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DESIGN AND DEVELOPMENT OF CURCUMIN NANOEMULSION BY APPLYING CENTRE COMPOSITE ROTABLE DESIGN RESPONSE SURFACE MODEL (CCRD-RSM)

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ABSTRACT

Curcumin (CRM) is a polyphenolic compound and it is used for treatment of several inflammatory diseases, Alzheimer‟s, and brain cancer. CRM having major activity to inhibit vascular endothelial growth factor (VEGF) is responsible for angiogenesis. Curcumin (CRM) loaded Nanoemulsion (NE) was fabricated by Vortexing technique and Centre composite rotable design response surface model (CCRD-RSM) was employed; three independent factors such as oil (A), surfactant (B) and co-surfactant (C) concentration were used. Optimised nanoemulsion (NE) (F19) had drug content of CRM (X) was 99.98 ± 0.54 (%) and droplet size (Y) 34.98 ± 0.25 (nm) respectively. This design, the best models such as linear model can be selected due to the analysis of variance (ANOVA) F-value and P value < 0.05 which is considered to be statistically significant. Response surface model was used to determine interaction pattern of independent and dependent variables. All concentrations of independent variables shows significant effect on dependent variables.

KEYWORDS

Curcumin, CRM, Nanoemulsion, CCRD-RSM, ANOVA, Linear model, RSM and *in vitro* release.

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INTRODUCTON

Curcumin (CRM) is a polyphenolic compound it belongs to class of flavonoids glycosides. CRM, a polyphenol known as diferuloylmethane, has been extensively studied for its therapeutic efficacy for many disorders including several inflammatory diseases, Alzheimer‟s, and brain cancer. The extensive research on CRM has revealed several of its important functions. It interacts with various proteins, inhibits the activity of various kinases, and controls the activation of transcription factors that are involved in cell proliferation and survival. CRM-induced apoptosis through down-regulation

of the NF-κB and Akt pathways. Colloidal drug delivery system has revolutionized drug delivery, allowing for therapeutic agents to be selectively targeted on an organ. Nanoemulsion (o/w type) is preferred than other formulations as it has several advantages to provides a better chance for the lipophilic drug to be solubilised easily in oily phase as well as rapidly absorbed by allowing a more intimate and prolonged contact between the drug and the mucosal membrane[1-4]. Curcumin loaded nanoemulsion (CRM-NE) were formulated by Vortexing technique by using Centre composite rotable design response surface model (CCRD-RSM) design applying three factors: oil concentration (A), surfactant concentration (B) and co-surfactant concentration (C) by optimizing the formula by observing the effect on three variables: drug content of CRM (%) and droplet size (nm).Analysis of variance (ANOVA) F-value and P-value < 0.05 which is considered to be statistically significant. Polynomial Equation shows the relationship between independent variables and response variables such as drug content of CRM(X) and droplet size (Y) respectively. The aim of present investigation was to develop CRM-NE by applying CCRD-RSM methodology.

MATERIAL AND METHODS

Materials

Curcumin (CRM) was obtained from Sunpure Extracts Ltd. (Delhi, India), Oleic acid was obtained from Soofi Traders Ltd. (Mumbai, India), Tween 20 and Polyethylene glycol 200 (PEG 200) was obtained from Loba Chemie Ltd. (Mumbai, India).

Method

Selection of oil phase

Curcumin (CRM) in various oils was determined by adding an excess amount of drug in selected oils (oleic acid, olive oil, castor oil) in a stopper vials, and mixed using a vortex mixer. The mixture vials were then kept at 25 ± 1.0 °C in an isothermal shaker. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and concentration of drug in oils was determined[5].

Selection of surfactant

To evaluate the capability of oils and surfactants (Tween 20) to form an emulsion spontaneously, selected oil was continually added into surfactant solution (20%, w/w) with vigorous vortex. If a uniform clear solution was visually obtained, the addition of the oil will be continued until the solution became cloudy[5].

Selection of Co-surfactants

The surfactant/oil mixture was diluted by distilled water. The resultant solution was titrated with increasing amount of co surfactant (PEG 200) until the system turned clear and add more co-surfactant until the transparent solutions changed cloudy again[5].

