Formulation and Process Optimization of polymeric microsphere formulation of Glibenclamide using solvent evaporation technique

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ABSTRACT:

Glibenclamide, a second generation sulphonyl urea, is to be more efficacious than first generation medications.A common oral anti-diabetic drug used to treat non-insulin-dependent diabetes mellitus is Glibenclamide. As a result of its 4-6 hour biological half-life, it needs to be administered more than once to maintain plasma concentration. This results in discomfort for the patient and changes in plasma medication concentration, which may result in diminished therapeutic effects or harmful effects.

With the aid of the solvent evaporation microencapsulation process, controlled release microspheres and PLGA polymer have been prepared. Effects of preparation conditions, drug: polymer ratio (X_1), concentration of poly (vinyl alcohol) (PVA) in aqueous phase (X_2), homogenization speed (X_3) upon the properties of PLGA microspheres containing Glibenclamide were examined. The most effective formulation was derived utilising response surface methodology and optimised polynomial equations. The microspheres were characterized for particle size distribution (PSD), surface morphology, drug excipient compatibility, drug entrapment, and *in vitro* drug release.

The antidiabetic effect of polymeric microsphere formulation of Glibenclamide demonstrated using Alloxan-induced diabetes mellitus model in rats. Glibenclamide microsphere formulation consistently reduced blood sugar levels up to the ninth day, indicating that drug release from the microsphere formulation was controlled up to the ninth day. Based on the findings, it is possible to conclude that Glibenclamide microsphere formulation has an anti-diabetic effect when administered intramuscularly. According to the results, a single dose of 'Glibenclamide microsphere formulation' normalised blood sugar levels in rats.

Keywords: Glibenclamide, microsphere, diabetes mellitus, blood glucose, alloxan

INTRODUCTION:

In the development Polymeric microspheres for parenteral drug delivery systems, sustained release of medicines for longer systemic therapeutic effects following subcutaneous (SC) or intramuscular (IM) administration is the main goal.

Microspheres are made from biocompatible and biodegradable polymers.The final product contains microspheres in a dry powder form that are either created as the result of the manufacturing process or by removing the solvent/dispersion liquid medium after the microspheres have been formed through lyophilization or filtration with final drying. A liquid diluent can be provided in a separate container or in the liquid compartment of a dual-chamber prefilled syringe. This liquid diluent is used to reconstitute a microsphere product before administration.

Many protein-loaded microspheres are available, including triptorelin (TrelstarTM Depot), octreotide acetate (Sandostatin® LAR), somatropin (Lupron Depot®), and leurpolide (Lupron Depot®). There are several small-molecule microspheres that have received US FDA approval for use as depot formulations to release medications over extended periods of time, ranging from weeks to months, including risperidone (RispersalConsta) and naltrexone (Vivtrol).[1–3]

The widespread development of long-acting parenteral formulations (LAPFs), with the goal of enhancing pharmaceutical pharmacokinetics and therapeutic efficacy, was spurred by the need for long-term treatments for chronic illnesses. [4] LAPFs have been demonstrated to improve treatment outcomes by increasing patient adherence and lengthening the half-life of medications. [5]

Diabetes mellitus (DM) is one of the world's most significant health issues at the moment. [5] It has been documented that the diabetes prevalence is steadily increasing, and that 6.4% of individuals in the world suffer diabetes, with 285 million persons estimated to have the disease in 2010 and perhaps 439 million by 2030. In developing countries, the prevalence of adult diabetes will rise by 69% between 2010 and 2030, while it will rise by 20% in wealthy nations. [6] By 2030, diabetes will be the seventh for the most common cause of death, according to WHO forecasts. [7]

The pancreas produces insulin, which is the main hormone responsible for elevated blood sugar levels used as a type of protection. In type 2 diabetes, insulin is not produced enough in response

to spikes in blood sugar, such as those that occur after meals. A common oral anti-diabetic drug used to treat non-insulin-dependent diabetes mellitus is Glibenclamide.

Glibenclamide, a second generation sulphonyl urea, is to be more efficacious than first generation medications. The primary pancreatic beta cells, which are in responsible of releasing insulin, are stimulated. The beta cells begin to produce more insulin as a result. Hence, patients with type 2 diabetes benefit from lowering blood sugar levels.As a result of its 4-6 hour biological half-life, it needs to be administered more than once to maintain plasma concentration. This results in discomfort for the patient and changes in plasma medication concentration, which may result in diminished therapeutic effects or harmful effects.

The manufacture of drug-containing microspheres from biodegradable polymers has been described using a variety of approaches. [8,9] The characteristics of the polymer and the medication, the region of pharmacological action, and the length of therapy all have a role in the approach that is chosen. [10]

Using the single-emulsion approach, emulsion solvent extraction and evaporation techniques include: It has been used to encapsulate hydrophobic medications via the o/w emulsification technique, albeit they may diffuse out or partition from the dispersed oil phase into the aqueous, resulting in poor encapsulation efficiencies. [11] The drug is dissolved or suspended in the polymer solution, which is dissolved in a volatile organic solvent that is immiscible with water, such as dichloromethane. The resulting mixture is emulsified in a sizable amount of water when an emulsifier is present. When the solvent in the emulsion is extracted in a substantial amount of water or evaporates at high temperatures, compact microparticles are created. The solvent removal rate reported to change the form of microparticles is calculated using the medium temperature, the polymer's solubility, and the solvent utilised. [12]

To analyse and comprehend the process factors and determine the ideal mix of them to produce a product with desired properties, experimental procedures like factorial designs can be utilised. In order to evaluate the influence of individual process variables, all elements were investigated in all conceivable combinations with the least amount of experimentation and effort. To achieve the best formulation, this optimisation technique creates model equations for the investigated responses over the experimental design (s). [13]

