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PREPARATION, CHARACTERIZATION AND OPTIMIZATION OF CARVEDILOL- β CYCLODEXTRIN LIPOSOMAL BASED POLYMERIC GEL

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ABSTRACT
Carvedilol-β-cyclodextrin loaded liposomal gel was developed for efficient incorporation and persistent release of carvedilol. Box-Behnken design was used to optimize the process parameters including Cholesterol (A), Poloxamer 188 (B) and Span 40 (C). Six dependent variables refractive index, globule size, viscosity, CDR at 12 hours, gel strength and spreadability were measured as responses. Mathematical equations and response surface plots were to relate the dependent and independent variables using Design-Expert software (DX11).
Keywords: Carvedilol, β-Cyclodextrin, Liposomes, Liposomal Gel, factorial design

INTRODUCTION

Liposomes are a novel drug delivery system (NDDS), they are vesicular structures consisting of bilayers which form spontaneously when phospholipids are dispersed in water. They are microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid bilayers. NDDS aims to deliver the drug at a rate directed by the needs of the body during the period of treatment and direct the place of action. (1) Liposomes are colloidal spheres of cholesterol non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins and drug molecules or it is also called vesicular system. (2) It differs in size, composition and charge and drug carrier loaded with variety of molecules such as small drug molecules, proteins, nucleotides or plasmids etc. (3) Few drugs are formulated as liposomes to improve their therapeutic index. (4) Hence a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes and pharmacosomes are developed. (5) The focus of this chapter is to the various method of preparation, characterization of liposomes, advantages and applications, etc. (6)

MATERIALS AND METHODS

Materials:

Carvedilol was purchased from (SM Pharmaceutical, Malaysia), Poloxamer 188 was purchased from (Merck), Span 80 was purchased from (Quicklab), cyclodextrin was purchased (HiMedia Laboratories Pvt Ltd.),diethyl ether from (Merck), Cholesterol purchased from (Sigma Aldrich, Germany), HPMC purchased from (Merck), Xantham gum purchased from (Merck).

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IN-VITRO EVALUATION

Refractive index:

The sample prism was cleaned by using distilled water then 1 to 2 drops of sample was transferred to the sample prism and closed of the prism gently.

Next, light source was adjusted until clear scale was determined. The scale needle was moved until get the half dark bright of the background colour by observing through the eyepiece of the Abbe Refractometer. The reading was noted by counting the scale number.

Particle size:

Particle size of carvedilol loaded SLNs was determined using Malvern particle size analyser (Zetasizer 4000S, Japan).

Viscosity:

Viscometer (Brookfield LVDV-II+P) is used to measure the viscosity of liposomes medium. The viscosity of liposomes medium was determined at 0.3 rpm and spinder number of 63.

The formulation was transfer in beaker of 50ml to make the depth of 4 to 5 cm. Then the spindle was adjusted and put into the formulation. Then the viscosity is determined.

Drug diffusion study:

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 leflon 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 method employed a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, 0.5% TEA: MeOH (30:70, v/v), and detection was made at 241 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation.

Preparation of carvedilol-β cyclodextrin loaded liposome:

