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FORMULATION AND EVALUATION OF POLYMERIC MICROSPHERES OF GLIPIZIDE USING BOX BEHNKEN DESIGN

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ABSTRACT

Glipizide is a hypoglycemic agent to treat Diabetes Mellitus type II. In the present investigation, Glipizide loaded polymeric microspheres has been formulated by single emulsion technique. A, three level Box-Behnken design was applied to investigate the different components of formulation and process variables on the formulation response using the numeric approach through the design expert version 7.0 software. All the formulations (F1 to F17) were characterized for the particle size, drug entrapment efficiency and flow properties. F13 was obtained as optimized formulation with minimum particle size (µm), maximum drug entrapment efficiency % and good flow properties. Further the F13 formulation was characterized for *In-vitro* release, Scanning Electron Microscopy (SEM), Fourier Transform Infra- Red Spectroscopy (FTIR) study and stability testing. The graphs of Zero-order, First-order, Higuchi model and Korsmeyer Peppas model were plotted. The polymeric microspheres provide a sustained release of the Glipizide for more than 12 hours, following zero order release and Korsmeyer Peppas model with non- Fickian diffusion.

Key words: - Glipizide, Korsmeyer Peppas Model, Scanning Electron Microscopy, Fourier Transform Infra-Red, Higuchi model, Regression, Fickian diffusion, single emulsion cross-linking technique.

INTRODUCTION

Diabetes mellitus is defined as a group of diseases characterized by high level of glucose that results from defects in the body's ability to produce or use insulin. [1-2] Several pathogenic processes are involved in the development of Diabetes mellitus.

Glipizide comes under the category, second -generation sulfonylurea with hypoglycaemic activity. Its short biological half-life, necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day. This class is used to control blood sugar level in patients with Type 2 Diabetes Mellitus.[4]

Polymeric microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio. [5] Poloxamer 407 and Carbopol 934 were selected as a polymers in the preparation of microspheres because of its good water solubility and surfactant properties. [6-7]

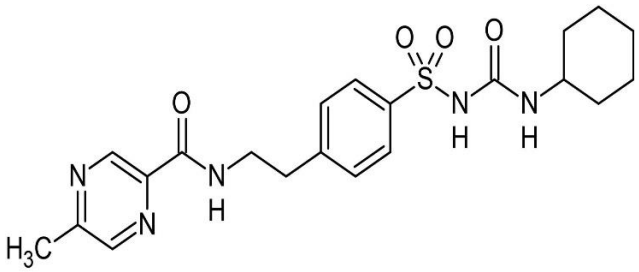

Fig No.1 : Structure of Glipizide

MATERIALS AND METHODS

Materials

Glipizide was obtained as a gift sample from micro labs, Ahmedabad (Gujrat, India). Poloxamer 407, Carbomer 934 and glutaraldehyde were procured from Merck Pvt.Ltd., Mumbai, India. All other reagents used were of analytical grade.

Methods

(1). Preformulation studies of Glipizide

1.1 Organoleptic properties of the drug

The physical appearance of Glipizide drug was examined by different organoleptic properties like colour, odour and appearance.

1.2 Differential scanning calorimetry

In this technique the sample and reference materials are subjected to precisely temperature change. The sample of pure drug (5mg) crimped in the pans made up of aluminium. There is press in differential scanning calorimetry used to crimp the aluminium pans. This press having set of dyes for the lower and upper part. These dyes are colour coated. Concave and flat dyes used in the press. Calibration of Differential Scanning Calorimetry was done by using Indium samples. The analysis on Differential Scanning Calorimetry was carried out over 50-250^{o}C at 5^{o}C/mi. [9-10] Differential Scanning Calorimetry was used in this research work to identify the appearance of the drug (crystalline and amorphous).

1.3 Absorption maxima of drug

The standard stock solution of Glipizide was prepared using 7.4 pH phosphate buffer. Accurately weighed 100mg of drug was dissolved in 100ml of phosphate buffer pH 7.4 in 100ml volumetric flasks with aid of sonication in bath sonicator for 20 min. The concentration of Glipizide was 100µg/ml and for the analytical purpose concentration of Glipizide was taken 10µg/ml.This sample was scanned under Ultra-Violet visible

spectrophotometer range from 200-400nm. From this spectrum of Glipizide drug, the wavelength with maximum absorbance was chosen for further analysis.

1.4 Calibration curve of Glipizide

Stock solution -I

100 mg of Glipizide was weighed accurately and dissolved in small amount of methanol. The volume of solution made up to 100ml.This solution marked as stock solution-I

Stock solution -II

From stock – I, 10 ml was taken and again volume made up to 100 ml. From this dilution having concentration 2µg/ml, 4µg/ml, 6µg/ml,8µg/ml,10µg/ml were prepared.[11] The absorbance of each concentration was measured using UV-Visible spectrophotometer at 223nm as λ max and the graph plotted against the concentration and absorbance.

(2). Preparation of polymeric microspheres of Glipizide

Polymeric microspheres of Glipizide were prepared by using single emulsion cross- linking technique. Poloxamer 407 and Carbopol 934 were used as polymers and glutaraldehyde was selected as cross-linking agent as per method described by Thanoo et al. [13] Polymers (Poloxamer 407 and Carbopol 934) were dissolved in 150 ml of 1% v/v aqueous acetic acid solution. 300 mg of drug was dispersed in this polymer solution. The resultant mixture was extruded through a syringe (No.20) in 1000 ml of liquid paraffin (heavy and light, 1:1 ratio) containing 0.2% Dioctyl sodium sulfosuccinate (DOSS) and stirring was performed by using the magnetic stirrer. After 30 minutes, glutaraldehyde was added and the stirring was continued. [13-16]

Box Behnken Design with 3 factors polymer concentration, stirring speed and cross-linking agent at 3 levels (low, middle and high). In all batches of Box Behnken these independent variables were at different levels. Microspheres thus obtained were filtered and washed several times with petroleum ether to remove the traces of oil. Then microspheres were finally washed with water to remove excess of glutaraldehyde. The obtained microspheres were dried at room temperature for 24 hours.

Table No. 1 Box Behnken Design Layout for Optimization of Polymeric Microspheres of Glipizide

Formulation code	Run order	Factor A (Polymer concentration) (mg)	Factor B (stirring speed) (rpm)	Factor C (Glutaraldehyde) (ml)
F1	1	100	750	0.75
F2	2	200	750	0.75
F3	3	100	750	0.75
F4	4	200	750	0.75

F5	5	100	500	0.50
F6	6	200	500	0.50
F7	7	100	1000	1.00
F8	8	200	1000	1.00
F9	9	150	500	0.50
F10	10	150	500	0.50
F11	11	150	1000	1.00
F12	12	150	1000	1.00
F13	13	150	750	0.75
F14	14	150	750	0.75
F15	15	150	750	0.75
F16	16	150	750	0.75
F17	17	150	750	0.75

2.1 Optimization of Glipizide Microspheres

The process of optimization formulation also predicted the results of different dependent factors such as particle size, drug entrapment efficiency, angle of repose, carr's index and Hausner' ratio of the microspheres.

Characterization of microspheres

3.1 Particle size determination

Polymeric microspheres of Glipizide were analysed by optical microscopy. Optical microscopy consists of two parts, ocular micrometer and stage micrometer. A stage micrometer is simply a microscope slide with a scale attached on the surface. Optical microscopy is based on counting method , is also called as number distribution method. The size of particle is presented in the form of projected diameter.

Least count of eye piece = N_2/N_1* 0.1mm

Where N_1 = divisions of eye piece and N_2 = divisions of stage micrometer

3.2 Drug entrapment efficiency

The microspheres were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 7.4 pH phosphate buffer. The resulting mixture was shaken by the magnetic stirrer for 24 h. the resultant mixture was filtered, and the filtrate was analysed for the drug content under the Ultra-Violet spectroscopy.

the drug entrapment efficiency was calculated using the following formula: -

Drug entrapment efficiency = Practical drug content / Theoretical drug content *100

3.3 Flow properties of microspheres

3.3.1 Angle of repose

Weighed quantity of microspheres (10gm) was passed through a funnel fixed on a stand at a specific height upon the graph paper. A static heap of powder with only gravity acting upon it was tending to flow form a conical mouth.

The height of heap (h) and the radius of the lower part of the conical were measured.

