematical models obtained for each response [25-26, 30-31]. The mathematical relationship generated using multiple linear regression analysis (MLRA) for the studied responses (% EE and % DR at 12 h) that were relating different response and independent variables are expressed as following equilibrium equations (quadratic model).

\[ Y_1 (\% \text{ EE}) = 76.63 \times 10^{-x_1} + 9.44 \times 10^{-x_2} + 3.18 \times 10^{-3} \times x_1^2 + 9.91 \times 10^{-3} \times x_2^2 + 0.393 \times x_1 \times x_2 + 6 \]  

\[ Y_2 (\% \text{ DR12 h}) = 64.74 \times 7.93 \times 4.63 \times 1.38 \times X_1 - 15.29 \times 5.82 \times 7 \]  

The above equations expose the quantifiable effect of the independent variables, a molar ratio of PL90G and CH on the responses as such % EE (Y1) and in vitro % DR at 12 h (Y2) as dependent variables. The fitted polynomial equation (quadratic model) related to % EE and percent in vitro % DR used to draw a conclusion after considering the coefficient and the mathematical sign it carries, i.e. positive and negative. The correlation coefficient (r) of the quadratic model (0.9736) for response Y1 (% EE) and (0.9779) for response Y2 (% DR) was found to be significant.

Response 1 (Percent entrapment efficiency) (% EE)

Regression analysis of above equation (6) of response Y1 (% EE) revealed that the coefficient of X1 was positive and X2 was negative, this indicated that as PL90G (X1) increased the % EE increased but as we further increased the PL90G (X1) to higher level the % EE decreased and on increasing cholesterol (X2) the % EE decreased. The higher concentration of cholesterol leads to rigidity in the vesicles [26] which in turn decreased the % EE. The % EE of different liposomal batches was in a range of 51.68 to 86.67%. The percent entrapment efficiency was in a range of 51.68 to 86.67%. The percent entrapment efficiency was in a range of 51.68 to 86.67%. The percent entrapment efficiency was in a range of 51.68 to 86.67%. The percent entrapment efficiency was in a range of 51.68 to 86.67%. The percent entrapment efficiency was in a range of 51.68 to 86.67%.

\[ \text{Response variables} = \begin{cases} 76.63 \times 10^{-x_1} + 9.44 \times 10^{-x_2} + 3.18 \times 10^{-3} \times x_1^2 + 9.91 \times 10^{-3} \times x_2^2 + 0.393 \times x_1 \times x_2 + 6 & \text{for } Y_1 \text{ (EE)} \\ 64.74 \times 7.93 \times 4.63 \times 1.38 \times X_1 - 15.29 \times 5.82 \times 7 & \text{for } Y_2 \text{ (DR12 h)} \end{cases} \]  

<table>
<thead>
<tr>
<th>Batches</th>
<th>Variable level in coaded form</th>
<th>Variable level in actual form</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>-1</td>
<td>1</td>
<td>75.65±1.48</td>
</tr>
<tr>
<td>L2</td>
<td>-1</td>
<td>1</td>
<td>6.42±2.01</td>
</tr>
<tr>
<td>L3</td>
<td>-1</td>
<td>1</td>
<td>51.68±0.75</td>
</tr>
<tr>
<td>L4</td>
<td>0</td>
<td>2</td>
<td>86.67±0.67</td>
</tr>
<tr>
<td>L5</td>
<td>0</td>
<td>2</td>
<td>78.69±1.61</td>
</tr>
<tr>
<td>L6</td>
<td>0</td>
<td>2</td>
<td>65.23±1.17</td>
</tr>
<tr>
<td>L7</td>
<td>1</td>
<td>3</td>
<td>77.12±1.53</td>
</tr>
<tr>
<td>L8</td>
<td>1</td>
<td>3</td>
<td>67.16±1.90</td>
</tr>
<tr>
<td>L9</td>
<td>1</td>
<td>3</td>
<td>65.89±1.32</td>
</tr>
</tbody>
</table>

*Values represented as mean±SD, n = 3, All baches contain drug 10 μM and 15 ml phosphate buffer (pH 7.4) for hydration.

