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DESIGN AND EVALUATION OF SELF-NANOEMULSIFIED DRUG DELIVERY SYSTEM (SNEDDS) OF DOCETAXEL BY OPTIMIZING THE PARTICLE SIZE USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT:

*The aim of the current study was to design an self-nanoemulsified drug delivery system (SNEDDS) of docetaxel by optimizing the particle size using response surface methodology. SNEDDS were prepared using surfactant, co-surfactant and oils/co-solvents. A IV optimal design for 3 factors at 3-level each was employed systematically optimize particle size. The particle size was taken as dependent variable. Cremophore-EL, polysorbate-80 and ethanol were taken as independent variables. The counter plot and 3D plot were drawn and optimum formulation was selected by feasibility and grid searches. The polynomial mathematical model generated for response were found to be Y = +53.66 +22.01 * A +41.76 * B -1.31 * C +17.79 * A * B +1.06 * A * C -2.76 * B * C 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.*

Keywords: *Response surface, docetaxel, response surface methodology, SNEDDS*

INTRODUCTION

Approximately 35-40% of new chemical entities have poor aqueous solubility. The oral drug delivery of such drugs is frequently associated with low dissolution and 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 tract (GIT). Nanoemulsions are preferred drug delivery system because of their stability and possibility of easy oral administration to improve drug self-emulsification in the gut [1].

Self-nanoemulsifying system is isotropic mixture of oil, surfactant and 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 glycerides 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 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 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].

Docetaxel is a clinically well established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer. Docetaxel binds to microtubules reversibly with high affinity and has a maximum stoichiometry of 1 mole docetaxel per mole tubulin in microtubules. Docetaxel 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 docetaxel by formulation of SNEDDS, which may result in increase in solubility and dissolution. 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 Docetaxel- SNEDDS. Particle size is particularly important since release rates are greatly influenced by particle size.

MATERIALS AND METHODS:

Materials

Docetaxel (DTL) was gifted by aptuit laurus laboratories, India. polyethoxylated castor oil (Cremophor®EL),Polysorbate-80,PEG400 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 docetaxel self-nano-emulsifying formulation:

Accurately weighed 20 mg of docetaxel was mixed with Cremophore-EL. Then in the blend add ethanol and mixed on a cyclomixer to get a uniform mixture. And afterword the mixture was sonicated until the complete solubilization of the docetaxel into the mixture.

Table 1: Composition of SNEDDS mixture

Factors(%)	Low	High
Cremophore-EL	10	85
Polysorbate-80	10	85
Ethanol	5	20

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

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

Mixture	Cremophore-EL	Polysorbate-80	Ethanol	Particle size (nm)
1.	10.000	85.000	5.000	48.25
2.	62.630	10.000	27.370	19.23
3.	10.000	85.000	5.000	52.36
4.	62.630	10.000	27.370	27.52
5.	28.088	48.860	23.052	19.24
6.	10.000	48.205	41.795	25.26
7.	65.326	29.669	5.005	26.25
8.	40.000	10.000	50.000	18.25
9.	10.000	48.205	41.795	16.25
10	28.730	66.270	5.000	85.25
11.	85.000	10.000	5.000	12.25
12.	40.000	10.000	50.000	18.25
13.	45.840	49.160	5.000	65.85
14.	43.761	28.739	27.500	18.32
15.	10.000	66.226	23.774	62.51
16	85.000	10.000	5.000	16.25

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 (PDI) 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 8.0) is as follows:

$$Yi = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2$$

Where, Yi is the dependent variable, b0 is the intercept, b_1 to b33 are the regression coefficients and X_1, X_2 and X_3 are the 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 DISCUSSIONS

Construction of phase diagram :

The phase diagram of Cremophor EL,Polysorbate-80 and Ethanol 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, polysorbate-80 and ethanol system has larger nanoemulsification region. These compositions had ability to solubilize various hydrophobic drugs and have potential to become platform systems.

