Research article

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FORMULATION AND OPTIMIZATION OF ETHYLCELLULOSE COATED TRIAMCINOLONE NANOPARTICLES USING 3-FACTOR, 2-LEVEL BOX-BEHNKEN FACTORIAL DESIGN AND IN-VIRO EVALUTION OF TRIAMCINOLONE LOADED NANOPARTICLES

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ABSTRACT:

The purpose of the present study was to prepare and optimize triamcinolone acetonidenanoparticles with prolonged drug release. Triamcinolone acetonide (TA)-loaded PMMAnanoparticles were prepared by solvent evaporation method. In this study, we present a formulation of these nanoparticles by siverson emulsifier. A 3-factor, 2-level Box-Behnken design was used to optimize the process parameters including surfactant % (A), speed (B) and time (C). Two dependent variables % of yield and particle size were measured as responses. Mathematical equations and response surface plots were used to relate the A,B,C and Y1,Y2. The optimization model predicted particle size of about 246.07 nm and % of yield of 83.41 % with A, B and C levels of 1.25%, 6500rpm and 120min respectively. The experiential responses were in near agreement with the predicted values of the optimized process. The prepared nanoparticle was characterized by SEM, Fourier transform infrared spectroscopy, DSC spectra and HPLC analysis. The optimized formulation of nanoparticles was loaded into gel and the in-vitro evaluation was performed.

Keyword: TA nanoparticles,Box-behnken, HPLC, TA nanoparticles loaded *in situ* gel

INTRODUCTION

Nanotechnology is a promising area involved in imaging, quantifying, modeling, creation and manipulation of materials at nanoscale level. According to the unique and unusual physical, chemical and biological properties of the nanoparticles, nanotechnology could assist to develop more practical devices [1]. Advances in nanotechnology and consideration of material properties at nanoscale level have resulted in the development of several novel drug delivery and therapeutic systems. Targeted and controlled delivery of the drug, delivery of poorly water-soluble drugs,decreased toxicity and side effects, as well as patient compliance are some of the advantages of novel drug delivery systems [2–4]. Nanocarriers as one of the considerable novel drug delivery systems have been investigated and profited for many years by virtue of their prominent features such as high surface area [5,6]. Nanoparticles can be plotted using a type of ingredients including polymers, lipids and viruses, inorganic and organometallic compounds. Polymeric nanoparticles are one of the most commonly used carriers in novel drug delivery systems [7]. Unique techniques have been established to prepare nanoparticles including emulsification solvent evaporation [8]. Triamcinolone acetonide (TA), a synthetic corticosteroid with potent anti-inflammatory effects, is administered as an injectable suspension for the treatment of different inflammatory conditions. TA nano formulations have been prepared using emulsification/solvent diffusion [9], high pressure homogenization [10], interfacial polymerization [11] and o/w emulsion-solvent evaporation [12]. Poloxamers are triblock copolymers of poly (ethylene oxide) and poly (propylene oxide). Using hydrophilic excipients, gelation temperature of poloxamer blends can be modulated so that they form in-situ gels at body temperature [13]. Further, several researchers have reported the use of thermosensitivepoloxamer gels in topical administration of drugs like Clotrimazole[14], fluconazole[15], itraconazole [16], ciclopriroxolamine[17] and loraditine[18]. Poloxamers

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have poor inherent mucoadhesive properties [16]. Hence, selectedmucoadhesive polymer is added along with poloxamers to make useful*in situ* gel formulation.The objective of this study was to develop and optimization of nanoparticles and in vitro evaluation of nanoparticles loaded thermosensitive gels of poloxamer for delivery of triamcinolone acetonide. For the study, carbopol 940 were selected as mucoadhesive polymer.

FACTORIAL DESIGN

The effects of the three factors (drug, polymer and surfactant) on the particle size and % of yield were tested. Through preliminary screening the drug, polymer and surfactant were identified as the most significant variables within the range of 1-1.5 %,5000-8000rpm, and 60-180 min, respectively. On the basis of the preliminary trials a 3-factor, 2-level Box-Behnken design was employed to study the effect of each independent variable on dependent variables (% of yield and mean particle size). This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The design consists of replicated center points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest [19]. The independent factors and the dependent variables used in the design are listed in Table 1**.** The experiments were conducted as for the design of experiments and the responses for the dependent variables were entered in Table 2. The response surfaces of the variables inside the experimental domain were studied using Stat-Ease Design-Expert software (DX9). Consequently, three additional confirmation experiments were showed to verify the validity of the statistical experimental strategies.