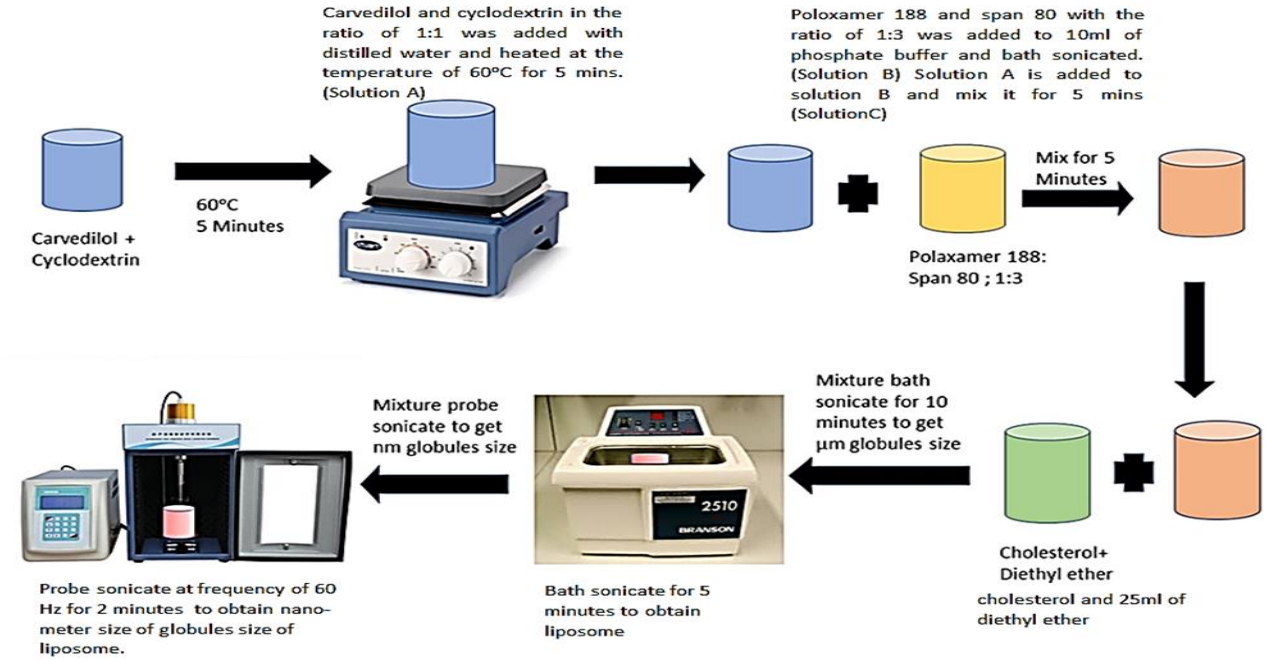

Figure 1-Show the preparation of liposome

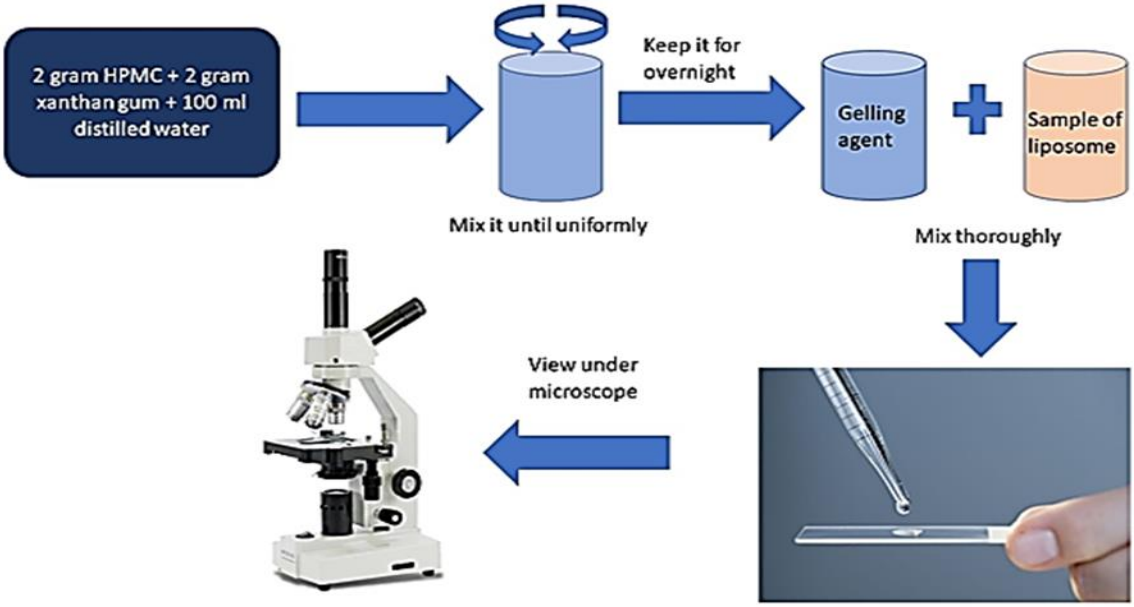

Figure 2-Show the preparation of liposomal gel

Gel strength:

The gel strength is the shear stress measured at low shear rate after a viscous liquid has set quiescently for a period of time. The cylinder was marked with 2 points of 5cm which is starting point and ending point. The cylinder was filled with 25ml of liposomes gel. The time taken for the tube to move from starting point to ending point is measured and recorded. The gel strength was recorded and determined.

Gel spreadability:

The assembly of apparatus is made up of two glass slides having two pans on both sides mounted on a pulley. Excess liposomal gel was up in between of two glass slides. 1 Kg of weight was up on the glass slides for 5 minutes to compress the liposomal gel samples into uniform thickness. Weight of 72g was added to the pan. The time taken to separate two glass slides was taken and recorded.

pH-Value:

pH is stands for 'potential of Hydrogen'. A measure of acidity or alkalinity of water soluble substances. The pH range is from 1 to 14, 7 is the neutral point. The electrode of pH meter was dipped into the sample to detect the pH of the sample.

RESULTS AND DISCUSSIONS

Experimental Design:

In this work, we report the successful effect on the formulation of carvedilol-β-cyclodextrin loaded liposomal gel. Through preliminary experiments the Cholesterol (A), Poloxamer (B) and Span 80 (C) were identified as the most identified as the most significant variables influence the refractive index, globules size, viscosity, CDR at 12 hours, gel strength and spreadability of liposomal gel. Among various design approaches, the Box-Behnken (BBD) has good design properties, little collinearity, rotatable; some have orthogonal blocks, insensitive to outliers and missing data. This Box-Behnken design is appropriate for exploring quadratic response surfaces and constructing second order polynomial models. The BBD consists of stimulated center points and the set of point lying at the midpoint of each edge of the multi-dimensional cube. Fifteen runs were essential for the response surface methodology based on the BBD. Based on the experimental design, the factor combinations produced different responses as presented in table 1. These results clearly indicate that all the dependent variables are strongly dependent on the selected independent variable as they show a wide variation among 15 runs. Data were analysed using Stat-Ease Design-Expert software (DX11) to obtain analysis of variance (ANOVA), regression coefficients and regression equation.

These equations represent the quantitative effect of cholesterol (A), Poloxamer (B) and Span 80 (C) and their interaction on refractive index (R1), globules size (R2), viscosity (R3), CDR at 12 hours (R4), gel strength (R5) and spreadability (R6) of liposomal gel. The values of the coefficient A, B and C are related to the effect of these variables on the responses R1, R2, R3, R4, R5 and R6. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively (table 2). A positive sign represents a synergistic effect, although a negative sign specifies an antagonistic effect. A backward elimination procedure was espoused to fit the data to the quadratic model. Both the polynomial equations were found to be statistically significant ($P<0.01$), as determined using ANOVA, as per the provision of Design Expert software (DX11).