RSM, or response surface methodology, is a method frequently employed in the development of drug delivery systems. The process, which is based on the design of experiments (DoEs) principle,

uses a variety of experimental designs, creates polynomial equations, and maps the response over the experimental domain to identify the best formulation (s). [14– 19]

Given the aforementioned information, glibeclamide was employed in the current investigation to treat diabetes using a microsphere formulation. The effect of formulativeparameters such as drug: polymer ratio (X_1), concentration of poly (vinyl alcohol) (PVA) in aqueous phase (X_2), homogenization speed (X_3) on responses particle size distribution (PSD), percentage yield, drug entrapment, and *in vitro* release kinetics were investigated through Box–Behnken design of response surface methodology. The optimal formulation was used to determine antidiabetic effect of polymeric microsphere formulation of Glibenclamide demonstrated using Alloxan-induced diabetes mellitus model in rats.

MATERIALS AND METHODS

Materials:

Biodegradable polymer, poly(lactide-co-glycolide), (Evonik Degussa, Mumbai, India). Glibenclamide API (GLI) was obtained as a gift sample from Acron Pharmaceuticals, India. Methylene chloride (DCM) (Finar Limited, India).N-Methyl-2-Pyrrolidone (Avantor Performance Materials India Limited, India).Poly vinyl alcohol (Merck Pvt. Ltd., Mumbai, India), Acetonitrile (Merck Pvt. Ltd., Mumbai, India).Chemicals and solvents of the highest standard for high-performance liquid chromatography (HPLC) were opted for the studies. Throughout the trial, distilled water that had just been prepared was used. 3^3Box–Behnken designwas conducted using Design-Expert® 12 software from state-ease, Inc.

Methods:

PreparationofGlibenclamidemicrospheres by Emulsion solvent evaporation/extraction Methods:

The solvent evaporation/extraction process using o/w emulsions was used to formulate the Glibenclamide microspheres.In brief, the appropriately weigh quantity 1 gm of Glibenclamide was dissolved in 4 mL N-Methyl Pyrrolidone (NMP) which used as miscible solvent for preparation of drug phase, PLGA quantity (in different ratio of drug : PLGA- 1:2; 1:3;1:4) dissolved in 32 mL dichloromethane. Drug phase prepared in NMP and polymer phase prepared in DCM. Drug phase is mixed with the polymer solution (O). The Drug polymer solution in organic solvents added to aqueous solution (W) containing different percentage of polyvinyl

alcohol (0.5, 1.0, 1.5 % w/v). Organic phase added through a long, narrow nozzle and agitated vigorously with a homogenizer(Silverson Machines Ltd, England) at different speed (1000, 2000, 3000 rpm). The resulting O/W emulsion was kept under stirring using magnetic stirrer to remove N-Methyl Pyrrolidone (NMP) by extraction and dichloromethane (DCM) by evaporation. The semi-dry microspheres are obtained. These microspheres filtered through 2 micron sieve, washed twice with water and collected. The microspheres dried using lyophilizer (Virtis by SP Scientific).The microspheres were transferred to glass vials and stored there at 2 to 8°C in preparation for further investigation.

Characterization of Glibenclamide microspheres:

UV spectrophotometry:

Standard stock solutions of GLI was prepared in acetonitrile and scanned spectrophotometrically over the range of 200–400nm with a double beam spectrophotometer (UV-1800, Shimadzu, Japan), against the respective blank, to determine the wavelength of maximum absorbance (λmax). The calibration curve was constructed at concentrations range 2-16 µg/ml. Each solution's absorbance was assessed at a wavelength of 230 nm. The calibration curve was constructed for GLI by plotting absorbance versus concentration at 230 nm wavelength.

Fourier transformsinfrared(FTIR) spectroscopic analysis:

FTIR is widely used to detect any drug–polymer interaction by matching the drug IR spectra with the reference standard spectra. Drug and polymer interaction is indicated by changes in frequency and peaks.[20]

FTIR sprecta of GLI and PLGA polymer obtained using FTIR sprectrometer. The measurement performed over the range of 400 – 4000 cm^{-1}. Around 5 mg of fine powder mixture placed on diamond crystal assembly. Pressure knob adjusted to produce pressure on sample for analysis. The sample spectrum analysed over the range of 400 – 4000 cm^{-1}.

Particle size distribution:

The microspheres sample was weighted in a glass vial and it wetted by addition of diluent. External sonication performed on suspended form of microsphere sample for 5 min.Dispersion sample was analyzed by using laser diffraction particle size analyzers (Malvern Mastersizer 3000).

Entrapment efficiency

GLI loaded microspheres were weighed into a volumetric flask. The microspheres were dissolved by adding small quantity of acetonitrile (ACN) to the flask. The obtained solution was further diluted to approximately 70% using ACN and sonicated for 15 min. Further, the volume was made up by ACN. Solution filtered through syringe filter (0.45-micometer PVDF) before analysis. To test the materials, the UV Spectrophotometer was employed (UV-1800, Shimadzu, Japan). The drug's concentration as estimated by the standard curve.

%Entrapment = (actual content/theoretical content) X 100

***In vitro* drug release-testing methods**

In-vitro drug release Drug release was carried out utilising a bottle rotation equipment (Make: Electro lab). Accurately weighed microspheres were added into glass vials. Dissolution media added to the above glass vials, stoppered using rubber stopper and sealed by crimper. Parameters were set and the apparatus was started on 12 RPM at 37°C (± 0.5°C). Device halted at a predetermined time. The bottle was removed from the assembly, and a bottle was kept to settle down the microspheres. Supernatant solution was withdrawn without disturbing settled microspheres. Solution filtered through syringe filter (0.45-micometer PVDF) before analysis. Fresh dissolution media added to the bottles & again fixed to assembly. The UV Spectrophotometer was used to evaluate the samples (UV-1800, Shimadzu, Japan). The concentration of drug determined using the standard curve.

