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Article

Application of Plackett-Burman Factorial Design in The Development of Curcumin Loaded Eudragit E 100 Nanoparticles

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Abstract

The present study was aimed to fabricate Curcumin loaded Eudragit E 100 polymeric nanoparticles and to study the effect of various manufacturing parameters on the average particle size, span, uniformity and surface area of the prepared polymeric nanoparticles by utilizing Plackett-Burman experimental designs. Curcumin loaded Eudragit E 100 nanoparticles were prepared by nanoprecipitation method and characterized using particle size analyser. Plackett-Burman design was implemented to study the influence of eight independent variables on three dependent variables. Twelve experimental trials involving 8 independent variables at higher and lower levels were generated by Design-Expert. Out of 12 trials, 4th and 9th trails were within the acceptable limits. Least average particle size can be obtained by increasing the concentration of poloxamer 188, increasing the volume of aqueous phase, increasing the sonication duration and decreasing the ethanol concentration. Similarly, span less than 1 can be obtained by increasing the concentration of poloxamer 188, increasing the sonication duration and decreasing the ethanol concentration. However, uniformity can be increased decreasing the ethanol concentration. Higher surface area can be obtained by increasing the concentration of Eudragit E 100, poloxamer 188 and increasing the volume of the aqueous phase.

Keywords: Curcumin, Eudragit E 100, Nanoprecipitation, Plackett-Burman Design, Polymeric Nanoparticles

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1. Introduction

Curcumin (an hydrophobic polyphenol isolated from powdered rhizome of turmeric) has been studied extensively and found to have wide range of pharmacological activities including anti-oxidant, anti-inflammatory and anti-bacterial properties and has a significant therapeutic potential in various ailments including acne, allergy, arthritis, atherosclerosis, diabetes mellitus, fever, gastric ulcer, inflammatory bowel disease, osteoporosis, psoriasis and wound. However, poor aqueous solubility of curcumin limits its clinical usefulness [1-5].

Various approaches have been implemented to enhance the aqueous solubility of hydrophobic drugs, which includes co-crystallisation, solid solution, co-solvency, cryogenic technique, eutectic mixture, floating granule, hydrotrophy, inclusion complex, miceller solubilization, microemulsion, micronization, pH adjustment, polymeric nanoparticle, self emulsifying drug delivery system, solid dispersion, solid lipid nanoparticle, sonocrystalisation, super critical fluid process, microsphere, microcapsule and liposome [6-9].

Of all approaches, we have preferred polymeric nanoparticle as it possess some significant advantages over other approaches which includes (a) Significant size reduction leading to improvement in the solubility and reactivity of the sized reduced drug towards its specific targets, (b) Provides stability to the encapsulated drug, (c) Choice of various route of administration, (d) Reduce the side effects of the encapsulated drug, (e) Ability to target the drug to the specific site and (f) Capacity to incorporate multiple drugs in a single polymeric matrix [10-14].

However, the selection of polymer for the preparation of polymeric nanoparticles mainly depends on the nature of the drug that needs to be encapsulated and release pattern of the polymer. We have preferred Eudragit E 100, as it is a cationic hydrophobic polymer expected to offer high zeta potential to the nanoparticles and expected to release Curcumin in gastric pH, where it is stable [15].

Polymeric nanoparticles can be prepared by at least eight techniques, which include solvent evaporation, salting out, nanoprecipitation, nano spray drying, desolva-

tion, supercritical fluid technology, ionic gelation and dialysis. However, we have preferred nanoprecipitation, as it is the most convenient and economical technique to fabricate polymeric nanoparticles [16-24].

Though, it is a simple technique, many process and formulation parameters influence the quality of the prepared polymeric nanoparticles. So we intend to implement Plackett-Burman experimental design, which is a two-level factorial design. In Plackett-Burman experimental design, the effect of each variable was determined by the following equation $E_{xi} = 2(\Sigma H_{xi} - \Sigma L_{xi})/N$, where Exi is the concentration effect of particular variable, Hxi is the response at the higher level, Lxi is the response at the lower level and N is the total number of trials. Positive sign in the model for a response indicates an effect that favours and negative sign indicates an inverse relationship between responses. The linear equation of Plackett-Burman experimental design is expressed as $R = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \ldots + b_nX_n$, where R is the response, b_0 is the constant and b_1, $b_2 \ldots b_n$ are the coefficients of the variables X_1, $X_2 \ldots X_n$ [25-29].