Experimental Design

To design the nanoemulsion (NE) formulation, Preliminary experiments revealed that the independent variables like Oil concentration (A), Surfactant concentration (B), Co-surfactant concentration (C) during preparation, were the main factors that affected the dependent variables such as, Drug content of CRM and droplet size. Centre composite rotable design response surface model (CCRD–RSM) was applied to systemically investigate the influence of these three decisive independent variables on three dependent variables of the nanoemulsion. It was applied with two-level factorial design points, axial points and centre points. All independent, coded and actual values of the variables of CCRD-RSM are given in Table No.1. This design, the best models such as linear model can be selected due to the analysis of variance (ANOVA) F-value and P-value < 0.05 which is considered to be statistically significant. Three dimensional RSM plots was used to determine relationship between variables and responses. For optimisation, the desirability function of drug content was in the maximum level while that droplet size was in the minimum level[6-7].

Preparation of Nanoemulsion (NE)

Nanoemulsion (NE) was prepared by using vortexing method in which drug (CRM) in oil phase was added into aqueous phase under vortexing for 30 min. to from O/W-CRM-NE (oil phase: Oleic acid and aqueous phase: tween 20 and PEG 200).

Drug Content

CRM from NE was extracted by dissolving 1 ml of NE in methanol. CRM content in the Methanolic extract was analyzed spectrophotometrically (UV 1700, Shimadzu, Japan) at 423 nm[8-9].

Droplet size analysis

Droplet size of prepared NE were determined by Zetasizer ZS 90, (Malvern Instruments Ltd., UK). The formulation was diluted with double distilled water and light scattering was monitored at 25˚C at a 90˚c angle[10-12].

RESULTS AND DISCUSSION

Selection of oil, surfactant and Co-surfactants phase

The solubility of CRM was found to be highest in Oleic acid as compared to other oils thus, oleic acid was selected as an oil phase for the development of NE formulation. Tween 20 solubilized the maximum amount of oleic acid, it was chosen as the surfactant for the NE development. Polyethylene glycol 200 (PEG 200) were selected as co surfactant which shows greater ability to reduce interfacial tension to form a good NE.

Experimental Design

Responses observed for twenty formulations prepared were fitted to various models using Design-Expert® software 7.0.1. CCRD-RSM methodology offers to investigate a high number of variables at different levels with limited number of experiments. To fit the data, a linear second-order polynomial model was chosen as the best model. Polynomial Equation shows the relationship between independent variables and response variables such as drug content of CRM (X) and droplet size (Y) respectively. All values of R^2, SD and % coefficient of variation and ANOVA are depicted in Table No.2.

$X= +99.98 +1.79*A + 0.79*B + 0.28*C.$

$Y=+34.98 + 0.092*A + 0.61*B + 0.51*C.$

Where, X=Drug content of CRM (%), Y =Droplet Size (nm), A = Oil concentration (mL), B= Surfactant concentration (mL) and C = Co-surfactant concentration (mL).

Response surface plots (RSP)

Response surface plots was important three dimensional surface curves for studying the interaction patterns. Three dimensional response surface plots generated at different levels by the Design-Expert® software. RSP showing effect of concentration oil (A) and surfactant (B) concentration on drug content of CRM (Figure No.1) and RSP showing effect of concentration surfactant (B) and co-surfactant (C) concentration on drug content of CRM (Figure No.2). RSP showing effect of concentration co-surfactant (C) and oil (A) concentration on drug content of CRM (Figure No.3). RSP showing effect of concentration oil (A) and surfactant (B) concentration on droplet size (Figure No.4) and RSP showing effect of concentration surfactant (B) and co-surfactant (C) concentration on droplet size (Figure No.5).RSP showing effect of concentration co-surfactant (C) and oil (A) concentration on droplet size (Figure No.6).

Drug content (%) of CRM and Droplet size (nm)

Drug content of CRM (%) and Droplet size (nm) are reported in Table No.1. drug content CRM in all twenty formulations were found to be in the range of 94.33 % to 99.98 % and droplet sizes of the all twenty formulations were found to be in the range of 34.98 nm to 39.23 nm respectively.

Effect of oil and surfactant concentration on the drug content of CRM (P)

The drug content was found to increases with increase in the oil concentration (directly proportional) and the drug content was found to decreases with decreasing in the oil concentration. The drug content was found to decrease with increase in the surfactant concentration (inversely proportional) and the drug content was found to increases with decreasing in the surfactant concentration. Concentration of oil and surfactant shows significant effect on drug content (Figure No.7 (P)).