The angle of repose was calculated using the following formula:

$$\tan\theta = h/r$$

3.3.2 Carr's index

It is a simple test that has been evaluate the flow ability of a powder by comparing the poured (fluff) density ($\rho_{Bmin)}$ and tapped density ($\rho_{Bmax)}$ of a powder and the rate at which it packed down.

Carr's index is determined by taking a small quantity of microsphere sample in 10ml measuring cylinder. The height of sample was measured before and after tapping indicates poured and tapped density respectively. It was calculated using following formula:

Carr's index (%) = Tapped – poured (bulk) density / Tapped density *100

3.3.3 Hausner's ratio

Hausner defined a similar index in 1967. Same method was employed for determination of poured and tapped density as in case of carr's index.

It was calculated using following formula :

Hausner's ratio = Tapped density / Bulk density

3.4 *In – Vitro* Drug Release Study

Release of Glipizide from the prepared polymeric microspheres were studied in phosphate buffer pH 7.4 (900 ml) using USP type II six station dissolution test apparatus (paddle type) at 50 rpm at the temperature of 37ºC. Samples of polymeric microspheres filled in capsule shell were used in each test. [14] Samples were withdrawn through a filter (0.2micron) at different time interval and were assayed at 223nm for Glipizide using U.V. spectrophotometer.

3.5 Drug Polymer Interaction Studies

It is necessary to confirm that drug is not interacting with polymers under experimental conditions and shelf life. The possible drug polymer interaction was studied by Fourier Transform Infra-red spectrophotometer.

Excipients can affect the stability of drugs in various ways, by direct chemical interaction, absorption of moisture or catalysis. [16-17] Drug polymer interaction studies were carried out to check the compatibility between the drug and various polymers. Apart from physical characteristics compatibility between a drug and polymer is a factor in determining the effectiveness of polymeric delivery systems.

The spectrum obtained from the Fourier Transform Infra- Red spectroscopy were compared with the spectrum of the pure Glipizide.

3.6. Scanning Electron Microscopy Analysis

The shape and surface morphology of microspheres samples were studied by SEM technique. Microspheres were dusted onto double sided carbon dust which was placed onto sample carrier (aluminium stubs having double adhesive tape) in the shape of a cylinder with 5 mm of height and 10mm of diameter and were coated with gold palladium mixture under vacuum with sputter coater to thickness of 50 mm.The basic principle is that a beam of electrons is generated by a suitable source. Typically, a tungsten filament or a field emission gun is used. This beam is generally accelerated through a high voltage (20kV). Then this beam scans the surface of the specimen.

3.7. Stability Studies

To evaluate stability profile of drug product for storage under refrigeration, room and accelerated temperature. The microspheres of glipizide were subjected to room temperature (25ºC), refrigeration temperature (4º C) and accelerated temperature conditions (40 ºC , 50 ºC , 60 ºC). Samples were withdrawn at predetermined time intervals of 15,30,45 and 60 days and analysed for physical appearance and drug content in Ultra- Violet spectrophotometer.

RESULT AND DISCUSSION

Preformulation

Glipizide was evaluated for organoleptic properties. It was obtained as off white in colour with characteristics odour.

The solubility of glipizide was determined in different solvent systems. The maximum solubility of Glipizide was obtained maximum in pH 7.4 phosphate buffer 650±0.32 µg/ml and minimum in water 51.9±0.32 µg/ml.

Differential Scanning Calorimetry (DSC) analysis

The DSC analysis of Glipizide showed a sharp endothermic peak at 208º C corresponding to the drug Melting point. The appearance of sharp endothermic peak is due to the crystalline nature of the drug Glipizide. The thermogram of Glipizide is shown in fig

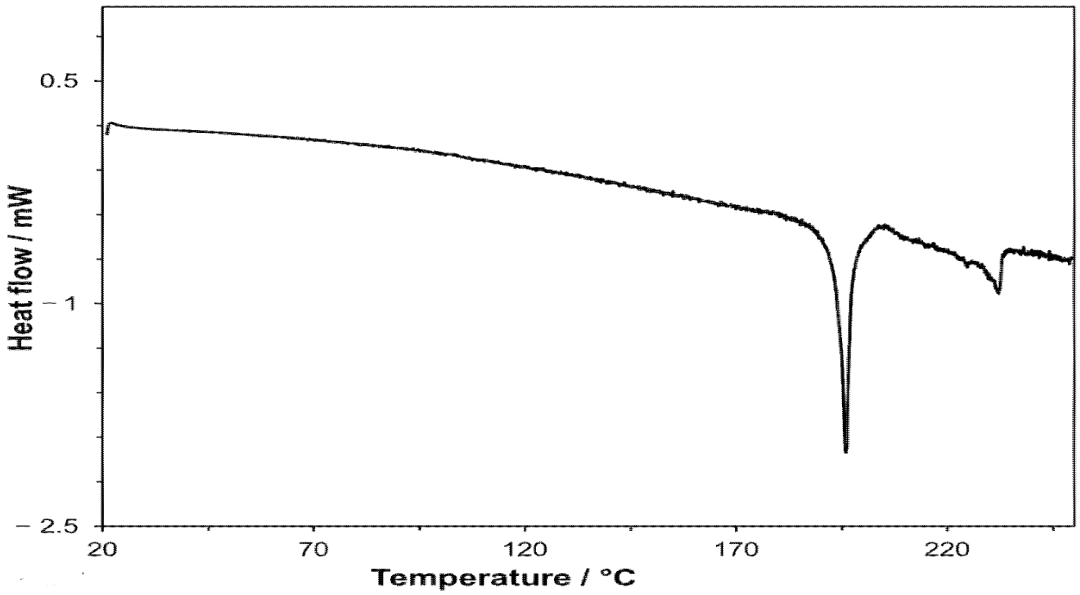

Fig No. 2: Differential Scanning Calorimetry of Glipizide

Determination of λmax

The solubility of Glipizide was maximum in pH 7.4 Phosphate buffer. The absorption maxima of the drug Glipizide were determined by running the spectrum of drug solution in double beam UV spectrophotometer**.** The absorption was highest at 223nm.

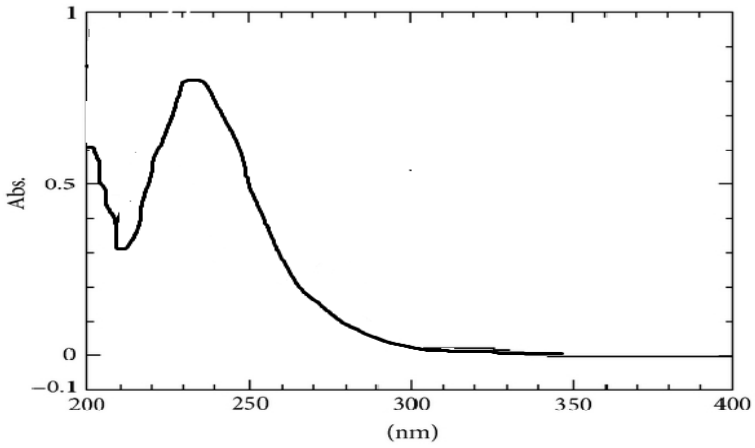

Fig No. 3: Determination of λmax of Glipizide

Determination of Calibration curve of Glipizide

The calibration curve of Glipizide was constructed using UV spectrophotometer and it was found to be obeying Beer – Lambert law over the concentration range of 2-10µg/ml.

