Research article

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FACTORIAL DESIGN OF NEW SURFACTANT "HIBISCUS ROSASINENSIS LEAF" FOR NANOPARTICLES: GLIMEPIRIDE VS HIBISCUS ROSASINENSIS

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ABSTRACT

Glimepiride is used for the treatment of type 2 diabetes mellitus, one of the third generation sulfonylurea drugs having poor aqueous solubility, slow dissolution rate and low elimination half-life. The objective of the present study was to prepare glimepiride loaded PMMAnanoparticles by using *Hibiscus rosasinensis*leaf extract as a natural surfactant. Prepared glimepiride nanoparticles were optimized for the size and yield by 3 factors 2 level factorial design.The prepared glimepiride formulations were further evaluated for particle size, % of yield, FTIR, DSC, SEM and RPHPLC analysis. The interactive effect of A and B on the practical yield (Y2) at fixed level of C. At low levels of A, Y2 increases from 60.2% to 67.5%. Similarly, at high levels of A, Y2 increases from 80.3% to 90.4%. The interaction between drug and polymer on particle size at a fixed level surfactant viscosity,at low levels of A, Y1 reduced from 246.75 to 241.16 nm. Similarly at high levels of A, Y1 reduced from 288.47 to 282.19 nm.

Keywords: *Hibiscus rosasinensis,* Glimepiride nanoparticles, RPHPLC, 3 factors 2 level factorial design

INTRODUCTION

*Hibiscus rosasinensis*flowers and leaves are available all over tropical countries. The qualitative phytochemical screening procedure was performed on each extract. Phytochemical study reveals that alkaloids, tannins, saponins, triterpenoids, coumarins, steroids, flavonoids were present in the three extracts [1].Concurring to the (BCS), nearly 40% of drugs in the industry pipeline belongs to the BCS class II having low aqueous solubility and high permeability and class IV having low solubility and low permeability. Drugs belong to these classes have low bioavailability [2-4]. Therefore, these drugs possess formulations and delivery problems [5]. For the treatment of diabetes, type 2, the use of oral anti-diabetic drugs increases rapidly [6]. Glimepiride is used for the treatment of type 2 diabetes, one of the third generation sulfonylurea drug having a poor aqueous solubility, slow dissolution rate and low elimination half-life (2-3 hrs) [7]. Glimepiride have a number of advantages over other members of sulfonylurea, currently in the market such as lower dosages, fast onset of action and lower C-peptide level of insulin, this is because of less secretionof insulin and more pronounced extra pancreatic effects [8].

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Glimepiride acts by binding to the specific site on pancreatic β-cells and block the ATP-Dependent potassium channels to stimulate the insulin release. Due to the short elimination half-life frequent dosing is required, which leads to the adverse effects such as headache and gastrointestinal disorders [9].Due to its short elimination half-life repeated doses are required which may cause different side effects to avoid the repeated dosing and enhance the bioavailability of glimepiride sustained release nanoparticles were developed.

Nanotechnology is promising application in drug delivery system that accounts for the main part of nanomedicine [10].The present aim was to formulate and optimization of glimepiride nanoparticles by using *Hibiscus rosasinensis* leaf extract for minimizing particle size and maximizing percentage of yield.

EXPERIMENTAL METHODS

Materials:

The leaves of Hibiscus rosa-sinensis was collected from AIMST Campus, Malaysia.Glimiperide was obtained as a gift sample from Ranbaxy (M) SdnBhd, (Malaysia). PMMA was purchased from Sigma Aldrich (USA).All other chemicals and reagents were of analytical grade. HPLC grade water was used throughout the studies.

Method:

The *Hibiscus rosasinensis*leaf extractwas subjected to saponification value and viscosity studies. The prepared nanoparticles were evaluated for particle size, % of yield.