Effect of surfactant and co-surfactant concentration on the drug content of CRM (Q)

The drug content was found to decrease with increase in the surfactant and co-surfactant concentration (inversely proportional) and the drug content was found to increases with decreasing surfactant and co-surfactant concentration. Concentration of surfactant and co-surfactant shows significant effect on drug content (Figure No.7 (Q)).

Effect of co-surfactant and oil concentration on the drug content (R)

The drug content was found to increases with increase in the oil concentration and drug content was found to decreases with increasing in the co-surfactant concentration. Concentration of co-surfactant and oil shows significant effect on drug content (Figure No.7 (R)).

Effect of oil and surfactant concentration on the droplet size (I)

The droplet size was found to decrease with increase in the oil concentration (inversely proportional) and the droplet size was found to increases with decreasing in the oil concentration (inversely proportional). The droplet size was found to decrease with increase in the surfactant concentration (inversely proportional) and the droplet size was found to increases with increasing in the surfactant concentration (directly proportional). Concentration of oil and surfactant shows significant effect on droplet size (Figure No.8 (I)).

Effect of surfactant and co-surfactant concentration on the droplet size (J)

The droplet size was found to decrease with increase in the surfactant and co-surfactant concentration (inversely proportional) and the droplet size was found to increases with increase in the surfactant and co-surfactant concentration (directly proportional). A higher surfactant and co-surfactant concentration reduces the surface as well as interfacial tension and facilitates partitioning during homogenisation. Decreasing droplet size is accompanied by a tremendous increase in the surface area. Droplet size of CRM loaded NE was decreases increases rate of extent of drug release. Concentration of surfactant and co-surfactant shows significant effect on droplet size (Figure No.8 (J)).

Effect of co-surfactant and oil concentration on the droplet size (K)

The droplet size was found to decreases with increase in the oil concentration and the droplet size was found to decreases with increasing in the co-surfactant concentration. Concentration of co-surfactant and oil shows significant effect on droplet size (Figure No.8 (K)).

Drug Content

Drug content of optimized batch (F19) was found to 99.98 ± 0.54 (%) for CRM (mean ± SD, n=3).

Droplet size analysis

The information on droplet size is particularly important for understanding the behaviour of NE. Droplet size of the prepared NE was determined and the results are shown in (Figure No.9) along with the polydispersity indices. A decrease in droplet size from formulation F19 were observed with the simultaneous increase in the surfactant and co-surfactant proportion, this is probably explained by stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil-water interface. From batch F19, droplet size increases which could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the high surfactant concentration and leading to ejection of oil droplets into aqueous phase. A varying polydispersity values shows that good droplet distribution had occurred in some batches while in some droplet distribution i.e. PDI > 0.5 which is quiet not suitable for NE. Moreover, it can be seen that formulation F19 had required. The formulation F19 was considered to be optimized as the droplet size (34.98 ± 0.25 nm) (mean ± SD, n=3) which is good in comparison to other formulations. The polydispersity index of formulation F19 is lowest having a value of 0.213 ± 0.04 (mean ± SD, n=3). Since the diameter of the dispersed oil droplets of the optimised NE (F19) was much smaller than the 50 nm, such droplet are considered to be suitable for targeting. The droplet size of the NE is important factor as this determines the rate and extent of drug release as well as absorption.

Table No.1: Independent variables along with their code, levels and respective droplet size (nm), drug content of CRM (%) of different batches of CRM loaded NE (n = 3). These results are mean ± standard deviation

S.No	Batch	Oil (A) in mL	Surfactant (B) in mL	Co-surfactant (C) in mL	Drug content (%) X	Droplet size (nm) Y
1	F1	10	15	6.636414339	99.22	34.98
2	F2	15	20	12	99.26	35.55
3	F3	10	15	10	99.89	34.98
4	F4	15	10	8	97.22	35.22
5	F5	10	15	10	99.89	34.98
6	F6	10	15	10	99.89	34.98
7	F7	10	15	10	99.89	34.98
8	F8	5	20	8	98.55	35.25
9	F9	5	10	8	96.22	36.33
10	F10	5	20	12	98.74	34.99
11	F11	15	10	12	94.33	39.23
12	F12	10	23.4089642	10	99.78	34.98
13	F13	10	15	10	99.89	34.98
14	F14	18.40896	15	10	94.88	34.98
15	F15	15	20	8	97.52	34.98
16	F16	10	15	13.36358566	96.47	36.33
17	F17	10	6.59103585	10	96.25	35.88
18	F18	1.591036	15	10	99.41	35.21
19	F19 (op)	10	15	10	99.98	34.98
20	F20	5	10	12	98.03	36.77

A =oil concentration in mL (high level-15, low level-10); B = surfactant concentration in mL (high level-20, low level-10); C =co-surfactant concentration in mL (high level-12, low level-8).

