Research article

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EFFECT OF INDEPENDENT VARIABLE ON THE ISRADIPINE NANOPARTICLE, SIZE AND DRUG LOADING CAPACITY EVALUATED BY MEANS OF DESIGN-EXPERT SOFTWARE

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ABSTRACT

Acacia concinna are widely used in the pharmaceutical cosmetic industry due to their primary cleansing agent**.** It belongs to herbal extracts category. It is standardized for saponins. Shikakai powder is an inexpensive natural detergent and used in cosmetics. The goal of this study is preparation and optimization of isradipine-loaded nanoparticles by using silverson emulsifier. A 3-factor, 2-level Box-Behnken design was used to optimize the process parameters including Surfactant (A), Stirring speed (B) and Stirring time (C). Two dependent variables size and drug loading capacitywere measured as responses. Regression equation, Y1 = 253.97-30.45A -2.95 B +0.84 C – 0.71 AB +1.05 AC + 0.44 BC + 8.64 A^2–1.25 B^2– 0.59C2, Y2= 61.12 + 10.15 A + 2.09 B + 0.96 C –0.43 AB - 0.98 AC –0.20 BC -2.76 A^2- 0.84B^2 -0.97 C^2. The interaction between A and B on particle size at a fixed level of C. At low levels of surfactant, Y1 reduced from 295.83 to 285.15 nm. Similarly at high levels of surfactant, Y1 reduced from 235.99 to 228.47 nm. The interactive effect of A and B on the drug loading capacity (Y2) at fixed level of C. At low levels of surfactant, Y2 increases from 45.10% to 52.4%. Similarly, at high levels of A, Y2 increases from 67.7% to 69.1%. The isradipine-loaded nanoparticles were capable of releasing upto 97.31% of drug in 24 h.

Key Words: Silverson emulsifier,Acacia concinna, isradipine-loaded PMMA nanoparticles

INTRODUCTION

The oral route remains the preferred route for drug administration due to its convenience, patient compliance and production costs. In order for a drug to be absorbed into the systemic circulation following oral administration, the drug must be dissolved in the gastric fluids. For poorly water-soluble drugs, this dissolution process acts as the rate-controlling step and therefore determines the rate and degree of absorption. As a result, much research has been conducted into methods ofimproving drug solubility and dissolution rates to increase the oral bioavailability of hydrophobic drugs [1,2]. These include (a) reducing particle size to increase surface area, thus increasing dissolution rate of drug.Isradipine (IS), is a dihydropyridine calcium channel blocker, was chosen as the model drug in this research.

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It is virtually insoluble in water but freely soluble in acetone [3,4] and may be degraded under the light. An IS formulation with improvement of dissolution and photo-instability hence should be investigated. In previous studies, we have successfully enhanced the dissolution rate and controlled release rate of IS using solid dispersion techniques [5, 6]. Recently, Park et al. has developed the inclusion complex of IS and b-cyclodextrin for improvement of photo-instability and dissolution profile [7]. However, there have been few of such studies on nano-sized formulation of IS apart from the research of Verger et al. [4]. In recent years, polymer nanoparticles have received considerable attention as a promising colloidal drug carrier [8]. 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 [9,10],

spontaneous emulsification-solvent diffusion [11,12], supercritical fluid [13], and emulsification-solvent evaporation techniques [14,15]. Polymerization approaches have been popularly studied in recent decades. However, critical problems relating to toxicity, caused by residual monomers or oligomers, and the drug-to monomer cross-reaction may limit potential use. Thus, well-defined pre-formed polymer-based techniques are generally preferred for pharmaceutical applications. Shikakai, (Acacia concinna) is a climbing shrub found in Asia and central and southern India [16, 17]. Pods of Shikakai have traditionally been used for washing hair. Seto Siris (Albizia procera) is a 30 m tall tree found in the sub-Himalayan tracts from Yamuna eastwards to West Bengal, Satpura Range and South India. Its leaves are used as a natural detergent [18].Saponins tend to have a foaming effect and are detergents hence they are soap like nature[19].The fruit is known in India as shikakai "fruit for hair" in its use as a traditional shampoo. In order to prepare it the fruit pods, leaves and bark of the plant are dried, ground into powder, then made into paste. Shikakai powders are non-toxic[20,21].The present endeavor was to develop isradipine nanoparticlesby using *Shikakai*for minimizing particle size and maximizing drug loading capacity.