Particle morphology using Scanning Electron Microscopy:

SEM results in a higher resolution compared to that possible using LM, and the images obtained are three-dimensional. The higher resolution of SEM is due to the use of electrons to provide topographic and 3-D images. [21] Double-sided adhesive conductive carbon tape was cut and affixed over sample holder. Glibenclamide -loaded PLGA microspheres were sprinkled over carbon tape. The excess sample removed by gently tapping of sample holder and using compressed air spray. An autofine coater (JFC-1100, JEOL Ltd.) was used to coat platinum vapours over the sample. We used an electron microscope to observe the GLI-loaded PLGA microspheres (SEM, JSM-6060LA, JEOL Ltd.).

Experimental design and optimization

Optimizing and designing experiments the variables in this study were optimised using a Box Behnken design. In this design, 3 factors: drug: polymer ratio (X_1), concentration of poly (vinyl alcohol) (PVA) in aqueous phase (X_2), homogenization speed (X_3) were evaluated, each at 3levels: low,medium and high (Table 1) and experimental trialswere performedat all 14 possible combinations as depicted. Various ratio of drug: polymer ratio (X_1) such as 1:2, 1:3, 1:4; concentration of poly (vinyl alcohol) (PVA) in aqueous phase (X_2) 0.5%, 1.0%, 1.5% and homogenization speed (X_3) such as 1000, 2000, 3000 were selected as independent variables. Percentage entrapment (Y_1),Mean particle size (Y_2) and % Drug release at day 1 (Y_3), day 5 (Y_4) and day 9 (Y_5) were selected as dependent variables.

With Design Expert software, data from all formulation trials was processed to create the study design and the response surface plots (Stat-Ease Inc., Minneapolis, Minnesota). Table 2 displays the design matrix with the causes and responses under investigation. Using the programme, polynomial models with linear, interaction, and quadratic terms were created for each response variable. Based on comparisons of numerous statistical characteristics, such as the regression coefficient (R^2), coefficient of variation (CV), and adjusted regression coefficient, Design Expert software gives the best-fitting model (adj. R^2). Using Design Expert software, perform an analysis of variance (ANOVA) to determine whether any particular factors had a significant impact on the response regression coefficients.

The mathematical model equation for optimisation including independent variables and their interactions for different measured responses produced by the Box Behnken design is:

When Y_i is the dependent variable, b_0 is the intercept, b_1 through b_{33} are the regression coefficients, and X_1, X_2, and X_3 are the independent variables, the equation is written as follows. [22]

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Software produced response surface plots were used to show how the dependent and independent variables interacted.Then, to create new formulations with the desired parameters, a numerical optimisation technique applying the desirability technique and a graphical optimisation technique employing overlay plots were applied.

Table 1 :Box-Behnken design input variables for GLI loaded PLGA microspheres

Design Summary			
Independent variables	**X_1**	**X_2**	**X_3**
Level	D:P Ratio	% PVA	Speed
-1	1:2	0.5%	1000
0	1:3	1.0%	2000
+1	1:4	1.5%	3000
Dependent variables			
Percentage entrapment (Y_1)			
PSD Mean particle size (Y_2)			
Drug release at day 1 (Y_3)			
Drug release at day 5 (Y_4)			
Drug release at day 9 (Y_5)			

Table 2: Obtained Responses for Box-Behnken Design of GLI loaded PLGA microspheres

Trial Run No.	**Actual Value**			**Responses**				
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5
	D:P Ratio	% PVA	Speed	%DE	PSD	Day-1	Day-5	Day-9
1	01:02	1	4000	41.34	11.15	15.99	78.54	108.00
2	01:04	1.5	3000	47.76	29.23	5.21	32.12	78.20
3	01:03	1	3000	48.31	25.20	10.71	58.23	100.80
4	01:04	1	2000	51.75	49.33	4.29	29.23	75.20
5	01:03	1	3000	47.46	27.45	10.58	58.47	101.40
6	01:02	0.5	3000	45.44	17.78	9.12	52.10	100.20
7	01:04	0.5	3000	46.02	25.73	3.52	20.34	68.20
8	01:04	1	4000	44.61	15.94	8.38	40.21	81.20
9	01:02	1.5	3000	46.77	15.52	14.12	69.84	105.20
10	01:02	1	2000	49.39	36.96	10.28	55.45	96.80
11	01:03	1.5	2000	46.82	48.96	9.41	50.20	86.10
12	01:03	0.5	2000	46.67	48.60	6.59	33.51	78.60
13	01:03	0.5	4000	42.01	15.53	13.40	59.62	102.60
14	01:03	1.5	4000	44.07	14.19	15.82	65.80	105.26

Study of*in-vivo* antidiabetic effect using Alloxan-induced diabetes mellitus model in rats:

The study was performed to provide information on the effect of Glibenclamide microsphere formulation and to investigate the therapeutic effect in Sprague–Dawley (SD) rats. The test item formulation was administered as a single dose intramuscularly. SD rats were divided randomly and assigned to one of five subgroups. Rats were given a single dosage of Alloxan to cause diabetes. The determination of blood glucose level after 48 hours confirmed diabetes. Blood glucose levels were measured up to 9 days after the test compound, standard drug and drug solution were administered in a single dose to each treatment group.

