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Research Article

The Study of Hypoglycemic effect of microencapsulated Glimepiride In Long Evans Rats

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Abstract

In this study glimepiride was used as a model drug to evaluate the hypoglycemic effect of microencapsulated or mucoadhesive drug delivery system. Sodium alginate based Microbeads of Glimepiride was prepared using Glycerine monostearate (GMS) for oral sustained release by Orifice-ionic gelation method. The microbeads were evaluated for physical appearance, floating properties, *In-Vitro* release study and *In-Vivo* evaluation. The microbeads were free flowing, spherical in shape and have the good content of uniformity of drugs. The spherical microbeads of 2 mm diameter were prepared by dropping sodium alginate incorporated with Glimepiride solution into aqueous solution of calcium chloride, causing the precipitation of calcium alginate. These Microbeads released the drug for a prolonged residence time of more than 2 hours. The hypoglycemic effect of the mucoadhesive floating drug delivery system is a valuable method for the long term delivery of Glimepiride on Long Evans Rats.

1. Introduction

Microbeads or microencapsulation is used to design and development of sustained release drug and enhances the residence time of the drug at the absorption site using mucoadhesive polymers like sodium alginate[1-4]. These mucoadhesivemicrobeads improve the bioavailability of drugs and reduce adverse effects[5, 6]. Microbeads are spherical in shape and small size. They facilitate the drug administration procedures and are stable, cheap and restraint. Glycerinemonostearate based microbeads are useful to release the drug in a controlled manner for more than 2 hours.

Glimepiride is an oral blood sugar-lowering drug as a third generation Sulfonylurea for the management of type-II diabetes mellitus. It is absorbed at the gastrointestinal tract[7]. In type-II diabetes, insulin is not essential to manage the blood sugar level[8]. Instead, diet, and oral drugs may be sufficient. Inadequate insulin secreted by the pancreas, the elevated blood sugar and Glimepiride lowers the blood sugar level in the blood by stimulating insulin to be secreted from the pancreas into the blood[8].

The sustained release Glimepiride formulation is useful to manage the blood sugar level for the prolonged period of time. It is suitable to design the dosage form due to its short biological half-life of 5 hours and advantageous for diabetic patients. The objective of this study was to investigate the hypoglycemic effect of encapsulated Glimepiride in biological condition.

2. Experimental:

2.1 Materials&Methods: Glimepiride, Glycerinemonostearate, Sodium alginateand calcium carbonate were provided by

Eskayef Bangladesh Ltd.

2.1.1 Preparation of microbeads: Glimepiride loaded mucoadhesivemicrobeads were prepared using Sodium alginate and Glycerinemonostearate by orifice-ionic gelation method. Glycerinemonostearate as a mucoadhesive polymers and Sodium alginate were dissolved in 50 ml of the purified water to form a homogenous polymer solution. The active ingredients 150 mg Glimepiride was added to the polymer solution and mixed well. The viscous solution was then added dropwise into the calcium chloride through a syringe of 22 gauge needle. The added droplets were retained in the calcium chloride solution and spherical microbeads were formed within a few seconds. 4000 mg, 8000 mg and 16000 mg Glycerinemonostearate and 5%, 10% (w/v) calcium chloride were used to prepare the microbeads formulations.

Table 1: Glimepiride microbeads formulations containing Mucoadhesive polymer

Formulations	GMS (mg)	CaCl2 10%	CaCl2 5%
F1	4000	10%	
F2	8000	10%	
F3	16000	10%	
F4	4000		5%
F5	8000		5%
F6	16000		5%

Table 2: Microbeads formation time

Formulations	Glycerinemonostearate (GMS) (mg)	%loading Efficiency	Time(seconds)
F1	4000	91	4
F2	8000	89	3
F3	16000	87	5
F4	4000	88	9
F5	8000	82	10
F6	16000	90	10

2.1.2 Estimation of Glimepiride: IV-visible spectrophotometric method (Shimadzu) was used to determine Glimepiride based on the measurement of absorbance at 229 nm in phosphate buffer pH 7.8 followed by the standard validation method and Beer–Lambert law with the concentration of 4-20 µg/ml.