EXPERIMENTAL

Preparation of isradipine nanoparticles:

Step I: Isradipine nanoparticles were prepared by using emulsion evaporation method. From which, the external phase – shikakai powder extract was first prepared. A measured quantity of shikakai powder was soaked using sufficient amount of distilled water to make up a volume of 100mL (external phase). The shikakai was later filtered 3 times using triple folded muslin cloth.

StepII:Additionally, the measured quantities of isradipine and PMMA were dissolved in 10 ml of dichloromethane. The volume of DCM used should be sufficient to produce a clear solution. The drug and polymer solution was used as internal phase in the process Table 1.

Step III:The clearshikakai solution was placed into a 200mL of beaker; the solution was subjected into high speed stirr (Silverson Emulsifier -removed base plate and emulsor screen). 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 run for 3 hours at 8000rpm.

Step IV:The formed nanoparticles then centrifuged using Lobofuge 200 Biofuge for 15 min at 5000 rpm. The sediment was placed on shallow evaporating dish. The suspension was allowed over hot plate with a constant temperature of 40±0.5ºC. Once the powder was dried, it was collected and packed in air tight container.

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 Isradipine loaded PMMAnanoparticles 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 time intervals of 0.15, 1, 2, 3, 4,5,6,8,10,12,16 and 24 h. 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 325nm. 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

In this design, we reported the successful result on the formulation of isradipine nanoparticles. Through preliminary experiments the Surfactant (A), Stirring speed (B) and Stirring time (C) were identified as the most significant variables influence the particle size and drug loading capacity. Design of experiments (DOE 9) has been used as a powerful approach to reduce the variation in a process and, ultimately, to produce high drug loading capacity with uniform particle size distribution. Among various design approaches, the Box-Behnken design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the process variables on the particle size and drug

loading capacity. 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. These designs are rotatable (or near rotatable) and require 3 levels of each factor.

Seventeen experiments were required for the response surface methodology based on the Box-Behnken design. Based on the experimental design, the factor combinations yielded different responses as presented in Table 2. These results clearly indicated that all the dependent variables were strongly dependent on the selected independent variables as they showed 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 using multiple linear regression analysis for the studied variables are expressed as shown in Table 3.

These equations represent the quantitative effect of Surfactant (A), Stirring speed (B) and Stirring time (C) and their interaction on Particle size (Y1) and Drug loading capacity (Y2). The values of the coefficient 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 terms and quadratic relationship respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. A backward elimination procedure was adopted to fit the data to the quadratic model. Both the polynomial equations were found to be statistically significant (P <0.01), as determined using ANOVA, as per the provision of Design Expert software (DX9).

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

Independent variable	**Levels**				
Variable	Name	Units	Low	Middle	High
A	Surfactant	mg	500	875	1250
B	Stirring speed	rpm	5000	6500	8000
C	Stirring time	h	1	2	3
Dependent variable	**Goal**				
Y1	Size	nm		minimize	
Y2	Drug loading capacity	%		100	

Table-2: Factorial design of isradipine nanoparticle formulations

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	A:Surfactant mg	B:Stirring speed rpm	C:Stirring time h	Size nm	Drug loading capacity %
1	1250	6500	3	230.31	69.3
2	1250	5000	2	235.99	67.7
3	875	8000	3	250.67	64.5
4	875	6500	2	253.14	60.3
5	500	8000	2	285.15	48.2
6	500	6500	1	295.83	47.4
7	875	6500	2	254.11	61.2
8	875	6500	2	254.88	62
9	875	5000	3	257.19	58.8
10	1250	8000	2	230.18	69.1
11	875	6500	2	253.57	61.3
12	875	8000	1	246.18	64.1
13	875	6500	2	254.14	60.8
14	875	5000	1	254.46	57.6
15	500	6500	3	293.47	52.4
16	1250	6500	1	228.47	68.2
17	500	5000	2	291.11	45.1

