ORIGINAL ARTICLE

Formulation and optimization of SNEDDS of gliclazide using response surface methodology

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The aim of the current study was to design an self-nanoemulsified drug delivery system (SNEDDS) of gliclazide by optimizing the particle size using response surface methodology. SNEDDS were prepared using oils, surfactant and co-surfactant. A D-optimal design for 3 factors at 3-level each was employed systematically optimize particle size. The particle size was taken as dependent variable. Oil such as Capryol 90, surfactant as Cremophor EL, co-surfactant as Akoline MCM were taken as independent variables. The counter plot and 3D plot were drawn and optimum formulation were selected by feasibility and grid searches. The polynomial mathematical model generated for response were found to be $Y = + 148.0778 + 8.1388*A - 0.2556*B - 0.2833*C - 0.2083*A*B - 0.25*A*C + 0.0083*B*C + 10.35A^2 + 2.2\ B^2 + 2.95C^2$ and that found to be significant ($P < 0.05$). Validation of optimization study performed using confirmatory runs, indicated very high degree of prognostic ability of response surface methodology with mean percent error (± S.D.) 5.11.

Key words: *D-optimal design, gliclazide, response surface methodology, SNEDDS*

INTRODUCTION

Approximately 35-40% of new drug candidates have poor aqueous solubility. The oral drug delivery of such drugs is frequently associated with low bioavailability, high inter-subject and intra-subject variability and lack of dose proportionality. Efforts are needed to enhance the oral bioavailability in the gastrointestinal (GI) tract. Nanoemulsion are preferred drug delivery system because of their stability and possibility of easy oral administration to improve dug self-emulsification in the gut.[1]

Self-nanoemulsifying system is isotropic mixture of oil, surfactant and hydrophilic co-surfactant which forms fine o/w nano-emulsion, when introduced in excess of aqueous phase under condition of gentle agitation. Agitation will be provided by body movement and GI movement *in-vivo*. Bases for self-nano-emulsifying system have been formulated using medium chain tri-glyceride oils and non-ionic surfactant, which are acceptable for oral ingestion.[2,3]

A literature search reveals that an exhaustive number of publications characterizing the self-emulsified drug delivery system. Reported studies use different method for *in vitro* evaluation such as self-emulsification time, cumulative percent release, low frequency dielectric spectroscopy, zeta potential measurement and surface tensiometry.[4] Particle size of self-nanoemulsified drug delivery system (SNEDDS) after dilution was selected as criteria for *in vitro* evaluation. Smaller the particle size of SNEDDS more is the release of drug with better bioavailability. Particle size around 20 nm gives total transparent system upon dilution, which acts as a solution. So, particle size was selected as criteria for optimization. Screening and optimizing SNEDDS could be further simplified by the use of statistical design that requires only a small number of experiments, thereby eliminating the need for time consuming, and detailed ternary phase diagrams. The statistical optimization design has been documented for the formulation of pharmaceutical solid dosage forms. Here SNEDDS were tried to optimize on the basis of particle size after dilution in double distilled water which are profoundly influenced by several formulation variables.[5]

In the development of a SNEDDS an important issue

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is to design an optimized formulation with an appropriate particle size with minimum number of trials. Statistical experimental design methodologies are powerful, efficient and systematic tools in design of pharmaceutical dosage forms, allowing rational study of the influence on formulation and/or processing parameters on the selected responses with a shortening of the experiment work. The main objective of the experimental design strategies is to plan experiments in order to obtain the maximum information regarding the considered experimental domain with the lowest numbers of experiments. Many statistical design have been recognized as useful techniques to optimize the process variables. For this purpose, a computer based optimization technique with a response surface methodology (RSM) utilizing a polynomial equation has been widely used. Different types of RSM design include 3-level factorial design, central composite design (CCD), Box Behnken design and D-optimal design. Response surface methodology (RSM) is used only a few significant factors are involved in optimization. The technique requires minimum experimentation and time, thus proving to be far more effective and cost effective than conventional methods of formulating SNEDDS.[6,7]