Preparation of triamcinolone acetonide:

Preparation of triamcinolone acetonide nanoparticles was prepared by emulsion solvent diffusion technique [20]. Drug and polymer were dissolved in the mixers of solvent ratio 20:10 (DCM and ethanol) by using sonicator(Table 1). This organic phase added (by using aerosol sprayer pressurized up to 200 PSI) to external aqueous phase containing various concentration of surfactant (PVA). The mixture was homogenized in the high pressure homogenizer at 8,000 rpm for 3 hours. The formed triamcinolone acetonide nanoparticles were recovered by centrifugation at 6,000 rpm for 5 min followed by washing twice with distilled water and washed nanoparticles were subjected to freeze drying.

Preparation of nanoparticles loaded in situ gel:

Preparations of nanoparticles loaded temperature induced oral gel were prepared by the cold method. Specified amount of poloxamer 188 (P188), poloxamer 407 (P407) and carbopol 940 (C940) were stirred in the calculated amount of cold distilled water (Table 7). The dispersions were kept at 4°C for overnight. Equivalent to 1 mg of nanoparticles (equivalent to 1 mg of triamcinolone acetonide) was added slowly in polymeric solution with continuous stirring (thermostatically controlled magnetic stirrer). Dispersions was stored in a refrigerator for overnight to get clear sol and eventually stored in a refrigerator so that it remains in sol form.

In Vitro Release Studies:

The diffusion medium used was phosphate buffer pH 5.5.The diffusion cell was designed as per the dimension given. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir maintained at 37±0.5ºC and was connected to water jacket of diffusion cell using rubber latex tubes.

The receptor compartment was filled with fluid. Then the cellophane membrane was mounted on the cell carefully so as to avoid the entrapment of air bubble under the mucosa. Intimate contact of mucosa was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment with the help of micropipette.Aliquots of samples were withdrawn at 15, 30, 60, 120, 180, 240, 300, 360, 420, 480 and 540 minutes intervals from sampling port of receptor compartment and same volume was replaced with receptor fluid solution in order to maintain sink condition. The samples were withdrawn and drug content was determined as per the above procedure.

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/ACN (pH 3.5) (50:50, v/v), and detection was made at 254 nm. 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 separation.

RESULT AND DISCUSSION

Optimization of process variables for the triamcinolone acetonidenanoparticles:

The most widely used method for formulation of the nanoparticlesis the solvent evaporation method, which usually requires high shear stress. In this work, we report the successful result on the formulation of TA nanoparticles. Through preliminaryexperiments the Surfactant (A), Speed (B) and Time (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 [21].

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 Surfactant (A), Speed (B) andTime (C) and their interaction on % yield (Y1) and Particle size (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 and 5), as per the provision of Design Expert software (DX9).

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

Independent variableLevels					
Variable	Name	Units	Low	Middle	High
A	Surfactant	%	1	1.25	1.5
B	Speed	rpm	5000	6500	8000
C	Time	time	60	120	180
Dependent variable Goal					
Y1	Yield	%		100	
Y2	Size	nm		minimize	

Table-2:Factorial design of triamcinolone nanoparticle formulations

Run	**Factor 1 A:surfactant %**	**Factor 2 B:speed rpm**	**Factor 3 C:time min**	**Response 1 P.Yeild %**	**Response 2 P.Size nm**
1	1	6500	60	71.41	218.41
2	1	5000	120	69.46	222.39
3	1.25	6500	120	83.41	246.18
4	1.5	6500	60	92.61	230.41
5	1	8000	120	71.77	218.11
6	1.25	6500	120	83.71	246.16