In-Vitro drug release studies:

Drug release studies of the glimepiridenanoparticles were performed, in a tablet (Electrolab tablet dissolution test apparatus) at 37±0.5ºC and stirring rate of 75 rpm. The release studies were carried out in a 900 mL dissolution medium of pH 1.2 for the first 2hrs, and continued in phosphate buffer pH 6.8 up to 12 hrs. Change in pH was made by the addition of 0.2M tribasic sodium phosphate. All dissolution media was contained 0.2% Sodium lauryl sulfate. Samples (5 ml) were collected periodically and replaced with equal volume of fresh dissolution medium on each occasion. 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, ACN 0.5%: TEA(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.

Formulation of glimepiride nanoparticles:

The nanoparticles were prepared by using emulsion evaporation method. A measured quantity of hibiscus leaves was crushed by hands using sufficient amount of distilled water to make up a volume of 100mL (external phase). The hibiscus extract was later filtered 3 times using triple folded muslin cloth (symmetrically twice). Additionally, the measured quantities of glimepiride and PMMA were dissolved in DCM. 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.The clear hibiscus extract was poured into the 200 mL beaker and mixed by using silverson emulsifier (with removed mixing head) with 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 formed nanoparticles then centrifuged using Lobofuge 200 Biofuge for 10 min at 4000 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 is dried, it was collected and packed in air tight container.

Table-1: List of dependent variable and independent variable in central composite 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	400	1700	3000
Dependent variable	**Goal**				
Y1	Size	nm		minimize	
Y2	Yield	%		100	

Table-2: Factorial design of glimiperide nanoparticle formulations

Run	Factor 1 A:Drug mg	Factor 2 B:Polymer mg	Factor 3 C:Surfactant viscosity cps	Response 1 Size nm	Response 2 yield %
1	75	200	3000	275.22	76.5
2	75	100	3000	262.33	70.4
3	100	150	3000	285.16	89.2
4	100	150	400	287.41	87.5
5	75	150	1700	268.14	72.8
6	50	150	400	246.75	63.1
7	50	150	3000	241.16	65.3
8	75	150	1700	267.91	72.4
9	75	150	1700	268.1	72
10	75	200	400	275.17	74.1
11	75	100	400	261.11	69.7
12	50	200	1700	251.12	67.5
13	75	150	1700	268.15	72.8
14	50	100	1700	243.13	60.2
15	75	150	1700	267.88	72.6
16	100	100	1700	282.19	80.3
17	100	200	1700	288.47	90.4

Table-3: Showing the polynomial equation of Y1 and Y2 response

Response	Regression equation
Y1	+267.02 + 20.13A + 5.15B -0.82C
Y2	+72.52 +11.41A + 3.49B + 0.88C + 0.70AB – 0.12AC + 0.43BC + 2.84A^2 -0.76B^2 +0.91C^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	3460.72	3	1153.57	204.62	< 0.0001	0.9793
A-Drug	3242.94	1	3242.94	575.23	< 0.0001	
B-Polymer	212.39	1	212.39	37.67	< 0.0001	
C- surfactant viscosity	5.40	1	5.40	0.96	0.3458	
Residual	73.29	13	5.64			

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

Source variations	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F	R^2
A-Drug	1188.16	9	132.02	92.50	< 0.0001	0.9917
B-Polymer	1041.96	1	1041.96	730.07	< 0.0001	
C- Surfactant viscosity	97.30	1	97.30	68.18	< 0.0001	
AB	6.13	1	6.13	4.29	0.0770	
AC	1.96	1	1.96	1.37	0.2796	
BC	0.063	1	0.063	0.044	0.8402	
A^2	0.72	1	0.72	0.51	0.4998	
B^2	33.96	1	33.96	23.79	0.0018	
C^2	2.43	1	2.43	1.70	0.2330	
Residual	3.53	1	3.53	2.47	0.1600	