Table 1. List of Independent variable and Dependent variables in Box-Behnken design

Independent Variable	Name	Units	Type	Changes	Low	High
A	Cholesterol	mg	Factor	Easy	90	110
B	Poloxamer188	mg	Factor	Easy	1000	1500
C	Span80	mg	Factor	Easy	3000	3500
Dependent Variable	**Name**	**Units**	**Types**	**Low**	**High**	
R1	Refractive Index		Response	1.337	1.342	
R2	Globule Size	µm	Response	1.24	2.32	
R3	Viscosity	cps	Response	53489	70185	
R4	CDR at 12 hour	%	Response	84.55	92.46	
R5	Gel Strength	seconds	Response	35.33	239.19	
R6	Spreadability	g.cm/sec	Response	25.63	58.16	

Table 2: Factorial Design of carvedilol Loaded Liposomal Gel

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6
Run	A:Cholestrol	B:Poloxamer188	C:Span80	Refractive Index	Globule Size	Viscosity	CDR at 12 hours	Gel Strength	Spreadability
	mg	mg	mg		µm	cps	%	seconds	g.cm/sec
1	100	1670.45	3250	1.34	1.6	63386	89.11	95.49	27.79
2	100	1250	3250	1.34	1.88	58088	89.15	78.42	43.77
3	116.818	1250	3250	1.34	1.44	57788	88.46	140.63	54.01
4	83.1821	1250	3250	1.339	1.76	55088	91.34	62.54	51.34
5	110	1500	3000	1.339	1.44	53489	84.55	62.42	31.18
6	90	1000	3000	1.342	2.08	63187	90.18	175.75	58.16
7	100	1250	3250	1.34	1.45	63346	89.76	80.64	53.34
8	110	1000	3000	1.337	1.52	61087	85.61	101.1	46.7
9	100	1250	3250	1.34	1.65	58961	89.66	94.76	49.62
10	100	1250	3250	1.34	1.7	59637	89.11	79.34	52.32
11	100	1250	3250	1.34	1.59	61987	89.13	69.38	52.87
12	100	829.552	3250	1.338	1.24	65486	87.62	61.25	31.5
13	100	1250	3670.45	1.34	1.48	57988	89.42	35.33	31.32
14	90	1500	3000	1.339	1.28	67886	90	239.19	25.63
15	110	1000	3500	1.338	2.32	65886	85.23	64.57	26.7
16	90	1000	3500	1.339	1.96	63286	92.46	114.61	35.39
17	100	1250	2829.55	1.34	1.52	63686	89.18	80.38	29.05
18	90	1500	3500	1.34	2.12	70185	90.75	81.87	39.86
19	100	1250	3250	1.34	1.8	62795	88.34	64.15	43.58
20	110	1500	3500	1.341	1.4	54588	85.16	45.36	38.86

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 fig 6 (a)(b)(c)(d)(e)(f).

The assumption of constant variance was tested by plotting externally studentized residual versus predicted values as illustrated in figures. The studentized residuals are located by dividing the residuals by their standard deviations. According

to evident from this figure 7 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 vs. Run were scattered randomly (figure 7). 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 Figure 9.

The Residuals vs. Predicted and Residuals vs. Run were scattered randomly. 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 transformation parameter, λ, is chosen such that it maximizes the log-likelihood function. The maximum likelihood estimate 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 value 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 10.

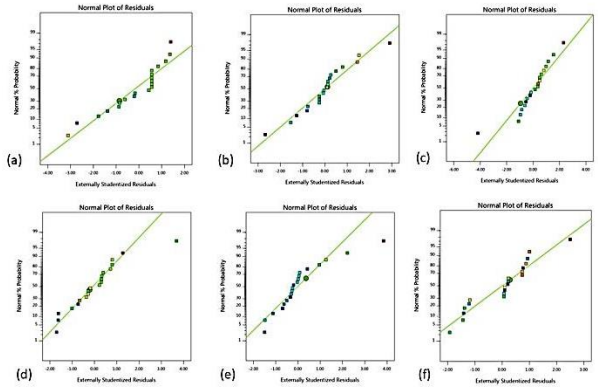

Figure 6: (a) Normal % probability plot of the externally studentized residuals (R1). (b)Normal % probability plot of the externally studentized residuals (R2). (c) Normal % probability plot of the externally studentized residuals (R3). (d) Normal % probability plot of the externally studentized residuals (R4). (e) Normal % probability plot of the externally studentized residuals (R5). (f) Normal % probability plot of the externally studentized residuals (R6)

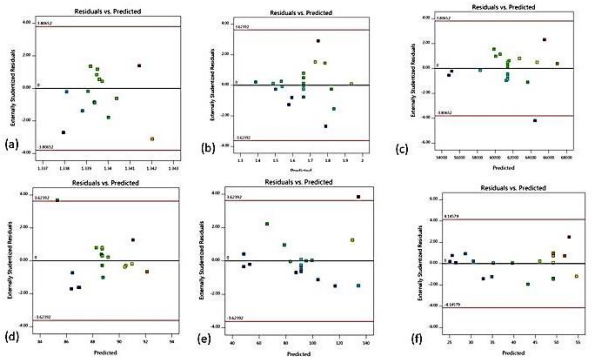

Figure 7: (a) Residual vs. Predicted (R1). (b) Residual vs. Predicted (R2). (c) Residual vs. Predicted (R3). (d) Residual vs. Predicted (R4). (e) Residual vs. Predicted (R5). (f) Residual vs. Predicted (R6)

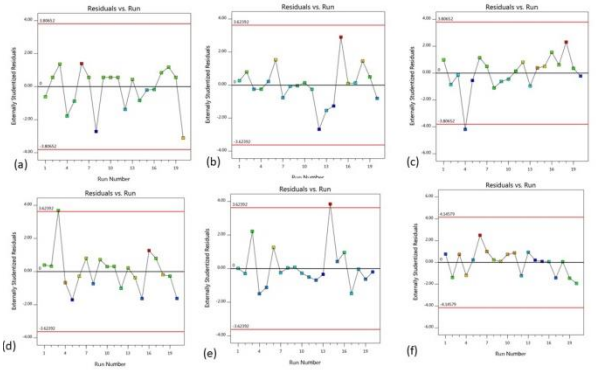

Figure 8: (a) Residuals vs. Run (R1). (b) Residuals vs. Run (R2). (c) Residuals vs. Run (R3). (d) Residuals vs. Run (R4). (e) Residuals vs. Run (R5). (f) Residuals vs. Run (R6)

