Research article

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DEVELOPMENT AND CHARACTERIZATION OF TERBUTALINE SO_4 NANOPARTICLES BY USING SILVERSON EMULSIFIER: A FACTORIAL DESIGN APPROACH

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ABSTRACT

The attention in the preparation and optimization of nanometer-sized materials is increasing due to their remarkable potential as a drug delivery system with wide range of applications.Terbutaline SO_4 (TS)-loaded PMMA nanoparticles were prepared by solvent evaporation method. In this study two dependent variables % of yield and particle size were measured as responses. The regression equation for the responses Y1= +285.13 + 6.42A+ 3.02B + 0.24C – 0.58 AB +1.12AC + 1.78BC – $9.91A^2$ + $0.65B^2$ – $0.72C^2$, Y2= + 86.10 + 4.65A + 1.03B + 0.83C + 0.15AB -0.15AC – 0.65BC + $0.87A^2$ – $0.58B^2$ – $1.12C^2$. At low levels of A (Drug), Y2 increases from 81.1% to 82.4%. Similarly, at high levels of A, Y2 increases from 89.4% to 93.3%.The prepared nanoparticle was characterized by SEM, Fourier transform infrared spectroscopy, DSC spectra and HPLC analysis.

Keywords: Terbutaline SO_4,Silverson emulsifier,Box-Behnken design

INTRODUCTION

Asthma is one of the most frequent diseases influencing the humanpursuit, with 610% of the adult population sufferingfrom asthma or related conditions [1]. Asthmahas a diurnal rhythm and in most of the patients, pulmonaryfunction gets reduced from midnight sustained up to 8 h.Thus a perfect therapeutic agent should have effectivemeasures in preventing bronchospasm for the period of 6–8 h during which most individuals sleep. Terbutaline sulfate;b-[(tert-butylamino) methyl]-3,5-dihydroxy-benzyl alcohol(C12H19NO3) is a synthetic b2-adrenoceptor(b2AR) agonist that is widely used as a bronchodilator in acuteand long-term treatment of bronchial asthma, chronic bronchitisand emphysema and other chronic obstructive pulmonarydiseases (COPD) with reversible bronchial hyper-reactiveconditions [2,3].TBS is a selective b2 adrenoceptor agonist. TBS is a short-actingbronchodilator which can be administered orally, parenterallyor by suitable inhalationsystems (DPI or nebulization).Orally administered terbutaline is absorbed incompletely.TBS undergoes high first-pass metabolism in the gut-wall and liver limits bio-availability up to 15% [3]. Peak plasma levels are 1.2 lg mL1 for everymg of an oral dose, reached within 2–3 h. Following inhalation,only about 10–20% of inhaled dose reaches the lungsbut after nanosizing the drug candidate >50% can be targeteddeeper to alveolarregion [1].In recent years, polymer nanoparticles have received considerableattention as a promising colloidal drug carrier [4]. They have been widely used for controlled drug delivery via intravenous, ocular, and oral administration routes. Depending on the desired route of administration, particle size and other physicochemical properties should be optimized to achieve targeted and extended drug delivery to the affected tissues. Several methods to produce polymer nanoparticles useful for drug delivery have been reported: in situ polymerization[5,6],spontaneous emulsification-solvent diffusion [7,8], supercritical fluid [9], andemulsification-solventevaporationtechniques [10,11].

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EXPERIMENTAL DESIGN

Initially, preliminary experiments (one factor at a time approach) were performed to determine the mainfactors and the appropriate ranges in which the optima lie. The effects of the three factors (drug, polymerand surfactant) on the particle size and % of yield were tested. Throughpreliminary screening the drug, polymer and surfactant viscosity were identified as themost significant variables within the range of 50-100 mg, 100-200 mg, 129-560cps, respectively. Onthe

basis of the preliminary trials a 3-factor, 2-level Box-Behnken design was employed to study theeffect of each independent variable on dependent variables (mean particle size and % of yield). Thisdesign is suitable for exploring quadratic response surfaces and constructing second-order polynomialmodels. The design consists of replicated center points and the set of points lying at the midpoint of eachedge of the multidimensional cube that defines the region of interest. The independent factors andthe dependent variables used in the design are listed in Table 1**.** The experiments were conducted as forthe design of experiments and the responses for the dependent variables were entered in Table 2. Theresponse surfaces of the variables inside the experimental domain were analyzed using Stat-Ease Design-Expert software (DX9). Subsequently, three additional confirmation experiments were conducted toverify the validity of the statistical experimental strategies.