Group	**Treatment**
I	Negative Control (10ml/kg p.o. Dist. water)
II	Positive control (Alloxan 150mg/kg i.p.)
III	(Alloxan 150mg/kg i.p.) + Glibenclamide Microsphere formulation i.m.
IV	(Alloxan 150mg/kg i.p.) + Glibenclamide drug solution i.m.
V	(Alloxan 150mg/kg i.p.) + Glibenclamide tablet p.o.

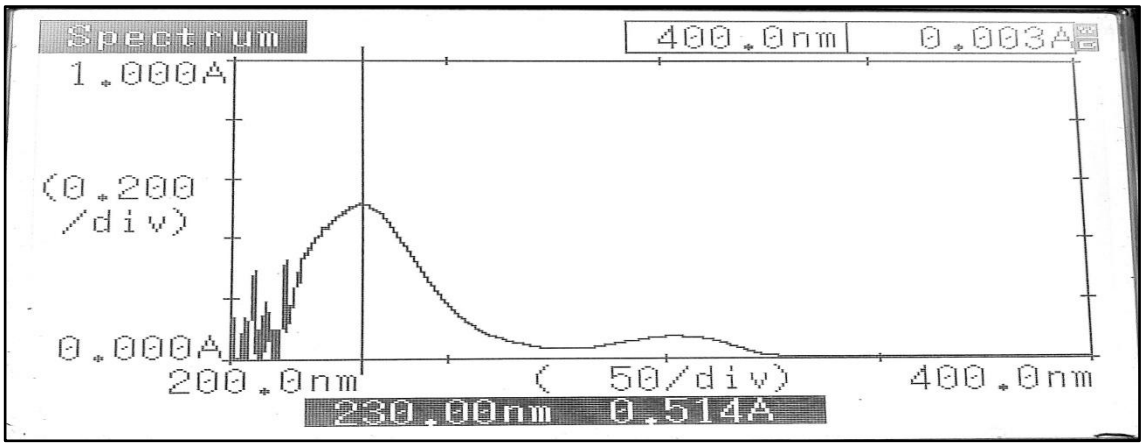
Blood sugar level was measured by employing a Dr.MorepanGluco*One*glucometer, All measurements were performed by the same operator, employing the same glucometer. Blood sugar level were measured on different time interval blood glucose level were measured at 0, 4, 10 hours and 1, 3, 5, 7, 9^{th} day after administration.

RESULTS AND DISCUSSION

UV spectrophotometry:

The maximum absorbance of GLI in acetonitrile was found at 230 nm as depicted in Fig. 1 which was similar to the literature on GLI. With reference to the obtained analytical data of solution, standard calibration curve was plotted which can be used to determine the drug release and % entrapment from the formulated microsphere. In acetonitrile, the regression analysis revealed a very strong association (r2=0.9993). These solutions obeyed Beer-Lambert's law and the linearity was found in the concentration range of 2-16 μg/mL in acetonitrile.

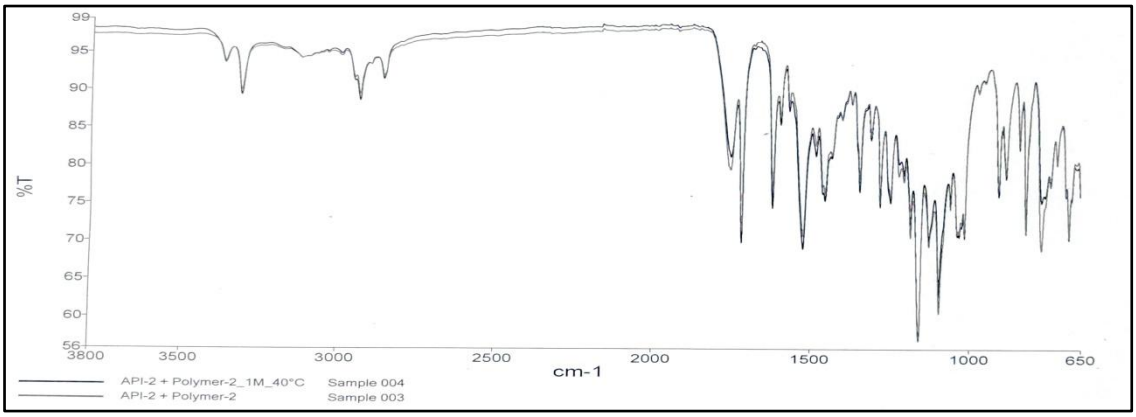
Figure 1: The maximum absorbance of GLI in acetonitrile



Fourier transform infrared spectroscopy (FT-IR)

The potential intermolecular interactions between the GLI and PLGA polymer were investigated using FT-IR techniques. The peaks of physical mixture of GLI and PLGA at initial time point and peaks of GLI and PLGA exposed to 40°C for 1 month period have been compared. There are no notable interactions between GLI, as evidenced by the same positions of the measured absorption bands.

Figure 2:FTIR of physical mixture of GLI and PLGA initial & 1 month at 40°C



Particle size distribution; Entrapment efficiency and *In vitro* drug release:

Mean particle sizes, entrapment efficiency and *In vitro* drug release-testing of all the formulations weredepicted in Table 2. Response surface plots, shown in Fig. 3, were used to clarify how the formulation's independent and dependent variables related to one another.