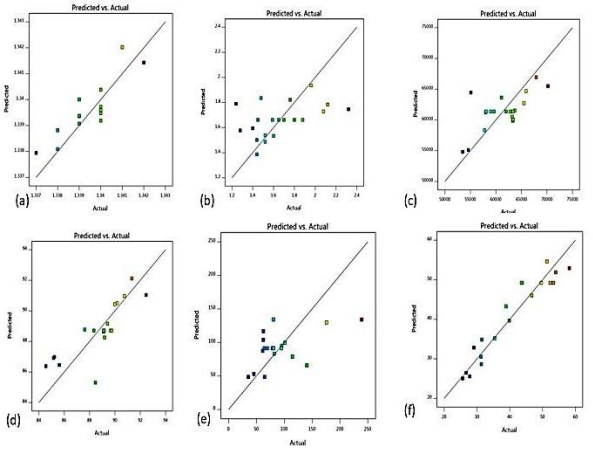

Figure 9:. (a)Actual Response vs. Predicted (R1). (b) Actual Response vs. Predicted (R2). (c)

Actual Response vs. Predicted (R3). (d) Actual Response vs. Predicted (R4). (e) Actual Response vs. Predicted (R5). (f) Actual Response vs. Predicted (R6)

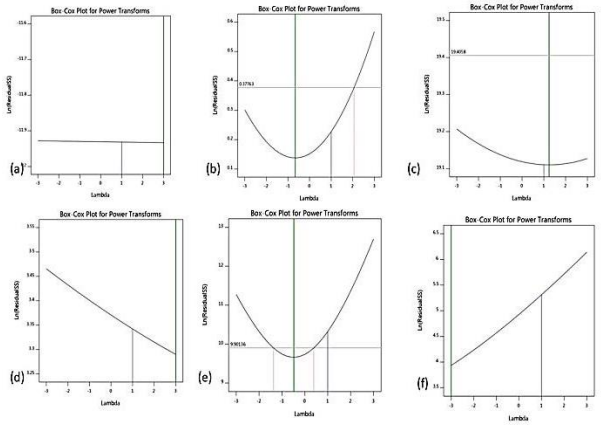

Figure 10: (a) Box-Cox Plot (R1). (b) Box-Cox Plot (R2). (c) Box-Cox Plot (R3). (d) Box-Cox Plot (R4). (e) Box-Cox Plot (R5). (f) Box-Cox Plot (R6)

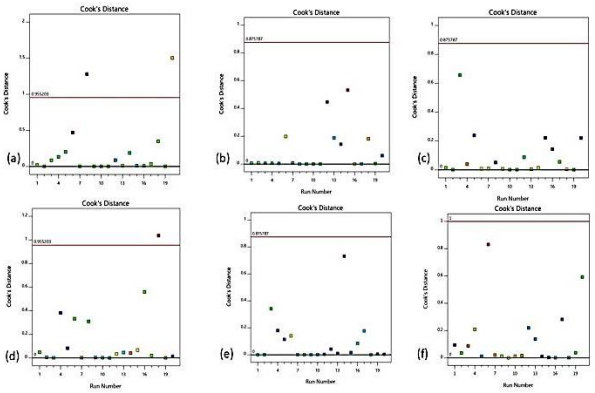

Figure 11: (a) Cook's Distance graph (R1). (b) Cook's Distance graph (R2). (c) Cook's Distance graph (R3). (d) Cook's Distance graph (R4). (e)Cook's Distance graph (R5). (f) Cook's Distance graph (R6)

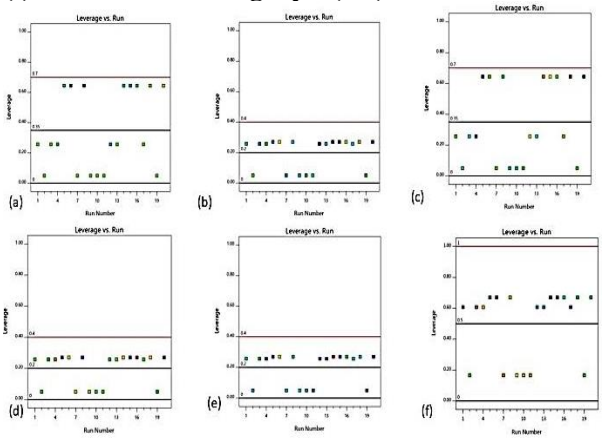

Figure 12: (a) Leverage vs. Run (R1). (b) Leverage vs. Run (R2). (c) Leverage vs. Run (R3). (d) Leverage vs. Run (R4). (e)Leverage vs. Run (R5). (f) Leverage vs. Run (R6)

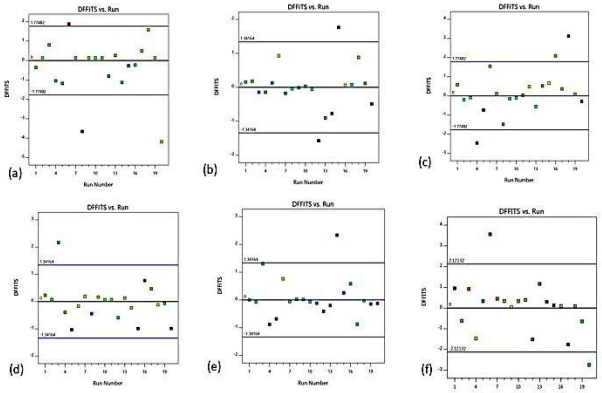

Figure 13: (a) DFFITS vs. Run (R1). (b) DFFITS vs. Run (R2). (c) DFFITS vs. Run (R3). (d) DFFITS vs. Run (R4). (e) DFFITS vs. Run (R5). (f) DFFITS vs. Run (R6)