The present study was aimed to fabricate Curcumin loaded Eudragit E 100 polymeric nanoparticles and to study the influence of various manufacturing parameters on the average particle size, span, uniformity and surface area of the prepared polymeric nanoparticles by utilizing Plackett-Burman experimental designs.

2. Experimental section

2.1 Material

Curcumin and β-cyclodextrin were purchased from Himedia Laboratories (India). Poloxamer 188 was purchased from Sigma Aldrich (India). Eudragit E 100 was obtained from Degussa (India). Analytical grade ethanol was purchased from Brampton (Canada).

2.2 Fabrication of Curcumin Loaded Eudragit E 100 Nanoparticles

Curcumin loaded Eudragit E 100 nanoparticles were prepared by nanoprecipitation method with slight modification. Briefly, a specified quantity of Curcumin and Eudragit E 100 (cationic polymer) were dissolved in specified volume of ethanol and sonicated for 5 minutes to ensure complete dissolution. Prepared organic phase was then emulsified with specified volume of aqueous phase containing poloxamer 188 (non-ionic surfactant) and β-cyclodextrin (stabilizer) under sonication (Lark, India) at 40 kHz for specified duration. Polymeric nanoparticles were formed spontaneously and centrifuged (Remi, India) at 19,000 rpm for about 45 minutes at −20°C. Curcumin loaded Eudragit E 100 nanoparticles were separated, washed and re-suspended in distilled water.

The average particle size, span, uniformity and surface area of the prepared polymeric nanoparticles were measured based on laser light scattering principle using Mastersizer (Malvern, UK). Briefly, prepared Curcumin loaded Eudragit E 100 nanoparticle formulation was added drop-wise in to the water maintained in the sample dispersion unit of particle size analyser, where the nanoparticles scattered using single shaft pump and stirrer and re-circulated continuously around the measurement zone of the particle size analyser. The surface morphology of the optimized trial was determined by transmission electron microscopy (Hitachi H7500) at 20,000 magnifications.

Table 1 Scheme for the fabrication of Curcumin loaded Eudragit E 100 nanoparticles using Plackett-Burman design.

Trails	A (mg)	B (mg)	C (mg)	D (ml)	E (%)	F (ml)	G (ml)	H min)
1	250	50	50	10	100	15	250	30
2	350	50	50	10	60	15	250	60
3	250	50	50	15	100	20	250	60
4	350	250	50	15	60	20	100	60
5	350	250	50	10	100	20	100	30
6	350	250	250	10	100	15	250	60
7	350	50	250	15	100	20	250	30
8	350	50	250	15	60	15	100	30
9	250	50	250	10	60	20	100	60
10	250	250	50	15	60	15	250	30
11	250	250	250	15	100	15	100	60
12	250	250	250	10	60	20	250	30

A: Concentration of Eudragit E 100;
B: Concentration of poloxamer 188;
C: Concentration of beta cyclodextrin;
D: Volume of organic phase;
E: Percentage of ethanol;
F: Volume of aqueous phase;
G: Volume of beaker used for sonication;
H: Sonication duration.

Plackett-Burman design was implemented to study the influence of independent variables such as concentration of Eudragit E 100 (A), concentration of poloxamer 188 (B), concentration of β-cyclodextrin (C), volume of organic phase (D), percentage of ethanol (E), volume of aqueous phase (F), volume of beaker used for sonication (G) and sonication duration (H) on the dependent variables such as average particle size (R1), span (R2), uniformity (R3) and surface area (R4) of the prepared polymeric nanoparticles. Twelve experimental trials (Table 1) involving 8 independent variables and 3 dummy variables at higher and lower levels were generated using Design-Expert® (Version 7.1.5; Stat-Ease, Inc. USA).

3. Results and Discussion

3.1 Fabrication of Curcumin loaded Eudragit E 100 nanoparticles

During nanoprecipitation method, addition of organic phase in to the aqueous phase leads to rapid miscibility of ethanol with water resulting in spontaneous growth of nanoparticles, which was initially controlled by sonication followed by adsorption of Eudragit E 100, which act as the barrier and inhibit the further growth of nanoparticles. Through out the experiment, concentration of Curcumin was maintained at 12.5 mg as this concentration produced

a minimum average particle size in the initial trials. The higher and lower levels of independent variables were selected based on previous studies.