The relationship between the formulation (independent variables and dependent variables) was elucidated using response surface plots presented

Analysis of data and optimization of design

The Box Behnken design was carried out in order to evaluate the effect of the selected critical variables on responses likepercentage entrapment (Y_1)Mean particle size (Y_2)and % Drug release at day 1 (Y_3), day 5 (Y_4) and day 9 (Y_5). Total 14 experimental runs were generated by Box Behnken design as a result of different variables and their subsequent responses are given in Table 2. The five dependent values, i.e., percentage entrapment (Y_1), Mean particle size (Y_2) and % Drug release at day 1 (Y_3), day 5 (Y_4) and day 9 (Y_5). ranged from 41.34 to 51.75 µm;11.15 to 49.33 %; 3.52 to 15.99 %; 20.34 to 78.54 % and 68.2 to 108 % respectively. Model selection for response analysis was performed based on the sequential model sum of squares, lack of fit test, and model summary statistics while ANOVA was applied to determine the significance of the variable effects.The mathematical equation for responses Y_1, Y_2, Y_3,Y_4 and Y_5 are as follows:

Final Equation in Terms of Coded Factors

%DE (Y_1)= + 46.32 + 0.9 * A + 0.66 * B- 2.83 * C

PSD (Y_2) =+ 26.33 + 4.85* A + 0.03* B –15.88 * C + 1.44 * AB- 1.90 * AC-0.43 * BC–3.87 * A^2-0.39 * B^2 + 5.89 * C^2

DR Day-1 (Y_3) = + 10.65+ 3.51 * A + 1.49 * B+ 2.88 * C - 0.83 * AB - 0.41 * AC -0.10 * BC - 2.11 * A^2 - 0.54 * B^2 + 1.20 * C^2

DR Day-5 (Y_4) = + 58.35 - 16.75 * A + 6.55 * B + 9.47 * C - 1.49 * AB - 3.03 * C -2.63 * BC - 8.09 * A^2 - 6.66 * B^2 0.59 * C^2

DR Day-9 (Y_5) = + 91.98 - 13.425 * A + 3.145 * B + 7.545 * C

Final Equation in Terms of Actual Factors:

%DE (Y_1) = + 50.77 + 0.90 * D:P ratio + 1.32 * % PVA - 0.003 * Speed

PSD (Y_2) = + 64.99 + 30.86 * D:P ratio - 2.89 * % PVA - 0.04 * Speed + 2.88 * D:P ratio * % PVA - 0.002 *D:P ratio * Speed -0.0009 * % PVA * Speed –3.87 * D:P ratio^2 - 1.57 * % PVA^2 + 5.89 * Speed^2

DR Day-1 (Y_3) = - 9.99375 + 12.02 * D:P Ratio + 12.88 * PVA sol - 0.009 * Speed - 1.66 * D:P Ratio * PVA sol - 0.0004 * D:P Ratio * Speed - 0.0002 * PVA sol * Speed - 2.11 * D:P Ratio^2 - 2.17 * PVA sol^2 + 1.20 * Speed^2

DR Day-5 (Y_4) = - 78.94 *+ 43.834 *D:P Ratio+91.10 * PVA sol+0.020 *Speed-2.98 * D:P Ratio * PVA sol-0.003 *D:P Ratio * Speed-0.005* PVA sol * Speed-8.09 * D:P Ratio^2-26.65*PVA sol^2+5.95*Speed^2

DR Day-9 (Y_5) = + 103.33 - 13.43 * D:P Ratio + 6.29 * PVA sol + 0.008 * Speed

Table 3: Analysis of variance (ANOVA) for Percentage Entrapment (Y_1)

Percentage Entrapment (Y_1)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	73.81	3	24.60	9.75	0.0026	significant
A-D:P Ratio	6.48	1	6.48	2.57	0.1401	
B-PVA sol	3.48	1	3.48	1.38	0.2672	
C-Speed	63.85	1	63.85	25.30	0.0005	
Residual	25.24	10	2.52			
Lack of Fit	24.87	9	2.76	7.65	0.2740	not significant
Pure Error	0.3613	1	0.3613			
Cor Total	99.05	13				

Table 4: Analysis of variance (ANOVA) for PSD Mean particle size (Y_2)

PSD Mean particle size (Y_2)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	2426.74	9	269.64	71.86	0.0005	significant
A-D:P Ratio	188.37	1	188.37	50.20	0.0021	
B-PVA sol	0.0084	1	0.0084	0.0023	0.9644	
C-Speed	2017.40	1	2017.40	537.62	< 0.0001	
AB	8.29	1	8.29	2.21	0.2113	
AC	14.36	1	14.36	3.83	0.1220	
BC	0.7225	1	0.7225	0.1925	0.6835	
A^2	47.86	1	47.86	12.76	0.0233	
B^2	0.4930	1	0.4930	0.1314	0.7353	
C^2	110.92	1	110.92	29.56	0.0056	
Residual	15.01	4	3.75			
Lack of Fit	12.48	3	4.16	1.64	0.5078	not significant
Pure Error	2.53	1	2.53			
Cor Total	2441.75	13				

Table 5: Analysis of variance (ANOVA) for Drug release at day 1 (Y_3)

Drug release at day 1 (Y_3)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	209.81	9	23.31	50.77	0.0009	significant
A-D:P Ratio	98.77	1	98.77	215.09	0.0001	
B-PVA sol	17.79	1	17.79	38.74	0.0034	
C-Speed	66.24	1	66.24	144.25	0.0003	
AB	2.74	1	2.74	5.96	0.0710	

Drug release at day 1 (Y_3)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
AC	0.6561	1	0.6561	1.43	0.2980	
BC	0.0400	1	0.0400	0.0871	0.7826	
A^2	14.26	1	14.26	31.06	0.0051	
B^2	0.9374	1	0.9374	2.04	0.2263	
C^2	4.62	1	4.62	10.06	0.0338	
Residual	1.84	4	0.4592			
Lack of Fit	1.83	3	0.6095	72.13	0.0863	not significant
Pure Error	0.0085	1	0.0085			
Cor Total	211.64	13				

Table 6: Analysis of variance (ANOVA) for Drug release at day 5 (Y_4)

