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FORMULATION AND OPTIMIZATION OF LOVASTATIN-B-CYCLODEXTRIN LOADED LIPOSOMAL GELS USING BOX-BEHNKEN DESIGN

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ABSTRACT
This study aims to prepare and optimize Lovastatin-β-cyclodextrin loaded liposomal gels using factorial design. The effect of major preparation variables, cholesterol, poloxamer 188 and span 80 on globule size, refractive index, drug release 24th hour, viscosity, gel strength and spreadability are studied with factorial design. Various parameters of liposomal gels were characterized which include globule size, refractive index, drug release 24th hour, viscosity, gel strength and spreadability. Optical microscope was employed to study the morphology of the prepared liposomes. FTIR studies were performed to assess the interaction between the excipients used and the drug due to its nature. HPLC analysis was demonstrated to confirm the identity of a drug and provide quantitative results. The ideal formulation has a globule size of 2.44692 μm, refractive index of 1.33395, drug release 24th hour of 81.3606 %, viscosity of 37343.2 cps, gel strength of 8.23646 seconds and spreadability of 49.7562 g.cm/sec. Optical microscope results indicate that the liposomes are spherical in shape. On the other hand, FTIR results showed that the components used in formulating the liposomes are compatible. HPLC chromatogram of lovastatin showed a sharp peak at 5.037 minutes.

Keywords: Lovastatin, β-cyclodextrin, FTIR, HPLC, Probe sonicator, Factorial design

INTRODUCTION

Liposome, a word derives from combination of two Greek words, "lipo-meaning fat" and "soma-meaning body" [1]. They are composed of an aqueous core that surrounded by one or more external shells consisting of lipids arranged in a bilayer configuration [2]. Generally, liposomes have been recognized as a promising novel drug delivery system (NDDS) in several different basic sciences [3]. They are acceptable superior carriers having capability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation [4]. They have the ability to serve as protection of drugs as well as providing sustain drug release mechanism [5]. Moreover, the purpose of NDDS is to deliver the drug to specific target side and thus increase efficacy and therapeutic index of the drug [6].

β-Cyclodextrin is cyclic oligosaccharides that consist of glucopyranose units [7]. It has the ability to form inclusion complexes with lipophilic drugs [8]. The ability of β-CD to increase drug solubility used to increase drug entrapment in aqueous compartment of liposomes and liposomes able to protect CD/drug inclusion complexes until drug release [9]. Lastly, the liposomal gel formulation could perform therapeutically better effects than the conventional formulations since it allow drug release in prolong period and also protect the lipid bilayer of liposome from leakage since it forms a double barriers [10].

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MATERIALS

Lovastatin was purchased from (SM Pharmaceutical, Malaysia), β–cyclodextrin was purchased from (HiMedia Laboratories Pvt. Ltd.), poloxamer 188 was procured from (Merck, Germany), span 80 was purchased from (Quicklab), gellan gum was purchased from (HiMedia Laboratories Pvt. Ltd.), cholesterol was procured from (Sigma Aldrich, Germany) and diethyl ether as purchased from (Merck). Phosphate buffer was used throughout the study.

METHODS

Preparation of Lovastatin-β-cyclodextrin loaded liposomes as shown in figure 1.

Preparation of Lovastatin-β-cyclodextrin loaded liposomal gels as shown in figure2.

IN-VITRO EVALUATION

Globule Size:

Globule size of Lovastatin-β-cyclodextrin loaded liposomal gels was determined by using Marvern particle size analyser (Zetasizer 4000S, Japan).

Viscosity:

The rheological studies were performed by using Brookfield viscometer. The formulations were transferred into a 50 ml beaker to make a depth of approximately 4 to 5 cm. The beaker filled with formulation was placed beneath the spindle and dipped into the formulations. Viscosity was determined at 0.3 rpm and spindle 63 and the readings were recorded.

pH:

The pH of the formulations was measured using a microprocessor pH meter by dipping the electrode into the formulations. The readings of pH were taken and the electrode was rinsed with distilled water under an empty waste beaker before measuring the subsequent formulations. In addition, pH evaluation was carried out in duplicate for all formulations.

Refractive Index:

Refractive index of the formulations was determined by using Abbe refractometer where the gel sample was sandwiched into a thin layer between an illuminating prism and a refracting prism. A detector placed on the back side of the refracting prism would show a light and a dark region by using rotating knob to adjust it. Lastly, the readings of refractive index for all formulations were taken.

Gel Strength:

A metal rod with metal discs on both ends and a metal cap through its body was set. The liposomal gel formulation was filled in a 25 ml measuring cylinder. The metal rod was placed in the measuring cylinder and allowed to fall to the bottom. Time taken for the metal disc to move at a distance of 5 cm was recorded. The mean readings of two trials as calculated to estimate the gel strength.

Spreadabilty:

Spreadability of formulations was calculated by using two glass slides having two pans on both sides mounted on a pulley. Excess gel sample was placed between the two glass slides and 1 kg weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. Weight with 72 g was added to the pan. The time in seconds required to separate the two glass slides was taken and recorded as a measure of spreadability. Generally, the spreadability of the formulations was measured by using the formula, S = M * L/T, where S: spreadability (gcm/s), M: weight in the pan (g), L: length moved by the glass slide (cm) and T: time taken to separate the slide completely from each other (s).

Drug Diffusion Studies:

Franz diffusion cell method was applied using phosphate buffer (pH 5.8) at room temperature for in vitro drug release studies. A cellophane membrane (dialysis membrane) was used to carry out the study and soaked overnight in phosphate buffer at room temperature to be prepared. The membrane was then placed between donor and receptor compartment of diffusion cell with an exposed membrane surface area of 2.97 cm2 to the receptor compartment. The receptor compartment was filled with 16.4 ml of freshly prepared phosphate buffer (pH 7.4) maintained at 35 ± 0.5°C with constant stirring using a Teflon coated magnetic stir bead. 2 g of liposomal gel formulation was placed on the membrane and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals, 2 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions. The amount of drug released from liposomal gel was determined by HPLC method. The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, 10mm phosphate buffer: methanol (35:65, v/v), and detection was made at 245 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use.