2.1.3 Efficiency of microbeads: Adequate amount of microbeads were crushed first and weighed. This powder wasthen suspended in methanol to extract the drug from the microbeads so that no material was lost in the procedure. After 24 hours, the methanol extract solution was filtered and measured spectrophotometrically at the 229 nm for drug content against methanol and blank by following formula:

$$\text{Microbeads efficiency} = \frac{\text{Practical Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

2.1.4 Analysis of particle size: Prepared microbeads were filtered through the standard sieve for 10 —15 minutes on a mechanical shake.

2.1.5 *In-Vitro* Dissolution Study: USP dissolution apparatus II (VEEGO-BDA-8DR-USP Standards and Electrolab UPS-XXII) was used to study release rate of Glimepiride microbeads with 900 ml of 0.2 M Phosphate buffer pH 7.8 at 75 rpm and 37 temperatures for 2 hours.At first most of the beads were floated on the dissolution medium.

A sample of the solution was withdrawn from the dissolution testing apparatus over 2 hours and the samples were replaced with fresh dissolution medium. The samples were diluted with 0.2 M Phosphate buffer pH 7.8 as the dilution factor 10 times. Absorbance of these solutions was measured at 229 nm wavelength using Shimadzu Spectrophotometer.

2.1.6 *In-Vivo* Test of Glimepiride Microbeads: Laboratory bred 1 month aged Long Evans Rats of either sex with 62-110 gm body weight were employed to made the diabetes using freshly prepared solution of Aloxen (Sigma Chem, Co., St. Louis, USA) through gavaging at a dose of 150 mg/kg dissolved in 2 mM Citrate Buffer (pH 3.0) after an overnight fasting. The rats were maintained at normal photo period (12 hour dark/12 hour light) with an ambient temperature of 23-26ºC under adequate light and ventilation. Commercial pellet diet and water were provided to the Rats. Long Evans Rats obtained from the animal house, International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B). All experimental studies were conducted as per the Ethical Guidelines of the National Institute of Health, USA and proper permissions obtained from the relevant authorities of The University of Asia Pacific and University of Development Alternative, both at Dhaka, Bangladesh prior to commencing the experiments.

After 1 week, the Rats with fasting blood glucose level of 300 mg/dL or more were considered diabetic and treated for this study. In this experiment a total number of 20 Rats were used. The Rats were divided into 4 groups, each group consisting of 5 Rats. In 4 groups of Rats each containing 2 male Rats and 3 female Rats with 62-110 gm body weight were used. Group 1 was kept as API of Glimepiride administration and group 2 and 3 were administered with 2 mg/kg body weight of mucoadhesive Glimepiride microbeads of F1, F2, F3 and F4, F5, F6 respectively. Group 4 was administered with commercially available dosage form of Glimepiride Tablet.

Blood samples were collected using the retro orbital puncture method at predetermined time at 1 hour interval up to 24 hour collected. Blood glucose levels were measured immediately by glucose oxidase method[13].

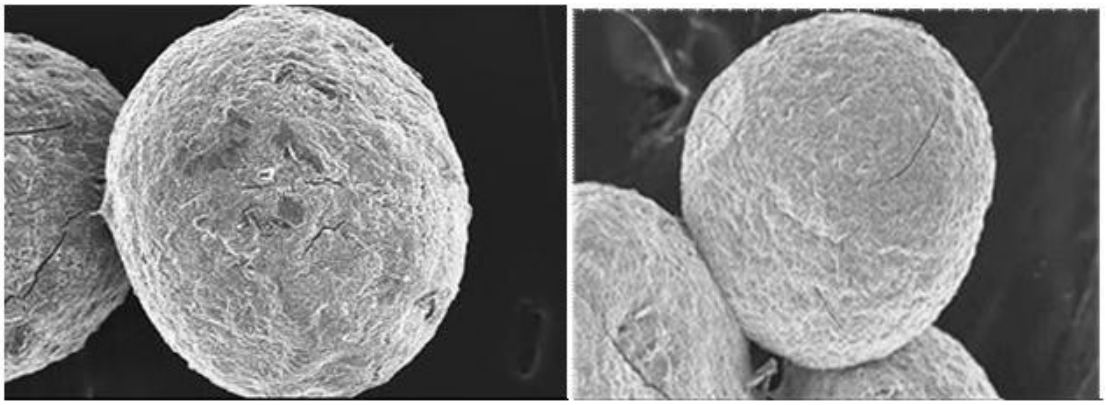
2.1.7 Kinetic Study: The in-vitro release mechanism of drug from floating tablets were determined on the basis of theoretical dissolution evaluations including zero order, first order, Higuchi kinetic model and Korsmeyer-Peppas kinetic model with goodness of fit test.