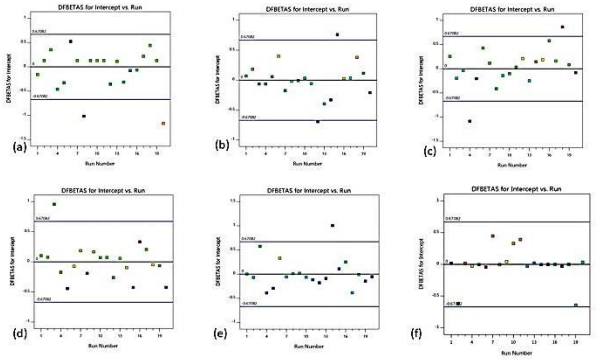

Figure 14: (a)DFBETAS for Intercept vs. Run (R1). (b) DFBETAS for Intercept vs. Run (R2). (c) DFBETAS for Intercept vs. Run (R3). (d) DFBETAS for Intercept vs. Run (R4). (e) DFBETAS for Intercept vs. Run (R5). (f) DFBETAS for Intercept vs. Run (R6)

REFRACTIVE INDEX

Refractive index analysis of carvedilol-β-cyclodextrin loaded liposomal gel was found to be in the range of 1.338 to 1.342 as shown in table 2 was found to be significant. The factorial equation for refractive index exhibited a good correlation coefficient (1.000) and the Model F value of 5.34 implies the model is significant. There is only a 0.56% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B, AB, AC, BC are significant model terms. Results of the equation indicate that the effect of B is more significant than A and C. A negative Predicted R^2 implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 9.689 indicates an adequate signal. This model can be used to navigate the

design space. The influence of the main and interactive effects of independent variables on the refractive index was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on refractive index are as shown in perturbation plots Figure 15(a). 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 15 (b) & (c) to depict the interactive effects of independent variables on response R1, one variable was kept constant whereas the other two variables diverse in a certain range. The stages of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The 3-D cube plots of Box-Behnken design are as shown in Figure 15 (d).

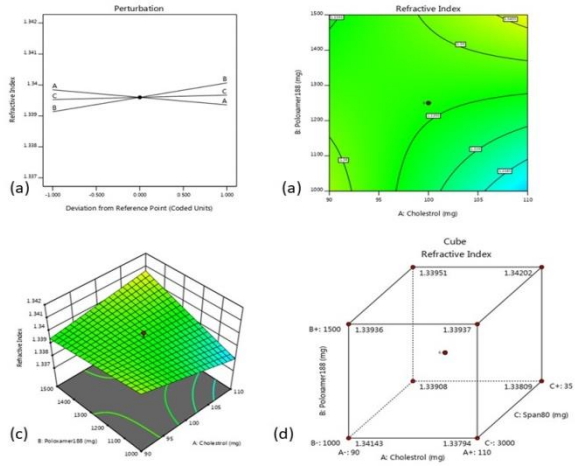

Figure 15. (a) Perturbation plot showing the main effect of cholesterol (A), poloxamer 188 (B) and span 80 (C) on refractive index. (R1). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the refractive index at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the refractive index at constant span 80 concentration. (d) 3-D plot of Box-Beknken design

GLOBULES SIZE

Globules size analysis of carvedilol-β-cyclodextrin loaded liposomal gel was found to be in the range of 1.4-2.32µm shown in table 2 and figure 15e. The factorial equation for globules size exhibited a good correlation coefficient (1.000) and the Model F value of 1.48 which implies the model is not significant relative to the noise. P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. The Lack of Fit F-value of 4.43 implies there is a 5.63% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling. The influence of the main and interactive effects of independent variables on the particle size was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on particle size are as shown in perturbation plots Figure 16(a). It is found that all the variables are having interactive effects for the response R2. The 2D contour plots and 3D response surfaces of the response R2 are shown in figure 16(b) & (c) to depict the interactive effects of independent variables on response R2, one variable was kept constant whereas the other two variables diverse in a certain range. The stages of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on particle size at a fixed level of C is shown in Figure 16 (c). The 3-D cube plots of Box-Behnken design are as shown in Figure 16(d).

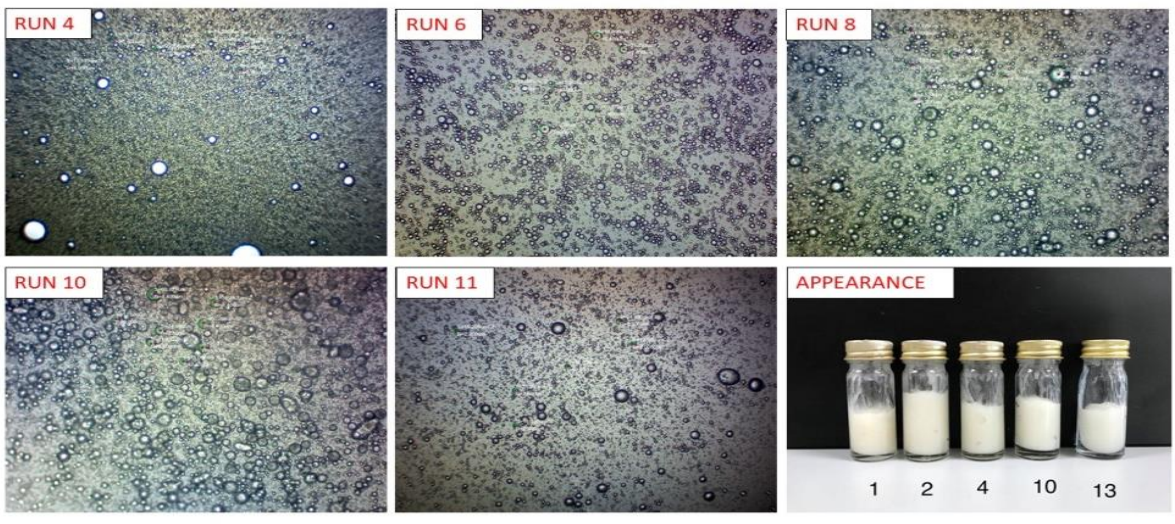

Figure 15: (e) Globule size analysis of carvedilol-β-cyclodextrin loaded liposomal gels.