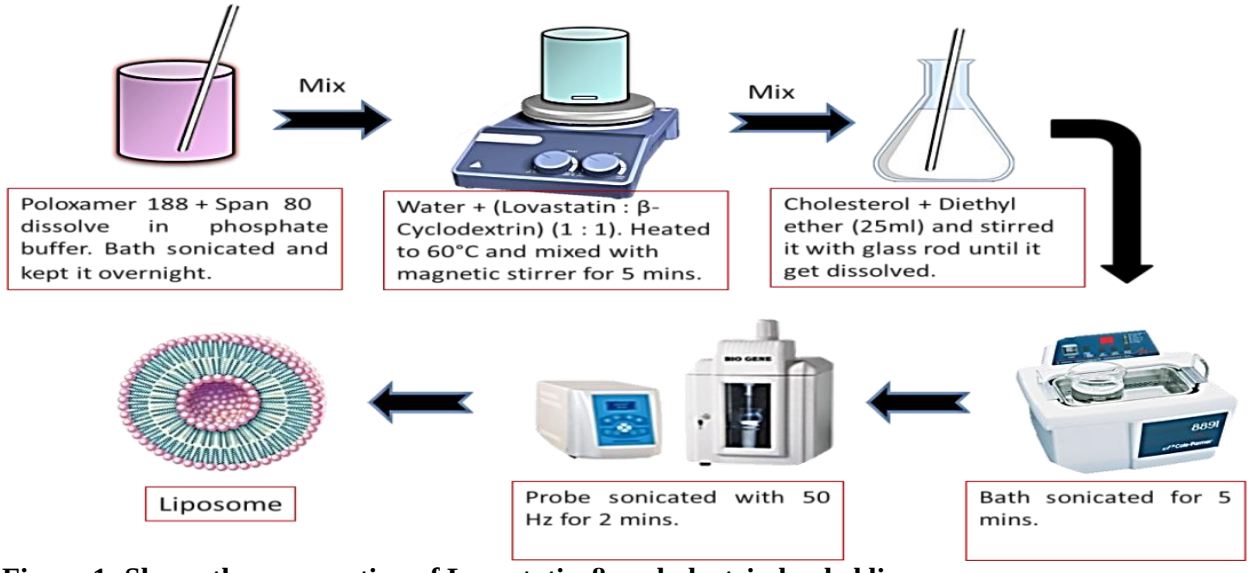

Figure 1: Shows the preparation of Lovastatin-β-cyclodextrin loaded liposomes.

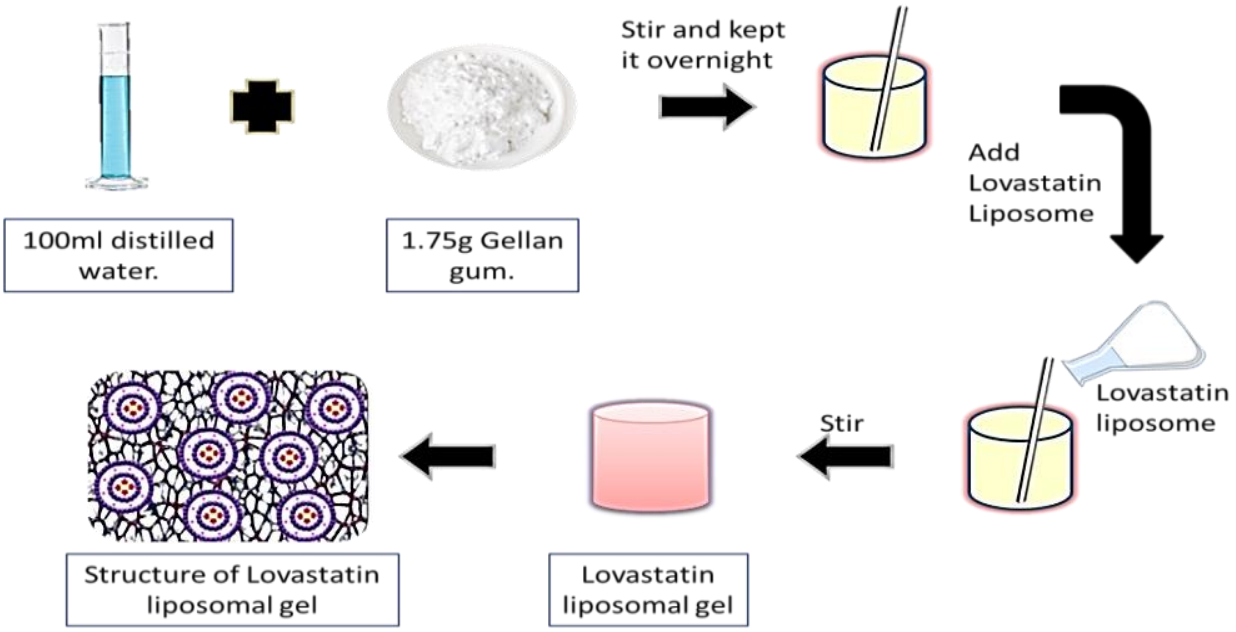

Figure 2: Shows the preparation of Lovastatin-β-cyclodextrin loaded liposomal gels.

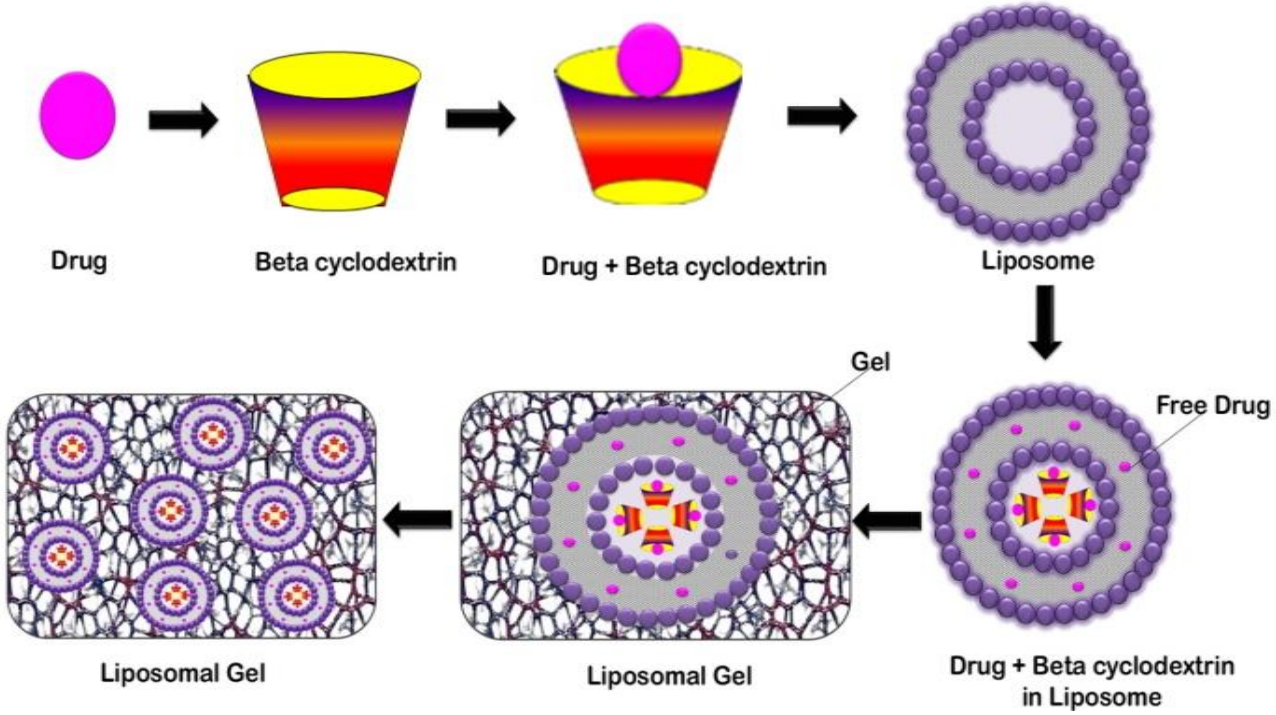

Figure 2a: Schematic diagram of lovastatin: β-cyclodextrin loaded Liposomal gel

RESULTS AND DISCUSSION

Experimental design:

In this work, we report the successful event on the formulation of Lovastatin-β-cyclodextrin loaded liposomal gels. Though preliminary experiments the Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) were identified as the most significant variables influence the globule size, refractive index, drug release 24th hour, viscosity, gel strength and spreadability. Among various design approaches, the Box-Behnken (BBD) has good design properties, little collinearity, rotatable or nearly rotatable; some have orthogonal blocks, insensitive to outliers and missing data. It does not predict well at the corners of the design space. Use when region of interest and region of operability nearly the same. This Box-Behnken design is appropriate for exploring quadratic response surfaces and constructing second order polynomial models. The BBD consists of simulated centre points and the set of points lying at the midpoint of each edge of the multi-dimensional cube.