Gliclazide is an antidiabetic drug. Gliclazide occurs as white amorphous powder. Gliclazide is practically insoluble in water and therefore absorbs poorly with irritation in gastric lining and hence shows bioavailability just 40%. Thus in order to improve its bioavailability, it is necessary to enhance its solubility and dissolution characteristics. It was decided to increase solubility of Gliclazide by formulation of SNEDDS, which may result in increase in solubility and dissolution with subsequent reduction in dose, as the dose of Gliclazide in conventional tablet is 40 mg.[2,8] The gliclazide is a weak acid (pKa = 5.9) practically insoluble in water and acidic environment but highly permeable (class II) according to Biopharmaceutical Classification Systems (BCS). The oral absorption is uniform, rapid and complete with a bioavailability of nearly a short biological half life (3.4 ± 0.7 h).[9]

Thus, the aim of the present paper was to evaluate, by means of response surface methodology, the influence of oil, surfactant and co-surfactant on the particle size from SNEDDS. As a part of optimization process, the main effects, interaction effects and quadratic effects of the formulation ingredients were evaluated for their effect on the particle size of Gliclazide SNEDDS. Particle size is particularly important since release rates are greatly influenced by particle size.

MATERIALS AND METHODS

Materials

Gliclazide was a received from Bal Pharma (Banglore, India) as donate sample. Akoline MCM (CAPMUL MCM) was received as a gift sample from ABITEC Corporation, Ohio, USA. Capryol 90 was received as a gift sample from Colorcorn India, Goa, India. Cremophor EL was received as a gift sample from BASF Ltd., Mumbai, India. All other chemicals/reagents were used of analytical grade and double distilled water used throughout the experiments.

Preparation of the gliclazide self-nano-emulsifying formulation

Accurately weighed 40 mg of Gliclazide was mixed with Capryol 90. Then in the blend add Cremophor EL, Akoline MCM and mixed on a cyclomixer to get a uniform mixture. And afterword the mixture was sonicated on the probe sonicator until the complete solubilization of the gliclazide into the mixture. Prepared formulations were filled in a transparent hard gelatin capsule (Size 0). Capsule was sealed with the help of gelatin band to avoid leakage. The formulations were shown in the [Tables 1-3].

Particle size analysis

For the study of particle size formulations were diluted with media like double distilled water. Visual observations were made immediately after dilution for assessment for self-nano-emulsification efficiency, appearance (transparency), phase separation and precipitation of drug. The mean globule size and polydispersity index (P.I.) of the resulting nano-emulsion were determined by PCS. Measurements were obtained at an angle of 90. Nanoemulsion were diluted with media for ensuring that the light scattering intensity (between 6E + 004 to 1E + 006), was within the instrument's sensitivity range. The resultant nanoemulsions were also allowed to stand for 6 hr at room temperature to assess dilution stability.

Experimental design

The traditional approach to developing a formulation is to change one variable at a time. By this method it is difficult to develop an optimized formulation, as the method reveals nothing about the interaction among the variables. In a mixture design where the composition is the factor of interest, the levels cannot be chosen arbitrarily. All fractions of component must sum to unity. In a design so constrained a simple lattice design is recommended. In three component mixture all mixture possible combinations can be graphically represented by the interior and boundaries of an equatorial triangle using simple lattice designs. Hence, a D-optimal statistical design with 3 factor, 3 levels and 27 runs was selected for optimization study. The experimental design consists of a set of points lying at the midpoint of each edge and replicated center point of the multidimensional cube. The independent and dependent variables are listed in Table 1. The polynomial equation generated by this experimental design (using Design expert software version 7.0) is as follows:

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where, Yi is the dependent variable, b_0 is the intercept, b_1 to b_{33} are the regression coefficients and X_1, X_2 and X_3 are the

Table 1: Experimental ranges for independent variables and constraints

Factors (%)	Experimental ranges		
	Low level (-1)	Middle level (0)	High level (+1)
A-Amount of Capryol 90	45	50	55
B-Amount of Akoline MCM	10	12.5	15
C-Amount of Cremophore EL	35	37.5	40

Dependent variable: Y-Particle size (nm) of the droplet after dilution with double distilled water

Table 2: Experimental matrix for the D-optimal 3 level, 3 factor design and result

Run	Variable factors			Result
	A-Capryol 90 (%)	B-Akoline MCM (%)	C-Cremophore EL (%)	Y-Particle size (nm)
1	50	10	35	165.6
2	45	15	37.5	153.5
3	50	15	35	152.5
4	45	15	35	155
5	45	12.5	40	158
6	50	10	40	152
7	45	12.5	37.5	155
8	50	12.5	40	149
9	45	12.5	35	152
10	50	12.5	35	151
11	50	15	40	148
12	55	10	37.5	170
13	55	10	35	168
14	55	12.5	40	172
15	55	10	40	173
16	45	10	40	153
17	55	12.5	37.5	160
18	50	12.5	37.5	145.8
19	55	15	37.5	172
20	45	10	37.5	152
21	55	15	35	173
22	55	15	40	172
23	50	15	37.5	149
24	45	15	40	155
25	55	12.5	35	170
26	45	10	35	150
27	50	10	37.5	151

independent variable that was selected from the preliminary experiments. The model generated contained quadratic terms which explained the non-linear nature of responses and multiple factor terms explaining effects between factors. The formulation was optimized with the help of response surface diagram.