Table-3: 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	7629.58	9	847.73	94.89	< 0.0001	0.9919
A-Surfactant	7236.65	1	7236.65	810.06	< 0.0001	
B-Stirring speed	88.25	1	88.25	9.88	0.0163	
C-Stirring time	5.61	1	5.61	0.63	0.4541	
AB	5.625E-003	1	5.625E-003	6.297E-004	0.9807	
AC	4.41	1	4.41	0.49	0.5050	
BC	0.77	1	0.77	0.087	0.7770	
A^2	287.78	1	287.78	32.21	0.0008	
B^2	11.16	1	11.16	1.25	0.3007	
C^2	0.20	1	0.20	0.022	0.8867	
Residual	62.53	7	8.93			
Lack of Fit	60.81	3	20.27	46.99	0.0014	

Table-4: ANOVA results of the quadratic model for the response drug loading capacity (Y2)

Source variations	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F	R^2
Model	909.75	9	101.08	44.97	< 0.0001	0.9830
A-Drug	824.18	1	824.18	366.64	< 0.0001	
B-Polymer	34.86	1	34.86	15.51	0.0056	
C-Surfactant	7.41	1	7.41	3.30	0.1123	
AB	0.72	1	0.72	0.32	0.5885	
AC	3.80	1	3.80	1.69	0.2346	
BC	0.16	1	0.16	0.071	0.7973	
A^2	32.07	1	32.07	14.27	0.0069	
B^2	2.94	1	2.94	1.31	0.2907	
C^2	3.92	1	3.92	1.74	0.2281	
Residual	15.74	7	2.25			
Lack of Fit	14.15	3	4.72	11.88	0.0184	

Particle size analysis of isradipine nanoparticles was found to be in the range of 230.31 – 295.83 nm as shown in Table 2. The factorial equation for particle size exhibited a good correlation coefficient and the Model F value of 94.89which implies the model was significant. Values of "Prob> F" less than 0.0500indicate model terms were significant. In this case A, B, C and the quadratic term of A is significant model terms as shown in Table 5. Results of the equation indicate that the effect of A (Surfactant) is more significant than B and 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 and interactive effects of independent variables on the drug loading capacity was further elucidated using the perturbation and 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 response Y1. The 2D response surfaces and the 2D contour plots of the response Y1 are shown in Figure 1 and 2 to depict the interactive effects of independent variables on response Y1, one variable was kept constant while the other two variables varied in a certain range. Variation of actual and predicted values of particle size of the Experimental Design is shown in Figure 3. The shapes of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on particle size at a fixed level of C is shown in Figure 2. At low levels of surfactant, Y1 reduced from 295.83 to 285.15 nm. Similarly at high levels of surfactant, Y1 reduced from 235.99 to 228.47 nm.

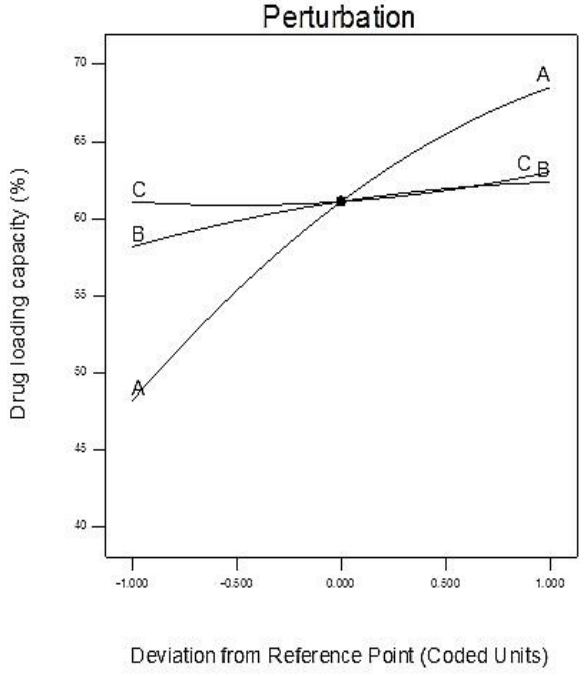

Figure-1: Perturbation plot showing the main effect of surfactant (A), stirring speed (B) and Stirring time(C) on size (Y1)