Table No.2: Summary of results of regression analysis for responses X and Y and analysis of variance for drug content of CRM (X) and droplet size (Y)

S.No	Parameter	DF	SS	MS	F	P value	R^2	SD	%CV
Drug content of CRM (X)									
1	Model	3	24.41	8.14	3.46	**0.0415 Significant**	0.9983	1.53	1.56
2	Residual	16	37.66	2.35	-	-	-		
3	Total	19	62.07	-	-	-	-		
Droplet Size (Y)									
4	Model	3	8.77	2.92	3.22	**0.0249 Significant**	0.9835	0.85	1.32
5	Residual	16	11.45	0.72	-	-	-		
6	Total	19	20.22	-	-	-			

DF, degrees of freedom; SS, sum of square; MS, mean sum of square; F, Fischer's ratio, pvalue, Probability value; SD, standard deviation; %CV, Coefficient of variation.

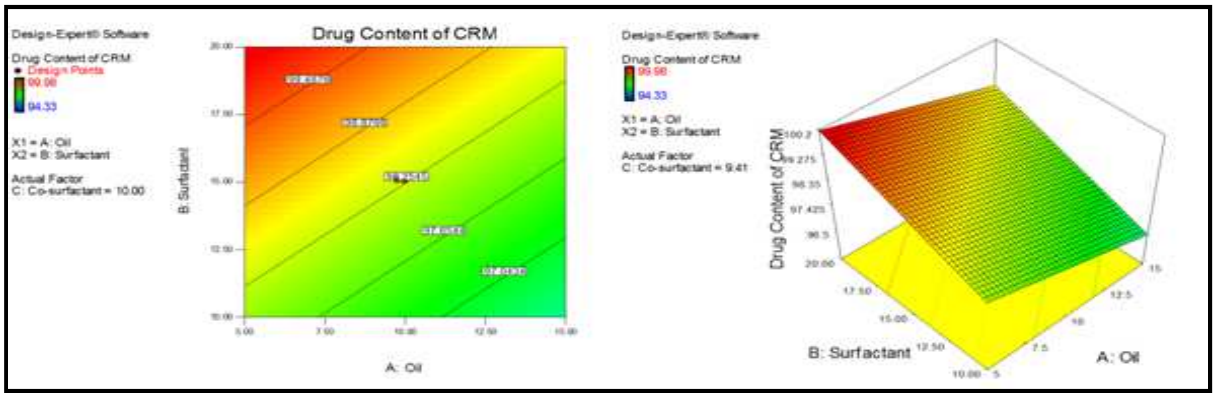

Figure No.1: RSP showing effect of concentration oil (A) and surfactant (B) concentration on drug content of CRM

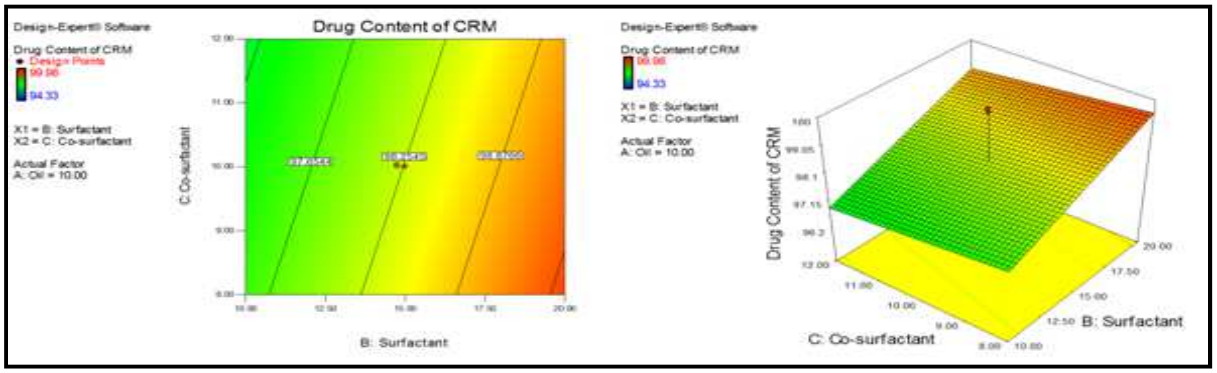

Figure No.2: RSP showing effect of concentration surfactant (B) and co-surfactant (C) concentration on drug content of CRM

