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Formulation and Evaluation of Gliclazide Microspheres.

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ABSTRACT

The objective of the present study was to develop Gliclazide microspheres in order to achieve an extended retention in GIT. Which may result in enhance the absorption and improve the bioavailability. The microspheres were prepared by emulsion solvent diffusion-evaporation method using different ratios of polymer poloxamer 407, Gliclazide is used in each formulation at constant ratio. The mixture of dichloromethane and ethanol at ratio of (1:1), with tween 80 as the surfactant. The prepared microspheres were evaluated for percentage yield, particle size, entrapment efficiency, shape and surface characterization, in vitro dissolution studies and drug release mechanism was interpretated by kinetic model. The effect of polymer concentration on these parameters was investigated. The studies revealed that increase in concentration of hydrophilic non-ionic polymer (Poloxamer 407) increased the drug release from the microspheres. The formulation F6 (Gliclazide:poloxamer 407 is 1:6) was selected as best formulation, and the entrapment efficiency 89.36%, drug content 98.16% and the *In-vitro* drug release 93.85% at 12^{th}hour.

Keywords:- Gliclazide , Microspheres , Poloxamer 407, Emulsion solvent diffusion-evaporation , Tween 80 .

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INTRODUCTION

Gliclazide is an anti-diabetic drug comes under second generation sulfonylurea, and is a good insulin sensitizer that has been widely used in management of NIDDM. It's half life is 5 hors, more than 99% bind to plasma proteins and is absorbed completely from the entire GIT. Since, Gliclazide is well absorbed from GIT controlled release formulation that retained in the GIT will be beneficial for effectively controlling diabetes. Several methods have been reported which can be used to retain the dosage form in the GIT, which results in the spreading the drug slowly over the absorptive surface in the GIT. A gastro retentive dosage form is one approach that will release the drug over a prolonged period of time in GIT thus enhancing the opportunity for absorption of drug. Considering the above factors, the present work is aimed to formulate and evaluate the Gliclazide microspheres for controlled release in the GIT [1].

MATERIALS AND METHODS

Gliclazide was purchased from Mahalakshmi chemicals pvt Ltd. Hyderabad., India. Poloxamer 407 was purchased from signet chemicals mumbai, India. Tween 80 was purchased from Loba chemie pvt.ltd, Mumbai, Dichloromethane, Ethanol was purchased from Medrich Pvt. Ltd and other ingredients used were of analytical grade.

Preparation of microspheres by emulsion solvent diffusion evaporation technique:

The formulations of different batches of Gliclazide microspheres are given in table 1. Accurately weighed amount of Gliclazide and poloxamer were dissolved in a mixture of Dichloromethane (DCM): Ethanol (ETN) (1:1) at room temperature. This solution was poured into 100ml distilled water containing 0.1% Tween 80 maintained at a temperature of 30^0-40^0C.The resultant emulsion was stirred with a propeller type agitator at 1200 rpm for 45 mins to allow volatile solvent to evaporate. The resultant microspheres were filtered and dried.

Table-1

Formulation Code	Drug: Polymer	Gliclazide (mg)	Poloxamer 407 (mg)
F1	1:1	10	10
F2	1:2	10	20
F3	1:3	10	30
F4	1:4	10	40
F5	1:5	10	50
F6	1:6	10	60

EVALUATION

PREFORMULATION STUDIES

The formulation of any drug substance in to dosage form, it is essential that drug and polymer should be chemically and physically characterized. Preformulation studies give the

information need to define the nature of drug substance and provide a frame work for a drug combination with pharmaceutical exciepients in the fabrication of a dosage form.