Average particle size, surface area and span determine the performance including solubility, dissolution, stability, circulation half-life, cellular uptake, drug release and bio-distribution. Hence, average particle size less than 200 nm, span less than 1 and surface area above 50 $m^2\ g^{-1}$ are required for maximum performance of the prepared polymeric nanoparticles. Similarly, particle size uniformity determines the consistency of performance of the prepared polymeric nanoparticles. Uniformity between 0.1-0.25 indicates narrow distribution and value above 0.5 indicates a broad distribution. Particles with broad distribution leads to difficulty in establishing conclusions about which sized particles are responsible for the biological effects of the prepared polymeric nanoparticles [30,31]. Curcumin loaded Eudragit E 100 nanoparticles were prepared by nanoprecipitation method as per the scheme and the observed responses of Plackett-Burman design are listed in table 2.

Table 2: Observed responses of Plackett-Burman screening design.

Trails	Average particle size (R1) (nm)	Span (R2)	Uniformity (R3)	Surface area (R4)($m^2\ g^{-1}$)
1	680	2.191	0.680	15.0
2	170	1.572	0.562	51.0
3	1218	2.801	1.360	14.5
4	122	0.841	0.260	54.1
5	294	2.112	0.678	32.6
6	408	1.951	0.408	22.0
7	641	2.514	0.781	17.9
8	134	1.057	0.329	52.0
9	126	0.837	0.262	52.6
10	125	0.986	0.306	54.3
11	1128	2.457	0.951	15.8
12	133	1.057	0.329	52.2
Acceptance Criteria	< 200	< 1.0	< 0.3	> 50

Out of 12 trails, 4th and 9th trail were within the acceptable limits. Fourth trail has produced a least average particle size of 122 nm with a span of 0.841, uniformity of 0.26 and surface area of 54.1 $m^2\ g^{-1}$ (Fig. 1) whereas, ninth trial has produced an average particle size of 126 nm with a span of 0.837, uniformity of 0.262 and surface area of 52.6 $m^2\ g^{-1}$ (Fig. 2). Hence, the 4th trial was considered as an optimized formulation for which transmission electron microscopy was taken at 20,000 magnifications (Fig. 3).

3.2 Influence of independent variables on the average particle size

Except β-cyclodextrin, all other independent variables significantly (P< 0.005) influence the average particle size (Table 3, Fig. 4). The linear model explaining the effects of various variables on the average particle size is given as [Average size = 428.08 − 133.24*A − 59.74*B + 133.26*D − 293.08*E − 5.74*F + 21.04*G + 100.59*H]. Moreover, observed values correlate well with predicted value (Table 4) and analysis of varaince showed a significant effect of independent variables (Prob > F, 0.0017) on the average particle size (Table 5). Variables such as concentration of Eudragit E 100, concentration of

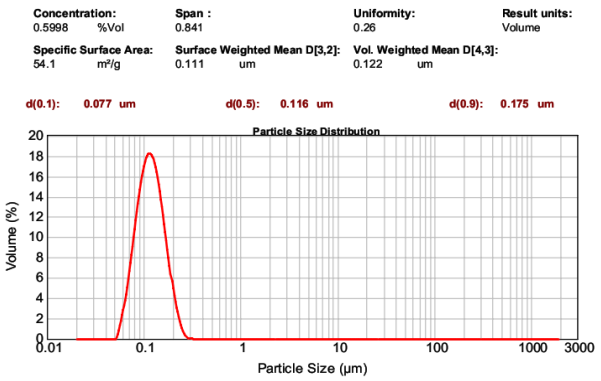

Fig. 1 Particle size distribution of Curcumin loaded Eudragit E 100 nanoparticles prepared by nanoprecipitation method with 12.5 mg of Curcumin, 350 mg of Eudragit E100, 250 mg of Poloxamer 188, 50 mg of Beta cyclodextrin, 15 ml of 60% ethanol as organic phase, 20 ml aqueous phase and 60 minutes sonication duration