3. Results and Discussion:

Microbeads of Glimepiride containing glycerinemonostearate and sodium alginate were discrete, spherical and free flowing without coating ingredients. Microbeads were uniform with their particle size and viscosity independent. Particle size was not affected by the polymer of the glycerinemonostearate. The SEM photograph (Figure 1) ofmicrobeadslooked as spherical and rough surface with minor crack. The range of the particle size was from 469 to 490 52 µm for all formulations.

Six formulations were prepared using only two polymers in different quantity to test the in-vitro drug release, loading efficiency and the adhering ability of the microbeads to tissue in phosphate buffer pH 7.8 by the in-vitro wash-off test. From the study, it was found that every tested parameter were dependent on the amount of the polymer. Particle size of the microbeads was not affected by the polymer. The six prepared formulations were used to carry out the in-vitro mucoadhesion test in phosphate buffer pH 7.8, which showed the results between the ranges from 26% to 79% within 6 hours. The percent of mucoadhesion was higher with the lower amount of glycerinemonostearate. The previously published study of Prajapati *et al*[9] and Mankala *et al*[10] showed a formulation containing different polymers admixtures and coating ingredients but this experimental study was done using single polymer based microbeads having no coating ingredients. Also,bioadhesiveproperties of the microbeads were sufficient in strength with the 79% to 68% at the 1 st hour for F1 to F6 microbeads.

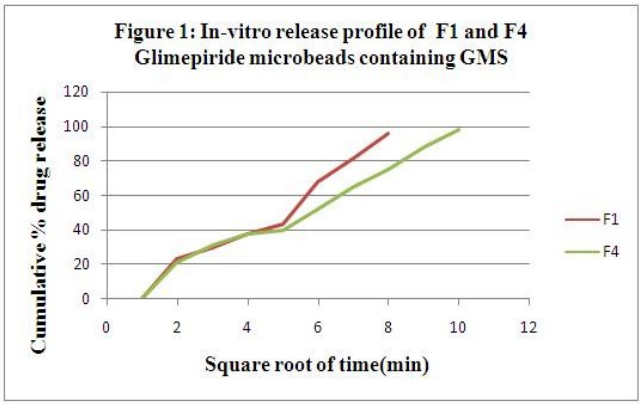
Figure 1: Scanning Electron Photomicrograph of Glimepiride Microbeads