According to BBD, twenty runs were essential for the response surface. The factor combinations produced different responses are illustrated in Table 1. These results clearly indicated that all the

dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 20 runs. Data were analysed using Stat-Ease Design-Expert software (DX11) to obtain analysis of variance (ANOVA), regression coefficients and regression equation. Mathematical relationship created via multiple linear regression analysis for the studied variables are stated in Table 2.

The normality of the data could be proved through the normal % probability plot of the externally studentized residuals. If the points on the plot lie on a straight line, the residuals are normally distributed as confirmed in Figure 3a, b, c, d, e and f.

The assumption of constant variance was tested by plotting externally studentized residual versus predicted values as illustrated in above figures. The studentized residuals are located by dividing the residuals by their standard deviations. According to evident from this Figure 4a,

b, c, d, e and f, the points are scattered randomly between the outlier detection limits -3.5 to +3.5 and -4.5 to +4.5.

The Residuals vs. Predicted and Residuals vs. Run were scattered randomly are shown in Figure 5a, b, c, d, e and f. From the results it can therefore be seen that the model is suitable for use and can be used to identify the optimal parameters. R1, R2, R3, R4, R5 and R6 results are quite satisfactory. Also, a high correlation between observed and predicted data indicates their low discrepancies.

The plot of predicted response versus actual responses performs the same function, albeit graphically and also helps to detect the points where the model becomes inadequate to predict the response of the system. This is the simplest graph which shows that the selected model is capable of predicting the response satisfactorily within the range of data set as shown in the Figure 6a, b, c, d, e and f.

The Box-Cox plot is a tool that helped to determine the most appropriate power transformation to apply to response data. The transformation parameter, λ, is chosen such that it maximizes the log-likelihood function. The maximum likelihood estimated of λ agrees to the value for which the squared sum of errors from the fitted model is a minimum. This value of λ is determined by fitting a numerous values of λ and choosing the value corresponding to the minimum squared sum of errors. t can also be chosen graphically from the Box-Cox normality plot. Value of λ=1.00 indicates that no transformation needed and produces results identical to original data shown in Figure 7a, b, c, d, e and f.

By plotting the residuals versus cholesterol (A) as illustrated in below figures, it was able to check whether the variance not accounted for by the model is different for different levels of a factor. Pronounced curvature may indicate a systematic contribution of the independent factor that is not accounted for by the model. According to evident from Figure 8a, b, c, d, e and f, the points are scattered randomly.

Cook's distance is a measure of how much the entire regression would change if the case is omitted from the analysis. It is a product of the square of the ith internally studentized residual and a monotonic function of the leverage. It is essentially the sum of differences in predictions at every point caused by leaving a point out for fitting the model. Figure 9a, b, c, d, e and f show the cook's distance plots for each response respectively.

The plot of leverage vs. run is a measure of how much each point influences the model fit. A run with leverage greater than 2 times the average is generally regarded as having high leverage. Such runs have few other runs near them in the factor space. The average leverage is the number of terms in the model divided by the number of runs in the design. According to the evident from Figure 10a, b, c, d, e and f, the points have leverage of 1.0, and thus the model exactly fits the observation at that point. That point controls the model.

The plot of DFFITS versus run is a measure of how much the prediction changes at the ith point when the ith point is not included for fitting the model. It measures the influence the ith observation has on the predicted value. It is the studentized difference between the predicted value with observation i and the predicted value without observation i. Figures 11a, b, c, d, e and f show the plots of DFFITS versus run for all responses.

The plot of DFBETAS for intercept versus run is a measure of how much a coefficient estimate changes when the ith point is not used to fit the model. There are separate DFBETA plots for each term in the model. This statistic is calculated for each coefficient at each run. The influence tool has a pull-down to pick which term's graph is shown. It shows the influence the ith observation has on each regression coefficient. The DFBETASj,i is the number of standard errors that the jth coefficient changes if the ith observation is removed. Figure 12a, b, c, d, e and f show the plots of DFBETAS for intercept versus run for all responses.

Table 1: Factorial design of Lovastatin liposomes formulations

	F1	F2	F3	R1	R2	R3	R4	R5	R6
Run	A: Cholesterol (mg)	B: Poloxamer 188 (mg)	C: Span 80 (mg)	Globule Size (μm)	Refractive Index	Drug Release 24th Hour (%)	Viscosity (cps)	Gel Strength (seconds)	Spreadability (g.cm/sec)
1	120	1000	3000	2.48	1.334	81.44	35792	11.8	52.53
2	110	1150	2750	2.48	1.336	85.79	37592	9.68	49.35
3	100	1300	2500	2.56	1.333	87.98	34293	6.67	50.14
4	120	1300	2500	2.04	1.334	80.15	37693	16.77	44.95
5	120	1300	3000	1.68	1.336	81.01	37492	16.68	44.18
6	110	1150	2750	2.49	1.337	85.88	37593	9.67	49.33
7	100	1300	3000	2.32	1.335	88.75	39991	18.04	55.66
8	100	1000	2500	1.92	1.334	88.16	40191	14.12	59.96
9	110	1150	2750	2.47	1.338	85.33	37594	9.66	49.34
10	110	1150	2750	2.46	1.335	85.21	37591	9.65	49.36
11	93.182	1150	2750	2	1.335	89.75	42491	24.38	57.32
12	100	1000	3000	2.36	1.334	88.34	43491	14.73	50.74
13	110	1150	2750	2.48	1.334	84.65	37590	9.69	49.37
14	126.81	1150	2750	1.6	1.334	78.23	44790	7.73	51.23
15	120	1000	2500	1.96	1.335	81.15	43691	16.53	50.69
16	110	1402.27	2750	1.52	1.335	85.24	44491	6.6	48.69
17	110	1150	3170.4	2.76	1.335	85.64	42498	14.53	47.09
18	110	1150	2329.5	2.56	1.334	84.02	42491	9.54	52.85
19	110	897.731	2750	1.88	1.335	84.11	40691	9.06	55.88
20	110	1150	2750	2.45	1.336	86.21	37592	9.68	49.35

Table 2: Regression equation for the response

Response Regression equation
$R1=+2.47-0.1225A-0.0531B+0.0510C-0.1650AB-0.0050AC-0.1950BC-0.2086A^2-0.2439B^2+0.0955C^2$
$R2=+1.34+0.0001A+0.0001B+0.0003C+0.0001AB-0.0001AC+0.0006BC-0.0006A^2-0.0004B^2-0.0006C^2$
$R3=+85.51-3.58A+0.0513B+0.0513C-0.2075AB+0.0250AC+0.1450BC-0.4988A^2-0.2566B^2-0.2018C^2$
$R4=+37726.06+41.62A-534.91B+66.62C+637.50AB-2137.25AC+1262.00BC+1262.31A^2+891.26B^2+857.14C^2$
$R5=+9.60-1.45A-0.2312B+1.14C+1.16AB-2.10AC+1.92BC+2.74A^2-0.1648B^2+1.32C^2$
$R6=+50.90-2.52A-2.28B-0.9019C$