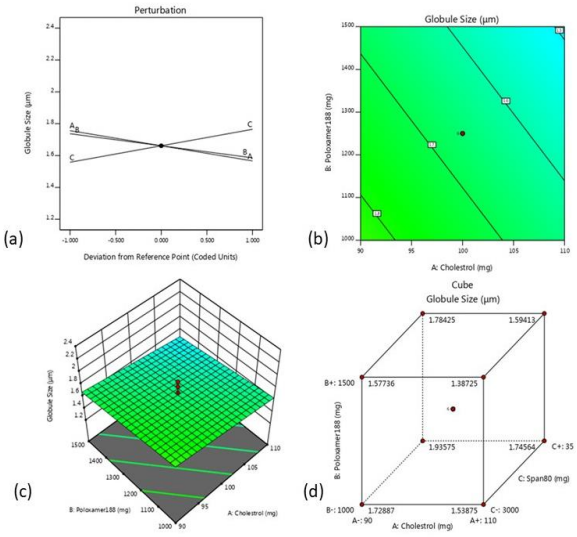

Figure 16. (a) Perturbation plot showing the main effect of cholesterol (A), poloxamer 188 (B) and span 80 (C) on globules size (R2). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the globules size at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the globules size at constant span 80 concentration. (d) 3-D cube plot of Box-Bechken design

VISCOSITY

Viscosity analysis of carvedilol-β-cyclodextrin loaded liposomal gel was found to be in the range of 53489 to 70185 cps as shown in table 2. The factorial equation for viscosity exhibited a good correlation coefficient (1.000) and the Model F value of 1.87 implies the model is not significant relative to the noise. P-values less than 0.0500 indicate model terms are significant. In this case AB is a significant model term. Values greater than 0.1000 indicate the model terms are not significant.The influence of the main and interactive effects of independent variables on the viscosity was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on viscosity are as shown in perturbation plots Figure 17(a). It is found that all the variables are having interactive effects for the response R3. The 2D contour plots and 3D response surfaces of the response R3 are shown in figure 17(b) & (c) to depict the interactive effects of independent variables on response R3, one variable was kept constant whereas the other two variables diverse in a certain range. The stages of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The 3-D cube plots of Box-Behnken design are as shown in Figure 17(d).

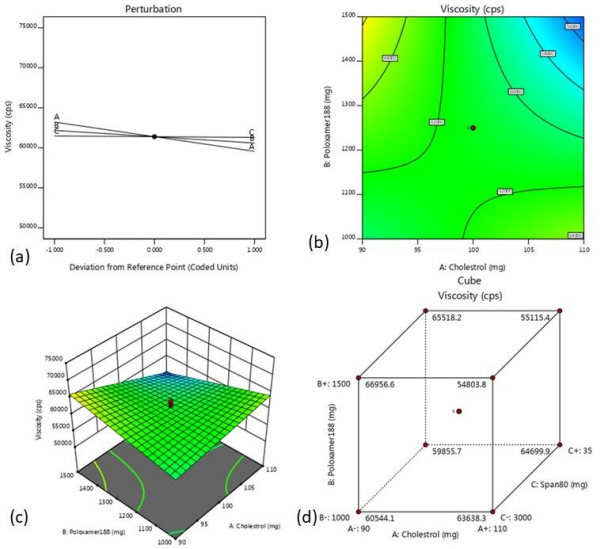

Figure 17: (a) Perturbation plot showing the main effect of cholesterol (A), poloxamer 188 (B) and Span 80 (C) on viscosity (R3). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting the viscosity at constant span 80 concentration. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting viscosity at constant span 80 concentration. (d) 3-D cube plot for Box-Behnken design

CDR at 12 hours

CDR at 12 hours analysis of carvedilol-β-cyclodextrin loaded liposomal gel was found to be in the range of 85.16-91.34% shown in table 2. The factorial equation for CDR at 12 hours exhibited a good correlation coefficient (1.000) and the Model F value of 10.77 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. The influence of the main and interactive effects of independent variables on the CDR at 12 hours was further elucidated using the perturbation and 3D response surface plots. The

Lack of Fit F-value of 4.57 implies there is a 5.54% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling. The individual main effects of A, B and C on particle size are as shown in perturbation plots Figure 18(a). It is found that all the variables are having interactive effects for the response R4. The 2D contour plots and 3D response surfaces of the response R4 are shown in figure 18(b) & (c) to depict the interactive effects of independent variables on response R4, one variable was kept constant whereas the other two variables diverse in a certain range. The stages of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on CDR at 12 hours at a fixed level of C is shown in Figure 18(c). The 3-D cube plots of Box-Behnken design are as shown in Figure 18(d).