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

Independent variables	**Values**	**Predicted values**		**Batch**	**Observed values**	
		P. Size (Y1)	**P.Yield (Y2)**		**P. Size (Y1)**	**P. Yield (Y2)**
Drug	75	267.024	72.52	D8	267.12	72.3
Polymer	150			D13	268.00	72.1
Surfactant viscosity	1700			D15	267.57	72.5

RESULT AND DISCUSSION

Optimization of process variables for the glimepiride nanoparticles:

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 viscosity of surfactant were identified as the most significant variables within the range of 50-100 mg, 100-200 mg and 400-3000 cps, 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 (mean particle size and % of yield). 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 [11]. 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 dependentvariables were entered in Table 2. The response surfaces of the variables inside the experimental domain were analyzed using Stat-Ease Design-Expert software (DX9). Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

In this work, we report the successful result on the formulation of glimepiride nanoparticles. Through preliminary experiments the Drug (A), Polymer (B) and Viscosity of surfactant (C) were identified as the 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 process and, ultimately, to produce high % yield 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% yield. 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 [12]. 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 indicate that all the dependent variables are strongly dependent 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 using multiple linear regression analysis for the studied variables are expressed as shown in Table 3.

The quadratic model equations represent the quantitative effect of Drug (A), Polymer (B) and Viscosity of surfactant (C) and their interaction on Particle size (Y1) and % yield (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 (Table 4 and 5), as per the provision of Design Expert software (DX9).

Response 1 analysis of glimepiride nanoparticles was found to be in the range of 241.16 – 288.47 nm as shown in Table 2. The factorial equation for particle size exhibited a good correlation coefficient (1.000) and the Model F value of 204.62 which implies the model is significant. Values of "Prob> F" less than 0.0500indicate model terms are significant. In this case A, B, C and the quadratic term of A and B are significant 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 and interactive effects of independent variables on the practical yield was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on particle size are as shown in Figure 2. It is found that all the variables are having interactive effects for the response Y1. The 3D 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. 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 A, Y1 reduced from 246.75 to 241.16 nm. Similarly at high levels of A, Y1 reduced from 288.47 to 282.19 nm.

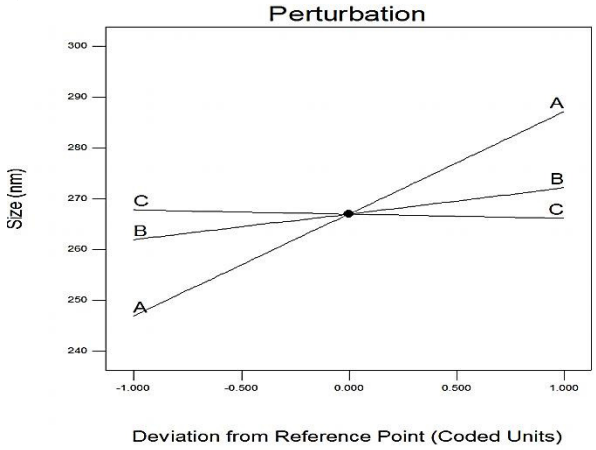

Figure-1: Perturbation plot showing the main effect of drug(A), polymer (B) and surfactant viscosity (C) on particle size (Y1)

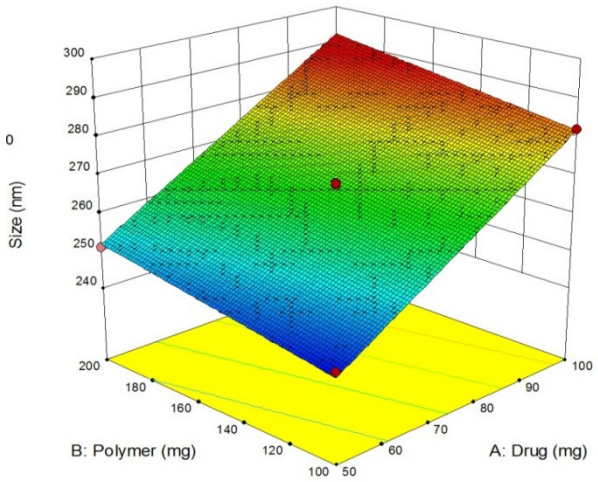

Figure-2: Response surface plot presenting the interactionbetween the drug and polymer affecting the particlesize at constant surfactant viscosity.