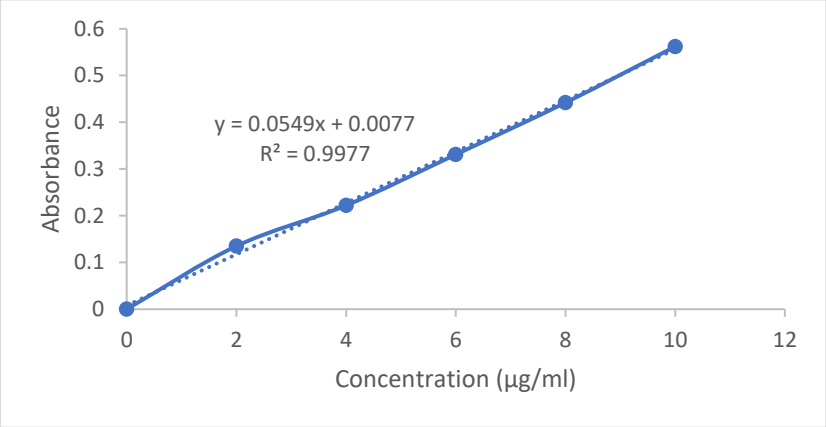

Fig No. 4: Calibration curve of Glipizide in pH 7.4 Phosphate Buffer

Particle Size Determination

Table No. 2 Result of 17 Runs for Particle Size Using Box Behnken Design

S.No.	Formulation code	Particle size (Response 1)
1	F1	112±1.20
2	F2	110±1.11
3	F3	154±1.23
4	F4	116±1.10
5	F5	112±1.12
6	F6	123±1.22
7	F7	142±1.23
8	F8	117±1.24
9	F9	107±1.20
10	F10	115±1.10
11	F11	116±1.22
12	F12	155±1.31
13	F13	99±1.10
14	F14	113±1.11
15	F15	134±1.13
16	F16	155±1.21
17	F17	102±1.22

Table No. 3 Results of Regression Analysis for Y1

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	C.V.%
Particle size (Y1)	**0.9383**	**0.8486**	**0.7585**	**10.127**	**7.19**	**5.87**

Response 1 (Y1): Particle Size

The following polynomial equation prevailed from the model for particle size of Glipizide polymeric microspheres.

$$\mathbf{Y1 = 120 - 1.25\ X1 - 22.87\ X2 + 2.88\ X3 + 0.25X1X2 + 1.25\ X1X3 - 2.50X2X3 - 0.75X1^2 + 12.00\ X2^2 - 7.50\ X3^2}$$

The concentration of polymers (X1) and stirring speed (X2) have negative effects on the size of particle size. The particle size decreased with increase in stirring speed, this is because the higher shearing stress breaks up the molecules to larger extent at higher stirring rates.

As the amount cross linking agent (X3) have positive effects on particle size with polymer concentration (X1), but increase in stirring speed it will decrease the particle size. Polymer concentration (X1) and stirring speed (X2) together have positive effect on particle size.

Response surface plots for particle size

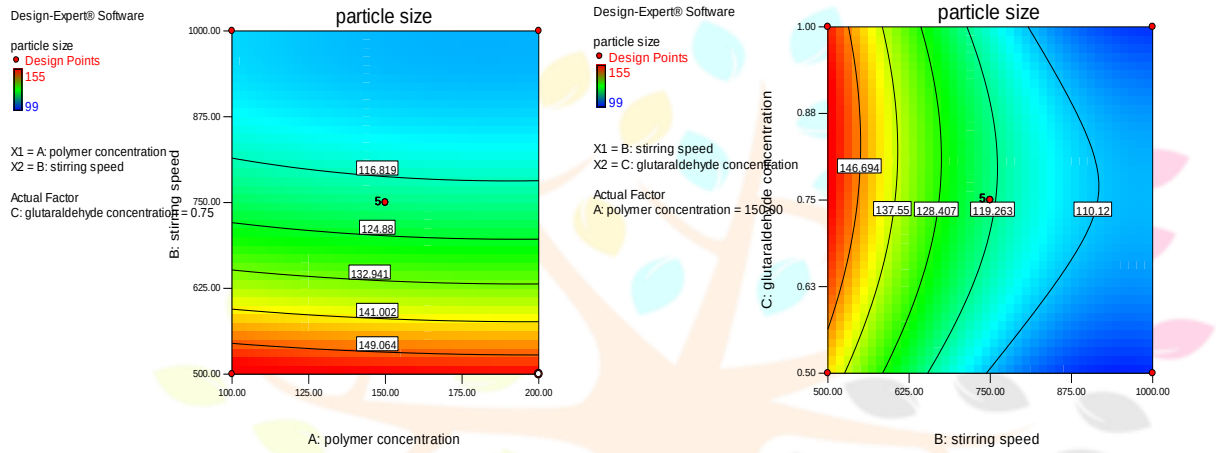

Fig No. 5 Contour Plot for particle size showing the effect of polymer concentration (X1), stirring speed (X2) and glutaraldehyde concentration (X3)

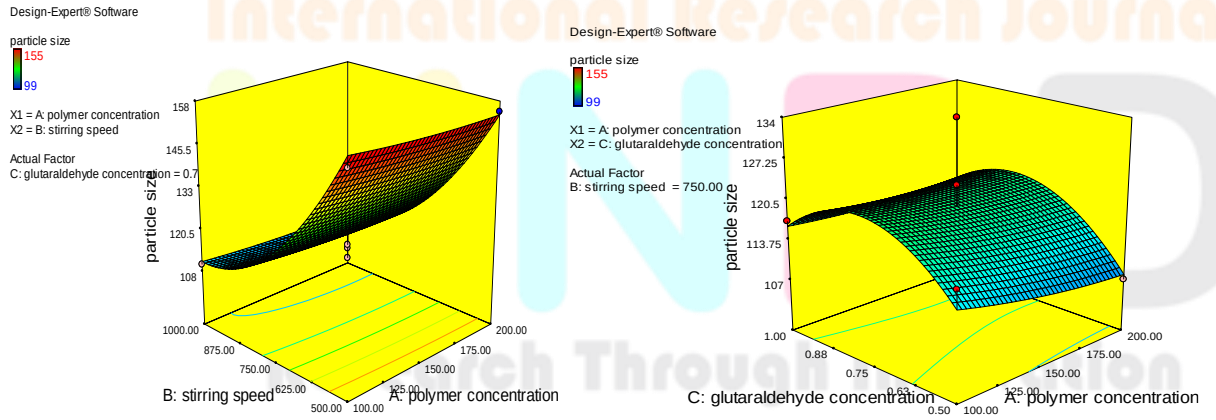

Fig No. 6: 3D surface plot for particle size showing the effect of polymer concentration (X1), stirring speed (X2) and glutaraldehyde concentration (X3)

Drug Entrapment Efficiency

Table No. 4: Result of 17 Runs for Drug Entrapment Efficiency of Glipizide microspheres

S.No.	Formulation code	Drug entrapment efficiency (response 2)
1	**F1**	**76**
2	F2	88
3	F3	58
4	F4	71
5	F5	83
6	F6	77
7	F7	45
8	F8	68
9	F9	66
10	F10	68
11	F11	72
12	F12	37
13	F13	89
14	F14	67
15	F15	70
16	F16	49
17	F17	81

Table No. 5 Results of Regression Analysis for Y2

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	C.V.%
Drug entrapment efficiency (Y2)	**0.9513**	**0.8886**	**0.4749**	**13.009**	**4.84**	**7.06**

Response 2 (Y2): Drug Entrapment Efficiency

$Y2 = 72.60 - 2.50X1 + 19.00X2 – 2.25X3 +1.00\ X1X2 +1.00\ X1X3 + 0.000\ X2X3 +0.95\ X1^2 - 4.05X2^2 - 5.55\ X3^2$

Polymer concentration (X1) and cross linking agent (X3) have negative effect on drug entrapment alone, but together they have positive effects. Both factors together will increase the amount of linking the drug. Stirring speed (X2) have a very high positive effect on drug entrapment. This factor when combine with polymer concentration (X1) and cross-linking agent (X3) similar effects on entrapment efficiency of microspheres.