COMPATIBILITY STUDIES

One of the requirement for the selection of suitable excipients or carrier for pharmaceutical formulation is its compatibility. Therefore in the present work a study was carried out by using FTIR spectrophotometer and Differential scanning calorimeter (DSC) to find out if there is any possible chemical interaction of Gliclazide with poloxamer, ethyl cellulose (EC)[2].

a) Fourier Transform Infrared Spectrophotometer (FTIR)

Compatibility study of drug with the excipients was determined by FTIR Spectroscopy using SHIMADZU- FTIR 410 model. The pellets were prepared at high compaction pressure by using KBr and the ratio of sample to KBr is 1:100.The pellets thus prepare were examined and the spectra of drug and other ingredients in the formulation were compared with that of the original spectra [3].

b) Differential scanning calorimeter (DSC)

Differential scanning calorimeter is used to measure the specific heat and enthalpies of transition. When a sample undergoes a thermal transition, the power to the heater is adjusted to maintain the temperature, and a signed proportional to the power difference is plotted on the second axis of the recorder is known as thermogram. The area under the resulting curve is direct measure of the heat of transition. Thermograms were obtained by using a differential scanning colorimeter at a heating rate 15^0C/min over a temperature range of 0 to 1000^0C.The sample was hermetically sealed in an aluminium crucible [4].

CONSTRUCTION OF STANDARD CURVE FOR GLICLAZIDE

Gliclazide can be estimated spectrophotometrically at 235 nm as it obeys Beer's-Lambert's law limit is the range of 2-10 µg/ml.

Preparation of reagents

Preparation of pH7.4 buffer

Place 50ml of 0.2 M potassium dihydrogen phosphate in a 200 ml of volumetric flask 39.1 volume of 0.2 M NaoH and then add water up to 200 ml.

Preparation of standard drug solution

Stock solution:

100 mg of Gliclazide was dissolved in 100 ml of pH7.4 to get a solution of 1000 µg/ml concentration.

Standard solution

10 ml of stock solution was made to 100 ml with pH7.4 thus giving a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml were transferred into 10 ml volumetric flask and were diluted up to the mark with pH 7.4 Thus the final concentration ranges from 2-10 µg/ml. Absorbance of each solution was measured at 235 nm against pH7.4 as a blank. A plot of concentrations of drug versus absorbance was plotted. The linear regression analysis was done on absorbance data points. A straight line equation was generated to facilitate the calculation of amount of drug [5].

Particle size analysis

The particle size of the microsphere is determined by using the optical microscopy method. Microspheres are counted for particle size using a calibrated optical microscope [6].

Shape and surface characterization

The shape and surface characterization of microspheres are observed under scanning electron microscope(SEM).The microspheres are mounted directly on the SEM sample stub, using double-sided sticking tape, and coated with gold film(Thickness 200mm) under reduced pressure (0.001 tort) and photographed[7].

Determination of drug content

Accurately weighed 10 mg of crushed microspheres were dissolved in pH 7.4 buffer solution and then transferred to 100 ml volumetric flask. The solution was filtered using Whatman filter paper no. 41. The samples were assayed for drug content using UV spectrophotometer at 235 nm[8].

Determination of percentage yield of microspheres

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below[9]

$$\text{percentage yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \text{X100}$$

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Encapsulation efficiency

Encapsulation efficiency of microspheres was calculated using the following formula[10];

$$\text{Encapsulation efficiency} = \frac{\text{Estimated drug content\%}}{\text{theoretical drug content\%}} \text{X100}$$

In-vitro dissolution study

The dissolution studies of the prepared microspheres were carried out using USP type I apparatus. Dissolution was performed in 900 ml of pH 7.4 buffer medium at 37±0.5°C and at 100 rpm. Aliquots samples were withdrawn at e1, 2, 3…….12 hours and analysed by UV spectrophotometer at 235nm. Sink condition was maintained throughout experiment by replacing with pH 7.4 buffer medium[11].