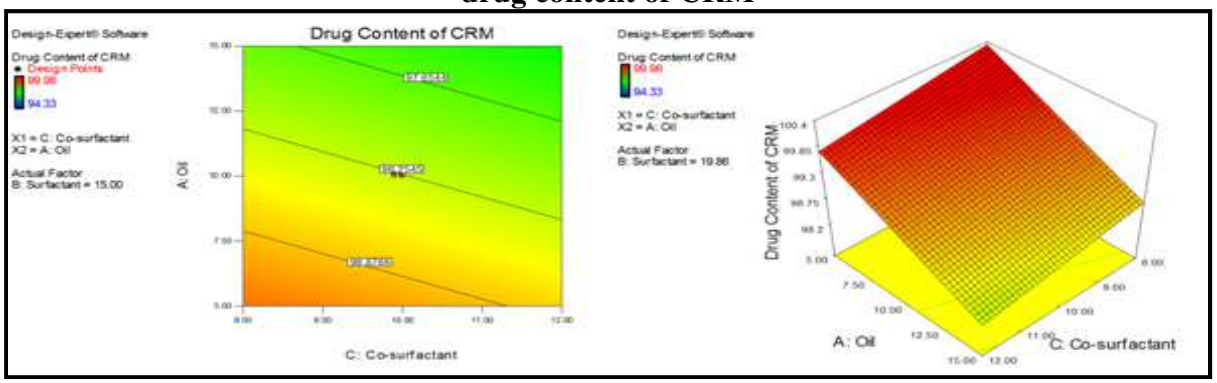

Figure No.3: RSP showing effect of concentration co-surfactant (C) and oil (A) concentration on drug content of CRM

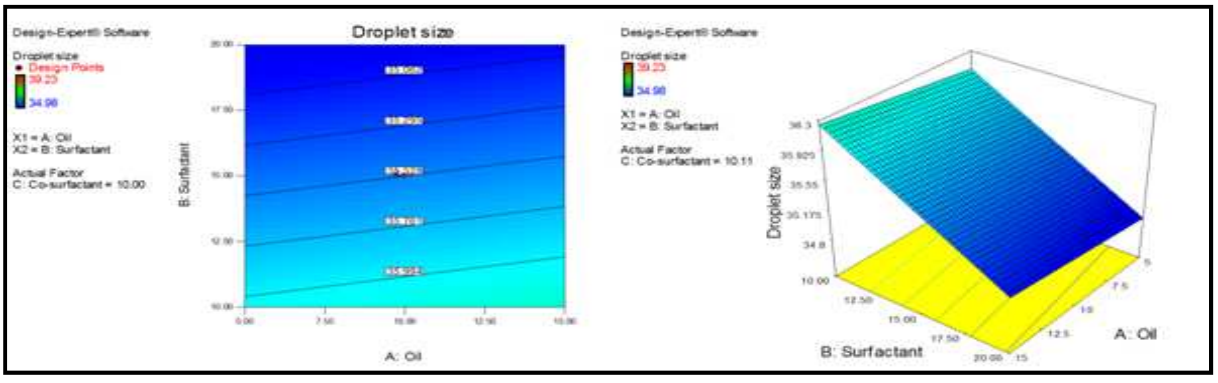

Figure No.4: RSP showing effect of concentration oil (A) and surfactant (B) concentration on droplet size