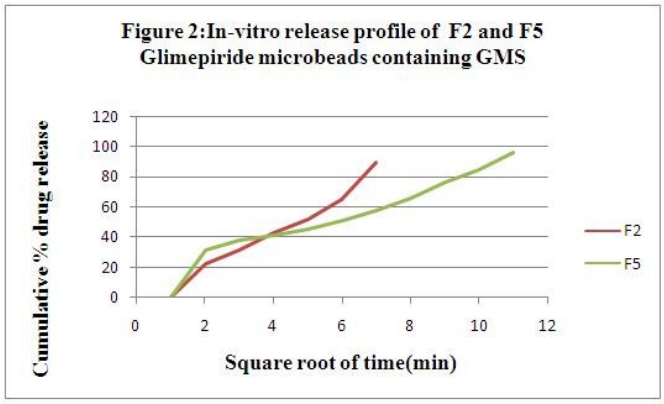


The efficiency of microbeadswas in the range of 88% to 91%. Microbeadscontaining lower amount of glycerinemonostearate with 10% calcium chloride (w/v) showed higher efficiencythan 5% calcium chloride (w/v). Efficiency of microbeadswas dependent on the amount of glycerinemonostearate and calcium chloride. The release rate of Glimepiride from the microbeads was varied according to the prepared formulations. The release of Glimepiride was slow and first in nature. The cause of such release may be for the type and amount of polymer. F1, F2, F3 microbeads containing glycerinemonostearate and 10% calcium chloride (w/v) showed first release and the release was completed within the 150 minutes. The release was highest at the amount of 8000 mg of glycerinemonostearate in F2. The release of Glimepiride microbeads of F4, F5, F6 containing glycerinemonostearate and 5% calcium chloride (w/v)was slow but the release was extended over a period of 6 hours. These microbeads were suitable to prepare the Glimepiride sustained releaseformulation over an extended period of time without coat technique.

Table 3: Kinetic and statistical parameters obtained from drug-release data of Glimepiride microbeads formulations containing Mucoadhesive polymer

Formulation	Zero-order Model		Higuchi Kinetic Model		Best fitted Models	Release Mechanism
	R2	K0	R2	Kh		
F-1	0.931	0.692	0.942	7.015	Higuchi Model	Diffusion & Erosion
F-2	0.923	0.783	0.953	7.13	Higuchi Model	Diffusion & Erosion
F-3	0.917	0.661	0.947	6.717	Higuchi Model	Diffusion & Erosion
F-4	0.892	0.510	0.971	6.164	Higuchi Model	Diffusion & Erosion
F-5	0.665	0.442	0.955	5.822	Higuchi Model	Diffusion & Erosion
F-6	0.914	0.239	0.940	4.094	Higuchi Model	Diffusion & Erosion





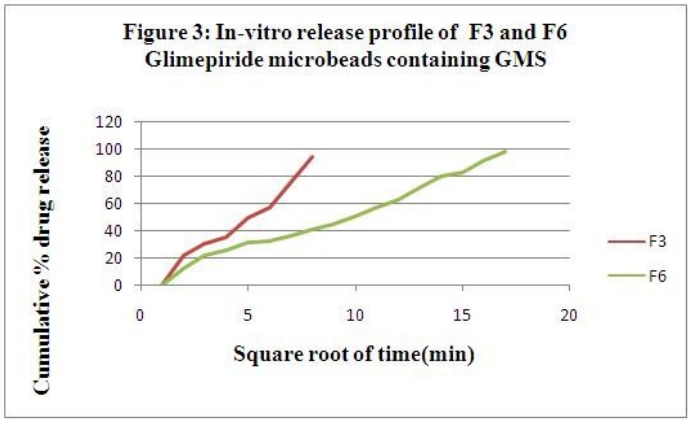
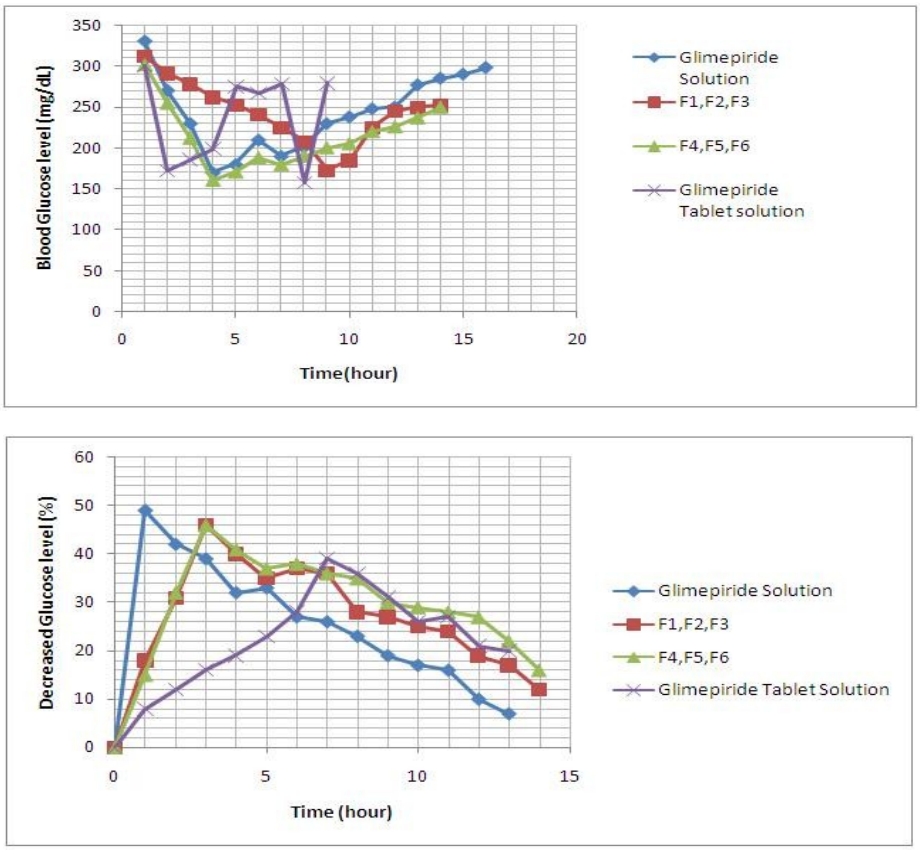