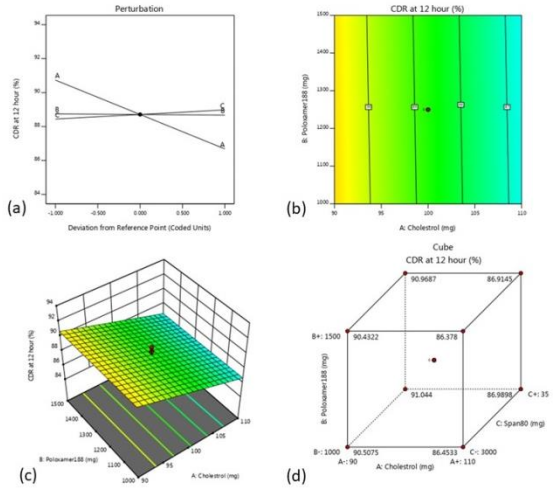

Figure 18. (a) Perturbation plot showing the main effect of cholesterol (A), poloxamer 188 and span 80 (C) on % CDR at 12 hours (R4). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting % CDR at 12 hours at constant Span 80. (c)Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting % CDR at 12 hours at constant Span 80. (d) 3-D cube plot of Box-Behnken design

Gel Strength

Gel Strength analysis of carvedilol-β-cyclodextrin loaded liposomal gel was found to be in the range of 35.33-239.19 seconds shown in table 2. The factorial equation for gel strength exhibited a good correlation coefficient (1.000) and The Model F-value of 2.11 implies the model is not significant relative to the noise. There is a 13.88% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case C is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The influence of the main and interactive effects of independent variables on the gel strength was further elucidated using the perturbation and 3D response surface plots. The Lack of Fit F-value of 24.43 implies the Lack of Fit is significant. There is only a 0.12% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit. The individual main effects of A, B and C on particle size are as shown in perturbation plots Figure 19(a). It is found that all the variables are having interactive effects for the response R5. The 2D contour plots and 3D response surfaces of the response R5 are shown in figure 19 (b) & (c) to depict the interactive effects of independent variables on response R5, one variable was kept constant whereas the other two variables diverse in a certain range. The stages of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on gel strength at a fixed level of C is shown in Figure 19(c). The 3-D cube plots of Box-Behnken design are as shown in Figure 19(d).

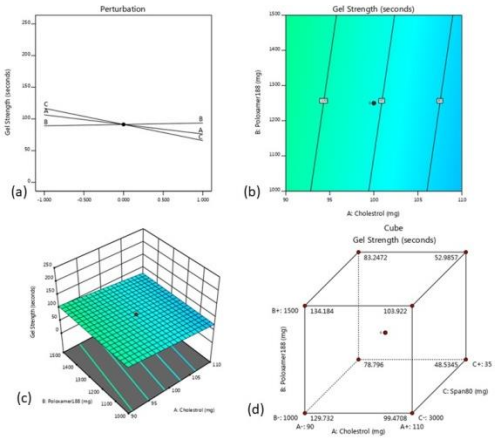

Figure 19. (a) Perturbation plot showing the main effect of cholesterol (A), poloxamer 188

and span 80 (C) on gel strength (R5). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting gel strength at constant Span 80. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting gel strength at constant Span 80. (d) 3-D cube plot of Box-Behnken design

Spreadability

Spreadability analysis of carvedilol-β-cyclodextrin loaded liposomal gel was found to be in the range of 25.63-54.01 g.cm/sec shown in table 2. The factorial equation for spreadability exhibited a good correlation coefficient (1.000) and the Model F value of 10.88 which implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case B, BC, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. B and C are more significant than A. 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 particle size was further elucidated using the perturbation and 3D response surface plots. The Lack of Fit F-value of 1.00 implies the Lack of Fit is not significant relative to the pure error. There is a 50.13% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit. The individual main effects of A, B and C on particle size are as shown in perturbation plots Figure 20(a). It is found that all the variables are having interactive effects for the response R6. The 2D contour plots and 3D response surfaces of the response R6 are shown in figure 20(b) & (c) to depict the interactive effects of independent variables on response R6, one variable was kept constant whereas the other two variables diverse in a certain range. The stages of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on spreadability at a fixed level of C is shown in Figure 20(c). The 3-D cube plots of Box-Behnken design are as shown in Figure 20(d).

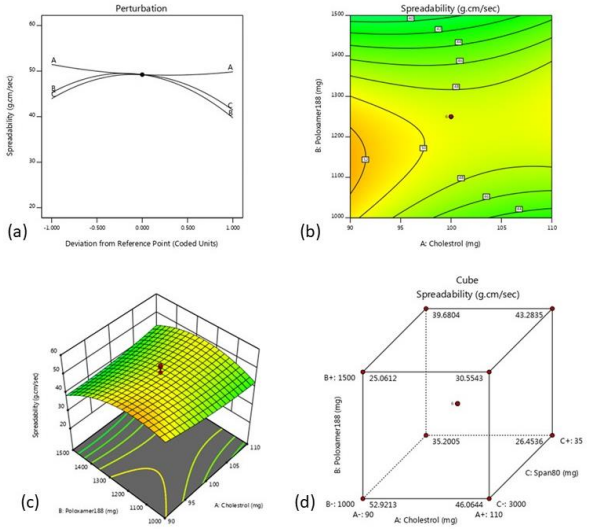

Figure 20. (a) Perturbation plot showing the main effect of cholesterol (A), poloxamer 188 and span 80 (C) on spreadability (R6). (b) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting spreadability at constant Span 80. (c) Response surface plot presenting the interaction between the cholesterol and poloxamer 188 affecting spreadability at constant Span 80. (d) 3-D cube plot of Box-Behnken design