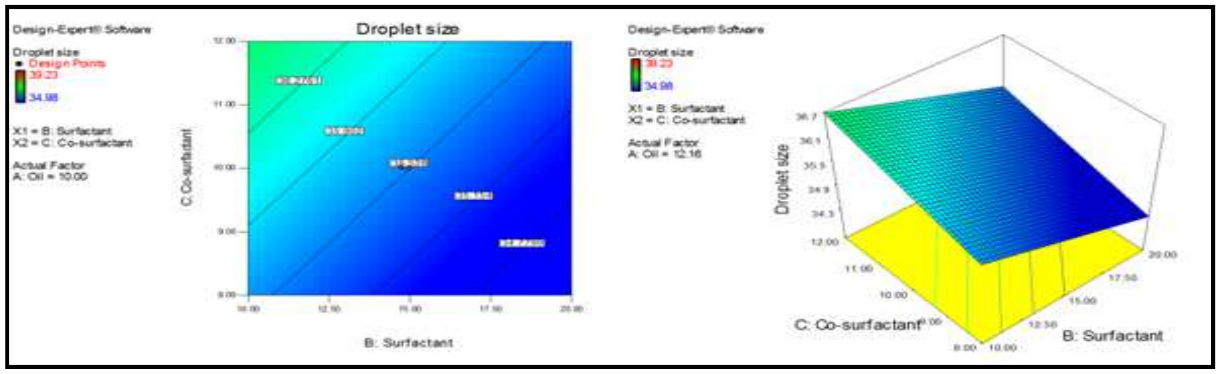

Figure No.5: RSP showing effect of concentration surfactant (B) and co-surfactant (C) concentration on droplet size

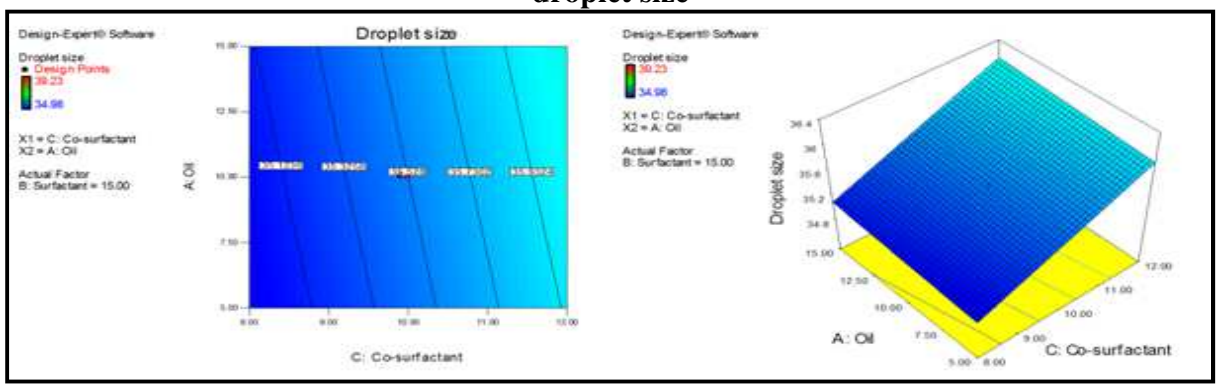

Figure No.6: RSP showing effect of concentration co-surfactant (C) and oil (A) concentration on droplet size