Response surface plot for drug entrapment efficiency

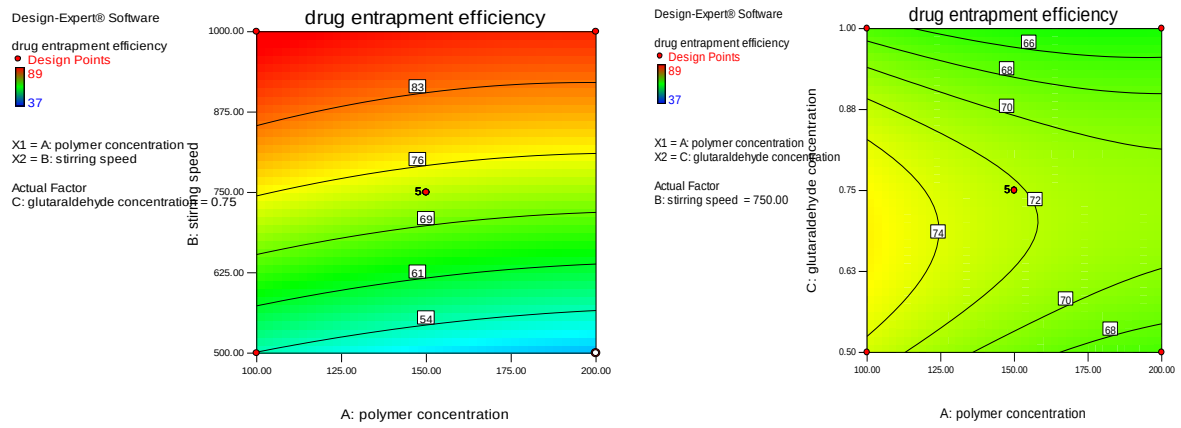

Fig No. 7: Contour plot for drug entrapment efficiency showing the effect of polymer concentration (X1) ,stirring speed (X2) and glutaraldehyde concentration

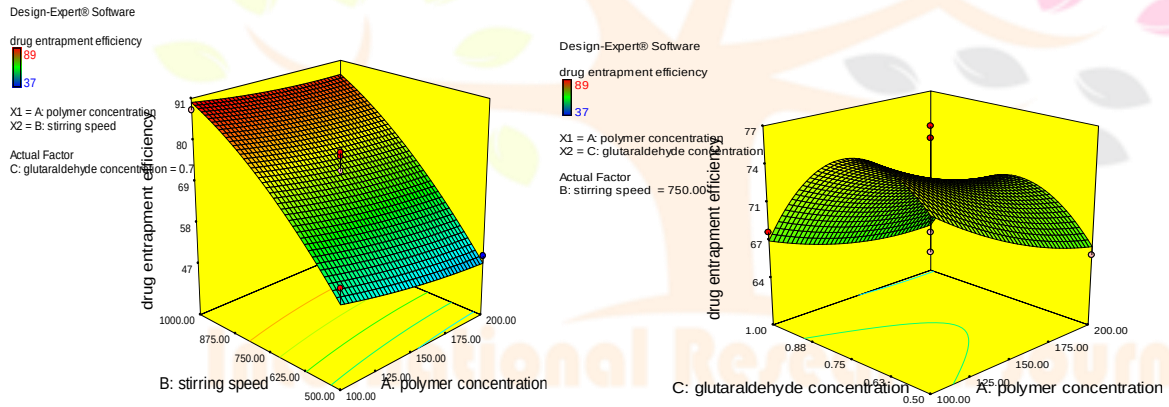

Fig No. 8 :3D surface plot for drug entrapment efficiency showing the effect of stirring speed (X2),polymer concentration (X1) and glutaraldehyde concentration (X3)

Flow properties of glipizide microspheres

Angle of repose

Table No. 6: Result of 17 Runs for angle of repose of Glipizide microspheres

Sr.No.	Formulation code	Angle of repose (Response 3)
1	F1	22.5 ± 1.12
2	F2	24±1.13
3	F3	21±1.40
4	F4	22±1.20
5	F5	24.5±1.23
6	F6	22±1.15

7	F7	22±1.18
8	F8	22±1.35
9	F9	22±1.28
10	F10	22±1.41
11	F11	24±1.22
12	F12	21±1.20
13	F13	21±1.11
14	F14	23.83±1.08
15	F15	22±1.34
16	F16	22±1.27
17	F17	25±1.23

.

Table No. 7 Results of Regression Analysis for Y3

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	C.V.%
Angle of Repose (Y3)	**0.8430**	**0.6412**	**0.2026**	**8.011**	**0.75**	**3.34**

Response 3 (Y3): Angle of Repose

$Y3 = 22.50 + 0.42\ X1 + 1.06\ X2 + 0.60X3 - 0.13\ X1X2 + 0.46X1X3 + 1.25\ X2X3 + 0.29\ X1^2 - 0.084X2^2 - 0.33\ X3^2$

All the factors polymer concentration (X1), Stirring speed (X2) and cross-linking agent (X3) have a positive effect on the angle of repose that is main parameter for flow properties of microspheres. These all factors when combine , have positive similar effects on angle of repose property. But the factor polymer concentration (X1) and stirring speed (X2) combine together have negative effect on the angle of repose.

Response surface methodology

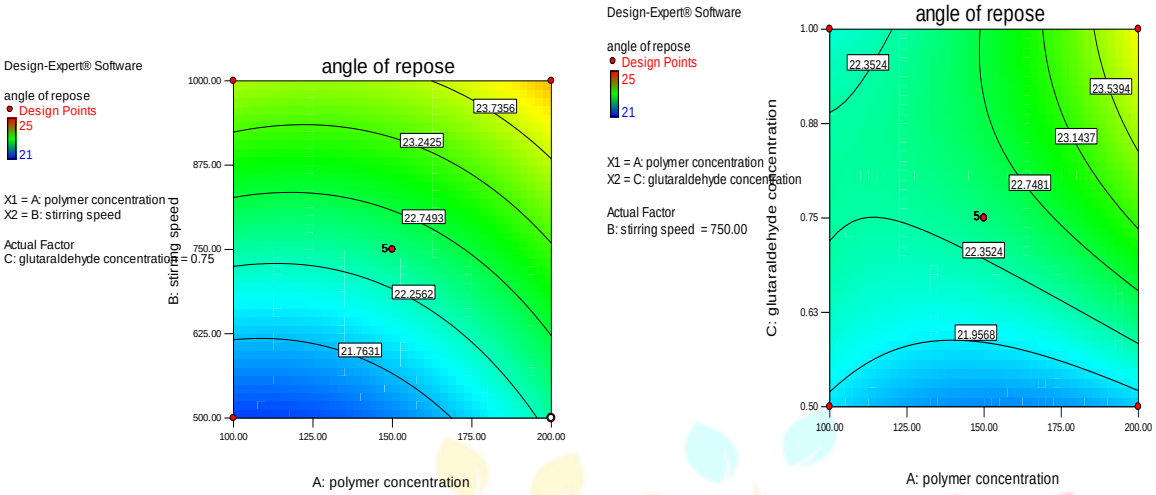

Fig No.9 :Contour Plot for Angle of Repose Showing effect of Polymer Concentration (X1), Stirring Speed (X2) and glutaraldehyde concentration (X3)

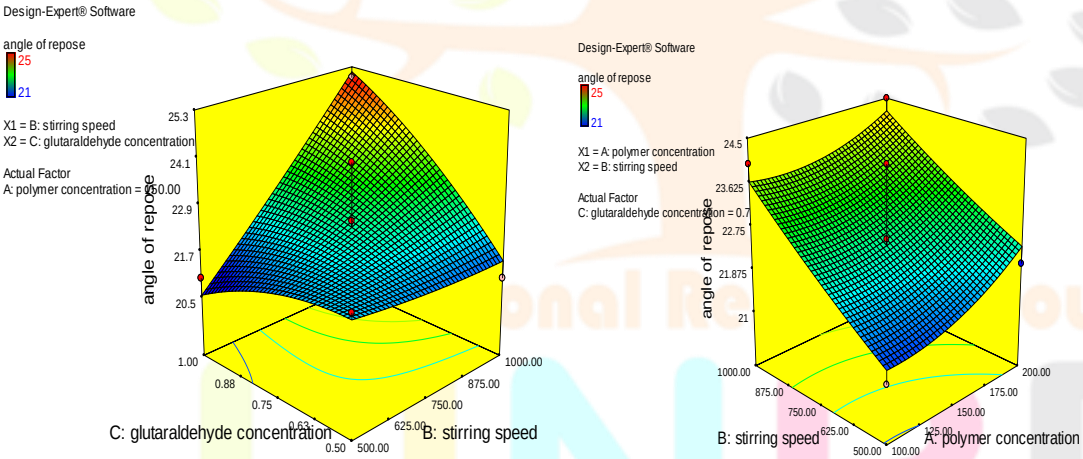

Fig No.10 : 3D Plot for Angle of Repose Showing effect of polymer concentration (X1) stirring speed (X2) and glutaraldehyde Concentration (X3)

Carr's Index

Table No. 8: Result of 17 Runs for Carr's Index of Glipizide microspheres

Sr.No.	Formulation code	Carr's Index (Response 4)
1	F1	10.2 ± 1.02
2	F2	10.13±1.03
3	F3	38.38±1.10
4	F4	11.41±1.27
5	F5	9.7±1.03

6	F6	12.18±1.17
7	F7	29.38±1.13
8	F8	10.41±1.01
9	F9	12.29±1.12
10	F10	11.45±1.32
11	F11	14.43±1.25
12	F12	16.63±1.22
13	F13	7.44±1.16
14	F14	16.21±1.34
15	F15	19.5±1.41
16	F16	23.04±1.21
17	F17	7.5±1.13

.