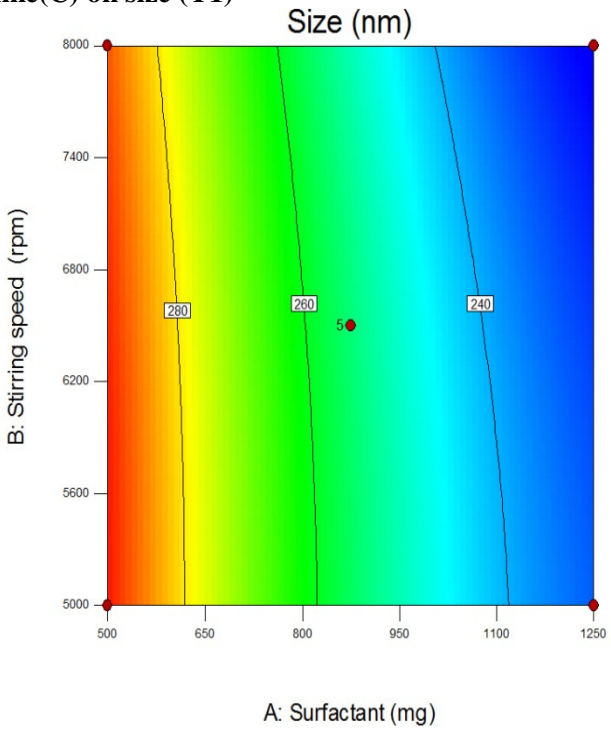

Figure-2: Response surface plot presenting the interaction between the surfactant and stirring speed affecting the particle size at constant stirring time.

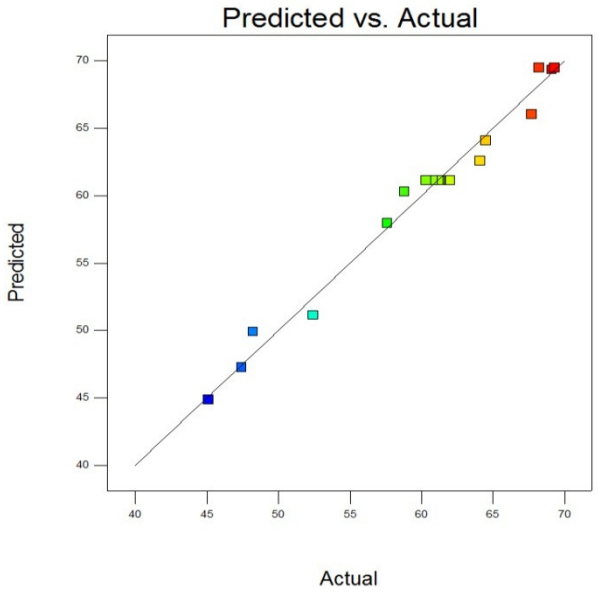

Figure-3: Plot showing the actual and predicted value of particle size

The polynomial equations relating the dependent and independent variables, the process was optimized for the responses. Numerical optimization using the desirability approach was employed to locate the optimal settings of the process variables to obtain the desired responses. Y1 = 253.97-30.45A -2.95 B +0.84 C –0.71 AB +1.05 AC + 0.44 BC + 8.64 A^2–1.25 B^2–0.59C^2, Y2 = 61.12 + 10.15 A + 2.09 B + 0.96 C –0.43 AB - 0.98 AC –0.20 BC -2.76 A^2- 0.84B^2 -0.97 C^2.Optimized conditions were obtained by setting constraints on the dependent and independent variables.

The mathematical model generated for drug loading capacity (Y2) was found to be significant with F-value of 44.97 ($p < 0.0001$) and R^2value of 0.9830. The independent variables A, B, C and the quadratic term of Ahave significant effects on the drug loading capacity, 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 effect of A is more significant than B and C. The influence of the main and interactive effects of independent variables on the drug loading capacity was further elucidated using the perturbation and 2D response surface plots. The perturbation plot (Figure 4) showing the main effects of A, B and C on the drug loading capacity (Y2) of isradipinenanoparticles. This figure clearly shows that A has the main and the major effect on Y2 followed by B which has a moderate effect on Y2 followed by C which has a little effect on Y2. Variation of actual and predicted values of % drug

loading capacity of the Experimental Design is shown in Figure 5. The relationship between the dependent and independent variables was further elucidated using response surface plots. Figure 6 shows the interactive effect of A and B on the drug loading capacity (Y2) at fixed level of C. At low levels of surfactant, Y2 increases from 45.10% to 52.4%. Similarly, at high levels of A, Y2 increases from 67.7% to 69.1%. Three batches of isradipine 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.