Drug release at day 5 (Y_4)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	3705.69	9	411.74	103.64	0.0002	significant
A-D:P Ratio	2245.51	1	2245.51	565.23	< 0.0001	
B-PVA sol	343.09	1	343.09	86.36	0.0007	
C-Speed	717.83	1	717.83	180.69	0.0002	
AB	8.88	1	8.88	2.24	0.2092	
AC	36.66	1	36.66	9.23	0.0385	
BC	27.62	1	27.62	6.95	0.0578	
A^2	209.30	1	209.30	52.69	0.0019	
B^2	142.04	1	142.04	35.75	0.0039	
C^2	1.13	1	1.13	0.2852	0.6216	
Residual	15.89	4	3.97			
Lack of Fit	15.86	3	5.29	183.59	0.0542	not significant
Pure Error	0.0288	1	0.0288			
Cor Total	3721.58	13				

Table 7: Analysis of variance (ANOVA) for Drug release at day 9 (Y_5)

Drug release at day 9 (Y_5)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1976.39	3	658.80	17.89	0.0002	significant
A-D:P Ratio	1441.85	1	1441.85	39.15	< 0.0001	
B-PVA sol	79.13	1	79.13	2.15	0.1734	
C-Speed	455.42	1	455.42	12.37	0.0056	

Drug release at day 9 (Y_5)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Residual	368.24	10	36.82			
Lack of Fit	368.06	9	40.90	227.20	0.0514	not significant
Pure Error	0.1800	1	0.1800			
Cor Total	2344.63	13				

Table 8: Model summary statistics of all dependent variables

Percentage Entrapment (Y_1)						
Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	**1.59**	**0.7452**	**0.6688**	**0.5007**	**49.46**	**Suggested**
2FI	1.85	0.7569	0.5486	-0.0947	108.42	
Quadratic	1.59	0.8982	0.6692	-0.5846	156.95	
Cubic	0.6010	0.9964	0.9526		*	Aliased
PSD Mean particle size (Y_2)						
Linear	4.86	0.9034	0.8744	0.7919	508.16	
2FI	5.51	0.9129	0.8383	0.5335	1139.19	
Quadratic	**1.94**	**0.9939**	**0.9800**	**0.9141**	**209.78**	**Suggested**
Cubic	1.59	0.9990	0.9865		*	Aliased
Drug release at day 1 (Y_3)						
Linear	1.70	0.8637	0.8228	0.7107	61.24	
2FI	1.91	0.8800	0.7771	0.3744	132.40	
Quadratic	**0.6776**	**0.9913**	**0.9718**	**0.8616**	**29.29**	**Suggested**
Cubic	0.0919	1.0000	0.9995		*	Aliased
Drug release at day 5 (Y_4)						
Linear	6.44	0.8884	0.8550	0.7933	769.17	
2FI	6.99	0.9081	0.8293	0.6504	1301.22	
Quadratic	**1.99**	**0.9957**	**0.9861**	**0.9318**	**253.91**	**Suggested**
Cubic	0.1697	1.0000	0.9999		*	Aliased
Drug release at day 9 (Y_5)						
Linear	**6.07**	**0.8429**	**0.7958**	**0.7307**	**631.31**	**Suggested**
2FI	7.06	0.8510	0.7233	0.4928	1189.09	
Quadratic	5.03	0.9568	0.8595	0.3090	1620.13	
Cubic	0.4243	0.9999	0.9990		*	Aliased