Table No.9 Results of Regression Analysis for Y4

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	C.V.%
Carr's Index (Y4)	**0.8506**	**0.6584**	**0.6802**	**8.142**	**4.82**	**31.55**

Response 4 (Y4): Carr's Index or % compressibility

$Y4 = 13.55 - 1.14\ X1 - 9.04\ X2 - 1.26\ X3 + 3.73\ X1X2 + 1.23\ X1X3 + 3.28\ X2X3 + 2.09\ X1^2 + 4.67\ X2^2 - 3.06\ X3^2$

All the factors polymer concentration (X1), Stirring speed (X2) and cross-linking agent (X3) have a negative effect on % compressibility that is another main parameter for flow properties of microspheres. These all factors the factor polymer concentration (X1) and stirring speed (X2) and the concentration of Glutaraldehyde (X3) when combine , have positive similar effects on % compressibility property. Positive effect means the amount of carr's index will be increased and the negative effect means the carr's index will be decreased.As the value of carr's index decreased , means the flow property of microspheres is good.

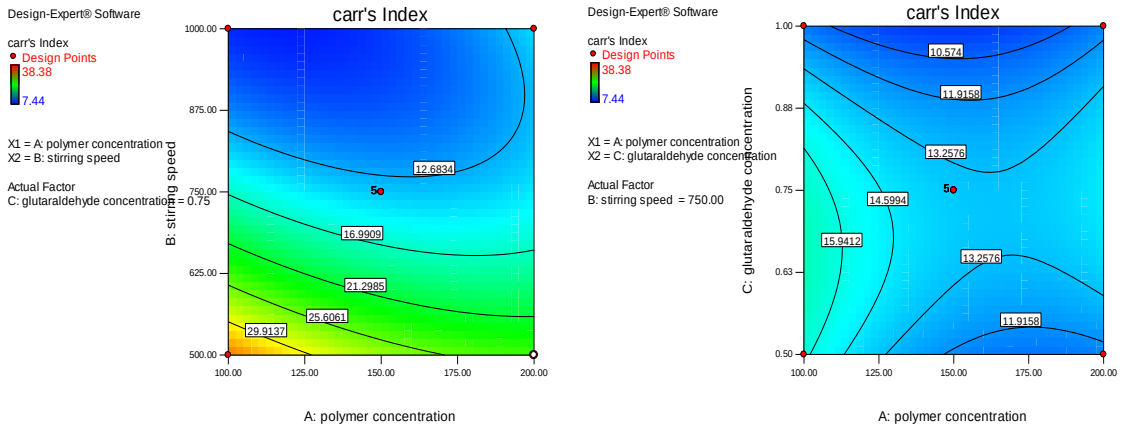

Fig No. 11 : Contour Plot for carr's index Showing effects Polymer Concentration (X1) stirring speed (X2) and Glutaraldehyde concentration (X3)

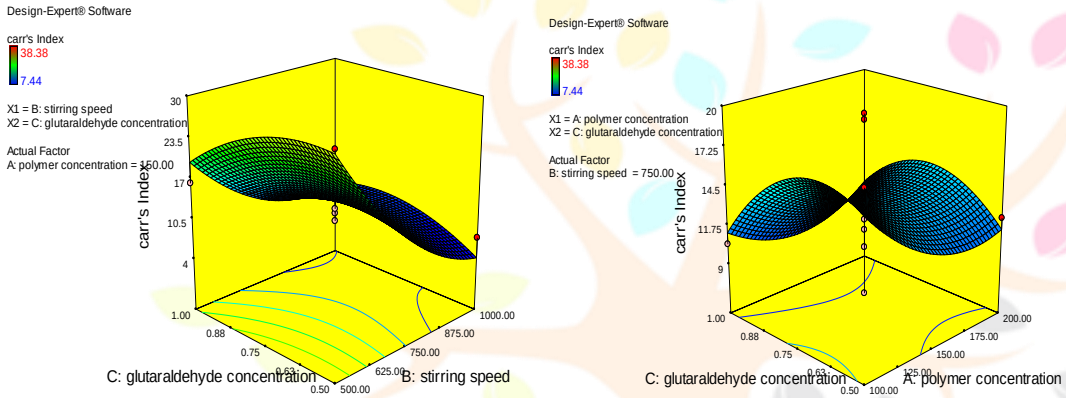

Fig No. 12: 3D surface Plot for carr's index Showing effects polymer concentration (X1) stirring speed (X2) and glutaraldehyde Concentration (X3)

Hausner's ratio

Table No.10: Result of 17 Runs for Hausner's ratio of Glipizide microspheres

Sr.No.	Formulation code	Hausner's Ratio (Response 5)
1	F1	1.141
2	F2	1.031
3	F3	1.192
4	F4	1.123
5	F5	1.031
6	F6	1.138
7	F7	1.185
8	F8	1.134
9	F9	1.14
10	F10	1.144

11	F11	1.121
12	F12	1.195
13	F13	1.023
14	F14	1.142
15	F15	1.111
16	F16	1.171
17	F17	1.024

Table No. 11 Results of Regression Analysis for Y5

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	C.V.%
Hausner's ratio (Y5)	**0.9778**	**0.9493**	**0.8615**	**16.852**	**0.013**	**1.17**

$Y5 = 1.13 + 5.00\ X1 - 0.079\ X2 + 3.00\ X3 + 5.25\ X1X2 – 2.25\ X1X3 – 2.25\ X2X3 + 1.625\ X1^2 – 0.026\ X2^2 + 2.125\ X3^2$

The factor A (polymer concentration) and the factor C (glutaraldehyde concentration) have the Positive effect on the Hausner's Ratio rather the factor B (stirring effect) have negative effect on Hausner's ratio. When polymer concentration is with the stirring speed, there is positive effect on Hausner's ratio but with the glutaraldehyde concentration have negative effects on Hausner's ratio.Factor B (stirring speed) and the glutaraldehyde concentration combinedly have negative effect on Hausner's Ratio.Positive effects mean the amount of Hausner's ratio will be increased and the negative effect means the Hausner's ratio will be decreased. As the value of Hausner's ratio decreased, means the flow property of microspheres is good.

Selection of the optimized formulation

From the values given in table it is evident that the model is significant with significant p value ($p < 0.0001$), lack of fit value ($p < 0.0063$) and R^2 values. Formulation F13 was found to have narrow particle size range, better drug entrapment and good flow properties. Based on these parameters F13 formulation was considered to be the optimized.

Table No.12 Box Behnken Design response for Formulation F13

Formulation code	Polymer concentration (mg)	Stirring speed (rpm)	Concentration of glutaraldehyde (ml)	Particle size (Y1) (µm)	Drug entrapment efficiency (Y2) (%)
F13	**13**	**150**	**750**	**99± 1.12**	**89±1.34**

Characterization of optimized formulation F13

FTIR Spectroscopy

The compatibility between drug and polymers was confirmed by using FTIR spectroscopy. Infra-red analysis for drug glipizide and mixture of drug- polymers (carbomer 934 and poloxamer 407) was carried out.