RESULTS AND DISCUSSION

Construction of phase diagram

The phase diagram of Cremophor EL, Akoline MCM and Capryol 90 system was shown in Figure 1. The outer parallelogram indicates the area, which explored for locating nanoemulsification region. The filled region indicated with NE indicates the region in which nanoemulsion of desired size were obtained. From figure, it is evident that Cremophor EL, Akoline MCM and Capryol 90 system has larger nanoemulsification region. Cremophor EL, Akoline MCM and Capryol 90 system yielded nanoemulsion for the compositions for the compositions that as high as 70% (w/w) of oily phase comprising of oil + lipophilic co-surfactant concentration. These compositions had ability to solubilize various hydrophobic drugs and have potential to become platform systems.

Fitting of data to the model

Different Gliclazide SNEDDS were obtained based on the experimental design [Table 2]. Particle size of SNEDDS was selected as a response for optimization. Particle sizes of all 27 formulations are shown in the Table 3.

The model was fitted to the data for a response, the normalizes coefficients of the fitted model are related in Table 4. In normalized form the coefficient are divided by the standard deviation of their respective response.

The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($P < 0.05$) effect of factors on response. The initial model was refined by excluding terms for which the level of significance was greater than 0.05 ($P \geq 0.05$). The remaining terms were used to refit the data and the resultant equation is given below:

Final equation in coded factor:

$$Y = + 148.0778 + 8.1388^{*}A - 0.2556^{*}B - 0.2833^{*}C - 0.2083^{*}A^{*}B - 0.25^{*}A^{*}C + 0.0083^{*}B^{*}C + 10.35A^{2} + 2.2\,B^{2} + 2.95C^{2} \quad (1)$$

Final equation in actual factor:

$$\text{Particle size} = + 177.8675 - 38.8139^{*}\text{Capryol 90} - 8.1188^{*}\text{Akoline MCM} - 4.53^{*}\text{Cremophor EL} - 0.0166^{*}\text{Capryol 90}^{*}\text{Akoline MCM} - 0.02^{*}\text{Capryol 90}^{*}\text{Cremophor EL} + 0.0013^{*}\text{Akoline MCM}^{*}\text{Cremophor EL} + 0.414^{*}(\text{Capryol 90})^{2} + 0.352^{*}(\text{Akoline MCM})^{2} + 0.472^{*}(\text{Cremophor EL})^{2} \quad (2)$$

Where, Y = particle size, A = Quantity of Capryol 90, B = Quantity of Akoline MCM, C = Quantity of Cremophor EL.

The above equation represents the quantitative effect of process variables (A, B, C) and their interaction on the response (Y). The values of the coefficients A, B and C related to the effect of these variables on the response Y. Coefficient with more than one factor term and those with higher order terms represent interaction term. A positive sign represent a synergistic effect, while a negative sign indicate

Table 3: Composition of gliclazide SNEDDS by factorial design

Batch	Ingredients					Result
	Gliclazide (mg)	Capryol 90 (mg)	Akoline MCM (mg)	Cremophore EL (mg)	Total (mg/capsule)	Y-Paricle size (nm)
1	40	260	52	182	534	165.6
2	40	234	78	195	547	153.5
3	40	260	78	182	560	152.5
4	40	234	78	182	534	155
5	40	234	65	208	547	158
6	40	260	52	208	560	152
7	40	234	65	195	534	155
8	40	260	65	208	573	149
9	40	234	65	182	521	152
10	40	260	65	182	547	151
11	40	260	78	208	586	148
12	40	286	52	195	573	170
13	40	286	52	182	560	168
14	40	286	65	208	599	172
15	40	286	52	208	586	173
16	40	234	52	208	534	153
17	40	286	65	195	586	160
18	40	260	65	195	560	145.8
19	40	286	78	195	599	172
20	40	234	52	195	521	152
21	40	286	78	182	586	173
22	40	286	78	208	612	172
23	40	260	78	195	573	149
24	40	234	78	208	560	155
25	40	286	65	182	573	170
26	40	234	52	182	508	150
27	40	260	52	195	547	151

