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Original Article

DEVELOPMENT AND *IN VITRO-IN VIVO* EVALUATION OF GLIPIZIDE LOADED MULTIUNIT PULSATILE FORMULATION FOR TREATMENT OF DIABETIC PATIENTS

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ABSTRACT

The aim of the present study was to develop oral multiparticulate pulsatile drug delivery system for hypoglycemic agent 'glipizide'. Time dependent rupturable system was selected for delivering glipizide in a pulsatile pattern. In the present study, two types of particles were prepared i. e. Type 1 (immediate release type) and Type 2 (delayed release type). Extrusion and spheronization process was selected to prepare particles, wherein lactose and microcrystalline cellulose mixture (2:1) was used as processing aid. Various parameters of extrusion and spheronization process were optimized in order to meet desired particle size distribution, shape and flow properties. Immediate release Type 1 particle was optimized to achieve more than 80% drug release within 30 min for which surfactant approach was employed to overcome the dissolution rate related issue of the glipizide. Delayed release pattern of Type 2 particles was achieved by coating hydroxypropylmethylcellulose and ethyl cellulose. Various coating parameters were optimized to attain efficient coating of the particles. Different concentrations of hydroxypropylmethylcellulose (5 and 3.5% w/w E-15 grade) and ethyl cellulose (5 and 3% w/w) were studied for release pattern for Type 2 particles. Final formulation was characterized using for particles size, flow properties and surface morphology. To examine the drug release, dissolution studies were performed. Pharmacokinetics studies in Sprague Dawley rats reveal improved oral bioavailability of glipizide following oral administration.

Keywords: Pulsatile delivery, Glipizide, Immediate release, Delayed release, Oral administration, Pharmacokinetic study.

INTRODUCTION

Diabetes mellitus (DM) is a group of diseases marked by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes can lead to serious complications and premature death, but people with diabetes can take steps to control the disease and lower the risk of complications. Type 1 DM account for 5 to 10 percent of all diagnosed cases of diabetes. Risk factors for Type 1 diabetes may be autoimmune, genetic, or environmental. Type 2 DM (non-insulin dependent diabetes mellitus) is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency [1].

Oral sulfonylurea's therapy remains a cornerstone for the treatment of diabetes. Sulfonylurea's acts through insulin release by inhibiting the K_{ATP} channel of the pancreatic β-cells [2]. Glipizide is a second generation sulfonylurea act by stimulating the release of insulin from the pancreas e reducing blood glucose level in humans [3]. Owing to its short biological half life (3-5h), there is an urgent need of such a delivery system which can overcome its multidosing per day through delayed release and extended half life. Thus, development of multiunit pulsatile dosage form would be the best option and advantageous. Various researchers have developed glipizide formulations by different methods [4-8]. Multiunit pulsatile dosage form can be prepared using various polymers and combination of polymers to control the release of the drug through wurster process [9].

Most widely used hydrophobic polymer in pharmaceutical film coating is ethyl cellulose (EC) due to its easy film forming nature, negligible toxicity and better physicochemical properties. EC is a best option for modified release coating [10]. To control the drug release from the formulation, polymers are used in combination with secondary polymer such as hydroxypropyl methylcellulose (HPMC). HPMC is hydrophilic in nature and alters its structure by virtue of pores and channels. Due to this property, drug molecule can diffuse out easily to control the release properties [11]. To prevent cracking of film and efficient film formation, plasticizers are used in EC containing formulations [12]. Moreover, plasticizers act as channeling agent through which drug release occur [13]. Thus, aim of the present investigation is to develop and *in vitro- in vivo* evaluation of novel glipizide loaded multiunit pulsatile formulation using HPMC and EC polymers by wurster process.

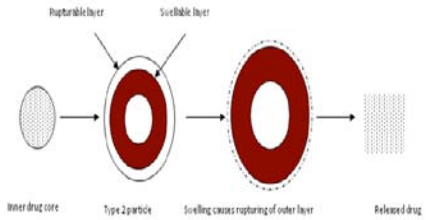

Fig. 1: Mechanism of drug release

MATERIALS AND METHODS

Materials

Glipizide was obtained as a kind gift sample provided by Supra chemicals, Mumbai and Consern Pharma, Ludhiana Punjab. Hydroxypropylmethylcellulose and Polyvinylpyrrolidone were obtained from Signet, Mumbai (India). Ethyl cellulose was obtained from Rohm, Germany. Methanol and other chemicals were obtained from Loba Chemie, Mumbai, India. All other chemicals were of analytical grade.