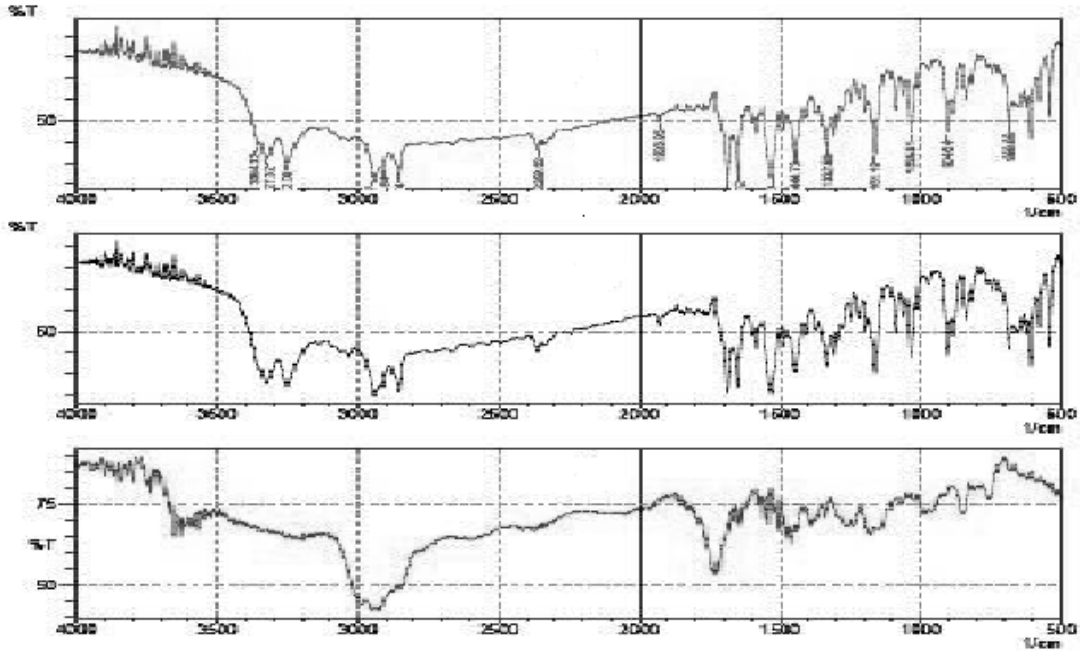

Fig No.13 :

1. **FTIR of Glipizide and carbomer 934**
2. **FTIR of Glipizide and poloxamer 407**
3. **FTIR of Glipizide and polymer (carbomer 934 and poloxamer 407) Microspheres**

Inference

The peaks observed in the FTIR spectrum of physical mixture and optimized formulation showed no shift and disappearance of characteristics peaks of glipizide as well as polymers (poloxamer 407 and carbomer 934). This suggests that there is no interaction between the drug and polymer. Hence it can be concluded that the drug glipizide maintains its identity without undergoing any chemical interaction with poloxamer 407 and carbomer 934.

Scanning Electron Microscope (SEM)

From the formulated batches of Glipizide microspheres, the formulation batch which showed in appropriate results including percentage release were examined for surface morphology using scanning electron microscope. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum

evaporator. The surface morphology of polymeric microspheres of Glipizide was studied by SEM.Microphotographs of glipizide microspheres were taken on different magnification was used for the surface morphology.

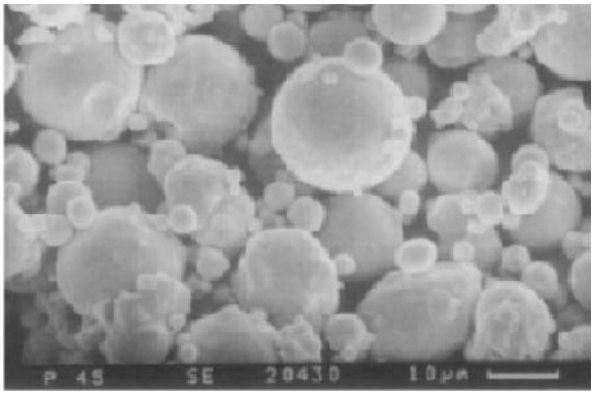

Fig No.14: Scanning electron morphology of Glipizide microspheres

***In – vitro* release kinetics of formulation F13**

Table No. 13: Release Kinetics Data of F13 of Glipizide Microspheres

Time	Square root of time	% cumulative release	Log % cumulative release	Cumulative % retained	Log cumulative % retained	Log time	Log (Mt/M)
1	1	1.27	0.103	98.73	1.994	0	1.188
2	1.414	2.26	0.354	97.74	1.990	0.301	1.438
3	1.732	4.43	0.646	95.57	1.980	0.477	1.730
4	2	8.82	0.945	91.18	1.959	0.602	2.029
5	2.236	14.162	1.151	85.84	1.934	0.699	2.235
6	2.449	20.65	1.314	79.35	1.899	0.778	2.399
7	2.645	32.19	1.507	67.81	1.831	0.845	2.592
8	2.828	43.656	1.640	56.34	1.750	0.903	2.724
9	3	58.54	1.767	41.46	1.617	0.945	2.851
10	3.162	68.142	1.833	31.86	1.503	1	2.917
11	3.317	72.48	1.860	27.52	1.439	1.041	2.944
12	3.464	82.32	1.915	17.68	1.247	1.079	3

Zero order kinetics

The graph is plotted between the time and % cumulative drug release

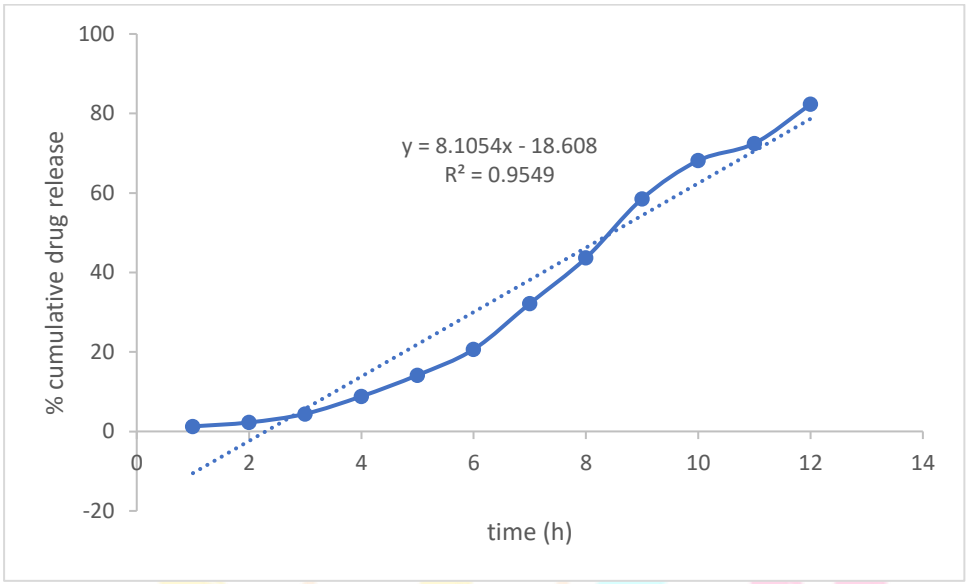

Fig No.15: Zero order release of F13

First order kinetics

The graph is plotted between the time and Log % cumulative drug release

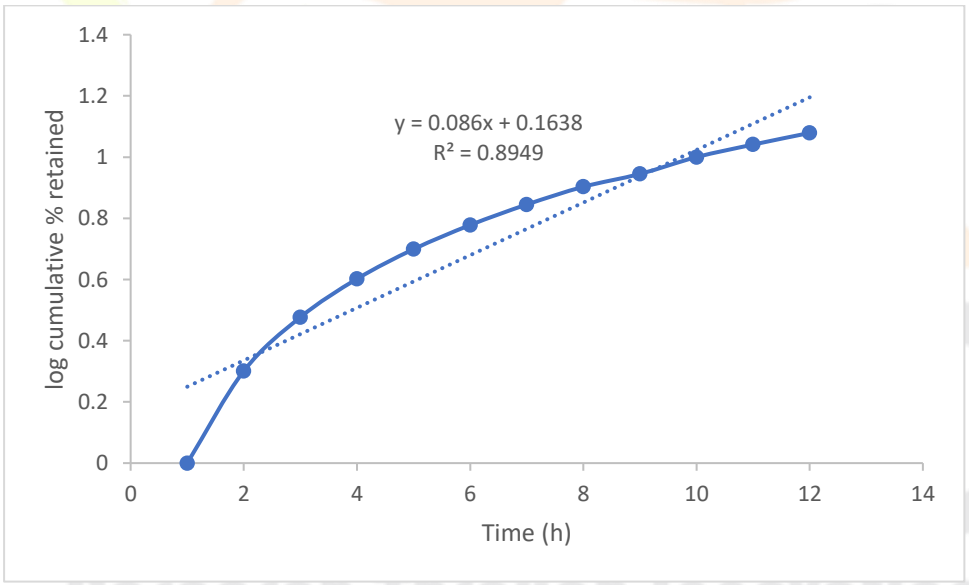

Fig No.16: First order kinetics for F13 after

Higuchi model

The graph is plotted between the square root of time and % cumulative drug release