The independent variables A, B, C and the quadratic term of A and Bhave significant effects on the % yield, since the P-values less than 0.0500 represent the significant model terms as shown in Table 5. The mathematical model generated for % yield (Y2) was found to be significant with F-value of 92.50 ($p < 0.0001$) and R^2 value of 0.9917. Results of the

equation indicate that the effect of A and B is more significant than C. The influence of the main and interactive effects of independent variables 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 (Y2) of glimepiridenanoparticles. 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. The relationship between 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 (Y2) at fixed level of C. At low levels of Drug, Y2 increases from 60.2% to 67.5%. Similarly, at high levels of A, Y2 increases from 80.3% to 90.4%.

D8, D13 and D15 batches code of glimepiride 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.

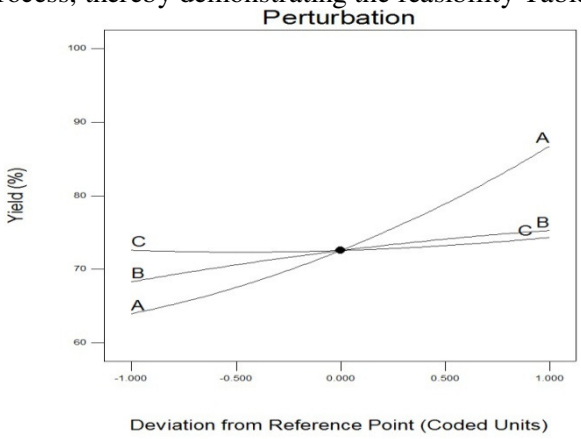

Figure-3: Perturbation plot showing the main effect of drug (A), polymer (B) and surfactant viscosity (C) on percentage of yield (Y2)

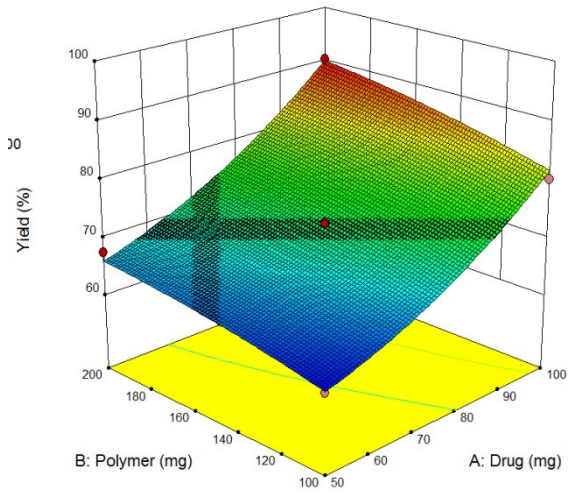

Figure-4: Response surface plot presenting the interaction between the polymer and drug affecting the percentage of yield size at constant surfactant viscosity.

The FTIR spectral analysis of glimepiride, pure drug alone, shows that the principal peaks were observed at wavenumber of (unit in cm^{-1}) 3369.18, 3289.00, 3134.81,2930.33, 2861.40, 1657.17, 1552.91, 1437.21, 1406.76,1392.71, 1340.42, 1272.14, 1240.92, 1208.19, 1149.96,1114.15, 1082.50, 1036.13, 1014.07, 999.15, 969.72,950.30,892.03,877.21,844.20,822.68,797.38,783.92, 761.68,746.55, 732.05, 709.81, 686.92, 622.70, 559.04. 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, 753.64. The FTIR spectral analysis of glimepiride and PMMA shows that principal peaks where observed at wavenumber of 3620.69, 3552.35, 3438.51, 3369.74, 3289.02,3133.11,2930.91,2842.96,2049.75,1708.02,1674.44,1542.91,1445.63,1346.52,1275.37,1153.53,1081.35,1036.86,999.56,877.71,687.52,617.36 (Figure 5).