7	1.25	8000	180	84.33	240.44
8	1.25	5000	60	82.01	250.11
9	1.25	6500	120	83.31	246.33
10	1.5	5000	120	92.55	230.46
11	1.25	5000	180	84.17	242.12
12	1.25	6500	120	83.41	246.12
13	1.25	6500	120	83.23	245.56
14	1.5	8000	120	92.42	232.16
15	1.5	6500	180	93.87	233.46
16	1	6500	180	71.57	217.56
17	1.25	8000	60	83.14	245.15

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

Response	Regression equation
	Y183.41+ 10.91A+ 0.43 B -0.60 C –0.61 AB +0.28AC–24 BC – 1.46 A^2–0.41 B^2 +41C^2
	Y2 246.07 + 6.25 A –1.15 B –1.31 C + 1.49 AB + 0.98 AC + 0.82 BC – 19.89 A^2– 0.40B^2– 1.22 C^2

Table-4: ANOVA results of the quadratic model for the response % of yield (Y1)

Source variations	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	R^2
Model	968.03	9	107.56	1069.26	< 0.0001	0.9993
A-surfactant	951.35	1	951.35	9457.52	< 0.0001	
B-speed	1.51	1	1.51	14.96	0.0061	
C-time	2.84	1	2.84	28.27	0.0011	
AB	1.49	1	1.49	14.80	0.0063	
AC	0.30	1	0.30	3.01	0.1265	
BC	0.24	1	0.24	2.34	0.1701	
A^2	8.92	1	8.92	88.70	< 0.0001	
B^2	0.70	1	0.70	6.98	0.0334	
C^2	0.70	1	0.70	6.93	0.0338	
Residual	0.70	7	0.10			

Table-5: ANOVA results of the quadratic model for the response particle size (Y2)

Source variations	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	R^2
Model	2050.84	9	227.87	46.45	< 0.0001	0.9835
A-surfacatant	312.75	1	312.75	63.76	< 0.0001	
B-speed	10.63	1	10.63	2.17	0.1846	
C-time	13.78	1	13.78	2.81	0.1376	
AB	8.94	1	8.94	1.82	0.2190	
AC	3.80	1	3.80	0.78	0.4078	
BC	2.69	1	2.69	0.55	0.4831	
A^2	1666.15	1	1666.15	339.65	< 0.0001	
B^2	0.67	1	0.67	0.14	0.7236	
C^2	6.24	1	6.24	1.27	0.2965	
Residual	34.34	7	4.91			

Table-6: Optimized values obtained by the constraints applies on Y1 and Y2

Independent variables	Values	Predicted values		Batch	Observed values	
		P. Yield (Y1)	P. Size (Y2)		P. Yield (Y1)	P. Size (Y2)
Surfacatant	1.25	83.414	246.07	TA3	83.38	246.12
Speed	65000			TA6	83.68	246.09
Time	120			TA12	83.40	246.11

Table-7: Composition of triamcinolone nanoparticles loaded *in situ* gel

Polymer (in 80mL)	D1	D2	D3	D4	D5	D6	D7	D8	D9
Poloxamer 188	41g	45g	49g	53g	57g	57g	57g	57g	57g
Poloxamer 407	-	-	-	-	-	6g	6g	6g	6g
Carbopol 904	-	-	-	-	-	-	0.1g	0.2g	0.4g
Temperature	-	-	43°C	41°C	35°C	25°C	27°C	28°C	36°C

Particle size analysis of triamcinolone acetonidenanoparticles was found to be in the range of 218.11 – 250.11 nm as shown in Table 2.The factorial equation for particle size exhibited a good correlation coefficient (1.000) and the Model Fvalueof 46.45 which implies the model is significant. Values of "Prob> F" less than 0.0500indicatemodel terms are significant. In this case A, B, C and the quadratic term of A and A^2 aresignificant model terms as shown in Table 5. Results of the equation indicate that the effect of A (Drug) is more significant thanBand C. All the three variables having the negative effect on the particlesize, which means these factors, are inversely proportional to the response. The influence of the main andinteractive effects of independent variables on the practical yield 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 1. It is found that all the variables are having interactive effects for the responseY2. The 3D response surfaces and the2D contour plots of the response Y2 are shown in Figure 1 and 2 to depict the interactive effects of independent variables on response Y2, 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. The interaction between A and Bon particle size at a fixed level of C is shown in Figure 1. At low levels of A, Y2 reduced from 218.11 to 222.11nm. Similarly at high levels of A, Y2 reduced from 230.41 to 230.46nm as shown in Figure3.