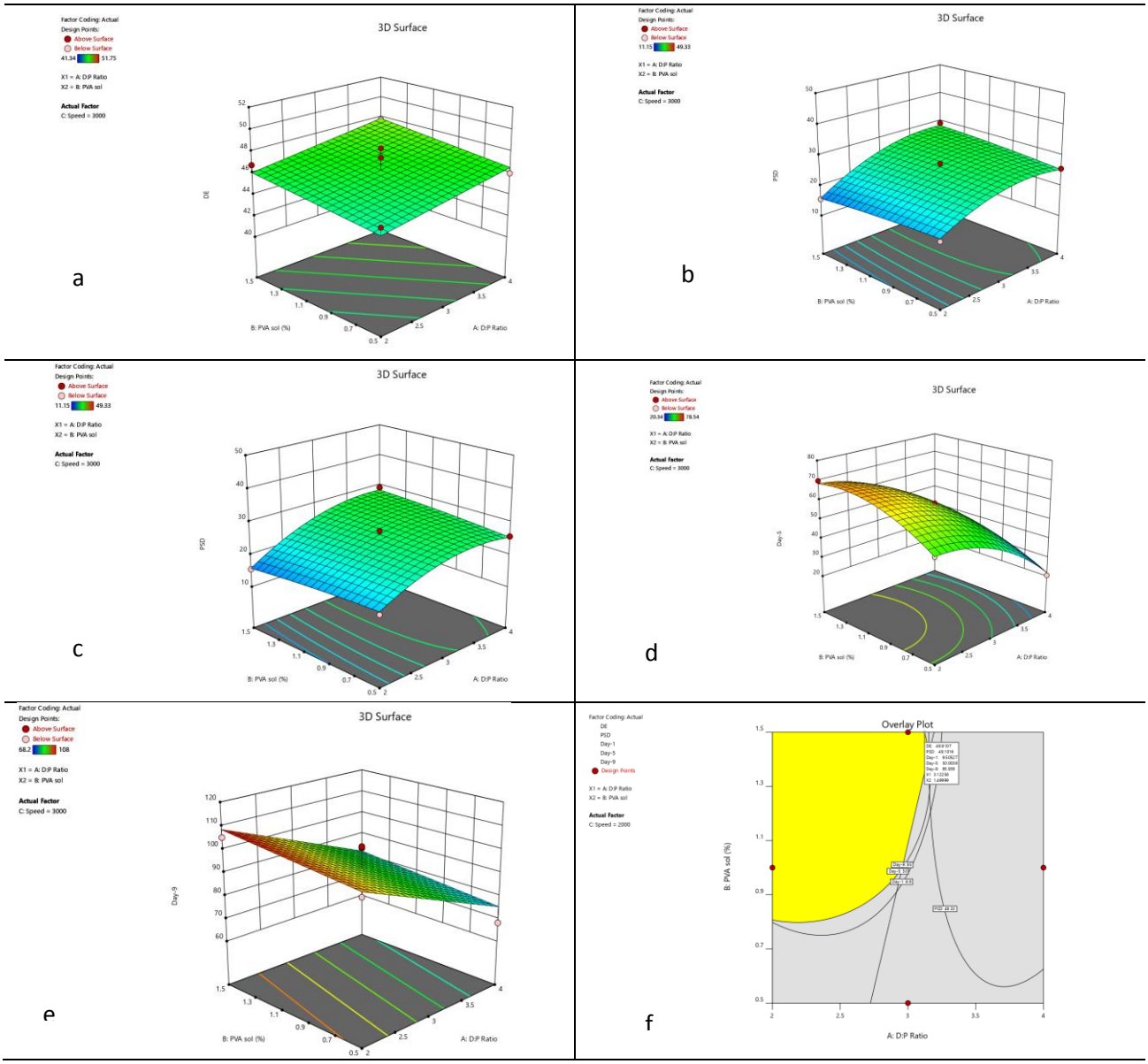


Figure 3: Response surface and contour plot showing interpretation of independent variables on Percentage Entrapment (a); PSD Mean particle size (b); Drug release at day 1 (c) ; Drug release at day 5 (d) ; Drug release at day 9 (e). Overlay plot of formulation composition (f)

Particle morphology using Scanning Electron Microscopy:

The SEM images of microspheres at different magnifications were observed with optical microphotograph to characterize the surface morphology of formulations as depicted in Fig. 5. The micrographs clearly showed the formation of sphere-shaped microspheres.

Study of *in-vivo* antidiabetic effect using Alloxan-induced diabetes mellitus model in rats:Glibenclamide microsphere formulation group significantly decrease the blood sugar level from 4th hour to 9th day after administration of formulation. Glibenclamide drug solution group significantly lowers blood sugar level on 4th hour up to 24th hour and Marketed Glibenclamide

tablet lowers level on 4th hour to 10th hour after drug administration and were compared with positive control group. Glibenclamide microsphere formulation was decreased blood sugar level consistently up to 9th day; it means that drug release from microsphere formulation was in controlled manner up to 9th day.

Table 9: Mean blood sugar level of all subgroups

Group	Time interval (Days)							
	0 hour	**4 hours**	**10 hour**	**24 hour**	**3rd**	**5th**	**7th**	**9th**
Negative Control	96.5 ±16.38*	93.3 ±14.92*	95.5 ±16.83*	86.8 ±13.11*	92.2 ±16.33*	97.0 ±11.71*	92.7 ±17.56*	97.3 ±12.08*
Positive Control	320.8 ±24.96*	335.7 ±23.48*	358.2 ±21.72*	393.5 ±21.73*	421.3 ±20.15*	447.8 ±20.86*	480.5 ±21.79*	515.7 ±32.83*
Glibenclamide Formulation	327.2 ±25.07*	296.0 ±21.39*	246.2 ±31.21*	171.8 ±3.74*	91.0 ±19.06*	90.5 ±10.97*	127.5 ±12.96*	289.2 ±13.29*
Glibenclamide drug solution	330.3 ±8.52*	249.0 ±23.65*	208.0 ±17.51*	286.3 ±11.41*	353.3 ±19.31*	381.8 ±30.79*	411.0 ±42.60*	442.3 ±60.47*
Glibenclamide tablet	321.0 ±15.82*	227.8 ±18.10*	278.3 ±15.88*	322.5 ±53.51*	352.0 ±46.01*	398.2 ±48.11*	454.3 ±54.45*	493.5 ±66.03*

*When compared to the Positive Control group, all values are expressed as mean + SEM (n=6) * p<0.05. All data are analysed using one-way ANOVA, and then Dunnett's test is performed.