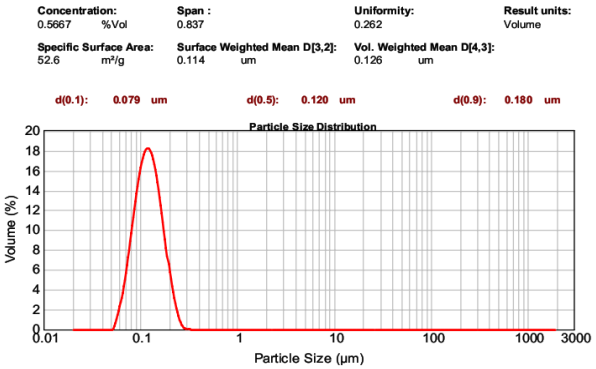

Fig. 2 Particle size distribution of Curcumin loaded Eudragit E 100 nanoparticles prepared by nanoprecipitation method with 12.5 mg of Curcumin, 250 mg of Eudragit E100, 50 mg of Poloxamer 188, 250 mg of Beta cyclodextrin, 10 ml of 60% ethanol as organic phase, 20 ml aqueous phase and 60 minutes sonication duration

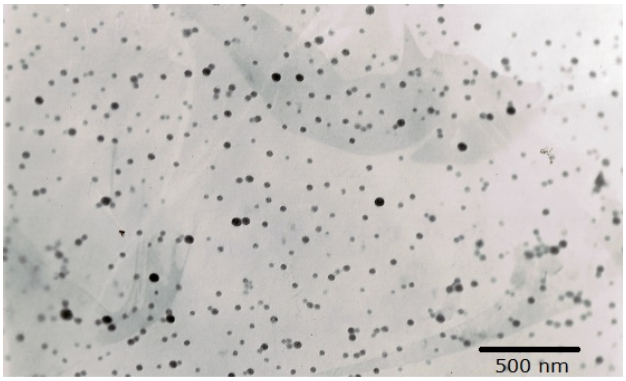

Fig. 3 Transmission electron microscopy of curcumin loaded Eudragit E 100 nanoparticles (4th trial) at 20,000 magnifications.

poloxamer 188, percentage of ethanol, volume of aqueous phase has inverse relationship with the average particle size whereas, variables such as volume of organic phase, volume of the beaker and sonication time has favourable effect on the average particle size (Fig. 4).

Table 3 Statistical analysis of average particle size, span, uniformity and surface area.

Variables	Average particle size		Span		Uniformity		Surface area	
	C[a]	P[b]	C[a]	P[b]	C[a]	P[b]	C[a]	P[b]
b_0	428.077	0.0017	1.675	0.0014	0.576	0.0072	36.337	0.0019
A	-133.24	0.0013	-0.0004	0.0500	-0.0725	0.1551	1.929	0.0044
B	-59.744	0.0028	-0.108	0.0024	-0.0868	0.1232	2.163	0.0039
C	0.2664	0.0500	-0.0294	0.0087	-0.0655	0.1547	-0.921	0.0092
D	133.256	0.0013	0.101	0.0025	0.089	0.1481	-1.571	0.0054
E	-293.08	0.0006	-0.617	0.0004	-0.234	0.0072	16.363	0.0005
F	-5.744	0.0295	0.0189	0.0135	0.0454	0.1327	0.9795	0.0087
G	21.039	0.0093	0.1387	0.0021	0.0454	0.2697	-1.023	0.0096
H	100.590	0.0017	0.0683	0.0037	0.0583	0.1476	-1.337	0.0063

[a] :Coefficient
[b] :P value

Table 4 Observed (O) and Predicted (P) value of average particle size, span, uniformity and surface area

Trails	Average particle size		Span		Uniformity		Surface area	
	O	P	O	P	O	P	O	P
1	680	680.30	2.191	2.191	0.68	0.810	15	15.015
2	170	170.21	1.572	1.572	0.562	0.341	51	50.984
3	1218	1218.21	2.801	2.800	1.36	0.810	14.5	14.484
4	122	122.38	0.841	0.841	0.26	0.341	54.1	54.115
5	294	294.38	2.112	2.112	0.678	0.810	32.6	32.590
6	408	407.69	1.951	1.951	0.408	0.810	22	22.010
7	641	640.69	2.514	2.514	0.781	0.810	17.9	17.910
8	134	133.80	1.057	1.057	0.329	0.341	52	51.910
9	126	125.80	0.837	0.837	0.262	0.341	52.6	52.615
10	125	125.21	0.986	0.986	0.306	0.341	54.3	54.310
11	1128	1127.80	2.457	2.457	0.951	0.810	15.8	15.790
12	133	132.70	1.057	1.057	0.329	0.341	52.2	52.185