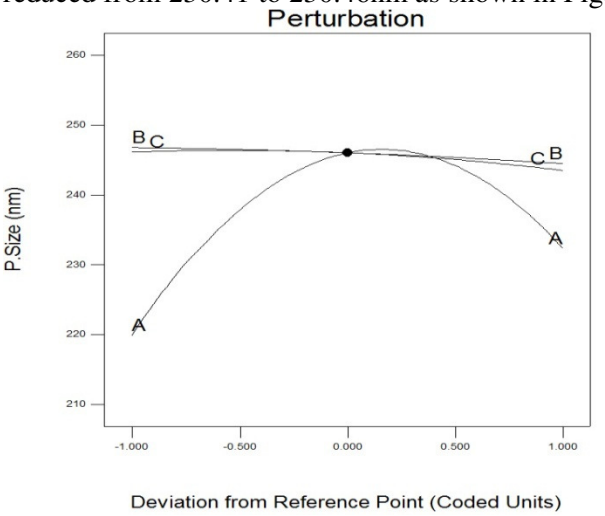

Figure 1: Perturbation plot showing the main effect of % surfactant (A), speed (B) and time (C) on particle size (Y2)

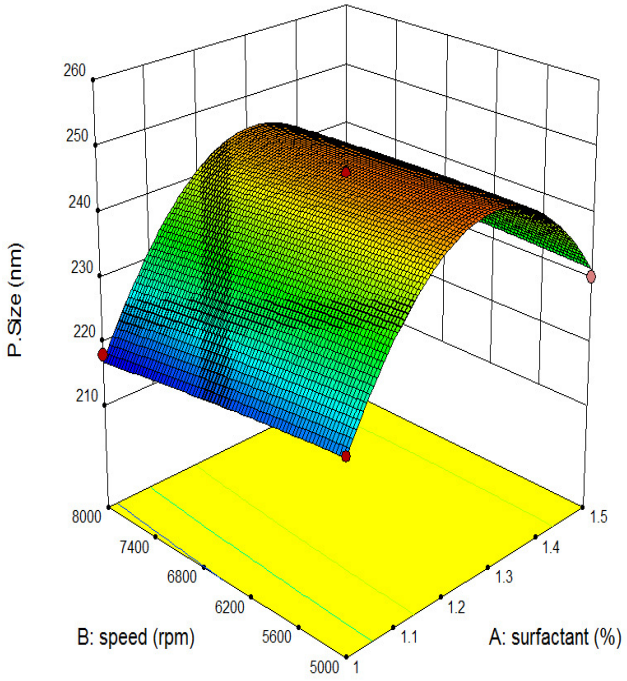

Figure-2: Response surface plot presenting the interaction between the % surfactant and speed affecting the particle size at constant time.