Preparation of terbutalineSO_4 nanoparticles:

The nanoparticles were prepared by using emulsion evaporation method. A measured quantity of emperor vegetable leaves was crushed by hand and sufficient amount of distilled water was added to make up a volume of 100 ml. The mucilaginous extract of emperor vegetable was squeezed out. The emperor vegetable mucilaginous extract was later filtered 4 times using muslin cloth. The viscosity of solution was measured by using Brookfield Viscometer.

The internal phase was prepared by dissolved measured quantities of terbutalineSO_4 in distilled water. On the other hand, measured quantity of PMMA was dissolved in DCM. The aqueous phase was added to the lipid phase and a quantity of methanol was added to the suspension. The Emperor vegetable mucilaginous extract was set into the Silverson Emulsifier (with removed base plate and emulsor screen) with resolution of 8000rpm. The internal phase was then added dropwise to the external phase. After 30 minutes, glutardialdehyde was also added dropwise to the mixture. The process was allowed to proceed for 3 hours.The suspension formed was then centrifuged using Lobofuge 200 – Centrifuge for 10 minutes at 4000 rpm. The sediment of the suspension was transferred to a shallow evaporating dish. The dish placed on a hot plate with a constant temperature of 40-41°C. Once the powder is dried, it was collected and packed and % yield was measured.

In vitro drug release:

In vitro release studies were performed using Franz diffusion cell. Dialysis membrane having pore size 2.4 nm, molecular weight cut off 12,000–14,000 was used. Membrane was soaked in double-distilled water for 12 h before mounting in a Franz diffusion cell. A volume of 1 ml of terbutaline SO_4PMMA nanoparticles was placed in the donor compartment and the receptor compartment was filled with 22 ml of dialysis medium consisting of phosphate buffer pH 7.4. An aliquot of 2 ml of sample was withdrawn from receiver compartment through side tube at specific time intervals. Fresh medium was replaced each time to maintain constant volume. Samples were analyzed by RP HPLC method.

The solution was determined by RP HPLC method. RP HPLC chromatographic separation was performed on 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: acetonitrile(pH 4.5) (40:60, v/v), and detection was made at 245nm. 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 separationFigure 13.

RESULTS AND DISCUSSION

Optimization of process variables for the terbutalineSO_4nanoparticles:

The most widely used method for formulation of the terbutalineSO_4nanoparticlesis the solvent evaporation method, which usually requires high shear stress. In this work, we report the successful result on the formulation of terbutalineSO_4 nanoparticles. Through preliminaryexperiments the Drug (A), Polymer (B) and Surfactant viscosity (C) were identified asthe most significant variables influence the particle size and% yield.Design of experiments (DOE) has been used as a powerful approach to reduce the variation in a processand, ultimately, to produce high % yield withuniform particle size distribution. Among variousdesign approaches, the Box-Behnken design was used to optimize and evaluate main effects, interactioneffects and quadratic effects of the process variables on the particle size and% yield. This design is suitable for exploring quadratic response surfaces and constructing second order polynomial models. Thedesign consists of replicated center points and the

set of points lying at the midpoint of each edge of themultidimensional cube. These designs are rotatable (or near rotatable) and require 3 levels of each factor.

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

Independent variable	**Levels**				
Variable	Name	Units	Low	Middle	High
A	Drug	mg	50	75	100
B	Polymer	mg	100	150	200
C	Surfactant	cps	129	344.5	560
Dependent variable	**Goal**				
Y1	Size	nm		minimize	
Y2	Yield	%		100	

Table-2:Factorial design of terbutaline nanoparticle formulations

	Factor 1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**
Run	A:Drug	B:Polymer	C:Surfactant viscosity	Size	yield
	mg	mg	cps	nm	%
1	50	150	129	270.15	81.1
2	75	200	560	290.77	85.4
3	100	200	344.5	285.26	93.3
4	50	100	344.5	265.33	79.8
5	100	150	560	281.1	90.3
6	100	150	129	279.66	89.4
7	75	200	129	285.43	84.6
8	50	200	344.5	272.47	82.4
9	75	100	129	282.91	82.1
10	75	150	344.5	285.15	85.3
11	50	150	560	267.11	82.6
12	75	100	560	281.13	85.5
13	75	150	344.5	285.49	86.2
14	75	150	344.5	285.17	86.3
15	75	150	344.5	284.98	86.4
16	75	150	344.5	284.86	86.3
17	100	100	344.5	280.43	90.1