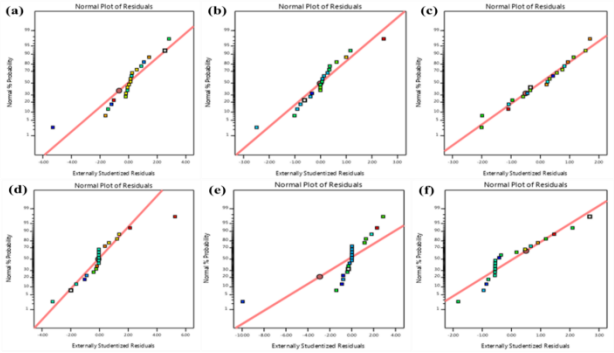

Figure 3: (a) Normal % probability plot of the externally studentized residuals (R1). (b) Normal % probability plot of the externally studentized residuals (R2). (c) Normal % probability plot of the externally studentized residuals (R3). (d) Normal % probability plot of the externally studentized residuals (R4). (e) Normal % probability plot of the externally studentized residuals (R5). (f) Normal % probability plot of the externally studentized residuals (R6).

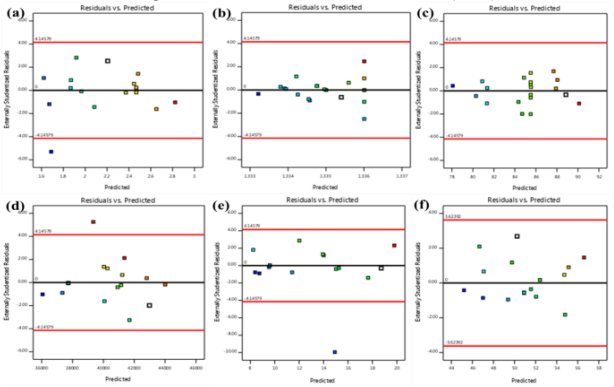

Figure 4: (a) Residuals vs. Predicted (R1). (b) Residuals vs. Predicted (R2). (c) Residuals vs. Predicted (R3). (d) Residuals vs. Predicted (R4). (e) Residuals vs. Predicted (R5). (d) Residuals vs. Predicted (R6).

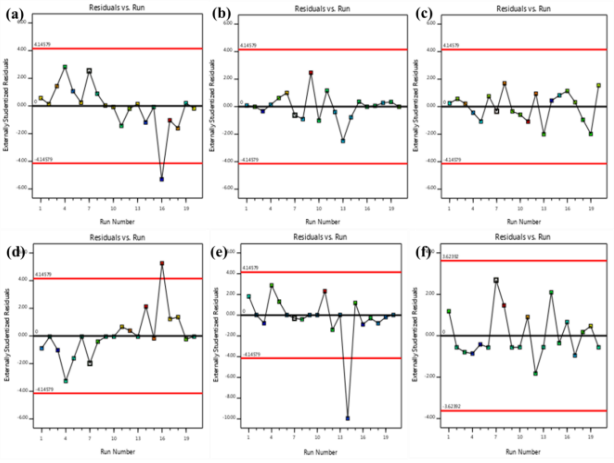

Figure 5: (a) Residuals vs. Run (R1). (b) Residuals vs. Run (R2). (c) Residuals vs. Run (R3). (d) Residuals vs. Run (R4). (e) Residuals vs. Run (R5). (f) Residuals vs. Run (R6).

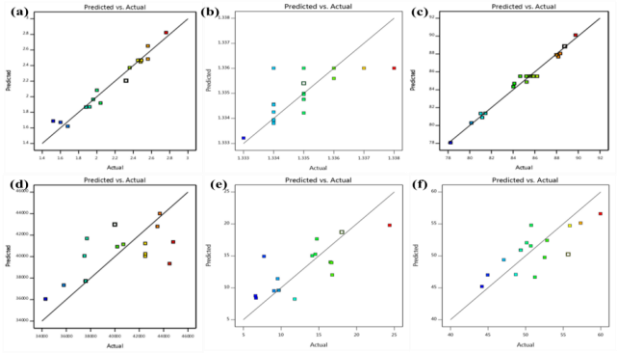

Figure 6: (a) Actual Response vs. Predicted (R1). (b) Actual Response vs. Predicted (R2). (c) Actual Response vs. Predicted (R3). (d) Actual Response vs. Predicted (R4). (e) Actual Response vs. Predicted (R5). (f) Actual Response vs. Predicted (R6).

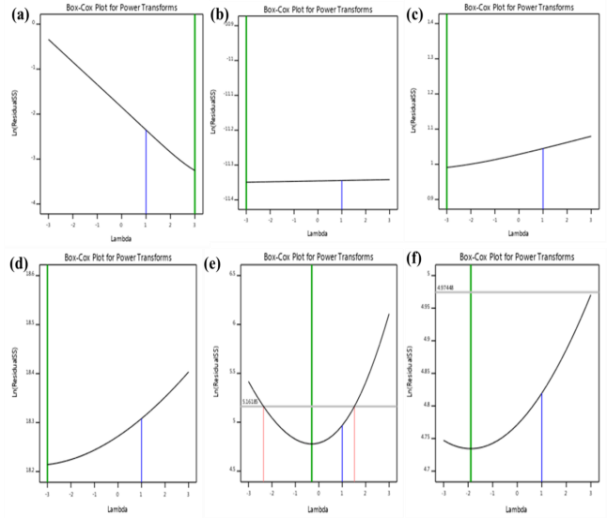

Figure 7: (a) Box-Cox Plot (R1). (b) Box-Cox Plot (R2). (c) Box-Cox Plot (R3). (d) Box-Cox Plot (R4). (e) Box-Cox Plot (R5). (f) Box-Cox Plot (R6).

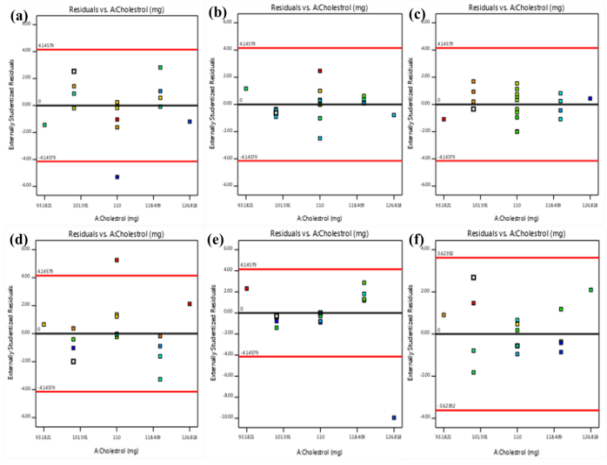

Figure 8: (a) Residuals vs. Cholesterol (R1). (b) Residuals vs. Cholesterol (R2). (c) Residuals vs. Cholesterol (R3). (d) Residuals vs. Cholesterol (R4). (e) Residuals vs. Cholesterol (R5). (f) Residuals vs. Cholesterol (R6).