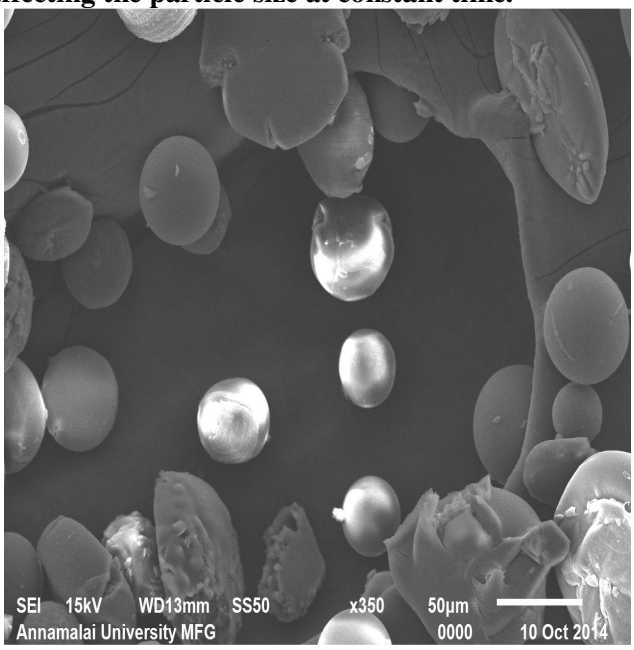

Figure -3: SEM photography of triamcinolone nanoparticles

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 (Y1) was found to be significant with F-value of 1069.26 ($p < 0.0001$) and R^2value of 0.9993. 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 4. 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 perturbation plot (Figure 3) showing the main effects of A, B and C on the percentage yield (Y1) of triamcinolone acetonide nanoparticles. This figure clearly shows that A has the main and the major effect on Y1 followedby C which has a moderate effect on Y1 followed by B which has a little effect on Y1. The relationshipbetween the dependent and independent variables was further elucidated using response surface plots.Figure 4 shows the interactive effect of A and B on the practical yield (Y1) at fixed level of C. At lowlevels of % surfactant, Y1 increases from 69.46% to 71.77%. Similarly, at high levels of A, Y1 increases from 230.41% to 230.46%.

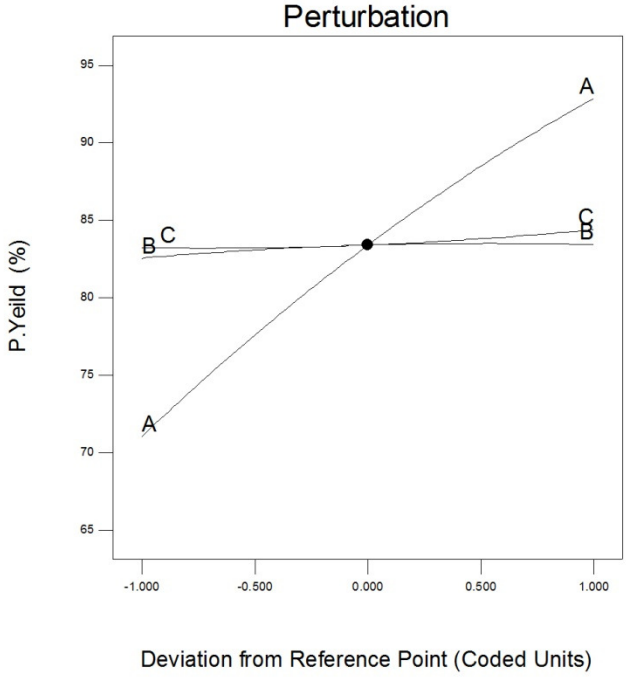

Figure-3: Perturbation plot showing the main effect of % surfactant (A), speed (B) and time (C) on % yield (Y1)

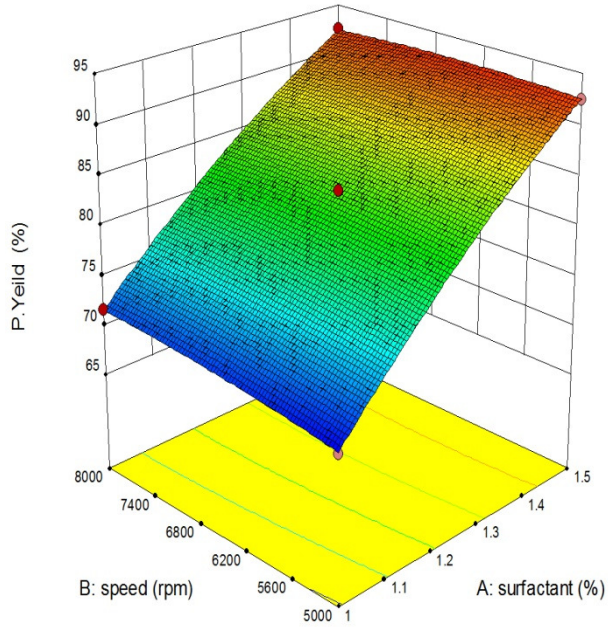

Figure-4: Response surface plot presenting the interaction between the % surfactant and speed affecting the % yield at constant time.

Three batches of triamcinolone nanoparticles were prepared according to these optimized levels. Observed responses were in close agreement with the predicted values of the optimized process, thereby demonstrating the feasibility Table 6.