Table-3: Regression equation for the responses YI & Y2

Response	**Regression equation**
Y1	$+285.13 + 6.42A + 3.02B + 0.24C - 0.58\,AB + 1.12AC + 1.78BC - 9.91A^2 + 0.65B^2 - 0.72C^2$
Y2	$+ 86.10 + 4.65A + 1.03B + 0.83C + 0.15AB - 0.15AC - 0.65BC + 0.87A^2 - 0.58B^2 - 1.12C^2$

Table-4: ANOVA results of the quadratic model for the response particle size (Y1)

Source variations	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	R^2
Model	842.11	9	93.57	109.73	< 0.0001	0.9930
A-Drug	330.12	1	330.12	387.15	< 0.0001	
B-Polymer	72.78	1	72.78	85.36	< 0.0001	
C-Surfactant viscosity	0.48	1	0.48	0.56	0.4774	
AB	1.33	1	1.33	1.56	0.2512	
AC	5.02	1	5.02	5.88	0.0457	
BC	12.67	1	12.67	14.86	0.0062	
A^2	413.19	1	413.19	484.59	< 0.0001	
B^2	1.77	1	1.77	2.08	0.1926	
C^2	2.18	1	2.18	2.55	0.1543	
Residual	5.97	7	0.85			
Lack of Fit	5.74	3	1.91	33.73	0.0027	

Table-5: ANOVA results of the quadratic model for the response % yield (Y2)

Source variations	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	R^2
Model	198.31	9	22.03	25.49	0.0002	0.9704
A-Drug	172.98	1	172.98	200.14	< 0.0001	
B-Polymer	8.41	1	8.41	9.72	0.0169	
C-Surfactant viscosity	5.45	1	5.45	6.30	0.0404	
AB	0.090	1	0.090	0.10	0.7564	
AC	0.090	1	0.090	0.10	0.7564	
BC	1.69	1	1.69	1.96	0.2047	
A^2	3.22	1	3.22	3.73	0.0947	
B^2	1.39	1	1.39	1.61	0.2450	
C^2	5.33	1	5.33	6.17	0.0420	
Residual	6.05	7	0.86			
Lack of Fit	5.23	3	1.74	8.50	0.0328	

Seventeen experiments were required for the response surface methodology based on the Box-Behnkendesign. Based on the experimental design, the factor combinations yielded different responses aspresented in Table 2. These results clearly indicate that all the dependent variables are stronglydependent on the selected independent variables as they show a wide variation among the 17 batches.Data were analyzed using Stat-Ease Design-Expert software (DX9) to obtain analysis of variance(ANOVA), regression coefficients and regression equation. Mathematical relationship generated usingmultiple linear regression analysis for the studied variables are expressed as shown in Table 3.These equations represent the quantitative effect of Drug (A), Polymer (B) andSurfactant viscosity (C) and their interaction on Particle size (Y1) and % yield (Y2). The values of thecoefficient A, B and C are related to the effect of these variables on the responses Y1 and Y2.Coefficients with more than one factor term and those with higher order terms represent interaction termsand quadratic relationship respectively. A positive sign represents a synergistic effect, while a negativesign indicates an antagonistic effect. A backward elimination procedure was adopted to fit the data to thequadratic model. Both the polynomial equations were found to be statistically significant (P <0.01), asdetermined using ANOVA (Table 4 &5), as per the provision of Design Expert software (DX9).

Particle size analysis of terbutalineso_4nanoparticles was found to be in the range of 265.33 –290.77nm as shown in Table 2.The factorial equation for particle size exhibited a good correlation coefficient (1.000) and the Model Fvalueof 109.73 which implies the model is significant. Values of "Prob> F" less than 0.0500 indicatemodel terms are significant. In this case A, B, C and the quadratic term of A and B aresignificant model terms as shown in Table 4. Results of the equation indicate that the effect of A (Drug) and Polymer (B) are

more significant than C. All the three variables having the negative effect on the particle size, which means these factors, are inversely proportional to the response. The influence of the main andinteractive effects of independent variables on the particle size was further elucidated using theperturbation and 3D response surface plots. The individual main effects of A, B and C on particle size are as shown in Figure 4. It is found that all the variables are having interactive effects for the responseY1. The 2D contour plots,actual and predicted value,factorial cube design and 3D response surfaces of the response Y1 are shown in Figure 1-5 to depict the interactive effects of independent variables on response Y1, one variable was kept constantwhile the other two variables varied in a certain range. The shapes of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. At low levels of A, Y1 reduced from 272.47 to 265.33 nm.