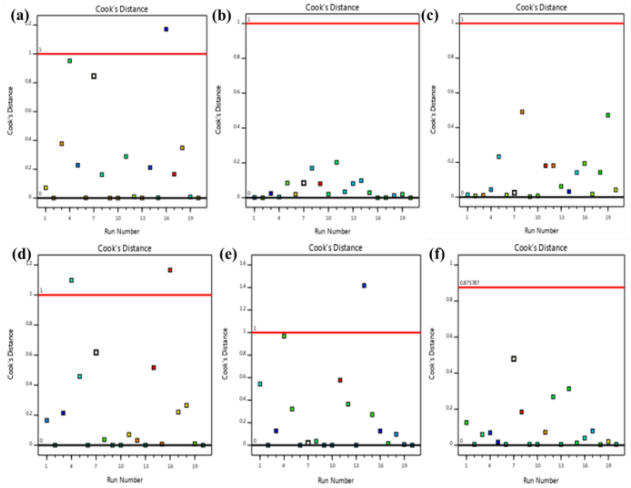

Figure 9: (a) Cook's Distance Plot (R1). (b) Cook's Distance Plot (R2). (c) Cook's Distance Plot (R3). (d) Cook's Distance Plot (R4). (e) Cook's Distance Plot (R5). (f) Cook's Distance Plot (R6).

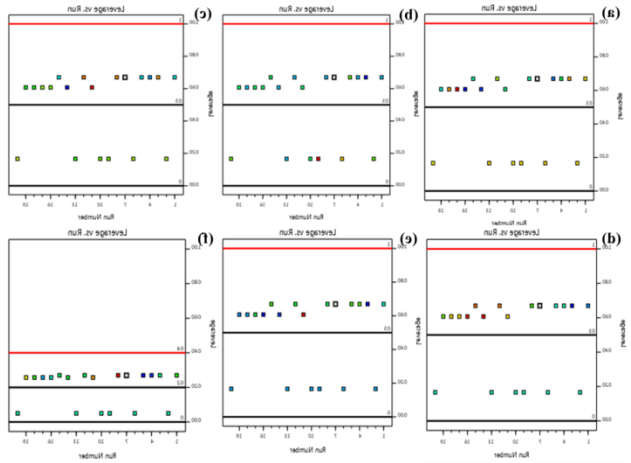

Figure 10: (a) Leverage vs. Run (R1). (b) Leverage vs. Run (R2). (c) Leverage vs. Run (R3). (d) Leverage vs. Run (R4). (e) Leverage vs. Run (R5). (f) Leverage vs. Run (R6).

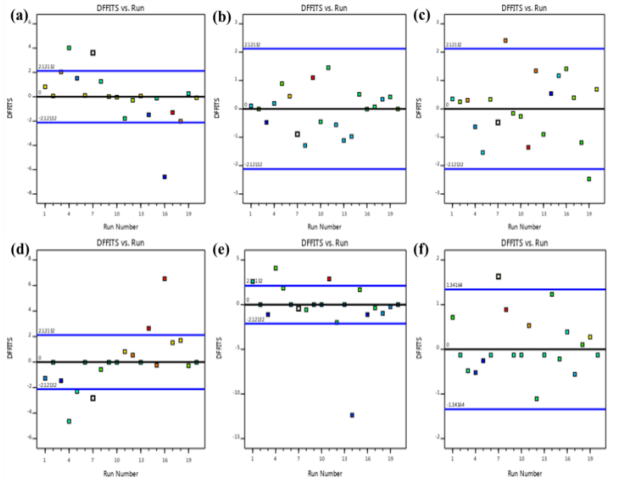

Figure 11: (a) DFFITS vs. Run. (R1). (b) DFFITS vs. Run. (R2). (c) DFFITS vs. Run. (R3). (d) DFFITS vs. Run. (R4). (e) DFFITS vs. Run. (R5). (f) DFFITS vs. Run. (R6).

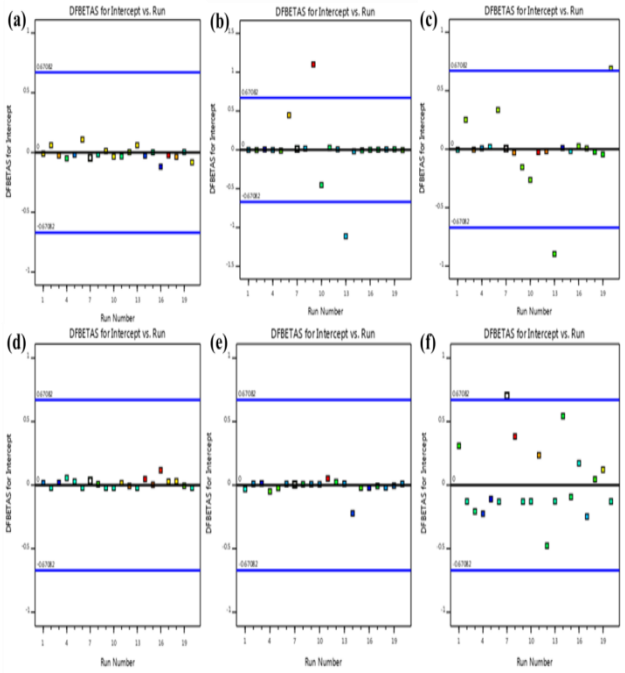

Figure 12: (a) DFBETAS for Intercept vs. Run. (R1). (b) DFBETAS for Intercept vs. Run. (R2). (c) DFBETAS for Intercept vs. Run. (R3). (d) DFBETAS for Intercept vs. Run. (R4). (e) DFBETAS for Intercept vs. Run. (R5). (f) DFBETAS for Intercept vs. Run. (R6).

Globule size analysis of Lovastatin-β-cyclodextrin loaded liposomal gels was found to be in the range of 1.52-2.76 µm as shown in Table 1 and Figure 12g. The factorial equation for globule size exhibited a good correlation coefficient (1.000) and the Model F value of 28.39 which implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, AB, BC, A^2, B^2, C^2 are significant model terms. Results of the equation indicate that the effect of (A) is more significant than B and C. The influence of the main and interactive effects of independent variables on the globule size was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on globule size are as shown in perturbation plot Figure 13a. It is found that all the variables are having interactive effects for the response R1. The 2D contour plots and 3D response surfaces of the response R1 are shown in figure 13b and c to depict the interactive effects of independent variables on response R1, one variable as kept constant whereas the other two variables diverse in a certain range. The shapes of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on globule size at a fixed level of C is shown in Figure 13c. The 3D cube plots of Box-Behnken design are as shown in Figure 13d.