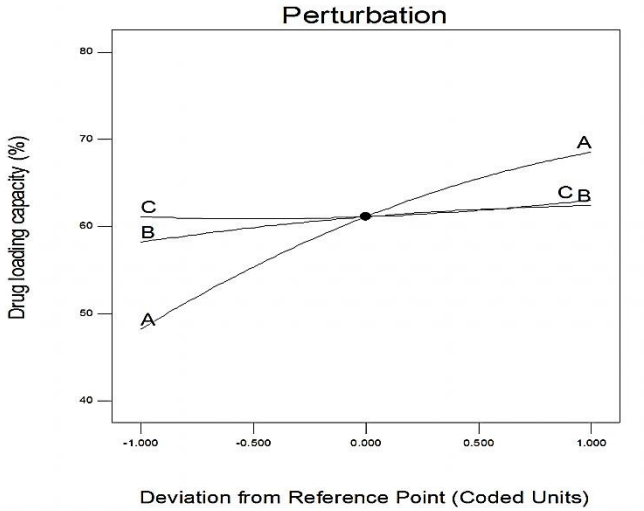

Figure-4: Perturbation plot showing the main effect of surfactant (A), stirring speed (B) and Stirring time (C) on % drug loading capacity (Y2)

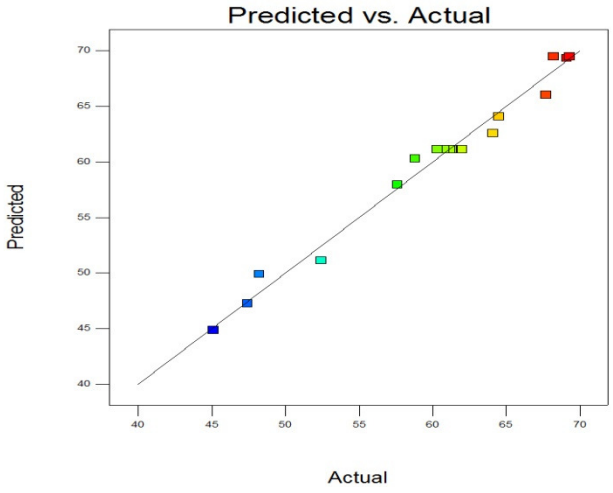

Figure-5: Plot showing the actual and predicted value of % drug loading capacity

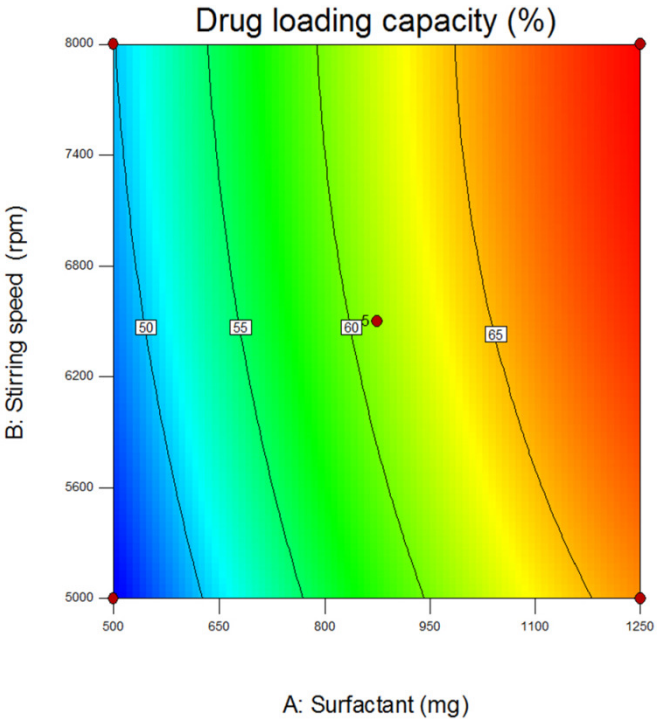

Figure-6: Response surface plot presenting the interaction between the surfactant and stirring speed affecting the % drug loading capacity at constant stirring time.