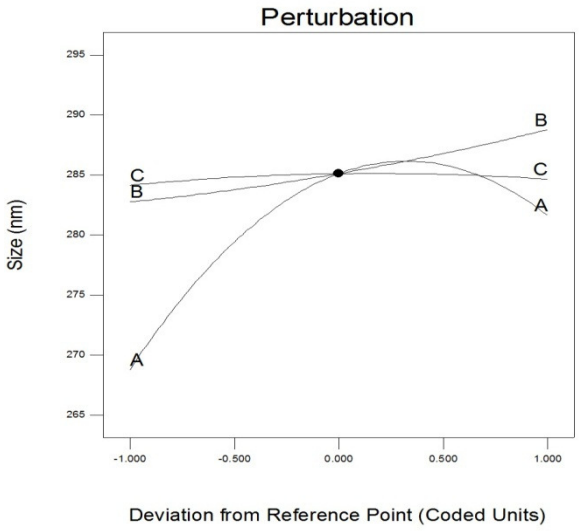


Figure-1: Perturbation plot showing the main effect of drug (A), polymer (B) and surfactant viscosity (C) on particle size (Y1)

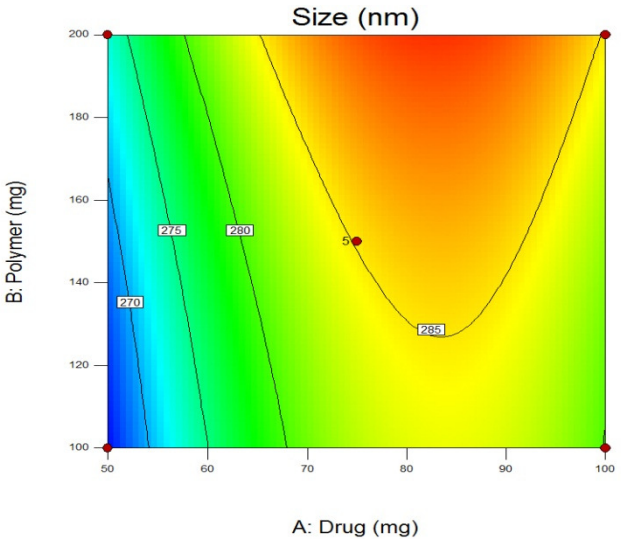


Figure-2: Response surface plot presenting the interaction between the drug and polymer affecting the particle size at constant surfactant viscosity.