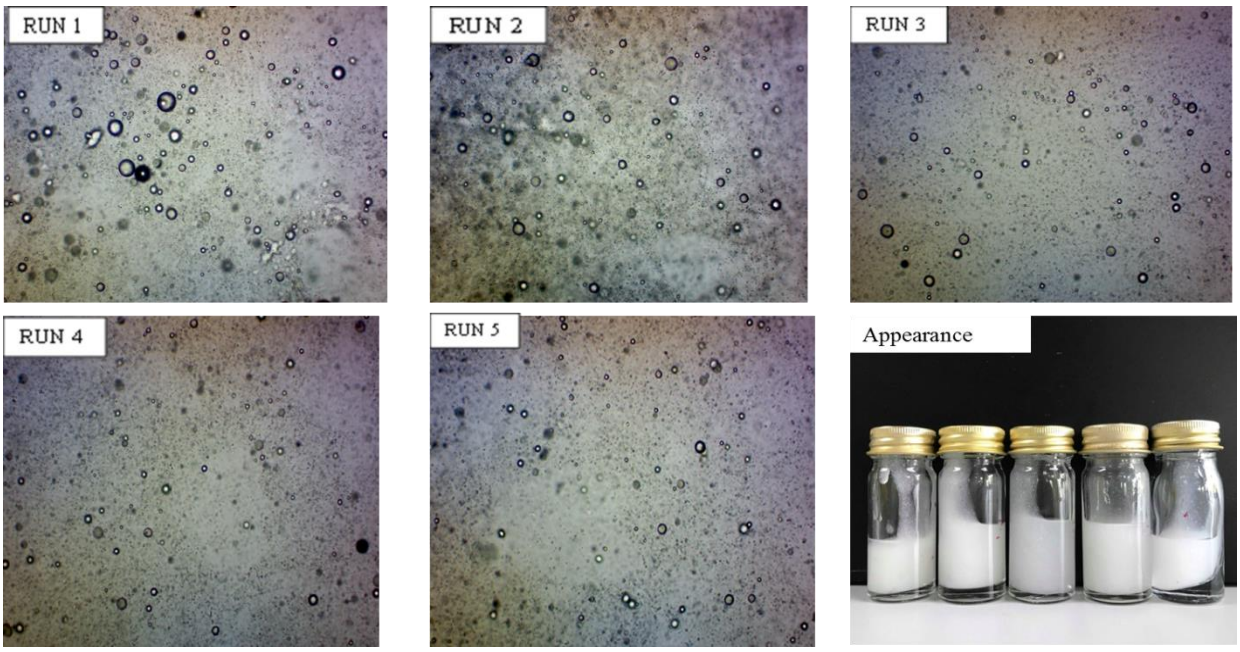

Figure 12g: Globule size analysis of lovastatin-β-cyclodextrin loaded liposomal gels.

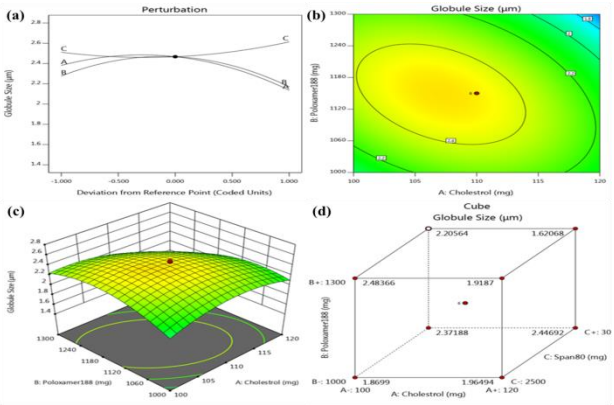

Figure 13: (a) Perturbation plot showing the main effect of Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) on globule size (R1). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the globule size at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the globule size at constant span 80 concentration. (d) 3D cube plot of Box-Behnken design.

The coefficient of determination, R-squared, is a measure of the fraction of the total squared error that is explained by the model. By definition the value of R2 varies between zero and one and the closer it is to one, the better. However, a large value of R2 does not necessarily imply that the regression model is good one. Adding a variable to the model will always increase R2, regardless of whether the additional variable is statistically significant or not. Thus it is possible for models that have large values of R2 to refractive index poor predictions of new observations or estimates of the mean response. To avoid this confusion, an extra statistic called the Adjusted R-squared statistic is needed; its value decreases if unnecessary terms are added. These two statistics can, when used together, imply the existence of extraneous terms in the computed model which is indicated by a large difference, usually of more than 0.20, between the values of R2 and Adj- R2. The amount by which the output predicted by the model differs from the actual output is called the residual. Predicted Residual Error Sum of Squares (PRESS) is a measure of how the model fits each point in the design. It is used to calculate predicted R2. Here, the Predicted R^2 of 0.7146 is not as close to the Adjusted R^2 of 0.9284 as one might normally expect; i.e. the difference is more than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. "Adeq precision" showed was 17.452 indicates an adequate signal respectively. This model can be used to navigate the design space. This statistics are used to prevent over fitting of model.

The mathematical model generated for refractive index (R2) was found to be not significant with F-value of 1.42 ($p < 0.0001$) and R2 value of 0.5608. P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms as shown in Table 4. Here, the "Pred R-Squared" of -0.0586, is a negative Predicted R^2 implies that the overall mean may be a better predictor of the response than the current model

with the Adj R-Squared of 0.1655. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. "Adeq precision" showed was 3.63 indicates an inadequate signal respectively. The influence of the main and interactive effects of independent variables on the refractive index was further elucidated using the perturbation plots and 3D response surface plots. The perturbation plot (Figure 14a) showing the main effects of A, B and C on the refractive index (R2) of lovastatin-β-cyclodextrin loaded liposomal gels. This figure clearly shows that A, B and C has the main and the major effect on R2. The relationship between the dependent and independent variables was further elucidated using 2D response surface plots; 3D response surface plot and 3D cube plot are shown in (Figure 14b, c and d). Figure 14c shows the interactive effect of A and B on the refractive index (R2) at fixed level of C.

The accurate model produced for drug release 24th hour (R3) was found to be significant with F-value of 70.93 ($p < 0.0001$) and R2 value of 0.9846. The independent variables A, B and C has significant effects on the drug release 24th hour, since P-values less than 0.0500 indicate model terms are significant as shown in Table 5. In this case A, C, A^2 are significant model terms. Here, "Predicted R-Squared" of 0.9352 is in reasonable agreement with the Adjusted R-Squared of 0.9707; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. "Adeq Precision" showed was 31.913 indicates an adequate signal respectively. The perturbation plot (Figure 15a) showing the main effects of A, B and C on the drug release 24th hour (R3) of lovastatin-β-cyclodextrin loaded liposomal gels. The correlation among the dependent and independent variables was elucidated using 2D response surface plots; 3D response surface plot and 3D cube plot are shown in (Figure 15b, c and d). Figure 15c shows the interactive effect of A and B on the drug release 24th hour (R3) at fixed level of C.

The accurate model produced for viscosity (R4) was found to be not significant with F-value of 1.17 ($p < 0.0001$) and R2 value of 0.5139. The independent variables A, B and C has not significant effects on the viscosity, since P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms as shown in Table 6. Here, "Predicted R-Squared" of -2.7075, is a negative Predicted R^2 implies that the overall mean may be a better predictor of the response than the current model with the Adjusted R-Squared of 0.0765; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. "Adeq Precision" showed was 3.76 indicates an inadequate signal respectively. The perturbation plot (Figure 16a) showing the main effects of A, B and C on the viscosity (R4) of lovastatin-β-cyclodextrin loaded liposomal gels. The correlation among the dependent and independent variables was further elucidated using 2D response surface plots; 3D response surface plot and 3D cube plot are shown in (Figure 16b, c and d). Figure 16c shows the interactive effect of A and B on the viscosity (R4) at fixed level of C.