RESULTS

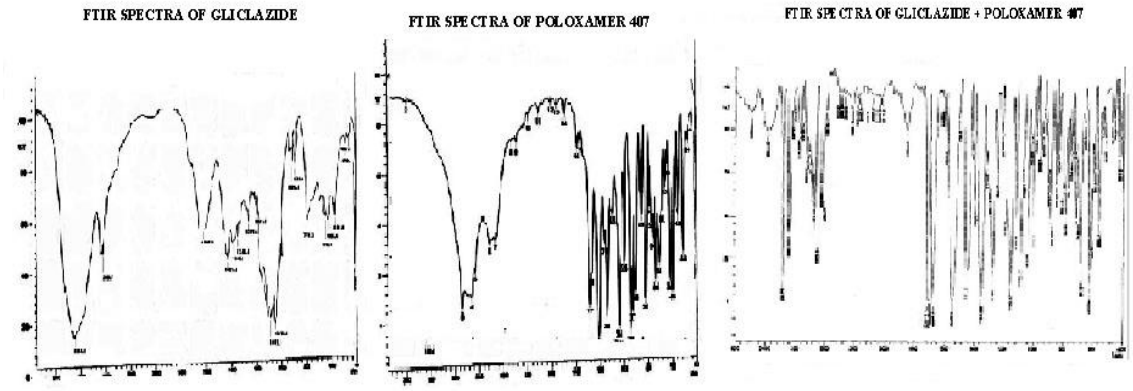

Figure 1: a) Fourier Transform Infrared Spectrophotometer (FTIR)

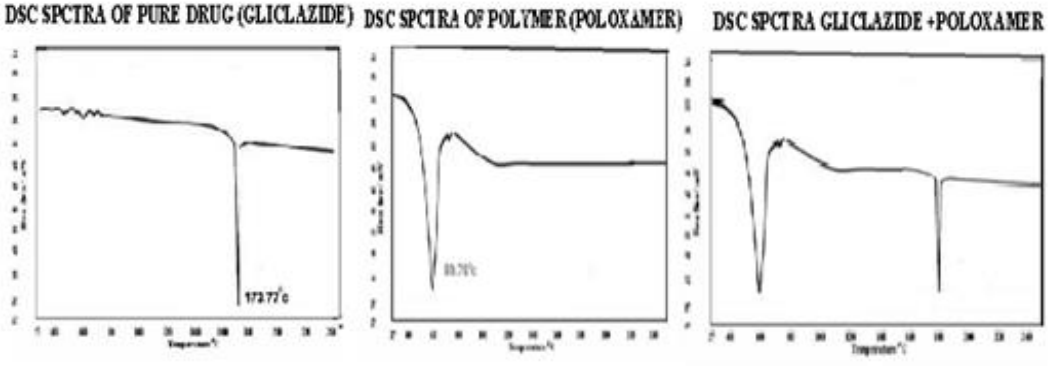

Figure 2: B)Differential scanning calorimeter (DSC)

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Table 2: Standard curve for Gliclazide

S.NO	Concentration in µg/ml	Absorbance at 235 nm
1	2	0.210
2	4	0.401
3	6	0.606
4	8	0.810
5	10	0.988
Slope		0.100
Correlation coefficient		0.999

CONSTRUCTION OF STANDARD CURVE FOR GLICLAZIDE:

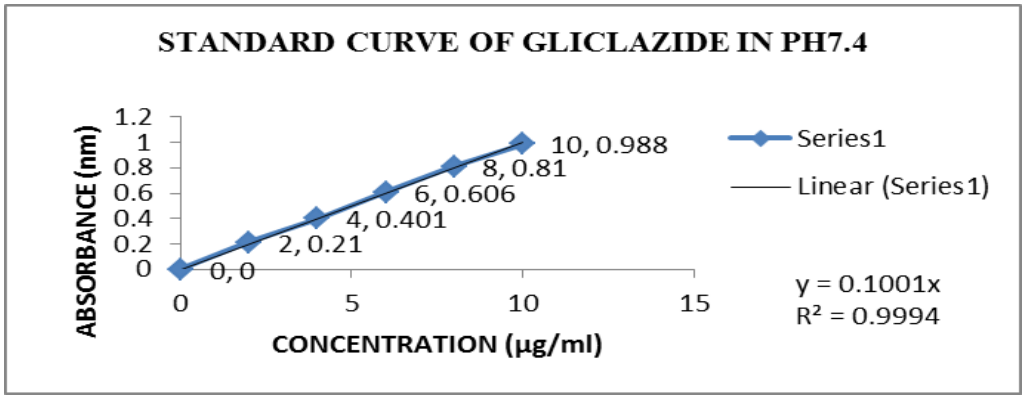

Table 3: Data for particle size of gliclazide microspheres

FORMULATION CODE	MEAN PARTICLE SIZE (µm)
F1	127±1.563
F1	157±2.039
F3	168±0.935
F4	217±1.178
F5	247±1.825
F6	265±1.509

SHAPE AND SURFACE CHARACTERIZATION OF THE PREPARED GLICLAZIDE MICROSPHERES (SEM)

Scanning electron micrograph (SEM) of the prepared microspheres of Gliclazide is showed in different magnifications. SEM images revealed that the microspheres were spherical in shape with a smooth surface morphology.

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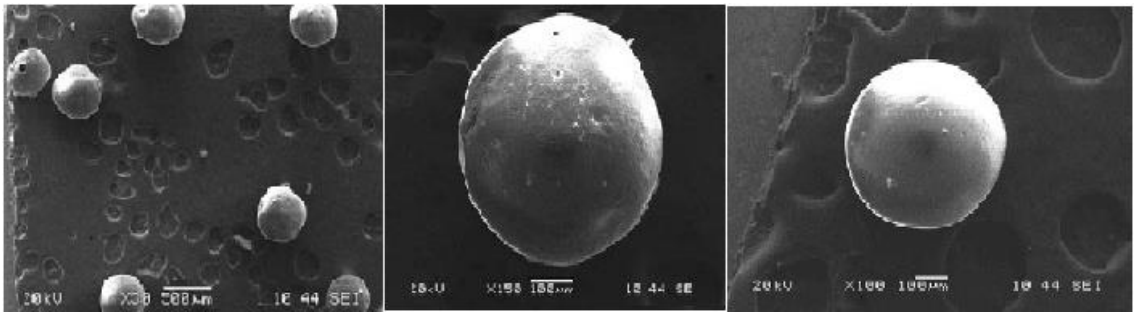

Figure3: Scanning electron micrograph (SEM) of the prepared microspheres of Gliclazide formulation

Table 4: Data for percentage drug content of gliclazide microspheres

FORMULATION CODE	PERCENTAGE DRUG CONTENT
F1	70.56±2.274
F2	75.71±0.991
F3	79.21±2.428
F4	84.83±1.541
F5	94.57±1.162
F6	98.16±1.357

Table 5: Data for percentage yield of gliclazide microspheres

FORMULATION CODE	PERCENTAGE YIELD
F1	61±1.862
F2	66±1.325
F3	69±1.472
F4	74±2.153
F5	79±1.025
F6	81±1.951

Table 6: Data for percentage drug entrapment efficiency ofgliclazide microspheres

Formulation code	Theoretical drug content in %	Practical drug content in %	Entrapment efficiency in %
F1	6.02	5.38	64.45±2.186
F2	8.15	6.73	72.44±2.38
F3	8.15	6.54	77.14±1.171
F4	9.45	7.29	80.24±1.436
F5	12.92	9.36	82.57±1.325
F6	15.25	9.83	89.36±2.428

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Table 7: DATA FOR *IN-VITRO* CUMULATIVE PERCENTAGE DRUG RELEASE OF F1 TO F6 FORMULATIONS