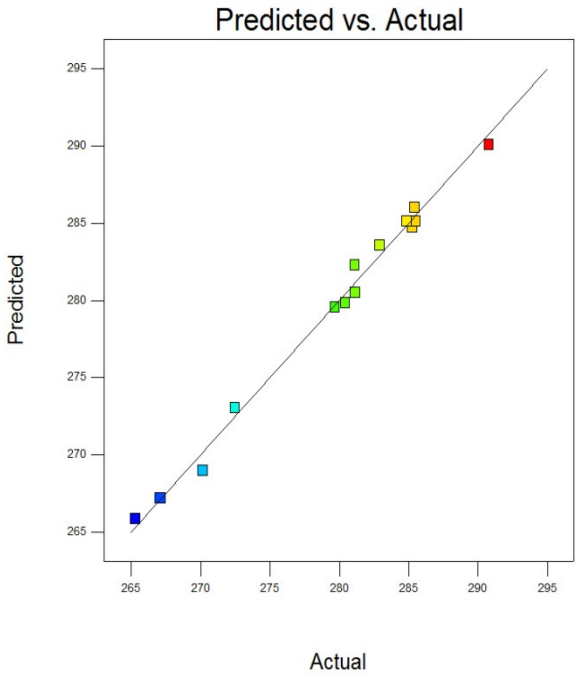


Figure-3: Plot showing the actual and predicted value of particle size

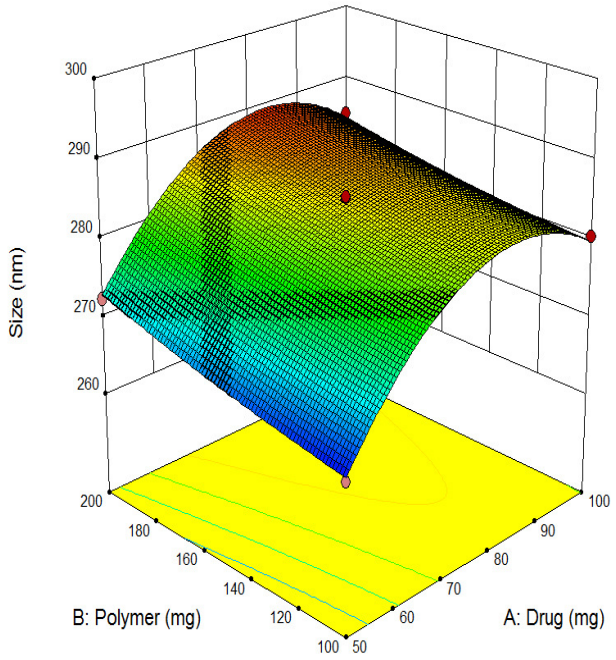


Figure-4: Response surface plot presenting the interaction between the drug and polymer affecting the particle size at constant surfactant viscosity.

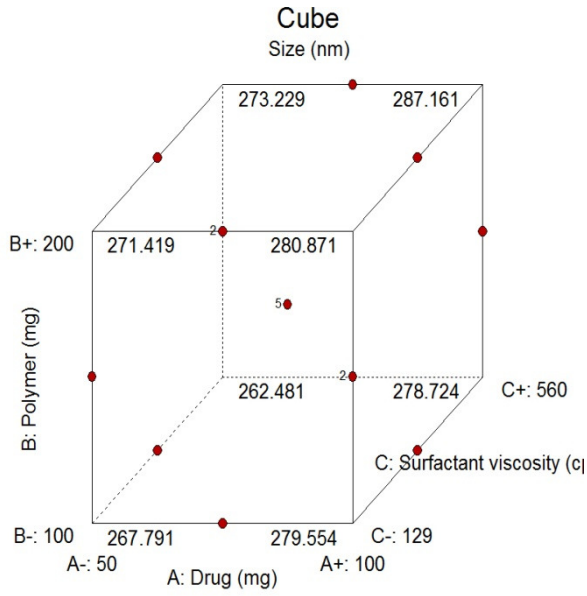


Figure-5: Factorial Cube design presenting the interaction between the drug and polymer affecting the particle size at constant surfactant viscosity.

After generating the polynomial equations relating the dependent and independent variables, the processwas optimized for the responses. Numerical optimization using the desirability approach was employedto locate the optimal settings of the process variables to obtain the desired responses. Optimizedconditions were obtained by setting constraints on the dependent and independent variables.

The mathematical model generated for % yield (Y2) was found to be significant with F-value of 25.49 ($p < 0.0001$) and R^2value of 0.9704. The independent variables A, B, C and the quadratic term of Ahave significant effects on the % yield, since the P-values less than 0.0500 represent the significant model terms as shown in Table 5. Results of the equation indicate that the effectof A is more significant than B and C. The influence of the main and interactive effects of independentvariables on the % yield was further elucidated using the perturbation and 3D response surface plots. The 2D contour plots,actual and predicted value,factorial cube design and 3D response surfaces of the response Y2 are shown in Figure 6-10 to depict the interactive effects of independent variables on response Y2. This figure clearly shows that A has the main and the major effect on Y2 followedby B which has a moderate effect on Y2 followed by C which has a little effect on Y2. The relationshipbetween the dependent and independent variables was further elucidated using response surface plots.Figure 9 shows the interactive effect of A and B on the practical yield (Y2) at fixed level of C. At lowlevels of A (Drug), Y2 increases from 81.1% to 82.4%. Similarly, at high levels of A, Y2 increases from 89.4% to 93.3%.