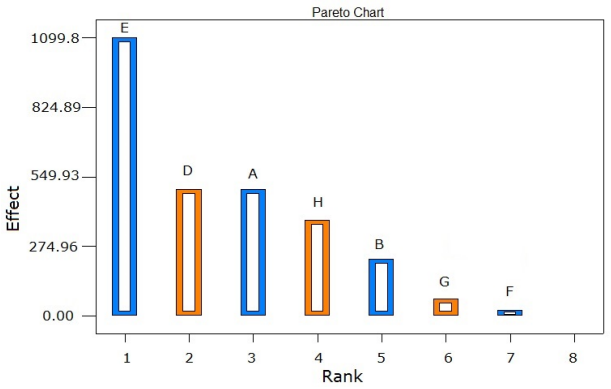

Fig. 4 Plackett-Burman Pareto-Plot for the average particle size using eight independent parameters. Blue colour column indicates the parameter has negative effects and orange colour with positive effects on the average particle size. The white column inside both blue and orange columns indicates that the parameter has significant effect on average particle size. Parameter β-cyclodextrin (C) does not have significant effect on average particle size.

3.3 Influence of independent variables on the span

Span (distribution width) has no relation with middle particle diameter but decides the performance of the prepared polymeric nanoparticles since it influence the solubility, dissolution, stability, circulation half-life, cellular uptake, drug release and bio-distribution. Except Eudragit E 100, all other independent variables significantly ($P < 0.005$) influence the span (Table 3, Fig. 5). The linear model explaining the effects of various variables on span is given as [Span = 1.67 − 0.11*B − 0.029*C + 0.10*D − 0.62*E + 0.019*F + 0.14*G − 0.068*H]. Simialry, a very good correlation was noticed between observed and predicted value (Table 4) and analysis of varaince showed a significant effect of independent variables (Prob > F, 0.0014) on span (Table 5). Varaibles such as concentration of poloxamer 188, concentration of beta cyclodextrin and percentage of ethanol has inverse relationship with span whereas, variables such as volume of organic phase, volume of aqueous phase, volume of the beaker and sonication time has favourable effect on span (Fig. 5).

Table 5 Analysis of variance results of average particle size, span, uniformity & surface area.

Variables	Source	Sum of Square	df	Mean of Square	F Ratio	Prob. > F*
Average Particle Size	Model	1757848	10	175784.8	214237.7	0.0017
	Residual	0.820513	1	0.8205		
	C. Total	1757849	11			
Span	Model	5.75735	10	0.5757	311856.5	0.0014
	Residual	0.000002	1	0.000002		
	C. Total	5.75735	11			
Uniformity	Model	0.658008	1	0.6580	11.3001	0.0072
	Residual	0.582304	10	0.05823		
	C. Total	1.240313	11			
Surface Area	Model	3527.625	10	352.7625	171971.7	0.0019
	Residual	0.002051	1	0.002051		
	C. Total	3527.627	11			

*Prob. > F is the significance level and a value less than 0.05 considered significant

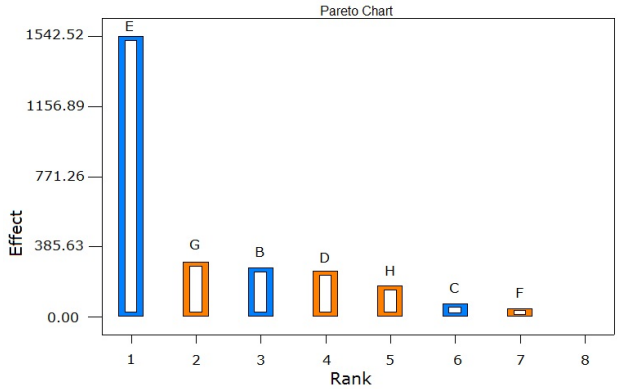

Fig. 5 Plackett-Burman Pareto-Plot for the span using eight independent parameters. Blue colour column indicates the parameter has negative effects and orange colour with positive effects on the average particle size. The white column inside both blue and orange columns indicates that the parameter has significant effect on span. Parameter Eudragit E 100 (A) does not have significant effect on span

3.4 Influence of independent variables on the uniformity

Particle size uniformity (distribution pattern) has relation with middle particle diameter and shows the extent of distribution deviating from the middle and also decides the consistency of performance of the prepared polymeric nanoparticles. Except percentage of ethanol, all other independent variables do not significantly (P> 0.005) influence the uniformity (Table 3, Fig. 6). The linear model explaining the effects of percentage of ethanol on uniformity is given as [Uniformity = +0.58 - 0.23*E]. However, there were no sufficient correlation between observed and predicted value (Table 4) and analysis of varaince showed a significant effect of percentage of ethanol (Prob > F, 0.0072) on uniformity (Table 5). Percentage of ethanol has favourable effect on the particle size uniformity (Fig. 6). Varaibles such as percentage of ethanol, concentration of poloxamer 188, concentration of Eudragit E 100 and concentration of beta cyclodextrin has inverse relationship with uniformity whereas, variables such as volume of organic phase, sonication time, volume of the beaker and volume of aqueous phase has favourable effect on uniformity (Fig. 6).