IN-VITRO EVALUATION OF NANOPARTICLES LOADED IN SITU GEL

Gelation temperature (GT) of nanoparticles loaded *in situ* gel decreased with increase in concentration of poloxamer 188 and poloxamer 407 (25°C) for a concentration of 57g and 6g, respectively. It was taken for further studies. However the concentration of poloxamer 118 & 407 showed a GT of 25°C which is less than body temperature and is not suitable for *in situ* gel formation. So, carbopol 940 was added in increasing concentration (0.1% to 0.4%) which led to the increase in gelation temperature from 27°C to 36°C for the formulae D7 to D9. The optimized concentration of carbopol 940 was found to 0.4% for (D9). The maximum viscosity of nanoparticles loaded *in situ* gel was found to 40000 cps as shown in Figure 5.

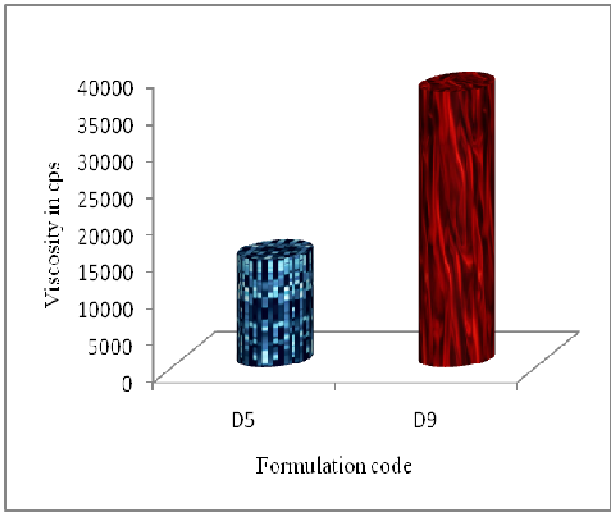

Figure-5: Viscosity of triamcinolonenanoparticles loaded *in situ* gel

The gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out from the site of administration. The gel strength of formulation D7 and D9 (150 and 159 sec) exhibited reliable gel strengthas shown inFigure 6.

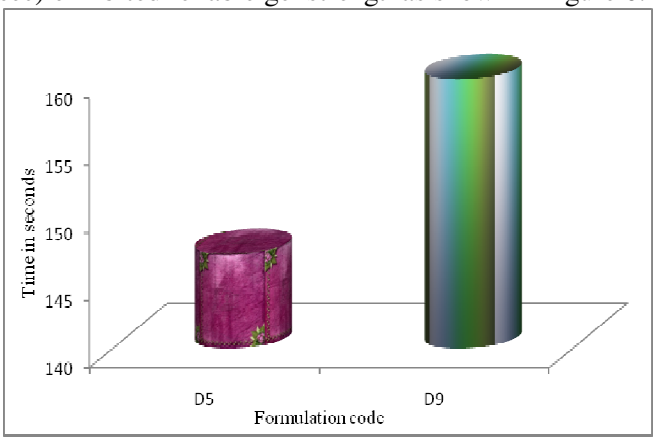

Figure-6: Gel strength of triamcinolone nanoparticles loaded *in situ* gel

The mucoadhesive force is an important physicochemical parameter of topical application. The mucoadhesive force was significantly increased from 7342.16 dynes/cm^2 to 9613.22 dynes/cm^2 for the formula D6 and D9, as the concentration of mucoadhesive polymer (carbopol 940) increased in formulation D9 and mucoadhesive force also increased as shown inFigure 7.

This also proved that carbopol has better mucoadhesive property than poloxamer combination.

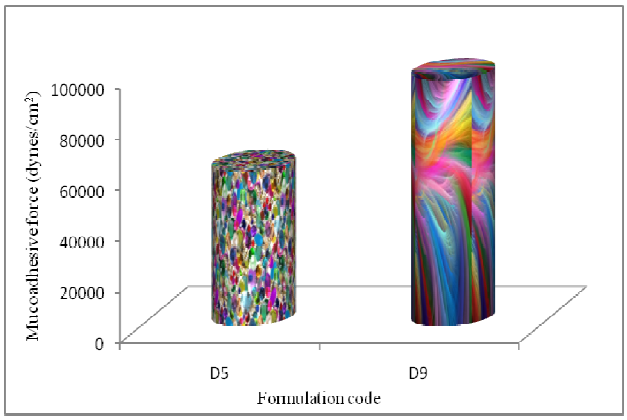

Figure-7: Mucoadhesive Force of triamcinolone nanoparticles loaded *in situ* gel

The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability of formulation D5 and D9was found to be (15.5 and 14.2 gm.cm/sec) as shown inFigure 8.