Figure 4 Comparative mean blood sugar level of among the different study groups

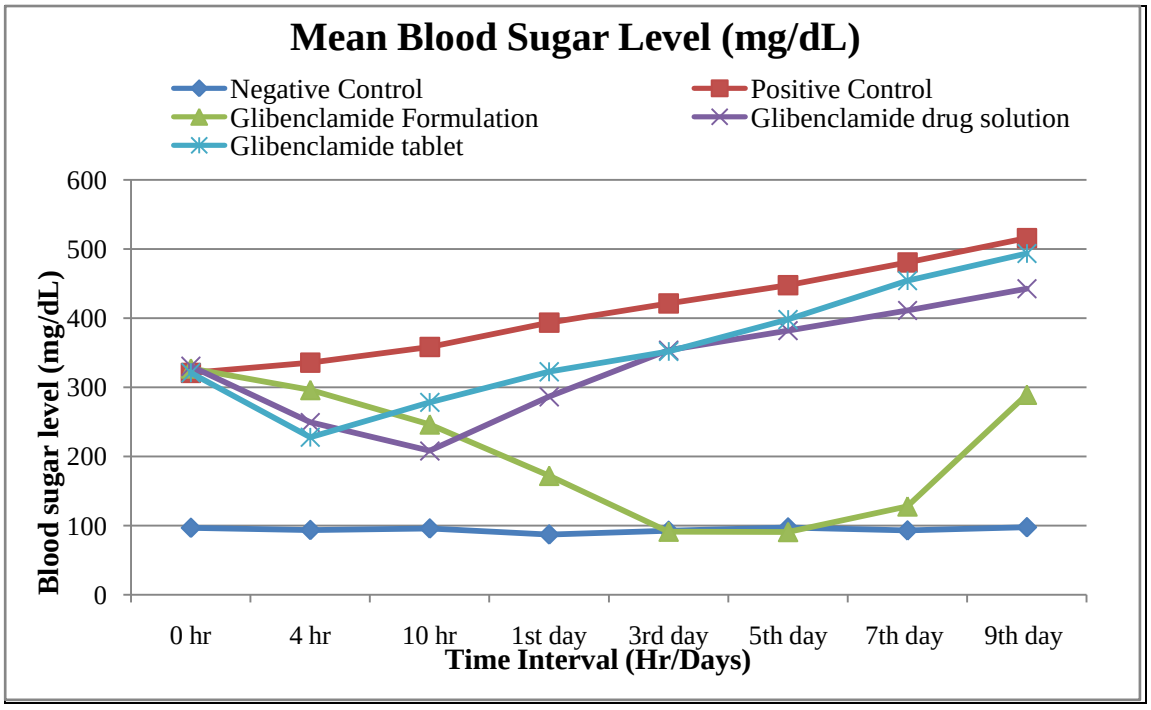
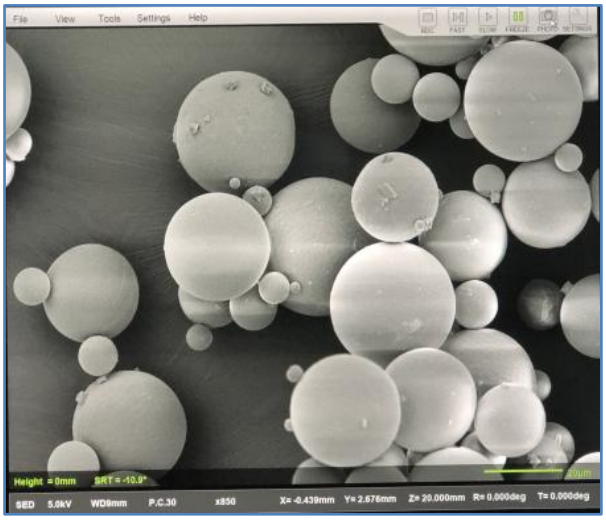
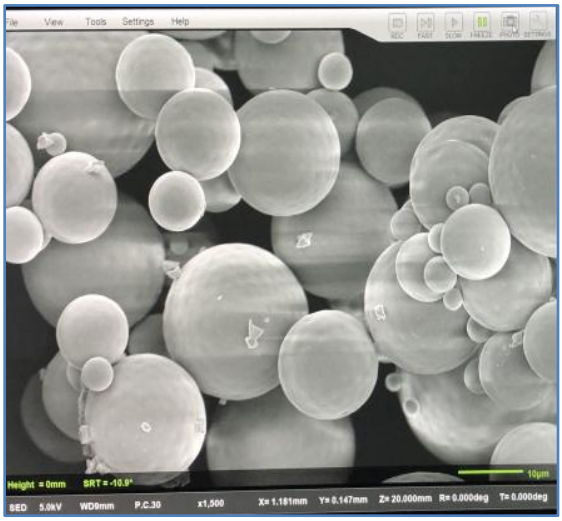


Figure 5Micrographs of Glibenclamide microsphere generated using scanning electron microscopy





CONCLUSION

The goal of the current work was to develop Glibenclamide controlled release microspheres using the solvent evaporation microencapsulation technology. The PLGA polymer-prepared microspheres had a nine-day extension of the drug release and could lower the frequency of administration.Release testing revealed that the highest concentration of polymer produced the most sustained effect, with release rate gradually decreasing as polymer concentration increases.On day 9, 68.2% and 108%, respectively, of the medication were released at their maximum and minimum levels. Mean particle size ranged from 11.15 to 49.33 µm, while the percentage of actual drug entrapment ranged from 41.34% to 51.75%.

The microspheres' smooth surface and good shape were confirmed by the SEM image.The drug and polymer interaction was previously validated by an IR investigation. Microspheres are effective at reducing variation within a therapeutic range, eliminating adverse effects, reducing dose frequency, and enhancing patient compliance.

Based *Invivo*studiesresults, it was concluded that the test item **'Glibenclamide microsphere formulation'** significantly decrease blood sugar level. It shows antidiabetic effect when administered through intramuscular route under the conditions and procedures followed in the study. Single dose administration of **'Glibenclamide microsphere formulation'** normalized blood sugar level in rats.Single dose administration of 'Glibenclamide microsphere formulation' normalized blood sugar level in rats. Glibenclamide microspheres may make a viable choice for a sustained drug delivery system for the treatment of type 2 diabetes, according to an *in vivo* study.

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CONFLICTSOFINTEREST

Theauthors declarenoconflict ofinterest.

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ETHICS STATEMENT

The study was conducted at Test Facility- Trans-Genica Services Pvt. Ltd.; Nagardeola, Tal-Pachora, Dist- Jalgaon 424104, Maharashtra, India.Study facility is certified to compliance to below national standards.CPCSEA, Government of India, Ministry of Environment & Forest, New Delhi: Certification for the purpose of "Research"

INFORMED CONSENT

The study was designed use to minimum number of animals to meet the scientific objectives, the goal of sponsor & in consideration of applicable regulatory requirements. Protocol for general procedures & use of animals for conducting this study was review & approved by Institutional Animal Ethics committee. All procedure related to animal experiment was performed as per recommendations of the Guide for the Care & Use of Laboratory Animals and committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines following all the ethical practices as laid down in CPCSEA guidelines for animal care.

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