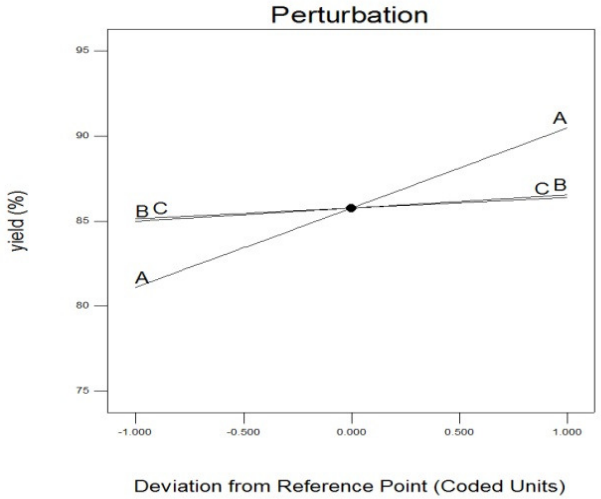


Figure-6 : Perturbation plot showing the main effect of drug (A), polymer (B) and surfactant viscosity (C) on % yield (Y2)

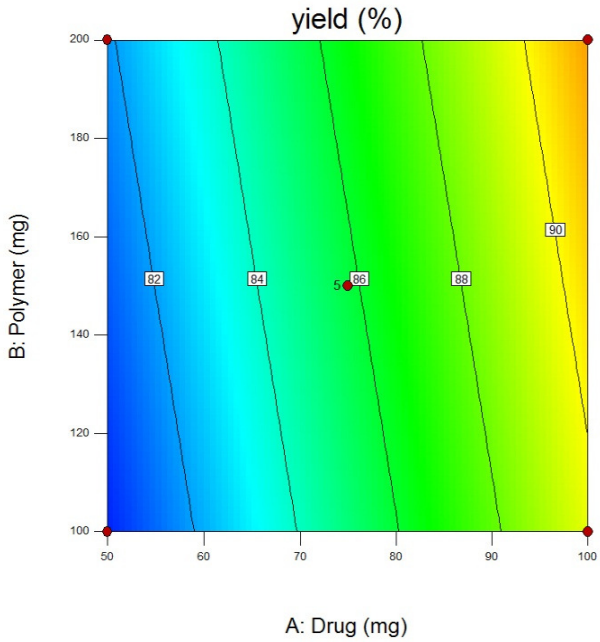
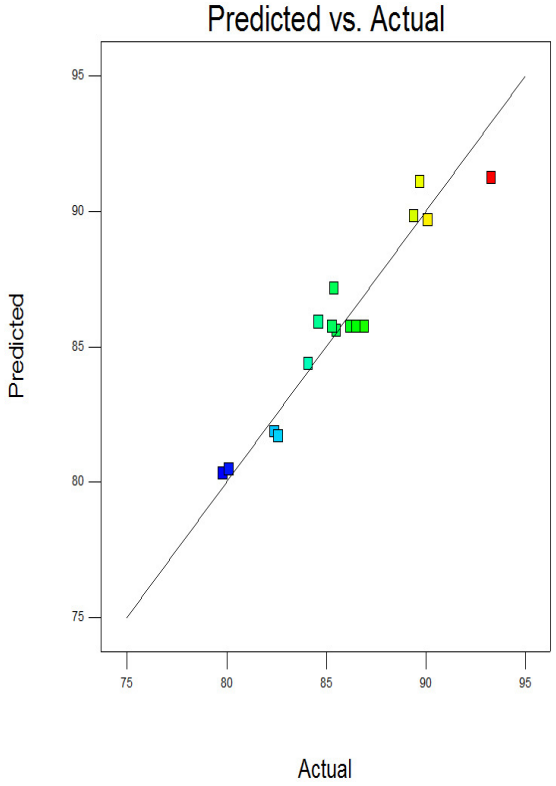


Figure-7: Response surface plot presenting the interaction between the drug and polymer affecting the % yield at constant surfactant viscosity.



Figuer-8: Plot showing the actual and predicted

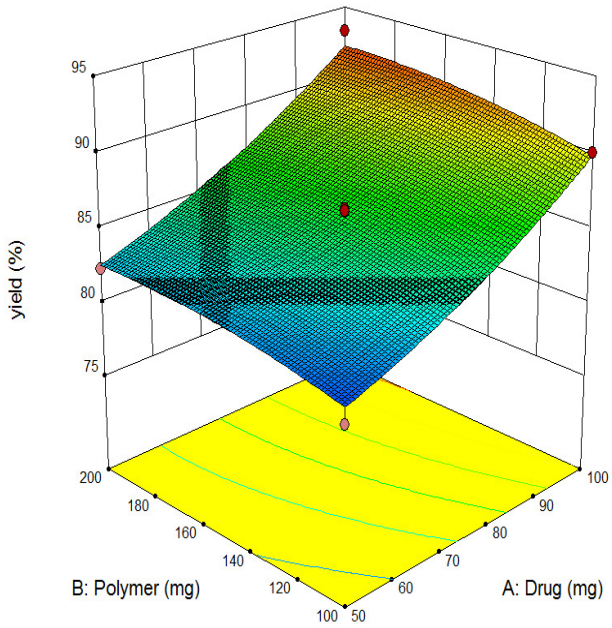


Figure-9: Response surface plot presenting the interaction between the drug and polymer affecting the % yield at constant surfactant viscosity.