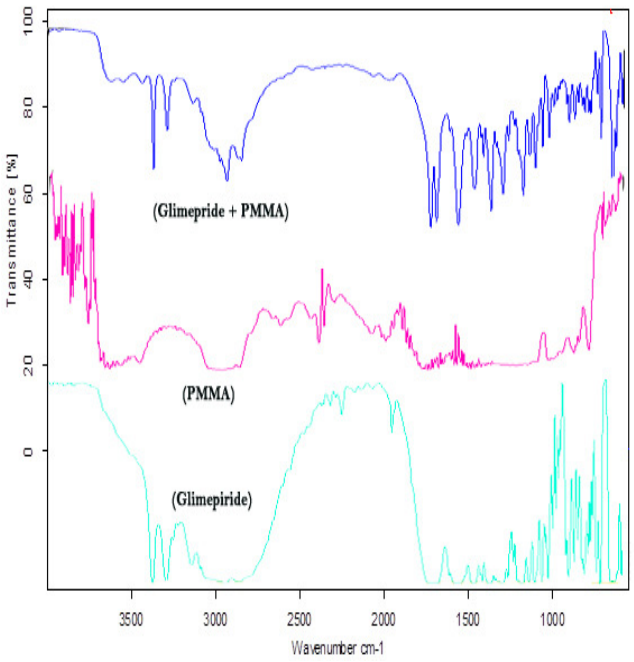

Figure-5: FTIR Spectra of physical mixture drug and drug + polymer

The DSC spectral analysis of glimepiride shows the endothermic peak at 215.17 ^{o}C. Pure PMMA shows the peak at 119.29. In the DSC spectral analysis of the combination of glimepiride and PMMA, it shows the endothermic peak at 211.72 ^{o}C. There is no significant change in the endothermic value of pure drug and drug plus polymer combination Figure 6. This indicates that the pure drug (glimepiride) is compatible with polymer (PMMA).

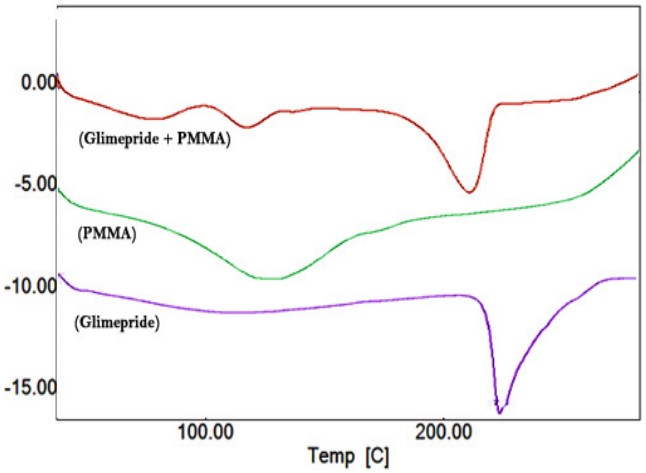

Figure-6: DSC Spectra of physical mixture and drug + polymer

Morphology of the prepared glimepride nanoparticles was examined by SEM analysis. The results show that glimepride loaded NPs were predominantly spherical. The distinct, porous nature and spherical shape of the NPs is evident from their SEM photographs (Figure 7).