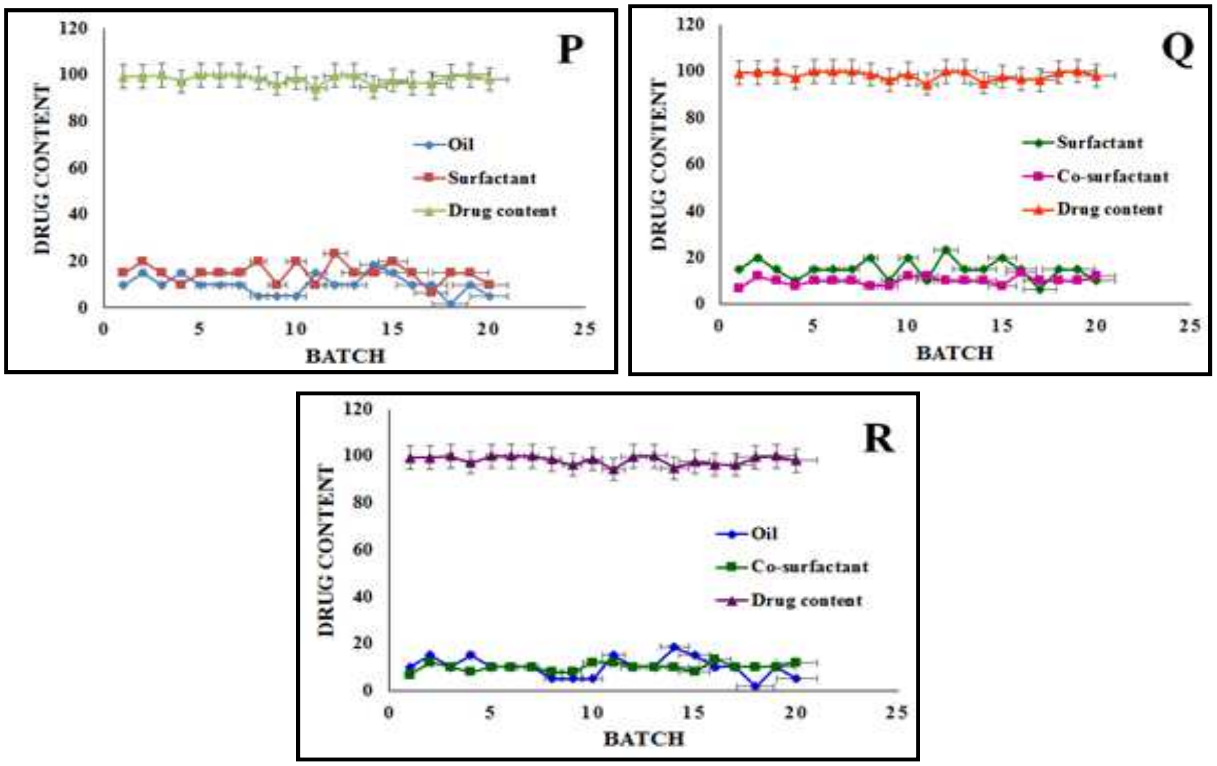

Figure No.7: Effect of oil and surfactant concentration on the drug content of CRM (P), Effect of surfactant and co-surfactant concentration on the drug content of CRM (Q), Effect of co-surfactant and oil concentration on the drug content (R)