Table 2: Composition 3^ full factorial design with measured responses of PTL formulation

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Table 3: Analysis of variance (ANOVA) table of % EE

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean squares</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>829.57</td>
<td>5</td>
<td>165.91</td>
<td>22.14</td>
<td>0.0142</td>
<td>Significant</td>
</tr>
<tr>
<td>X₁ - Phospholipid (PL90G)</td>
<td>57.72</td>
<td>1</td>
<td>57.72</td>
<td>7.70</td>
<td>0.0692</td>
<td></td>
</tr>
<tr>
<td>X₂ - Cholesterol (CH)</td>
<td>534.68</td>
<td>1</td>
<td>534.68</td>
<td>71.36</td>
<td>0.0035</td>
<td></td>
</tr>
<tr>
<td>X₁ X₂</td>
<td>40.58</td>
<td>1</td>
<td>40.58</td>
<td>5.42</td>
<td>0.1024</td>
<td></td>
</tr>
<tr>
<td>X₁²</td>
<td>196.35</td>
<td>1</td>
<td>196.35</td>
<td>26.20</td>
<td>0.0144</td>
<td></td>
</tr>
<tr>
<td>X₂²</td>
<td>0.24</td>
<td>1</td>
<td>0.24</td>
<td>0.032</td>
<td>0.8693</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>22.48</td>
<td>3</td>
<td>7.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor-total</td>
<td>852.05</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For estimation of the significance of the model, the analysis of variance (ANOVA) was executed. From the ANOVA data, the model F-value of response (Y₁) (22.14) indicated that the model is significant shown in table 3. There is only a 1.42% chance that an F-value this large could occur due to noise. Values of “prob>F” less than 0.0500 indicate model terms are significant. In these case X₂, X₁² are significant model terms. Values greater than 0.1000 indicate that model terms are not significant.

Table 4: Parameter of selected quadratic model of % EE

<table>
<thead>
<tr>
<th>Std. dev.</th>
<th>R-squared (r²)</th>
<th>0.9736</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>70.26</td>
<td>0.9296</td>
</tr>
<tr>
<td>C. V. %</td>
<td>3.90</td>
<td>0.7315</td>
</tr>
<tr>
<td>PRESS</td>
<td>228.77</td>
<td>15.693</td>
</tr>
</tbody>
</table>

The predicted R-squared value of 0.7315 is in reasonable agreement with the adjusted R-squared of 0.9296; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable and the result of adequate precision was 15.693 indicates an adequate signal. So this model can use to navigate the design space.

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. The contour (fig. 1) and 3D response surface plots (fig. 2) of % EE clearly indicated that X₁ and X₂ highly influenced the response 1 (% EE). The change in % EE as a function of X₁ and X₂ was depicted in the form of contour and response surface plots based on full factorial design (³). So, middle level of X₁ and low level of X₂ was found to be favorable conditions for obtaining higher % EE.

Response 2 (Percent drug release at 12 h) (% DR)

The effect on drug release at 12 h (% DR) (Y₂) was observed to be significant (P<0.05) by ANOVA and the polynomial equation (7) revealed that the coefficient of β₁ was positive and β₂ was negative, this indicated that as PL90G (X₁) increased the % DR increased and on increasing cholesterol (X₂) the % DR decreased. The % DR increased with increased concentration of lipid and at a certain level the percent release is retarded above that and the release was decreased at higher levels of cholesterol. This is because cholesterol at higher levels makes the lipid bilayers more rigid and retards the release of the drug. This was evident by the higher cholesterol concentration of vesicles showed around 50 % of the release except for (L₆) formulations. The L₄ formulation found to have 63.43 % DR at 12 h (table 2) with the composition of PL90G: CH (2:1 molar ratio) (0,-1). At lower concentration of phospholipid and cholesterol, the drug release was very less due to the formation of stagnant layer [26].
Fig. 2: Response surface plot showing the effect of phospholipid (PL90G) (X1) and cholesterol (CH) (X2) on % EE (Y1) of PTL