The accurate model produced for gel strength (R5) was found to be not significant with F-value of 1.94 ($p < 0.0001$) and R2 value of 0.6364. P-values less than 0.0500 indicate model terms are significant. In this case A^2 is a significant model term as shown in Table 7. In this case A, C, A^2 are significant model terms. Here, "Predicted R-Squared" of -1.7787, is a negative Predicted R^2 implies that the overall mean may be a better predictor of the response than the current model with the Adjusted R-Squared of 0.3092; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. "Adeq Precision" showed was 4.312 indicates an adequate signal respectively. The perturbation plot (Figure 17a) showing the main effects of A, B and C on the gel strength (R5) of lovastatin-β-cyclodextrin loaded liposomal gels. The correlation among the dependent and independent variables was elucidated using 2D response surface plots; 3D response surface plot and 3D cube plot are shown in (Figure 17b, c and d). Figure 17c shows the interactive effect of A and B on the gel strength (R5) at fixed level of C.

The accurate model produced for spreadability (R6) was found to be significant with F-value of 7.25 ($p < 0.0001$) and R2 value of 0.5762. P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms as shown in Table 8. Here, the Predicted R^2 of 0.2509 is not as close to the Adjusted R^2 of 0.4968 as one might normally expect; i.e. the difference is more than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. "Adeq Precision" showed was 9.155 indicates an adequate signal respectively. The perturbation plot (Figure 18a) showing the main effects of A, B and C on the spreadability (R6) of lovastatin-β-cyclodextrin loaded liposomal gels. The correlation among the dependent and independent variables was elucidated using 2D response surface plots; 3D response surface plot and 3D cube plot are shown in (Figure 18b, c and d). Figure 18c shows the interactive

effect of A and B on the spreadability (R6) at fixed level of C.

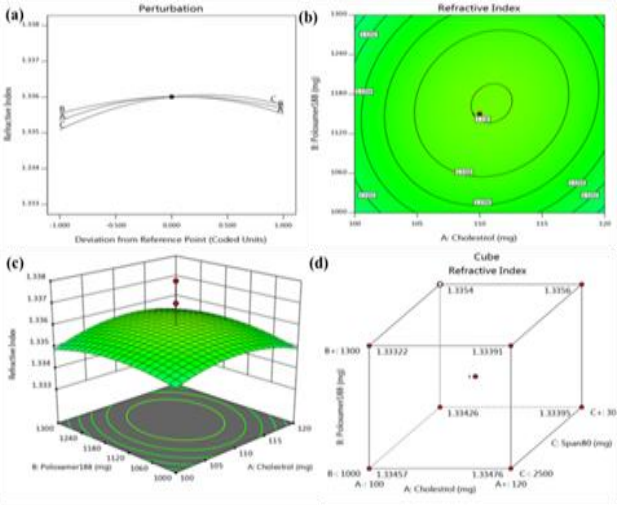

Figure 14: (a) Perturbation plot showing the main effect of Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) on refractive index (R2). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the refractive index at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the refractive index at constant span 80 concentration. (d) 3D cube plot of Box-Behnken design.

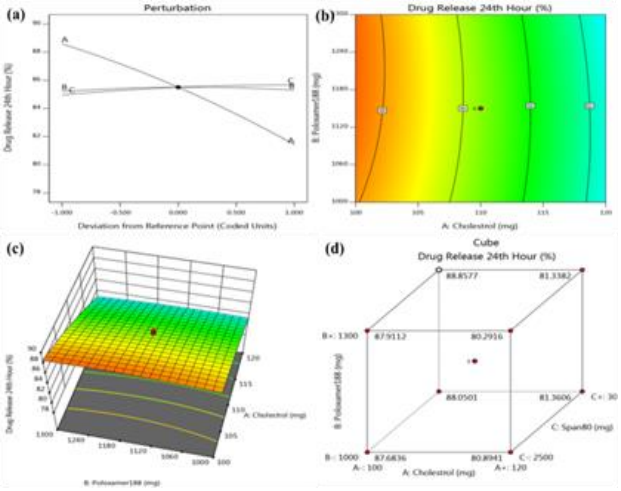

Figure 15: (a) Perturbation plot showing the main effect of Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) on drug release 24th hour (R3). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the drug release 24th hour at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the drug release 24th hour at constant span 80 concentration. (d) 3D cube plot of Box-Behnken design.

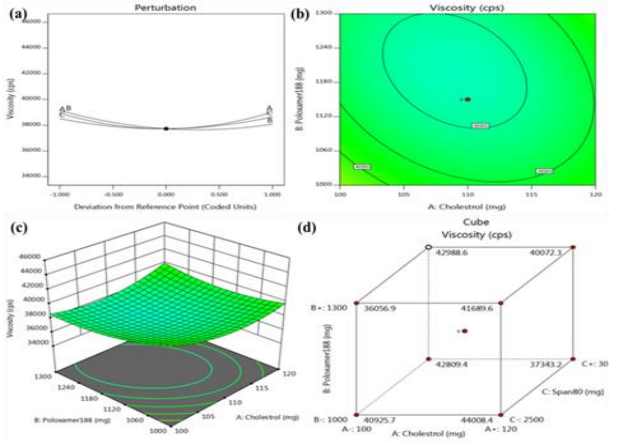

Figure 16: (a) Perturbation plot showing the main effect of Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) on viscosity (R4). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the viscosity at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the viscosity at constant span 80 concentration. (d) 3D cube plot of Box-Behnken design.

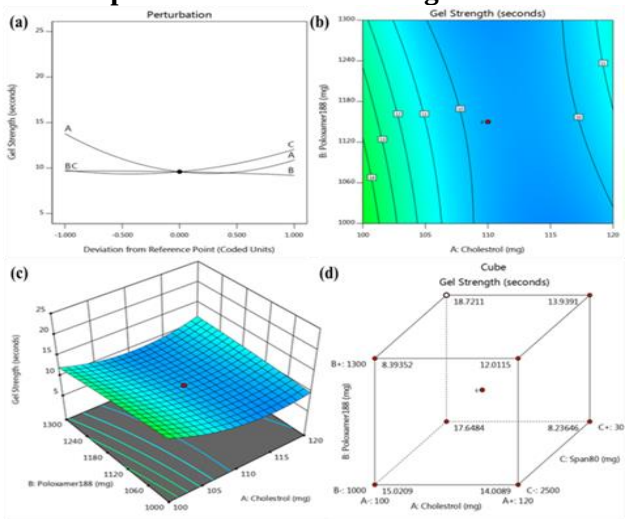

Figure 17: (a) Perturbation plot showing the main effect of Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) on gel strength (R5). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the gel strength at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the gel strength at constant span 80 concentration. (d) 3D cube plot of Box-Behnken design.

Table 8: ANOVA results of Quadratic Model for response spreadability (R6).