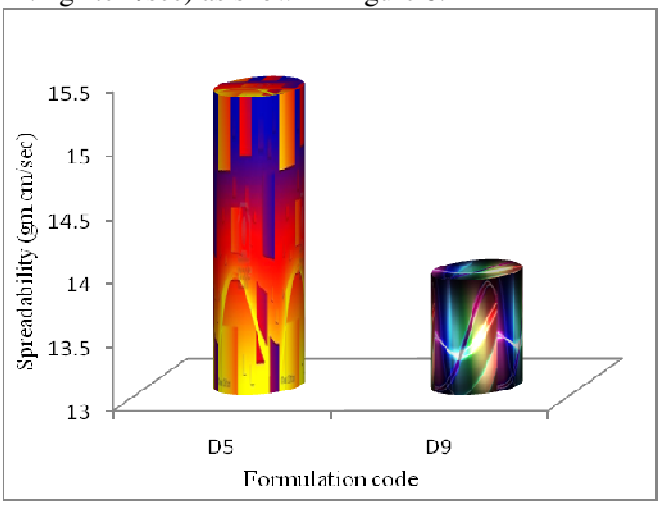

Figure-8: Spreadability of triamcinolone nanoparticles loaded *in situ* gel

The drug release from formulation D7 was less may be due to presence of poloxamer 188& 407 in the gel which retards the drug release rate owing to reduction in dimension of water channel. While diffusion of drug through formulation D9 was found to be more which may be due to presence of carbopol 940, which undergoes rapid swelling and helps in faster diffusion as shown inFigure 9 and10**.**