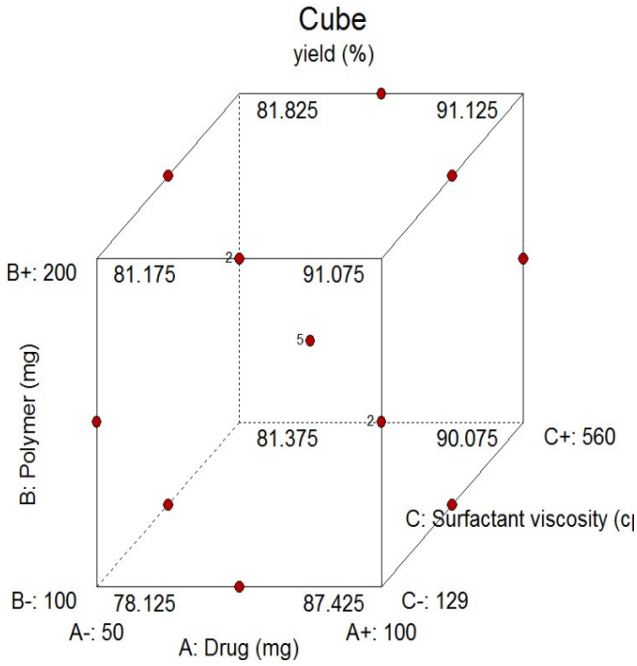


Figure-10: factorial cube design presenting the interaction between the drug and polymer affecting the % yield at constant surfactant viscosity.

The spectra analysis of terbutalineso_4showed that, the principal peaks were observed at wave numbers of3935.55,3906.84,3856.16,3808.56,2976.40, 2679.28,2491.94,2457.46, 2320.11,2140.35, 2073.13, 2012.59, 1840.46, 1737.44, 1608.53, 1482.44, 1346.50, 1106.72, 921.02, 838.79, 759.28, 698.04 and 618.14 (unit in cm^{-1}).The FTIR spectral analysis of PMMA alone showed that principal peaks where observed at wavenumber of 3922.66, 3890.44, 3874.42,3859.47,3845.97,3788.55,3767.18,3756.13,3729.00,3705.98,3694.52,3665.54,3639.14,3606.08,3572.92,3456.43,2961.35,2609.27,2423.74,2379.79,2349.09,2285.44,2059.23,1976.99,1930.51,1900.93,1877.34,1853.27,1836.83,1802.23,1756.15,1725.37,1708.61,1691.72,1678.79,1659.46,1642.67,1629.75,1583.50,1565.69,1549.01,1528.86,1514.08,1500.70,1481.07,1464.46, 1443.31, 1403.71, 1231.80, 989.14, 848.90, 813.62 and 753.64 (unit in cm^{-1}).

The FTIR spectra analysis of terbutalineso_4 with PMMA showed that, the principal peaks were observed at wave numbers of 3335.71,2975.22, 2665.54,2503.27,2456.84,2068.91,1974.37,1736.62,1609.51, 1483.98, 1109.03, 854.94, 758.04,696.02 and619.45 (unit in cm^{-1}). Finally the FTIR studies of mixture of polymer and drug does not show any significant change. This result indicating that there is no any interaction between drug and selected polymer.