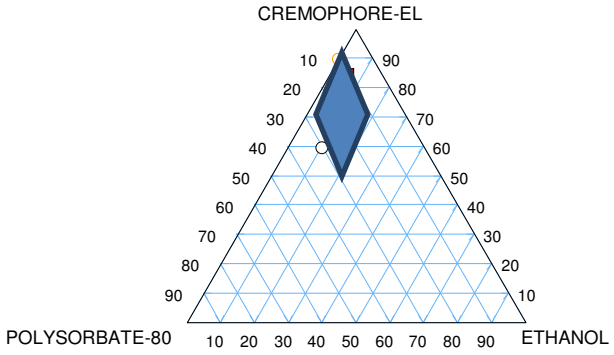

Figure 1: Ternary phase diagram of Cremophore-EL,Polysorbate-80 and Ethanol

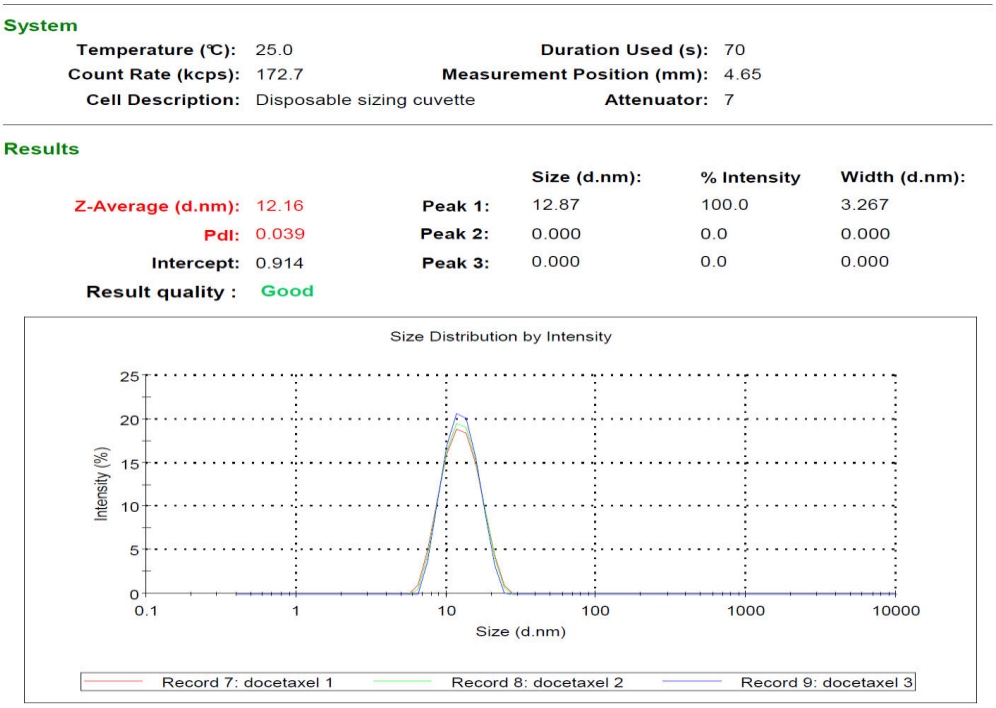

Figure-2 Droplet size upon 25 times dilution

Fitting of data to the model

Different Docetaxel SNEDDS were obtained based on the experimental design Table-2. Particle size of SNEDDS was selected as a response for optimization.

The model was fitted to the data for a response, the normalizes coefficients of the fitted model are related in Table-3. In normalized form the coefficient are divided by the standard deviation of their respective response.

Table 3: Analysis of variance for particle size of docetaxel SNEDDS

Source	Sum of Square	df	Mean Square	F Value	p-value Prob > F
Model	6050.43	6	1008.40	4.15	0.0236 significant
A-CREMOPHORE-EL	*542.81*	*1*	*542.81*	*2.23*	*0.1660*
B-POLYSORBATE-80	*2000.92*	*1*	*2000.92*	*8.23*	*0.0167*
C-ETHANOL	*11.05*	*1*	*11.05*	*0.045*	*0.8355*
AB479.54	*1*	*479.54*	*1.97*	*0.1904*	
AC7.92	*1*	*7.92*	*0.033*	*0.8604*	
BC68.33	*1*	*68.33*	*0.28*	*0.6075*	
A^2	*0.000*	*0*			
B^2	*0.000*	*0*			
C^2	*0.000*	*0*			
Residual	2430.81	10	243.08		
Lack of Fit	*2339.41*	*5*	*467.88*	*25.60*	*0.0014 significant*
Pure Error	*91.40*	*5*	*18.28*		
Cor Total	8481.24	16			

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:

GLOBULE SIZE(Y) =
+53.66 +22.01 * A +41.76 * B -1.31 * C +17.79 * A * B +1.06 * A * C -2.76 * B * C

Where, Y = Globule size, A = Quantity of cremophore , B = Quantity of polysorbate-80 C = Quantity of ethanol 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 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 infigure-2 Plots showed the points fairly close to straight lines indicating good model.