The formulation was optimized for further studies, on the basis of particle size and high drug loading capacity. The obtained nanoparticles were spherical, uniform sizeand tough in nature (Figure. 7).

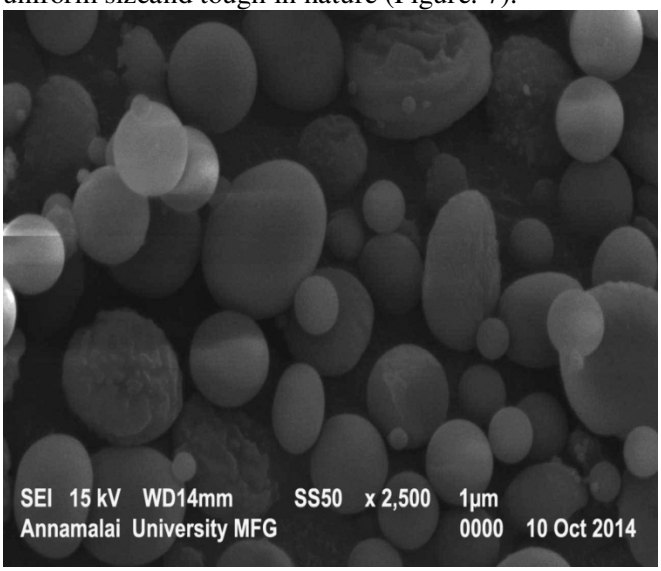

Figure-7: SEM photography of isradipine nanoparticles

The *in vitro* drug release profile of isradipine from nanoparticles from formulations ISN4, ISN 7 and ISN11 were conducted in dissolution medium.The formulations ISN4, ISN 7 and ISN11 containing constant surfactant ratio, stirring speed and stirring speed showed 97.31%, 95.18% and 96.46% release in 24 hours is shown in Figure 8 and 9 .