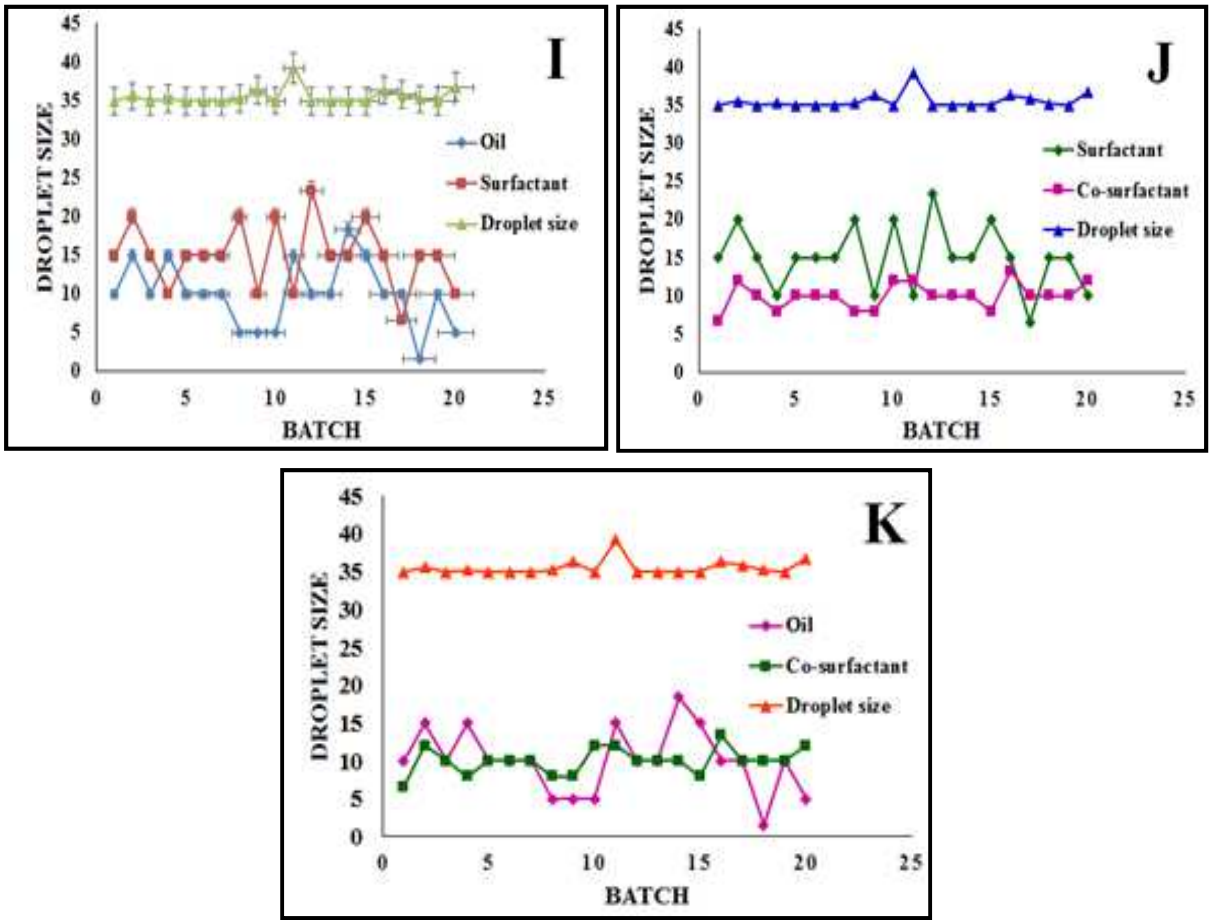

Figure No.8: Effect of oil and surfactant concentration on the droplet size (I), Effect of surfactant and co-surfactant concentration on the droplet size (J), Effect of co-surfactant and oil concentration on the droplet size (K)

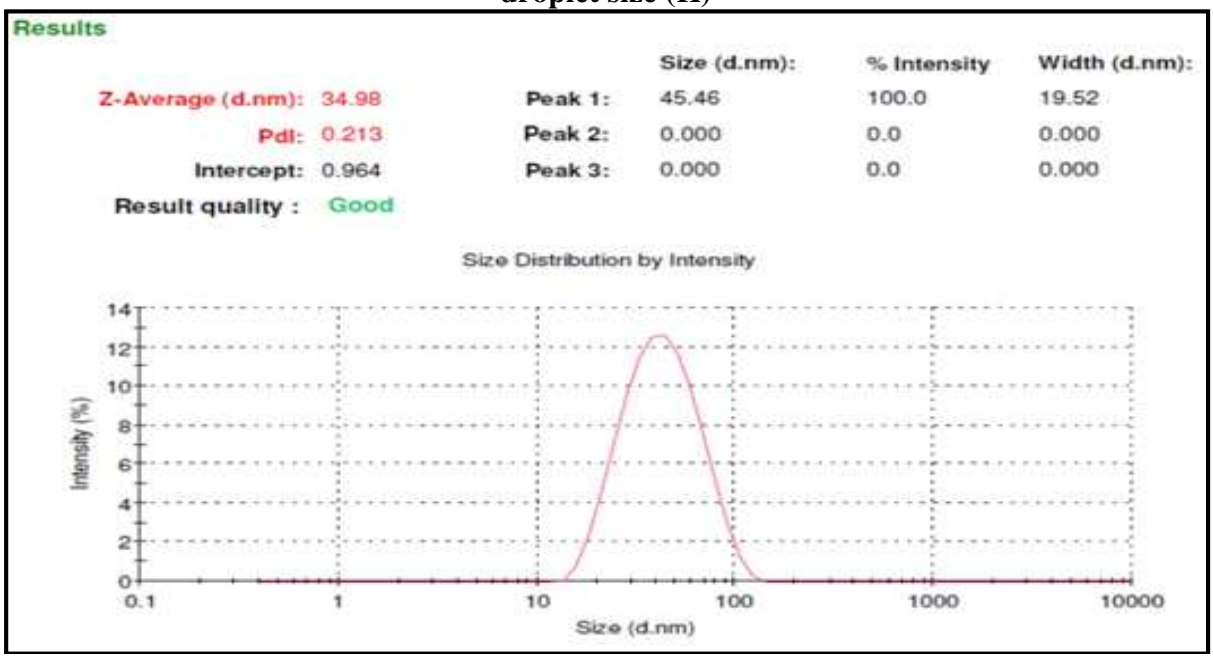

Figure No.9: Droplet size graph of optimized nanoemulsion (NE) (F19)

CONCLUSION

CRM is successfully incorporated into NE by Vortexing method with high drug content due to its high lipophilicity. The CRM loaded NE were formulated using central composite rotable design response surface methodology (CCRD-RSM) by fitting linear model to the response data. The result of present investigation shows that the independent variable shows significant effect on that dependent variable having maximum drug content of CRM loaded NE and minimum droplet size.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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