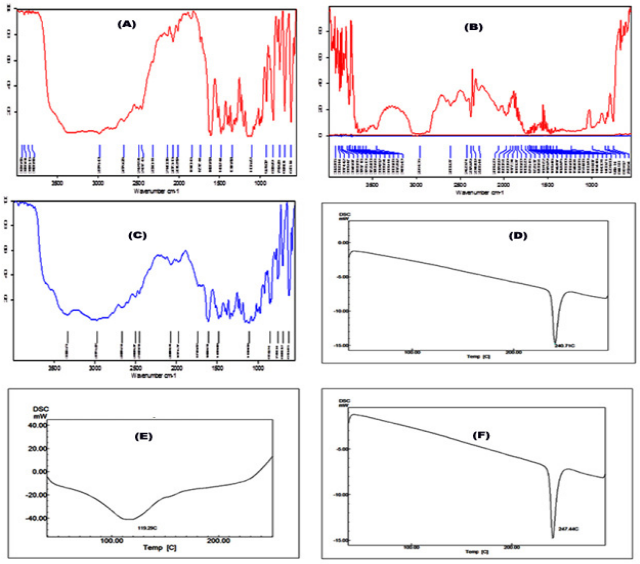


Figure-11: FTIR Spectra of Terbutaline (A) PMMA (B) Terbutaline + PMMA (C) DSC Spectra of Terbutaline (D) PMMA (E) Terbutaline + PMMA (F)

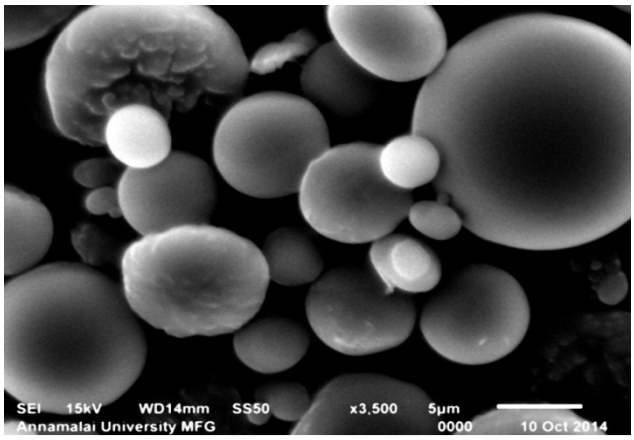


Figure -12: SEM photography of terbutaline nanoparticles

The DSC spectral analysis of terbutalineso_4 showed the endothermic peak at 240.71 °C. The DSC spectral analysis of PMMA showed the endothermic peak at 119.29 °C. The physical mixture of terbutalineso_4 and polymer (PMMA) has no major change which at 247.44 °C. From the DSC Studies, it can be concluded that there was no significant change in the peak value in comparison with pure drug which revealed that the polymer is compatible with drug in Nano formulation.

The nanoparticle size and shape for the formulations are represented in Figure 12. The result shows that the nanoparticle diameter increases with increasing ratio of drug ratio.

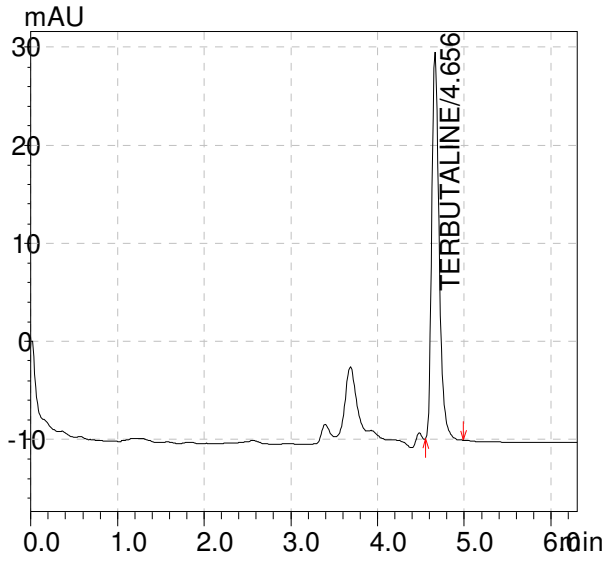


Figure- 13: Typical chromatogram of terbutaline SO_4

CONCLUSION

From the above findings it is evident that,polymeric system of terbutaline so_4PMMA loaded nanoparticleshas achieved the objectives of particle size and yield.Particle size analysis of terbutalineso_4nanoparticles was found to be in the range of 265.33 –290.77nm.The factorial equation for particle size exhibited a good correlation coefficient (1.000) and the Model F value of 109.73 which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant.The mathematical model generated for % yield (Y2) was found to be significant with F-value of 25.49 ($p < 0.0001$) and R^2 value of 0.9704. The independent variables A, B, C and the quadratic term of Ahave significant effects on the % yield, since the P-values less than 0.0500 represent the significant model

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