TIME in Hrs	FORMULATION CODE AND CUMULATIVE PERCENTAGE OF DRUG RELEASE					
	F1	F2	F3	F4	F5	F6
1	10.47	16.21	13.25	14.21	15.87	17.42
2	13.92	21.42	16.67	19.50	18.27	25.93
3	19.63	25.32	21.36	24.63	27.90	35.59
4	24.20	31.82	24.68	32.8	32.62	47.46
5	28.14	37.21	28.18	38.73	38.85	56.58
6	35.81	43.81	34.41	43.21	43.17	60.22
7	42.16	48.27	41.37	49.98	51.39	67.64
8	48.33	54.31	47.54	54.72	60.42	75.27
9	54.42	61.88	54.83	61.24	68.25	82.19
10	59.56	67.21	62.74	66.65	74.76	87.33
11	63.39	71.58	69.14	72.37	80.56	91.43
12	66.25	73.82	76.87	79.96	84.73	93.85

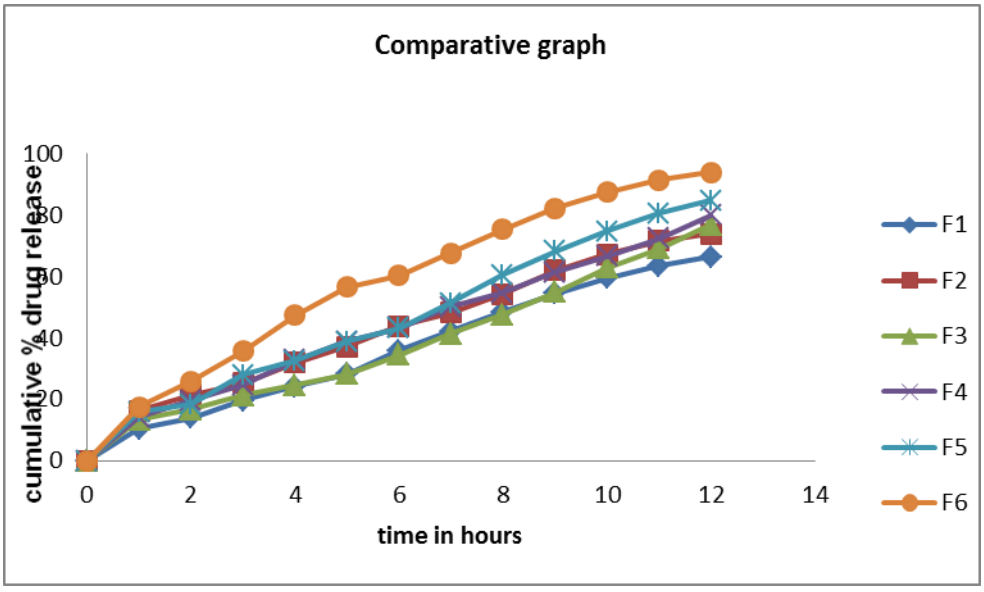

Figure:4: For comparative *in-vitro* cumulative percentage drug release of f1 to f6 formulations

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Table-8: *In-vitro* kinetic data of f1 to f6 formulations

Formula Code	Zero-order Plots	First- order Plots	Higuchi's Plots	Koresmeyer- Peppa's plot		Possible Drug Release mechanism
	Regression Coefficients (R^2)	Regression Coefficients (R^2)	Regression Coefficients (R^2)	Slope (n)	Regression Coefficients (R^2)	
F1	0.993	0.983	0.933	0.806	0.909	Zero-order Non-Fickian release
F2	0.985	0.981	0.961	0.82	0.923	Zero-order Non-Fickian release
F3	0.985	0.923	0.899	0.84	0.934	Zero-order Non-Fickian release
F4	0.992	0.961	0.955	0.85	0.957	Zero-order Non-Fickian release
F5	0.992	0.946	0.938	0.736	0.967	Zero-order Non-Fickian release
F6	0.968	0.961	0.979	0.708	0.934	Zero-order Non-Fickian release

DISCUSSION

Gliclazide is an anti-diabetic drug comes under the category of second-generation sulfonylurea and is very potent drug. It acts as insulin sensitizer that has been widely used in management of NIDDM. Its half-life is 5 hrs and more than 99% bind to plasma proteins. It is absorbed from entire GIT and is mainly excreted through urine remaining through feces. Gliclazide should be administered with breakfast or the first main meal. and recommended dose is minimum 20mg to maximum 80mg per day. In the present work efforts have been made to develop microspheres for controlled drug delivery of gliclazide by emulsion solvent diffusion-evaporation technique using various proportions of poloxamer 407 as a polymer.