Table 4: Analysis of variance for particle size of gliclazide SNEDDS

Source	Sum of squares	Degree of freedom	Mean square	Model F value	Model *P value*	Model prediction
Model	1920.229	9	213.35	10.2542	<0.0001	Significant
A	1192.347	1	1192.347	57.3053	<0.0001	Significant
B	1.1755	1	1.1755	0.0564	0.815	Nonsignificant
C	1.445	1	1.445	0.0694	0.7953	Nonsignificant
AB	0.5208	1	0.5208	0.02503	0.8762	Nonsignificant
AC	0.75	1	0.75	0.03604	0.8517	Nonsignificant
BC	0.0008	1	0.0008	4.01	0.995	Nonsignificant
A^2	642.735	1	642.735	30.8904	<0.0001	Significant
B^2	29.04	1	29.04	1.3956	0.2537	Nonsignificant
C^2	52.215	1	52.215	2.5095	0.1316	Nonsignificant
Residual	353.7172	17	20.8069			
Core total	2273.947	26				

an antagonistic effect. The values of the coefficient A, B and C were substituted in the equation to obtain the theoretical values of Y.

To show the quality of fit of the model, residual plots of the observed values verses the predicted values were depicted in Figure 2. Plots showed the points fairly close to straight lines indicating good model.

The model term for the particle size was found to be significant with high value of r^2 0.8444 which indicates the adequate fitting to a quadratic model. The model F-value of 10.25 implies the model is significant.

Also, the "Pred R-Squared" values of 0.6047 is in reasonable agreement with the "Adj R-Squared" value of 0.7620. The relationship between the dependent variable and

independent variables was elucidated using contour and response surface plots.

The resultant equations 1 or 2 which represents the quantitative effect on formulation parameter on particles size. The effect of A and B and their interaction on Y (Particle size) at a fixed level of C are given in Figure 3. Figure illustrate the corresponding response surface and counter plot of the model. It was found that, at low level of A (amount of Capryol 90, 45%), Y decreases from 155 to 150 nm, as amount of Akoline MCM increases from 10 to 15%. At higher level of A, Y remains approximately constant (at 173 and 160 nm) because quantity of surfactant at this level cannot provide required HLB value to emulsify lipophile, therefore produce emulsion of large particle size.

The effect of A and B and their interaction on Y (Particle size) at a fixed level of C are given in Figures 4-6. Figure illustrate the corresponding response surface and counter plot of the model. It was found that, at low level of B (10% Akoline MCM), Y decreases from 172 to 150 nm as the amount of Capryol 90 decreases from 55 to 45%. At the middle level of B, Y changes from 150 nm to 145.8 nm as the B changes from 10-15%. Particle size of the droplet was found minimum 145.8 nm at a ratio of Capryol 90 to Akoline MCM 4:1, when Cremophor EL at a Middle level (50%). Here quantity of Cremophor EL and Akoline MCM are critical. Emulsion gives minimum particle size at a critical concentration of surfactant system.

The effective formulation obtained from the factorial design run no. 18 containing Capryol 90 (50%), Akoline MCM (12.5%) and Cremophor EL (37.5%) showed the possible result from the expected values of ANOVA. Therefore run no. 18 taking further for model validation.

Model validation (Optimization)

The two formulations were prepared for the model validation. The values of response predicted from obtained model are shown in Table 5, along with result obtained by experimentation. The close resemblance between observed and predicted response values assessed the robustness of the predictions. These values indicate the validity of the generated model.

CONCLUSIONS

A method to obtain good experimental mixture designs when the experimental factor space is not a simplex, is to use