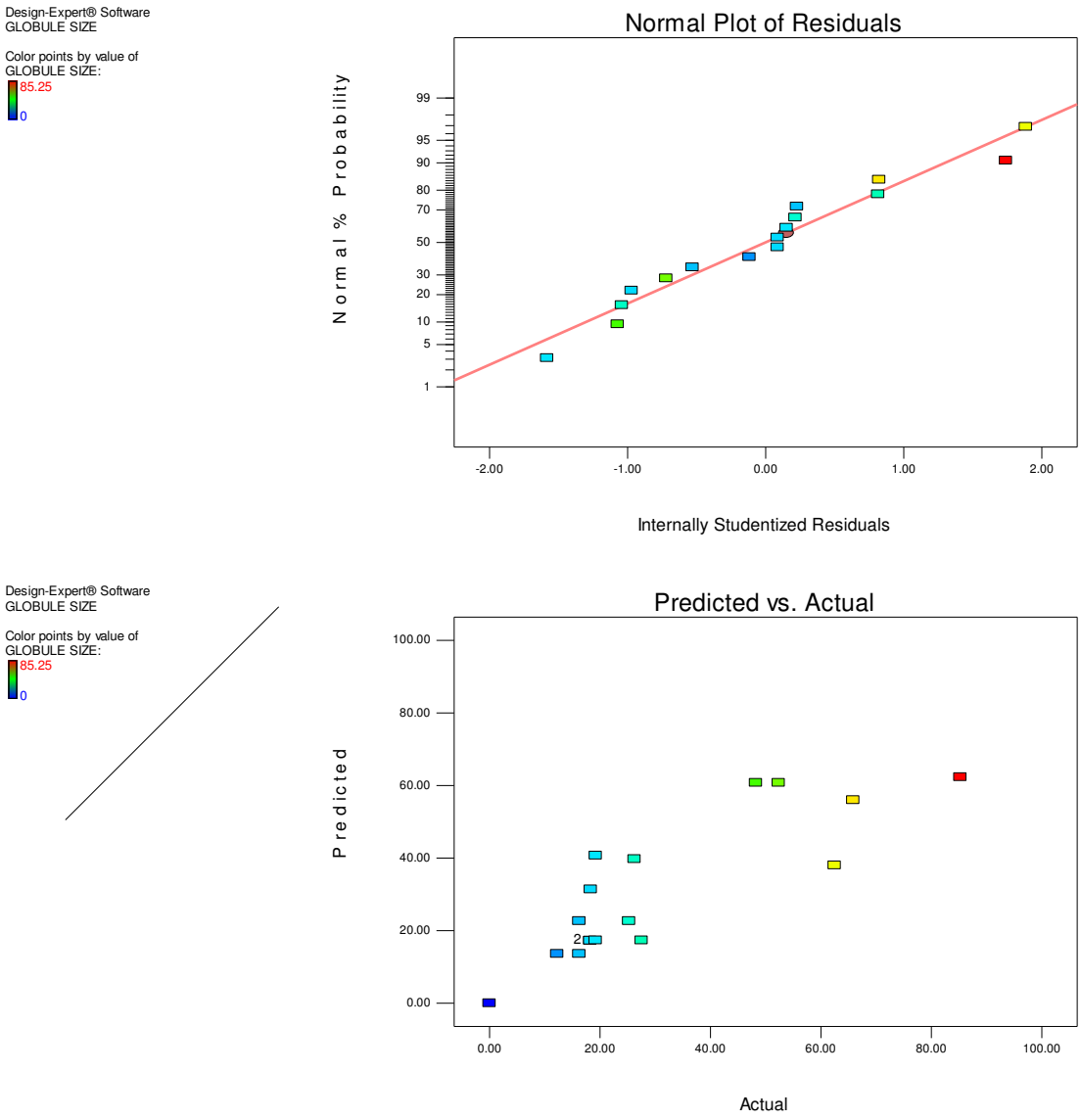

Figure 2: Normal residual plot and predicted plot

The model term for the particle size was found to be significant with high value of r^2 0.7134 which indicates the adequate fitting to a quadratic model. The model F-value of 4.15 implies the model is significant .

Also, The "Pred R-Squared" of 0.3026 is not as close to the "Adj R-Squared" of 0.5101.The relationship between the dependent variable and independent variables was elucidated using contour and response surface plots.

The resultant equations 1 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 . Figures-3 and 4 illustrate the corresponding response surface and counter plot of the model. It was found that, at high level of A (amount of Cremophore-EL 85%), Y increases the particle size, as amount of Polysorbate-80 decreases from 85 to 15%.