Table 4: Percentage of microbeads adhering to tissue in phosphate buffer pH 7.8

	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
F1	79	69	58	50	47	39
F2	72	65	52	40	34	29
F3	68	60	51	42	36	30
F4	75	62	53	46	35	26
F5	73	64	57	49	38	31
F6	77	67	59	47	36	27

Glimepiride microbeads of F1, F2, F3 and F4, F5, F6 were used for In-Vivo test in Long Evans Rats. The drug were administered at a dose of 2 mg/kg body weight of Glimepiride solution and after oral administration a rapid reduction in blood glucose level was observed and maximum reduction was found at 49% within 1 hour. After 14 hour, the blood glucose level was recovered and 70-99 mg/dLwas considered for normal blood glucose level was considered for normal blood glucose level. Maximum 46% reduction was found after administration of mucoadhesive Glimepiride microbeads after 3 hour and sustained reduction was obtained for a long period of time. The significant reduction was considered at 25% blood glucose level. Blood glucose level was maintained for 10 hour after the treatment with Glimepiride but Glimepiride microbeads showed this maintenance for 16 to 20 houras sustained manner. The Glimepiride microbeads formulation showed a sustained hypoglycemic effect due to a slower release and absorption of drug from the microbeads over a longer period of time. Hence, this newly formulated Glimepiride microbeads dosage form is a significant and potential than immediate release glimepiride dosage form for the treatment of type-II Diabetes.

Figure 4: Comparative *In-Vivo* Study of Glimepiride Microbeads, and commercial dosage form.



From the in-vitro drug release data, it was found that drugs were released for 2 to 6 hours with the highest rate of 98%. The release data were tested with the kinetic models and found fitted with Higuchi Kinetic Model[11]. The release was non-Fickian transport and the R^2 value was found 0.971.

4. Conclusion

Sustained release Glimepiride with single polymer of glycerine monostearate could be prepared by orifice-ionic gelation method as it necessitates the administration of 2 to 3 doses per day. The performed in-vitro release study was sufficient to release the drug in a desired controlled manner from mucoadhesive Glimepiride microbeads. The results were showed that the choice of combination polymers or single polymers will be a potential method for the designing and development of Glimepiride loaded mucoadhesive microcapsules for easy, reproducible and oral controlled drug delivery that is also reported by Ahad et al[12].The obtained formulation was cost effective instead of using HPMC and further study may be suggested to improve the clinical efficiency.

Declaration of Interest

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