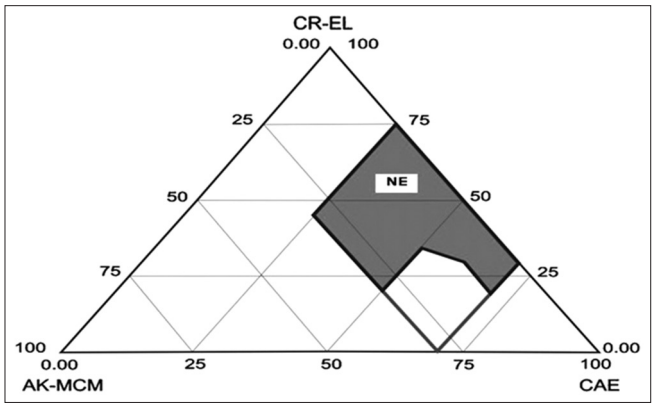

Figure 1: Ternary phase diagram of CR-EL, AK-MCM and capryol 90

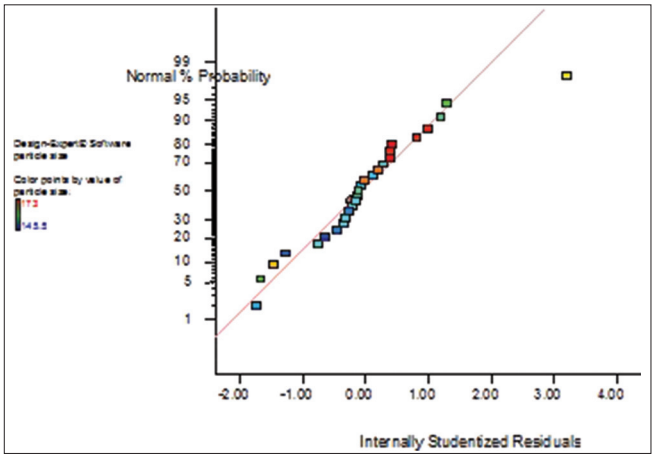

Figure 2: Normal residual plot

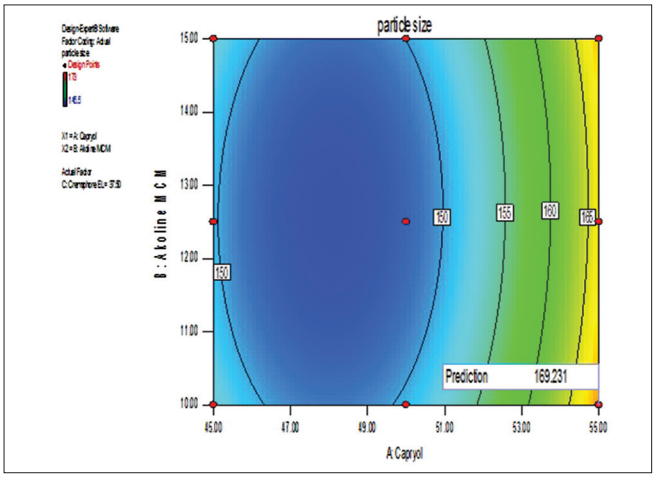

Figure 3: Counter plot for response particle size

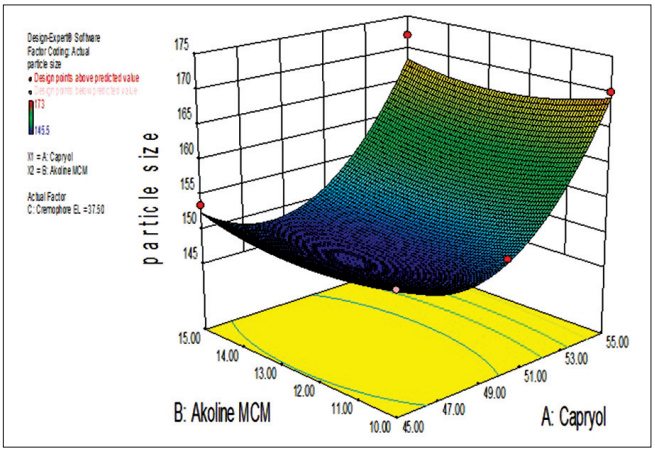

Figure 4: Response surface plot for particle size (Cremophore EL = 37.50%)

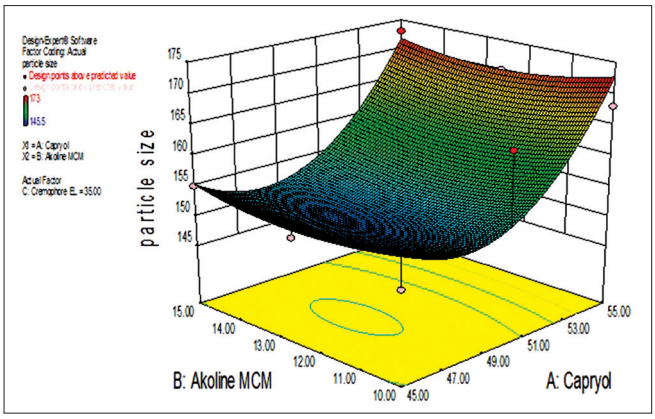

Figure 5: Response surface plot for particle size (Cremophore EL = 35%)