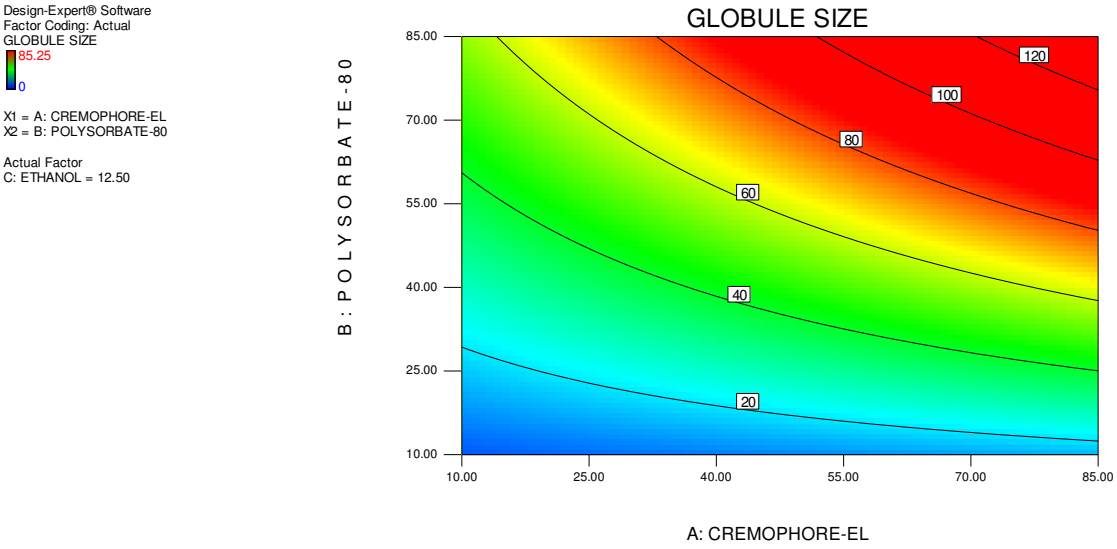

Figure 3: Counter plot for response particle size

The effect of A and B and their interaction on Y (Particle size) at a fixed level of C are given in Figure -3 and 4 illustrate the corresponding response surface and counter plot of the model.

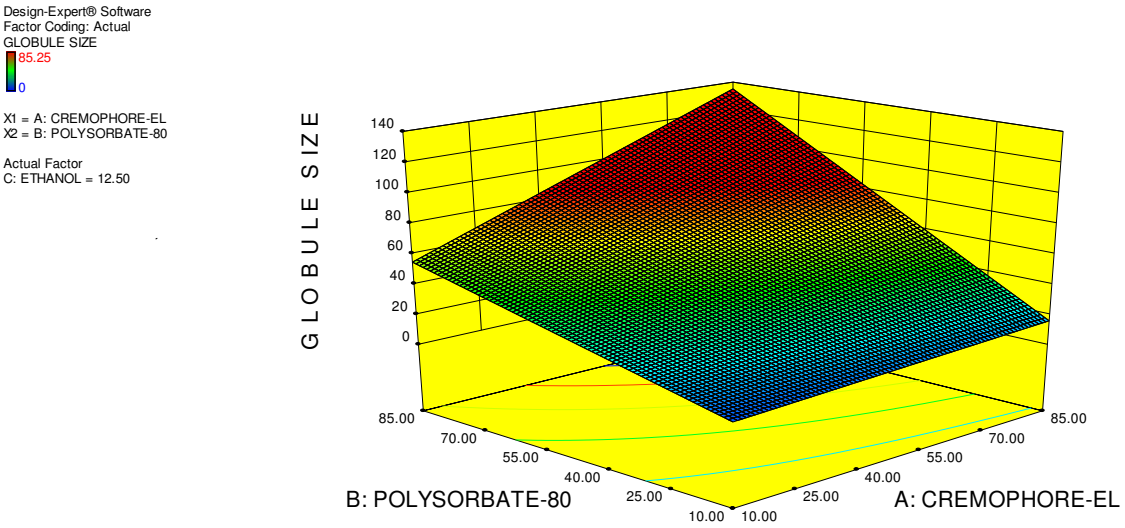

Figure 4: Response surface plot for particle size

The effective formulation obtained from the factorial design run no. 11 containing Cremophore-EL (85%), Polysorbate-80(10%) and Ethanol (5%) showed the possible result from the expected values of ANOVA. Therefore run no. 11 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

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

S.No	Weight Fraction of Excipient (%)			Droplet size	
Trials	X1	X2	X3	Predicted	Measured
1	85	10	5	13.61	12.25

.

Conclusion:

A method to obtain good experimental mixture designs when the experimental factor space is not a simplex, is to use 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 docetaxel was performed using 3 factors, 3 level design. The dependent variable used A-Cremophore-EL (85%), B-Polysorbate-80 (10%) and C-Ethanol (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 12.16 nm. The resultant formulation shows the effective results because of the concentration of surfactant present in the formulation having greater impact on the co-surfactant and co-solvents 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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