Polymer concentration is the major factor for controlling the drug release. Poloxamer 407 formulations led to enhanced solubilisation of poorly water-soluble drugs and prolonged release profile for many galenic applications (e.g., oral, rectal, topical, ophthalmic, nasal and inject able preparations). Poloxamer 407 formulations having the dissolution follow azero-order kinetics due to the rapid dissolution of Poloxamer 407 in the receptor fluid and present advantages of promoting stabilization and water dissolution of many pharmacological drugs. New trends suggest combining Poloxamer 407 with other copolymers (e.g., thickening agents, other types of poloxamers). The FTIR and DSC spectral analysis showed that there was no appearance or disappearance of any characteristic peak of pure drug, physical mixture of drug and polymer, which confirms the absence of chemical interaction between the drug and polymer. Microspheres were prepared using a gradually increasing polymer concentration in combination with a fixed dose concentration of the drug to assess the effect of polymer concentration on the size of the microspheres. The mean particle size or average diameter of the microspheres significantly increased with increasing polymer concentration. Larger particles

developed due to increased viscosity of the medium with an increasing higher polymeric concentration. This is because at higher viscosities there is enhanced interfacial tension and diminished shearing efficiency. Thus, the higher polymeric concentrated microspheres influence the particle size and drug release of the microspheres. The surface morphology was observed by scanning electron microscopic photographs, which showed that the fabricated microspheres were spherical with a smooth in surface.

The percentage yield and drug entrapment efficiency were determined for all the formulations. As the concentration of polymer increases both percentage yield and entrapment efficiency are also increased. The drug content was depends up on the concentration of polymers, as the concentration of polymer increases the drug content also increases due to the rapid dissolution of Poloxamer 407 in the receptor fluid. The prepared microspheres then subjected to dissolution test for evaluating the *in-vitro* drug release studies. The result of dissolution studies indicates that cumulative percentage release of microspheres significantly increased with increasing polymer concentration. The increased density of the polymer matrix at higher concentration results in an increased diffusion path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres formed at a lower polymer concentration and having a larger surface area when exposed to the dissolution medium showed a faster drug release and the polymer concentration maintain the controlled drug release of the drug.

The data obtained for *in-vitro* release kinetics were fitted into equations for the zero-order, first-order, Higuchi and peppa's release models. The interpretation of the data was based on the value of the resulting regression coefficients. The *in-vitro* drug release showed the highest regression coefficient values for the zero order release kinetics, indicating that the polymer shows the controlled release of the drug, and the slope (n) value of peppa's mechanism of drug release was found to be non-fickian diffusion mechanism. The release profile of formulations F5 and F6, were best fitting with USFDA guidelines for extended drug release for 12hrs, and release of the drug not more than 20% in 1^{st} hr and not less than 80% in 12^{th} hr. Based on the parameters like drug content 98.16%, entrapment efficiency-89.36%, and the cumulative percentage drug release rate 93.85% at 12^{th} hrs, and follows zero order drug release kinetics and non-fickian diffusion mechanism. Hence the formulation F6 was considered as the best one among all the formulations.

CONCLUSION

The study concluded that Gliclazide microspheres can be developed with poloxamer 407 polymer by Emulsion solvent diffusion-evaporation method and the results revealed that the formulation F6 shows desired release characteristics in the polymer ratio of (1:6) to achieve the controlled drug release of drug up to 12 hours. Further *in-vivo* studies to be carried out to confirm the formulation.

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