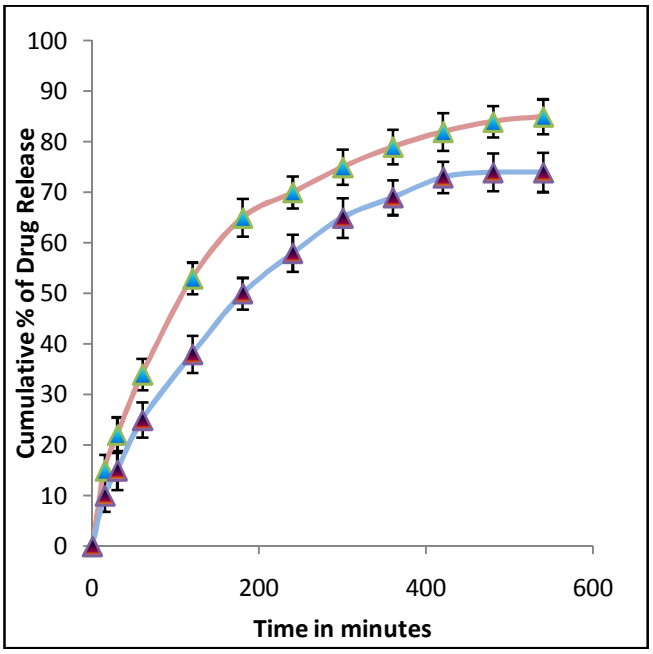

Figure-9: Drug release from nanoparticles loaded *in situ* gel

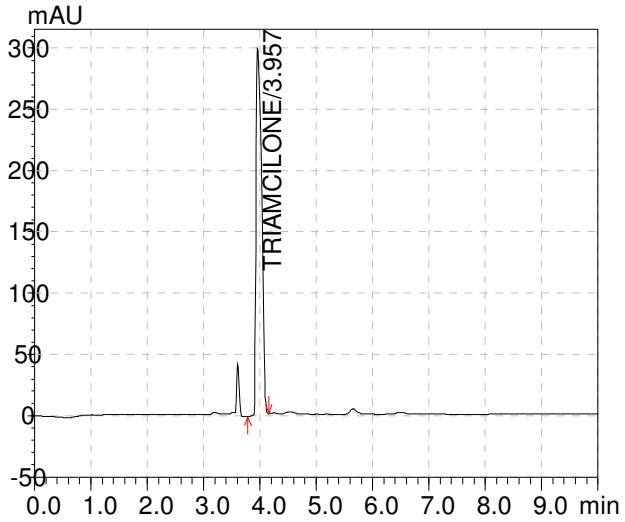

Figure 10: Typical chromatogram of triamcinolone

In FTIR spectra no extra bindings or chemical shifts were observed, indicating that there is no strong interaction between drug and polymer in the formulation as shown inFigure 11. The DSC results revealed that the matrix micro devices have sufficient stability and there is no indication of chemical interaction between drug and polymer as shown inFigure 12.

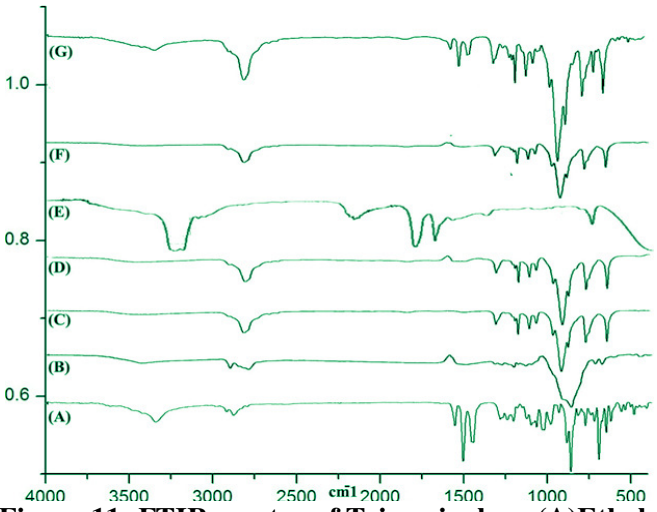

Figure-11: FTIR spectra of Triamcinolone (A)Ethyl Cellulose(B)P 407 (C)P 188(D)C 940 (E) EC+P 188+P 407+C 940 (F)Triamcinolone + EC+P 188+P 407+C 940 (G)

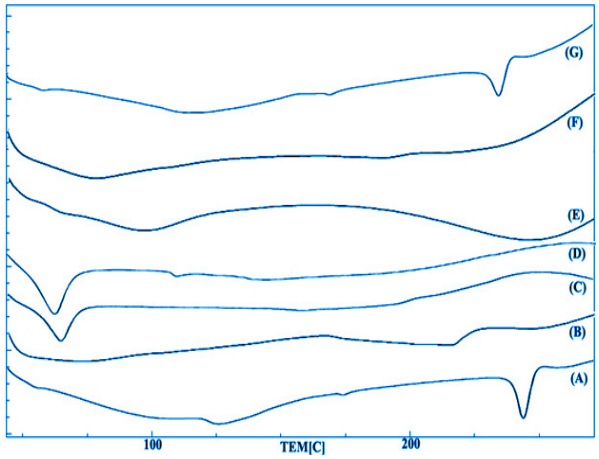

Figure-12: DSC spectra of Triamcinolone (A) Ethyl Cellulose (B)P 407 (C) P 188(D)C 940 (E) EC+P 188+P 407+C 940(F) Triamcinolone + EC+P 188+P 407+C 940 (G)

CONCLUSION

In this manuscript we revealed thathighly effective triamcinolone nanoparticles via high pressure homogenizer. From preliminary experiments, the surfactant%, speed and timewere identified as the most significant variables which influence the % yield and mean particle size. The quantitative effect of these factors at different levels was predicted by using polynomial equations. Response surface methodology was then used to predict the optimum levels of these factors to attainnanoparticles with higher yield and uniform particle size. Three batches of triamcinolone nanoparticles were prepared according to these optimized levels. Observed responses were in close agreement with the predicted values of the optimized process, thereby demonstrating the feasibility.The drug release from formulation D7 was less may be due to presence of poloxamer 188 and 407 in the gel which retards the drug release rate owing to reduction in dimension of water channel.

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