Fig. 3: Counter plot showing the effect of phospholipid (PL90G) (X1) and cholesterol (CH) (X2) on % DR at 12 h (Y2) of PTL
To envisage the effect of an independent factor on the response ($Y_2$) the contour plot (fig. 3) and 3D-response surface plots (fig. 4) of % DR at 12 h was obtained with predicted models and the experimental model were shown in table 8. The percent relative error was obtained using equation 4. The percent relative error (PRE) for response $Y_1$ (% EE) and $Y_2$ (% DR at 12 h) were found to be (–0.290) and (0.058) respectively. The maximum PRE value was (–0.290). However, the values were found to be<2 % and hence it confirmed the suitability of experimental design. The results showed good agreement on preparation properties with theoretical properties.

Table 7: Characteristics of optimum formula

<table>
<thead>
<tr>
<th>Objects</th>
<th>Phospholipid (PL90G) in moles ($X_1$, W)</th>
<th>Cholesterol (CH) in moles ($X_2$, W)</th>
<th>% EE ($Y_1$, %)</th>
<th>In vitro % DR at 12 h ($Y_2$, %)</th>
<th>Desirability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td>1.996</td>
<td>1.000</td>
<td>86.419</td>
<td>63.527</td>
<td>0.996</td>
</tr>
<tr>
<td>Actual (L4)</td>
<td>2</td>
<td>1</td>
<td>86.67±0.67</td>
<td>63.49±1.21</td>
<td>Selected</td>
</tr>
</tbody>
</table>

The optimization parameter of desirability was determined by regulating the optimum input variables to obtain one or more optimal parameters. The desirability value ranged between 0 and 1, where a value of 1 is perfect, i.e., the ideal parameter value [30]. The PTL desirability plot was shown in fig. 6. The optimizing desirability of PTL formulation was 0.996. This value was near to ideal value (1), meaning that the predicted parameters were desired parameter values. The composition of the predicted formulations was matching with L4 liposomes (table 7).

Fig. 5: Overlay plot of % EE and % DR at 12 h
Figure 6: PTL desirability plot

Table 8: Validation of predicted and experimental PTL batch

<table>
<thead>
<tr>
<th>Response</th>
<th>Experimental values</th>
<th>Predicted value</th>
<th>% Relative error (PRE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y₁ (% EE)</td>
<td>86.67±0.67</td>
<td>86.419</td>
<td>-0.290</td>
</tr>
<tr>
<td>Y₂ (% DR at 12 h)</td>
<td>63.49±1.21</td>
<td>63.527</td>
<td>0.058</td>
</tr>
</tbody>
</table>

Vesicle morphology, percent drug content, particle size, PDI and zeta potential (ζ) of developed PTL formulation

Vesicle morphology of developed PTL formulation was observed by Motic Digital Microscope (type DM-1802). The liposomes were spherical in shape with a smooth surface shown in fig. 7. The developed liposomal percent drug content was found to be 98±1.0 %, (mean±SD, n = 3). Percent drug content indicated that the PT was uniformly distributed in vesicular dispersions and percent drug content near to 100 % indicated no loss of the material during the preparation.

Figure 7: Microphotograph of PTL by motic image plus 2.0 ML software

Figure 8: The particle size (A) and Zeta potential (B) of developed PTL formulation
The present study, the response surface methodology i.e.3 full factorial design was successfully employed for the optimization of PTL formulations. The PTL was prepared by thin film hydration method. The results of above optimization study displayed that the phospholipids (PL90G) and cholesterol (CH) with a molar ratio (2:1) showed an enhancement in rate and extent of in vitro release of PT from design-optimized PTL formulations. Thus, we conclude that the proposed RSM could be useful for the preparation and optimization of paclitaxel-based liposomal formulations.

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AUTHORS CONTRIBUTION
This work was carried out in collaboration between all the four authors in the concept and design of the work, collection, assembly, analysis and interpretation of data, writing, critical revision and approval of the final manuscript.

CONFLICT OF INTERESTS
All authors have none to declare

REFERENCES