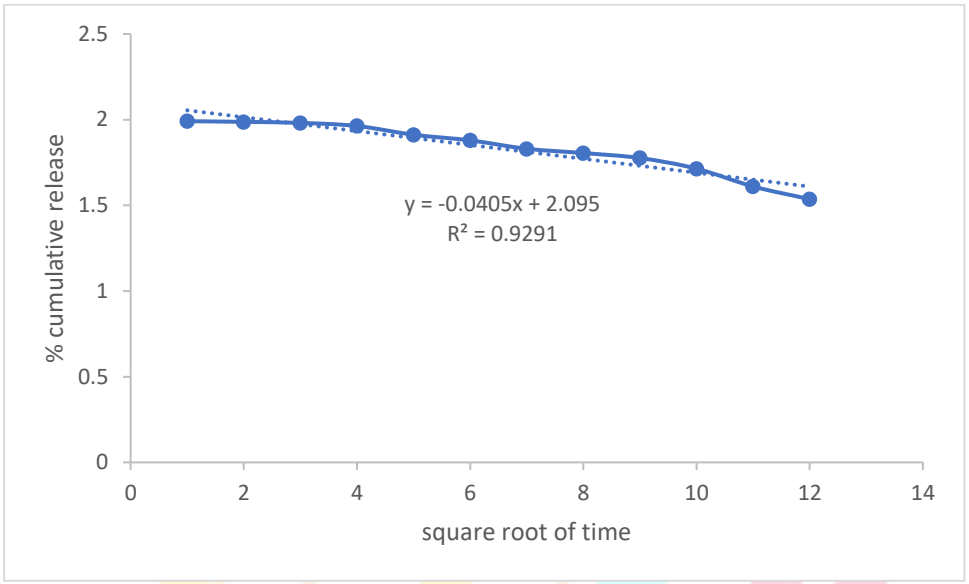

Fig No.16 : Higuchi model for F13

Korsmeyer Peppas model

The graph is plotted between log time and log cumulative drug release

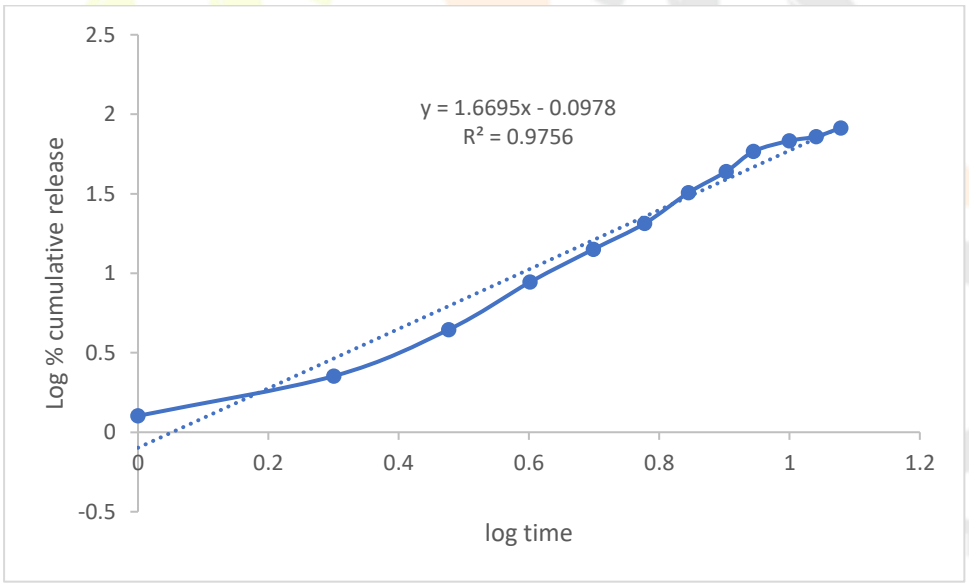

Fig No.17 Korsmeyer Peppas model for F13

Table No. 14 :Release kinetics of polymeric microspheres of Glipizide after stability testing

Formulation Code	Zero-Order R^2	First – Order R^2	Higuchi Matrix R^2	Korsmeyer - Peppas R^2	Diffusion Component 'n' value
F	0.9549	0.8831	0.8774	0.9756	1.66

The result of the *in-vitro* drug release study obtained from optimized batch were plotted using kinetic models. Zero-order kinetics, first order kinetics, Higuchi matrix and Korsmeyer -Peppas model were used to evaluate the release mechanism from Glipizide microspheres.

The kinetic model showing highest correlation coefficient was considered as the most appropriate model for the dissolution data. The best fit with the highest correlation coefficient was observed in Korsmeyer - Peppas model and Zero -order kinetics followed by Higuchi model as described in table no. the value 'n' diffusion constant of formulation was found to be 1.66 indicating that the drug release was followed by anomalous (non-Fickian) diffusion.

Stability Studies of Glipizide Microspheres

The Optimized formulation was studied for stability profile at normal and accelerated conditions as per ICH guidelines. The formulation was placed separately in amber coloured borosilicate screw capped glass container and stored at normal room temperature (25±2 °C), freezing temperature (5-8 °C) and for accelerated testing at oven temperature (40±2 °C/75±5% RH) respectively for a period of 3 months.

Change in colour was visualized and size of the formulation was determined by optical microscopy using an ocular micrometer.

Table No. 15 Temperature dependent stability studies of optimized Glipizide microspheres performed at different temperature

Formulation Code of Optimized Formulation	Drug content (mg/g)											
	Temperature (40° C)				Temperature (50° C)				Temperature (60° C)			
F13	Time (days)				Time (days)				Time (days)			
	0	30	60	90	0	30	60	90	0	30	60	90
	410	398	390	384	410	395	388	381	410	397	385	380

Table No. 16 Temperature dependent stability studies of optimized Glipizide microspheres performed at different temperature

Formulation Code of Optimized Formulation	Drug content (mg/g)											
	Room Temperature (25± 2 ° C)				Refrigerator Temperature (2-8° C)				Temperature (Humidity chamber) (40°C ± 2 / 75 ± 5%RH)			
F13	Time (days)				Time (days)				Time (days)			
	0	30	60	90	0	30	60	90	0	30	60	90
	410	398	386	385	400	399	389	386	410	395	392	388

***In -vitro* release study of F13 after stability conditions**

Table No. 17: Release Kinetics Data Optimized formulation of Glipizide Microspheres

Time	Square root of time	% cumulative release	Log % cumulative release	Cumulative % retained	Log cumulative % retained	Log time	Log (Mt/M)
1	1	1.834	0.263	98.166	1.991	0	1.446
2	1.414	2.834	0.452	97.166	1.987	0.301	1.635
3	1.732	4.24	0.627	95.76	1.981	0.477	1.810
4	2	8.16	0.911	91.84	1.963	0.602	2.094
5	2.236	18.28	1.261	81.72	1.912	0.699	2.445
6	2.449	24.24	1.384	75.76	1.879	0.778	2.567
7	2.645	32.25	1.508	67.75	1.830	0.845	2.691
8	2.828	36.15	1.558	63.85	1.805	0.903	2.741
9	3	40.24	1.604	59.76	1.776	0.945	2.787
10	3.162	48.22	1.683	51.78	1.714	1	2.866
11	3.317	59.24	1.772	40.76	1.610	1.041	2.955
12	3.464	65.58	1.816	34.42	1.536	1.079	3

The *in-vitro* release data of optimized formulation after stability conditions was fitted into Zero -order , First -order , Higuchi equation and Korsmeyer – Peppas model.