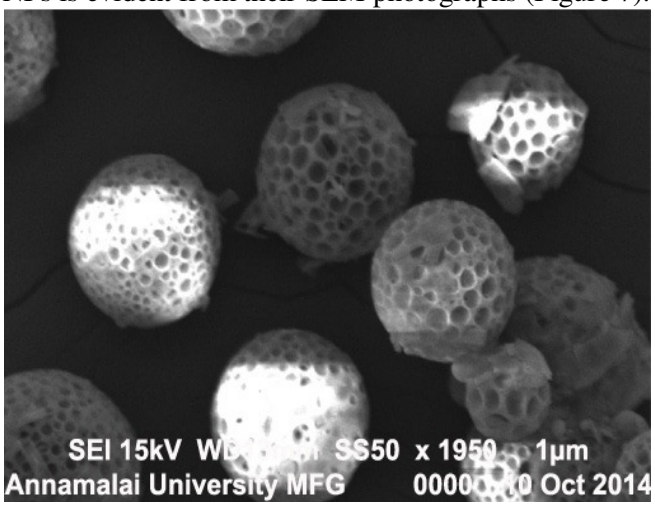

Figure-7: SEM photography of glimiperide nanoparticles

The in vitro *drug release of glimepiride* from the *nanoparticles*formulations D8, D13 and D15 released 80.98%, 85.72% and 87.33% at the end of 24th hour(Figure 8 and 9).

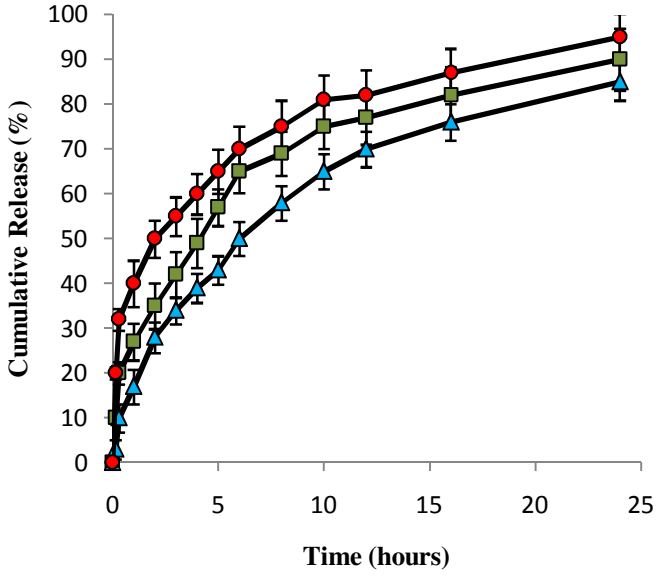

Figure-8: Showing the Drug Release of Glimepiride Nanoparticles

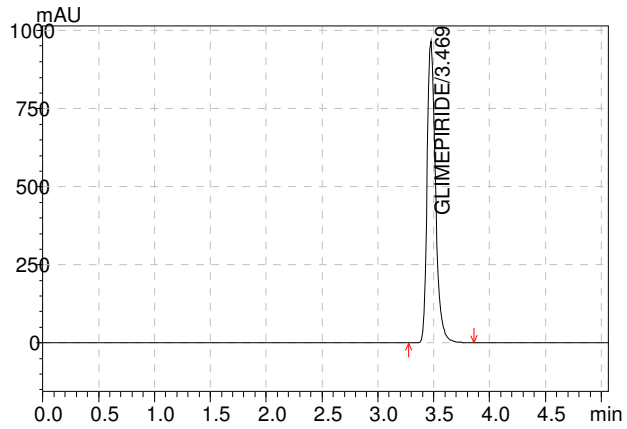

Figure-9: Typical chromatogram of Glimepride

CONCLUSION

Glimepiride loaded nanoparticles were prepared by the solvent evaporation method. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size and high % yield. Drug: polymer ratio and surfactant viscosity were found to influence the particle size and % yield of glimepiride loaded PMMA nanoparticles but the Drug:Polymer ratio had greater influence on both dependent variables (Particle size and % yield) as compared to surfactant viscosity. In vitro drug release study of selected factorial formulations (D8, D13, and D15) showed 80.98 %, 85.72%, and 87.33% release respectively up to 24 hrs.

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