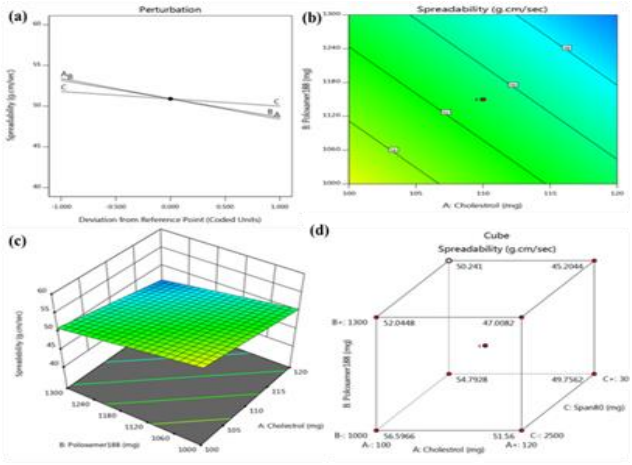

Figure 18: (a) Perturbation plot showing the main effect of Cholesterol (A), Poloxamer 188 (B) and Span 80 (C) on spreadability (R6). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the spreadability at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the spreadability at constant span 80 concentration. (d) 3D cube plot of Box-Behnken design.

Run 9, Run 10 and Run 20 code of Lovastatin-β-cyclodextrin loaded liposomal gels were prepared according to the optimized levels .The conditions of optimization were acquired by setting constraints on both the independent and dependent variables. The observed responses were in close agreement with the predicted values of the optimized process. This was described in Table 3.

Table 3: Optimized values obtained by the constraints applies on R1 to R6

Independent variables	Value	Predicted values						Code	Observed values					
		Globule Size (R1)	Refractive Index (R2)	Drug Release 24th Hour (R3)	Viscosity (R4)	Gel Strength (R5)	Spreadability (R6)		Globule Size (R1)	Refractive Index (R2)	Drug Release 24th Hour (R3)	Viscosity (R4)	Gel Strength (R5)	Spreadability (R6)
Cholesterol	110	2.44692	1.33395	81.3606	37343.2	8.23646	49.7562	RUN 9	2.47	1.338	85.33	37594	9.66	49.34
Poloxamer 188	1150							RUN 10	2.46	1.335	85.21	37591	9.65	49.36
Span 80	2750							RUN 20	2.45	1.336	86.21	37592	9.68	49.35

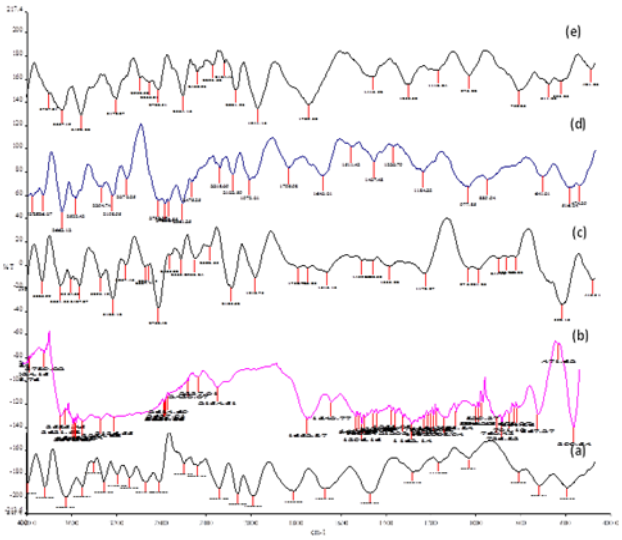

Figure 19: (a) FTIR spectrum of lovastatin. (b) FTIR spectrum of β-cyclodextrin. (c) FTIR spectrum of cholesterol. (d) FTIR spectrum of Poloxamer 188. (e) FTIR spectrum of mixture of lovastatin, β-cyclodextrin, cholesterol and Poloxamer 188.

Figure 19a, b, c, d and e shows the FT-IR spectra of pure lovastatin, β-cyclodextrin, pure cholesterol, pure Poloxamer 188 and mixture of lovastatin, β-cyclodextrin, cholesterol and Poloxamer 188. The spectrum of pure lovastatin showed principal peaks at wavenumbers (cm^{-1}) of 3989.95, 3834.69, 3642.95, 3490.21, 3383.98, 3294.42, 3170.40, 3058.56, 2913.20, 2786.05, 2562.58, 2438.38, 2235.60, 2065.66, 1962.98, 1779.26, 1635.93, 1430.25, 1236.14, 1116.60, 978.41, 750.76, 656.31 and 529.82. The spectrum of pure cholesterol shows peaks at wavenumbers (cm^{-1}) of 3823.99, 3661.33, 3564.63, 3295.19, 3183.18, 3067.46, 2887.71, 2861.15, 2768.42, 2665.82, 2563.07, 2438.24, 2302.65, 2105.32, 1943.75, 1750.70, 1706.05, 1616.13, 1459.05, 1408.30, 1333.08, 1172.07, 976.03, 931.23, 841.19, 808.76,762.28, 552.16 and 413.51. The spectrum of pure Poloxamer 188 shows peaks at wavenumbers (cm^{-1}) of 3927.05, 3836.07, 3662.12, 3533.43, 3294.74, 3198.95, 3070.35, 2783.88, 2723.04, 2688.85, 2561.25,2475.35, 2218.09, 2102.59, 1973.01, 1795.98, 1640.01,

1511.43, 1407.48, 1320.79, 1184.23, 977.85, 889.94, 641.01, 516.34 and 474.39. The peaks shown by mixture of lovastatin, cholesterol and poloxamer 188 are at wavenumbers 3787.01, 3667.19, 3492.88, 3172.27, 2952.50, 2866.01, 2788.51, 2564.16, 2430.93, 2293.62, 2184.46, 2081.93, 1941.13, 1709.30, 1413.52, 1253.30, 1118.04, 976.22, 750.26, 611.88, 555.83 and 421.03 cm-1.

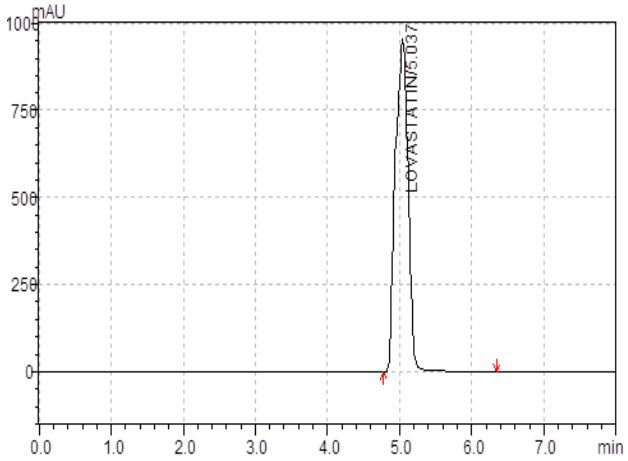

Figure 20: HPLC chromatogram of lovastatin.

CONCLUSION

In this study, lovastatin-β-cyclodextrin loaded liposomal gels were successfully developed and optimized with the use of stat-ease design-expert software (DX11). Optical microscopy images showed uniform morphology of lovastatin-β-cyclodextrin loaded liposomal gels with globule size analysis was found to be in the range of 1.52-2.76µm. Lovastatin-β-cyclodextrin loaded liposomal gels were analysed. The parameters examined were globule size, refractive index, drug release 24th hour, viscosity, gel strength and spreadability. Optimization was done based on the results obtained and the results of the prepared lovastatin-β-cyclodextrin loaded liposomal gels coincide with the expected values of various parameters.

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