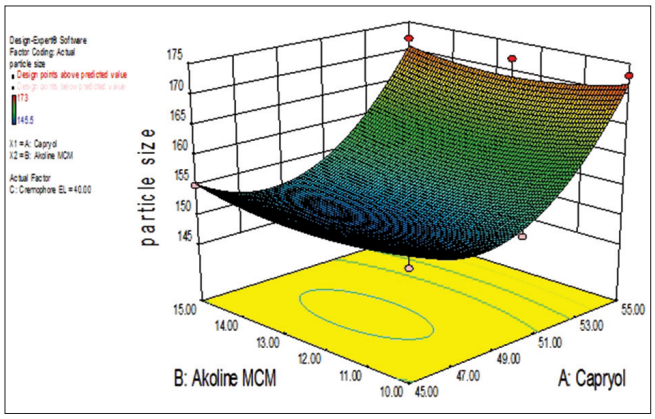

Figure 6: Response surface plot for particle size (Cremophore EL = 35%)

Table 5: Optimized values obtained by applying constraints on variables and responses

Variables	Quantity (%)	Expected particle size (nm)	Observed particle size (nm)
A-Capryol 90	50	145.8	148.08
B-Akoline MCM	12.5		
C-Cremophore EL	37.5		

D-optimum criterion where a given number of experiments is selected out of many possible mixtures, in order to give a statistically optimized design.

Examination of the contour plots led to the determination of the regions where acceptable values of the response are obtained. Optimum region respecting all the constraints applied to the results was found in the interior of this optimum zone by non-linear programming methods using the method of Lagrenge multipliers.

Optimization of the self-nano-emlusifying formulation of gliclazide was performed using 3 factors, 3 level design. The dependent variable used A-Capryol 90 (50%), B-Akoline (12.5%) and C-Cremophor-EL (37.5%) showed significant effect on the response i.e., particle size and physical appearance of the resultant nanoemulsion on dilution with double distilled water. The quantitative effect of factor at different level was predicted using polynomial equation. Response methodology was then used to predict the levels of one factor A, B and C requires to obtain an optimum formulation with particle size 145.8 nm. The resultant formulation shows the effective results because of the concentration of oil present in the formulation having greater impact on the surfactant and co-surfactant which reduces the particles size in the effective ranges.

The information obtained on the influence of the different excipients would be expected to prove useful further development when formulations of different particle size characteristics might be required.

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REFERENCES

1. Shaji J, Lodha S. Response surface methodology for the optimization of celecoxib self microemulsifying drug delivery system. Indian J Pharm Sci 2008;70:585-90.
2. Wankhade VP, Tapar KK, Pande SD, Bobade NN. Design and evaluation of self-nanoemulsifying drug delivery systems for gliglazide, scholar research library. Der Pharmacia Lettre 2010;2:132-43.
3. Deshmukh A, Nakhat P, Yeole PG. Formulation and evaluation of self emulsifying drug delivery system for fursemide, scholar research library. Der Pharmacia Lettre 2010;2:94-106.
4. Nazzal S, Khan MA. Response surface methodology for optimization of ubiquinone self-nanoemulsified drug delivery system. AAPS Pharm Sci Tech 2002;3:E3.
5. Nazzal S, Smalyukh II, Lavrentovich OD, Khan MA. Preparation and *in vitro* characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiquinone: Mechanism and progress of emulsion formation. Int J Pharm 2002;235:247-65.
6. Holm R, Jensen IH, Sonnergaard J. Optimization of self-microemulsifying drug delivery systems using a D-optimal design and the desirability function. Drug Dev Ind Pharm 2006;32:1025-32.
7. Bodea A, Leucuta SE. Optimization of hydrophilic matrix tablets using a D-optimal design. Int J Pharm 1997;153:247-55.
8. Finsher JH. Particle size of drugs and its relationship to absorption and activity. J Pharm Sci 1968;57:1825-35.
9. Shivkumar HN, Patel PB, Desai BG, Ashok P, Arulmozhi S. Design and statistical optimization of gliclazide loaded liposphere using response surface methodology. Acta Pharm 2007;57:269-85.

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