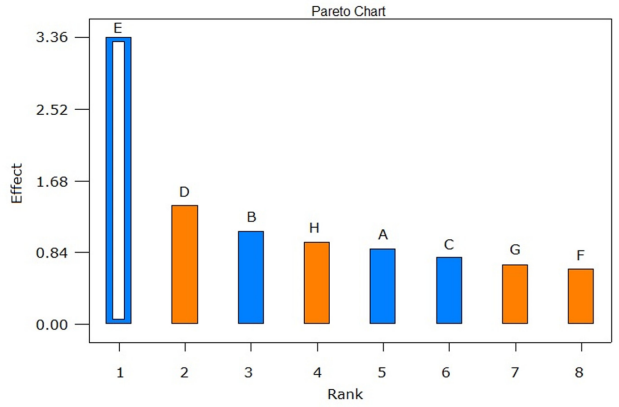

Fig. 6 Plackett-Burman Pareto-Plot for the uniformity using eight independent parameters. Blue colour column indicates the parameter has negative effects and orange colour with positive effects on the average particle size. The white column inside both blue and orange columns indicates that the parameter has significant effect on uniformity. Parameters without white column inside both blue and orange columns do not have significant effect on uniformity

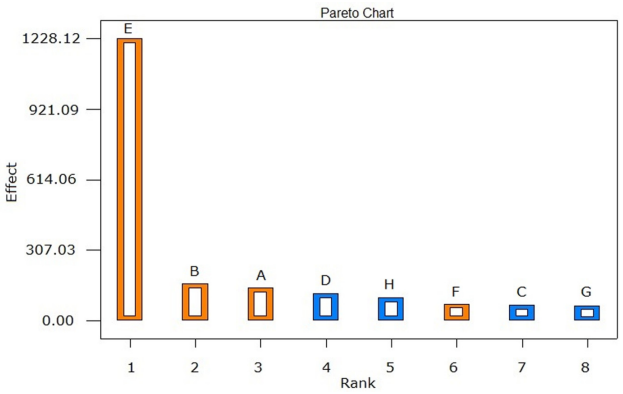

Fig. 7 Plackett-Burman Pareto-Plot for the surface area using eight independent parameters. Blue colour column indicates the parameter has negative effects and orange colour with positive effects on surface area. The white column inside both blue and orange columns indicates that the parameter has significant effect on surface area.

3.5 Influence of investigated parameters on the surface area

All eight independent variables significantly (P< 0.005) influence the surface area (Table 3). The linear model explaining the effects of various variables on surface area is given as [Surface area = 36.34 + 1.93*A + 2.16*B − 0.92*C − 1.57*D + 16.36*E + 0.98*F − 1.02*G − 1.34*H]. Moreover, observed values correlate well with predicted value (Table 4) and analysis of varaince showed a significant effect of independent variables (Prob > F, 0.0019) on surface area (Table 5, Fig. 7). Varaibles such as concentration of beta cyclodextrin, volume of organic phase, volume of the beaker and sonication time has inverse relationship with the surface area whereas, concentration of Eudragit E 100, concentration of poloxamer 188, percentage of ethanol and volume of aqueous phase has favourable effect on the surface area (Fig. 7).

4. Conclusion

In the present study, effect of various manufacturing variables on the average particle size, span, uniformity and surface area of the prepared Curcumin loaded Eudragit E 100 nanoparticles were studied by Plackett-Burman design. Least average particle size can be obtained by increasing the concentration of poloxamer 188, increasing the volume of aqueous phase, increasing the sonication duration and decreasing the ethanol concentration. Similarly, span less than 1 can be obtained by increasing the concentration of poloxamer 188, increasing the sonication duration and decreasing the ethanol concentration. However, uniformity can be increased decreasing the ethanol concentration. Higher surface area can be obtained by increasing the concentration of Eudragit E 100, poloxamer 188 and increasing the volume of the aqueous phase.

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