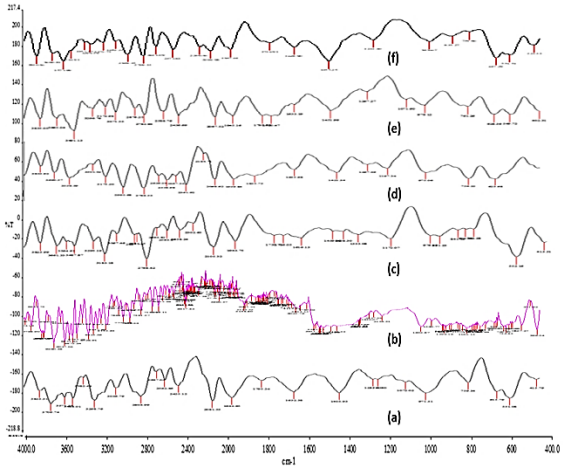

Figure 21. (a) FTIR spectrum of carvedilol (b) FT-IR spectrum of β-cyclodextrin (c) FTIR of cholesterol. (d) FTIR spectrum of poloxamer 188. (e) FTIR spectrum of cholesterol and poloxamer 188 (f) FTIR spectrum of carvedilol, cyclodextrin, cholesterol and poloxamer 188

Figure 21 (a) shows the FT-IR spectra of pure carvedilol. The spectrum of pure carvedilol showed principle peaks at wavenumbers (cm1) of 3823.31, 3709.74, 3571.24, 3488.01, 3382.44, 3268.76, 3066.79, 2804.99, 2547.04, 2562.45, 2425.12, 2081.55, 1942.65, 1795.24, 1632.38, 1406.99, 1072.02, 971.51, 759.34, 615.79, 551.08, 415.79 (b) Shows the FT-IR spectra of β-cyclodextrin. (c) shows the FT-IR spectra of pure cholesterol. The spectrum of pure cholesterol shows peaks at wavenumbers (cm-1) of 3823..99, 3661.33, 3564.33, 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 (d) Shows the FT-IR spectra of pure poloxamer 188. The spectrum of pure poloxamer 188 shows peaks at wavenumbers (cm-1) 3833.92, 3690.57, 3301.02, 3173.20, 2995.68, 2788.53, 2636.08, 2558.98, 2467.34, 2361.92, 2184.72, 2068.62, 1939.84, 1835.75, 1635.68, 1421.34, 1267.49, 1167.34, 975.06, 759.22, 623.68. (e) Shows the FT-IR spectra of pure cholesterol and poloxamer 188. The spectrum of pure cholesterol and poloxamer 188 show peaks at wavenumbers (cm-1) 3835.05, 3665.04, 3491.13, 3299.76, 3172.98, 3071.55, 2876.25, 2782.94, 2588.76, 2436.69, 2067.52, 1945.16, 1792.30, 1633.19, 1451.99, 1267.27, 1071.60. 979.12, 761.36, 628.36, 549.72, 402.31. (f) shows the FT-IR spectra of pure carvedilol + cyclodextrin + cholesterol + poloxamer 188. The spectrum of pure carvedilol + cholesterol + poloxamer 188 shows peaks at wavenumbers (cm-1) 3840.16, 3680,81, 3571.99, 3491.91, 3357.35, 3299.39, 3172.73, 3051.21, 2929.35, 2769.14, 2641.09, 2473.83, 2206.46, 2089.98, 1947.04, 1749.91, 1628.90, 1455,25, 1233.40, 949.67, 835.27, 749.86, 617.29, 551.51, 424.33.

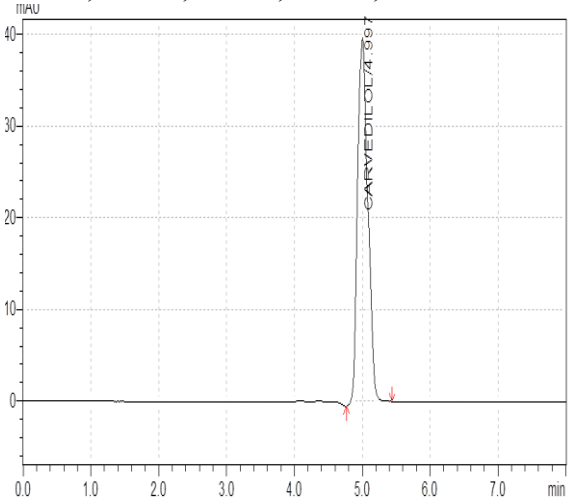

Figure 22: HPLC chromatogram of Carvedilol

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

Independent variables	Values	Predicted values						Code	Observed values					
		Refractive index	Globule size	Viscosity	CDR At 12 hours	Gel Strength	Spreadability		Refractive index	Globule size	Viscosity	CDR At 12 hours	Gel Strength	Spreadability
Cholesterol	100	1.33971	1.73608	62820	90.1859	98.7737	49.8194	4	1.339	1.76	55088	91.34	62.54	51.34
Poloxamer 188	1250							9	1.34	1.65	58961	89.66	94.76	49.62
Span 80	3250							10	1.34	1.7	59637	89.11	79.34	52.32

Code 4, 9 and 10 batches of carvedilol-β-cyclodextrin loaded liposomal gels were prepared according to these optimized levels. Observed responses were in close agreement with the predicted values of the optimized process was shown in table 3, thereby demonstrating the feasibility. The cumulative drug release from liposomal gel at the end of 12th hour was shown in table 2.

CONCLUSION

The results of the carvedilol-β-cyclodextrin loaded liposomal gel has succeeded the objectives of refractive index, globule size, viscosity, CDR at 12 hours, gel strength and spreadability. The factorial equation for particle size exhibited a good correlation (1.000) and the Model F of 10.77

implies the model is significant. There is only a 0.04% 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 this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. Carvedilol-β-cyclodextrin loaded liposomal gel was successfully developed and optimized with the use of stat-ease design-expert software (DX11). Statistical methods based on experimental designs of tests, regression analysis and optimization techniques can be used to carry experimental designs of tests, regression analysis and optimization techniques can be used to carry out this task more effectively and efficiently. The developed carvedilol-β-cyclodextrin loaded liposomal gel demonstrated ideal particle size range of 1.4-2.32µm size.

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