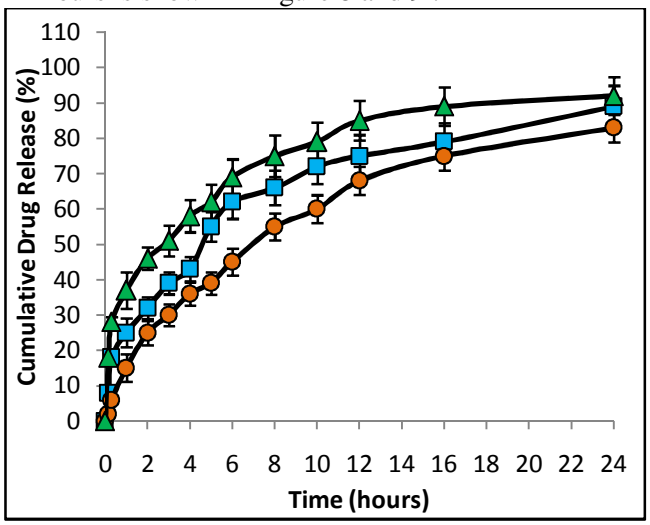

Figure-8: Showing the Drug Release of Isaradipine Nanoparticles

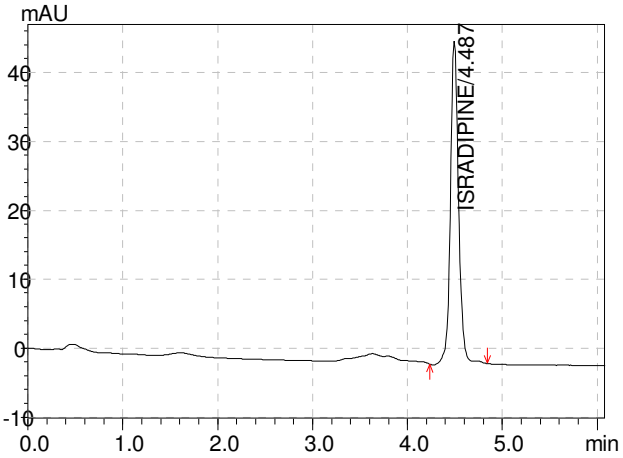

Figure-9: Typical chromatogram of isradipine

The FTIR Spectra analysis of isradipine alone showed that principal peaks where observed at wave number of 3348.51,3244.15,310.48,2978.05, 2945.02, 2796.19, 2109.03,1961.05,1894.41, 1821.36, 1703.03, 1648.70, 1491.41,1366.94, 1309.32,1225.10, 1110.90, 1019.25, 998.30, 866.97, 802.11, 745.14, 669.92, 621.10. FTIR spectra 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, 753.64. The FTIP spectra of PMMA and Isradipine showed that principal peaks where observed at wavenumber of 3626.22, 3526.12, 3439.499, 3348.84, 3244.40, 2925.90, 2595.17, 2413.85, 2050.81, 1962.44, 1648.88, 1225.75, 756.06, 669.41, and 621.95as shown in Figure 10.

The DSC spectra ofisradipine showed endothermic peaks at 164.60 ^{o}C. The polymer PMMA peaks at 119.29 ^{o}C. The physical mixture of isradipine & polymer PMMA has no major changes which at 158.31 ^{o}C (Figure 10).

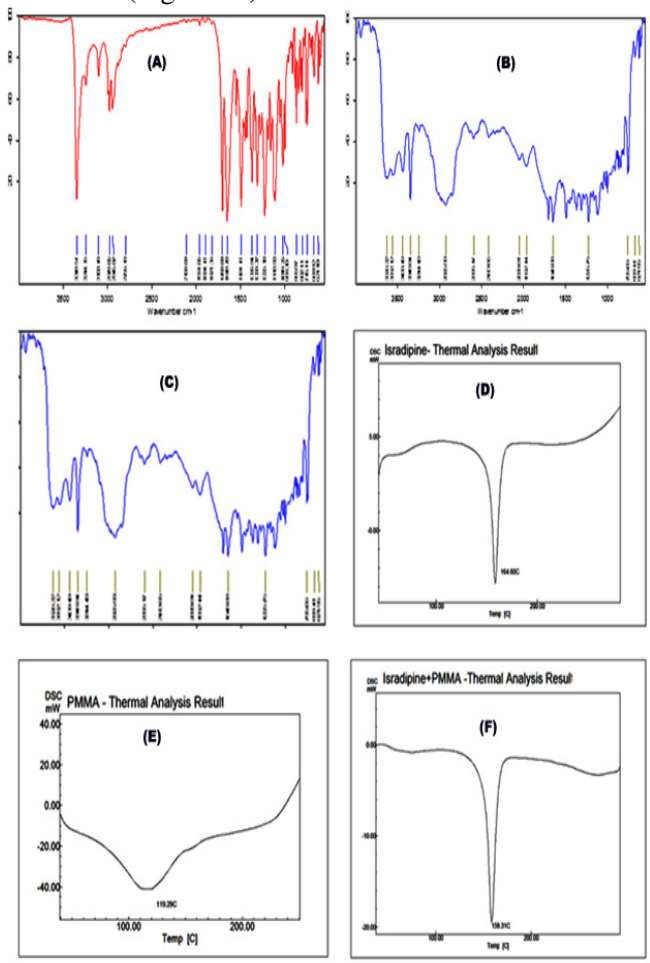

Figure-10: FTIR Spectra of isradipine (A) PMMA (B) isradipine + PMMA (C)DSC Spectra of isradipine (D) PMMA (E) isradipine + PMMA (F)

CONCLUSION

Isradipine loaded PMMA nanoparticles were prepared by the emulsification solvent evaporation method. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size and drug loading capacity. The factorial equation for particle size exhibited a good correlation coefficient and the Model F value of 94.89which implies the model is significant. Values of "Prob> F" less than 0.0500indicate model terms are significant. The mathematical model generated for drug loading capacity (Y2) was found to be significant with F-value of 44.97 ($p < 0.0001$) and R^2value of 0.9830. The independent variables A, B, C and the quadratic term of Ahave significant effects on the drug loading capacity, since the P-values less than 0.0500 represent the significant model In vitro drug release study of optimized formulation (ISN4, ISN7and ISN11) showed 97.31%,95.18% and 96.46% release in 24 hours. These results indicate thatisradipine loaded PMMA nanoparticles could be effective in sustaining drug release for a prolonged period.

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