Zero order kinetics

The graph between the %cumulative drug release and time (h)

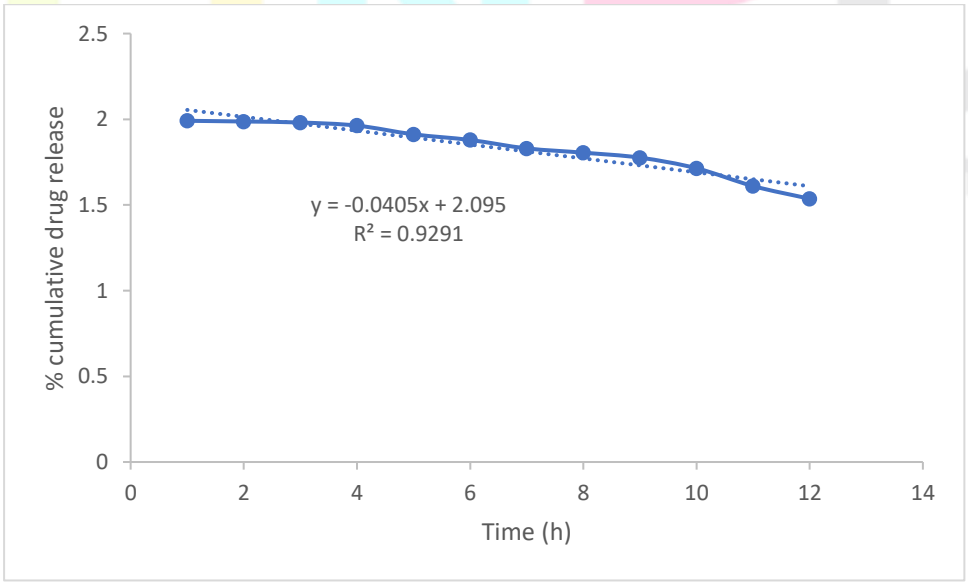

Fig No.18: Zero order kinetics for optimized formulation after stability testing

First Order kinetics

The graph of first order kinetics plotted between Time and log % cumulative retained

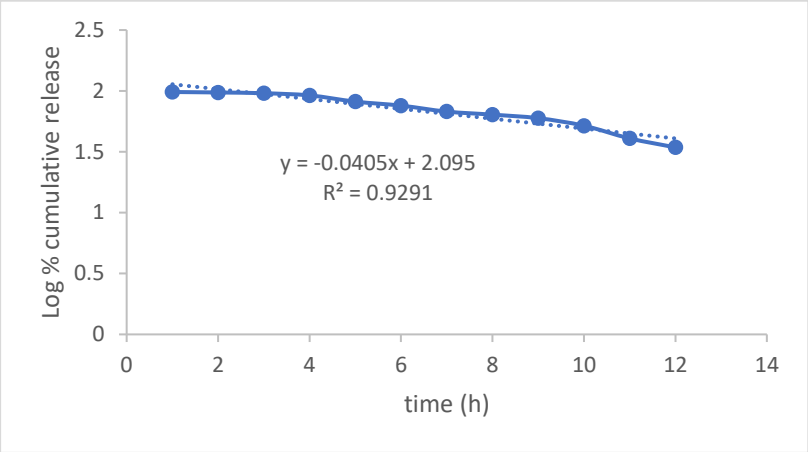

Fig No.19: First order kinetics for optimized formulation after stability condition

Higuchi model

The graph of Higuchi model plotted between square root of time and % cumulative drug release

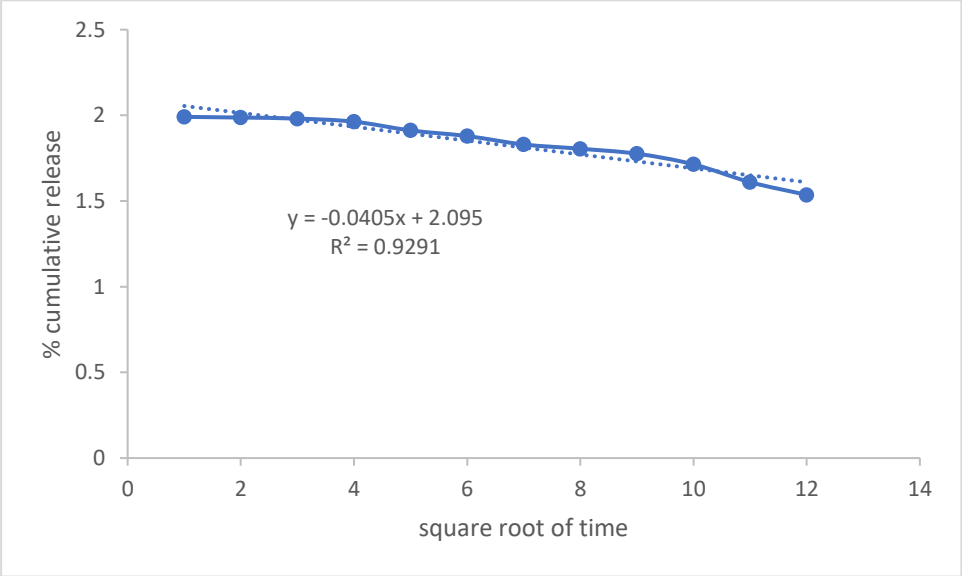

Fig No.20: Higuchi model for optimized formulation of Glipizide microspheres after stability conditions

Korsmeyer Peppas Model

The graph of Korsmeyer Peppas Model plotted between log time and log cumulative drug release

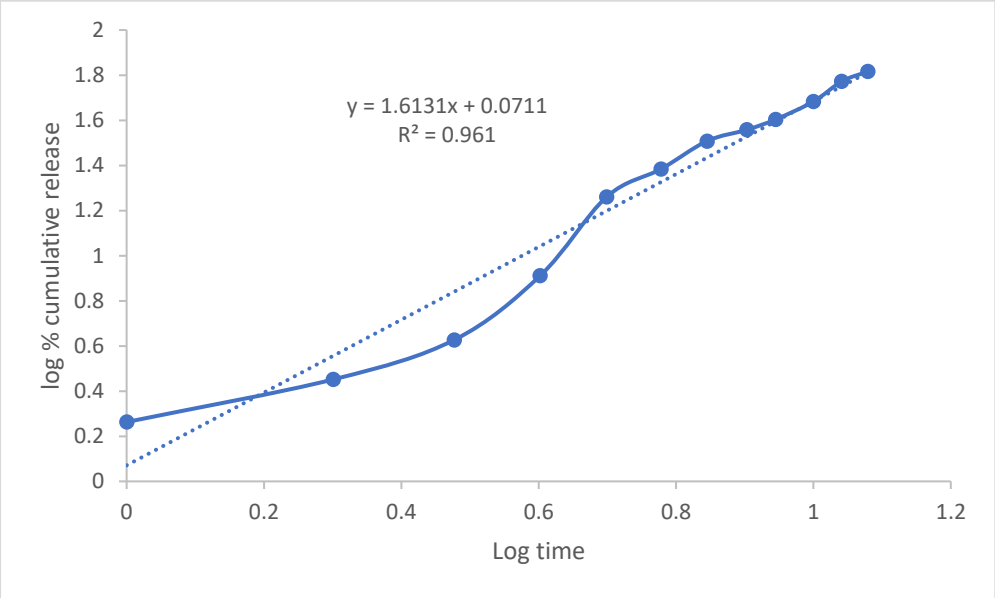

Fig No.21: Korsmeyer Peppas model for optimized formulation of Glipizide microspheres

Table No.18 Release kinetics of polymeric microspheres of Glipizide

Formulation Code	Zero-Order R^2	First – Order R^2	Higuchi Matrix R^2	Korsmeyer - Peppas R^2	Diffusion Component 'n' value
F	0.9763	0.9291	0.9159	0.961	1.613

The result of the *in-vitro* drug release study obtained from optimized batch were plotted using kinetic models. Zero-order kinetics, first order kinetics, Higuchi matrix and Korsmeyer -Peppas model were used to evaluate the release mechanism from Glipizide microspheres.

The kinetic model showing highest correlation coefficient was considered as the most appropriate model for the dissolution data. The best fit with the highest correlation coefficient was observed in Korsmeyer -Peppas model and Zero -order kinetics followed by Higuchi model as described in table no. the value 'n' diffusion constant of formulation was found to be 1.248 indicating that the drug release was followed by anomalous (non-Fickian) diffusion.

CONCLUSION

The polymeric microspheres of Glipizide were successfully formulated by single emulsion cross linking technique using Poloxamer 407 and Carbopol 934 as polymers. The important formulation parameters including particle size, drug entrapment efficiency and flow properties have been optimized and analyzed

against different independent variables by three levels of three factorial Box-Behnken designs. Based on the result of Box-Behnken design F13 was obtained as optimized formulation.

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