Preparation of Type 1 (immediate release) and Type 2 particles (delayed release)

The required quantity of drug and excipients was mixed in a pestle and mortar. Type 1 particles was prepared by extrusion and spheronization. Subsequently, these particles were evaluated for various parameters like particle size distribution by sieving, flow properties and friability. Type 2 particles were prepared by coating optimized Type 1 particles using Mini Glatt coater (Germany). Type 1 particles was coated initially with HPMC E-15 followed by EC polymer to obtain the Type 2 particles. The coating process was optimized to obtain a desired release pattern. Type 1 and Type 2

particles were then mixed in a proper proportion to obtain a pulsatile release profile with a lag period of 6-8 h.

Characterization of Type 1

Particles size analysis

Particle size analysis was performed by mechanical sieving using sieves of different sieve number (18, 28, 40, 60 and 100). The plot of sieve number vs. Percent particle by weight retained on each sieve was plotted. Correlation between the particle diameter and sieve number is given in Table 1.

Table 1: Correlation between particle diameter and sieve number

Sieve number	Diameter of particles retained on the sieve (µm)
18	900 and above
28	600-900
40	450-600
60	250-450
100	150-250
Pan	150 and below

Flow ability

Flow ability was assessed by measuring angle of repose and Carr's Index (CI). Angle of repose and tap density were measured by using granulator tester and tap density tester, respectively [14]. Mean of six determinations was reported. The CI was calculated from bulk and tap densities.

$$\text{Tan}\,\alpha = {}^{\text{Height}}/_{0.5 * \text{Base}} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{CI} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} * 100 \quad \ldots. \quad (2)$$

Friability test

10 g particles were weighed and friability test was performed by using Roche friabilator up to 100 counts.

Evaluation of final formulation

Dissolution studies

Final pulsatile release formulation was prepared by combining Type 1 and Type 2 particles in proper proportion. Type 2 particles were selected based on the release profile obtained previously. Final formulation was prepared by mixing the Type 1 and Type 2 particles equivalent to 5 mg of drug, respectively.

Pharmacokinetic studies

Pharmacokinetic study was carried out to determine the oral bioavailability of glipizide formulation. For *in vivo* pharmacokinetic study, male S. D. rats weighing 250–300 g (n=4) were used as per IAEC approved protocol number IAEC/10/72. Animals were kept on fasting overnight before start of experiment. Glipizide suspension in sodium CMC (2.5% w/v) at a dose of 5 mg/kg body weight, and final formulation with dose of 5 mg/kg of body weight was orally administered to rats with the help of oral gavage.

Blood samples (0.5 ml) were collected from the retro-orbital plexus under mild ether anesthesia into heparinized microcentrifuge tubes (containing 20 µl of 1000 IU heparin/ml of blood). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h. After each sampling, 1 ml of normal saline was administered to prevent changes in the central compartment volume. Plasma was separated by centrifuging the blood samples at 10000 g for 10 min, at 15°C.

To 200 µl of plasma, 50 µl of tolbutamide (10 µg/ml) and methanol (500µl) were added and vortexed for 5 min. Finally, it was centrifuged at 10,000 g for 10 min and organic layer was separated which contained glipizide and tolbutamide. Supernatant was collected and analyzed using validated HPLC bioanalytical method.

RESULT AND DISCUSSION

Preparation and characterization of Type 1 and Type 2 particles

Optimized parameters for preparation of Type 1and Type 2 particles are given in Table 2 and 3 respectively. For preparation of Type 2 particles, Type 1 particles were coated with optimized concentrations of 3.5 and 2% w/v of HPMC and EC, respectively. Optimized formula for Type 1 particles is presented in Table 4.

Table 2: Process parameter for extrusion and spheronization

Parameter	Value
Volume of binder solution	12 ml
Concentration of binder solution	2% w/v
Speed of extruder	50%
Speed of spheronizer	3000 rpm
Spheronization time	5 min
Time for drying	2 h

Table 3: Optimized parameters for film coating process

Parameter	HPMC	EC
Inlet air temperature	60^0C	40^0C
Feed solution concentration	3.5% w/v	2% w/v
Flow rate	1.5 ml/min	1.5 ml/min
Fluidization pressure	0.22 MPa	0.22 MPa
Atomization pressure	0.32 MPa	0.31 MPa

* Batch size - 5 g

Table 4: Optimized formula for Type 1 particles

Name of the ingredient	% Quantity
Glipizide	3%
Microcrystalline cellulose	31%
Lactose	62%
SLS	3%
Binder solution (2% w/v) PVP	12%

Characterization

Particles size distribution

Particle size distribution analysis was performed using sieving method. Type 1 particles exhibited maximum retention on the sieve number 28. While, Type 2 particles prepared with 20%w/w HPMC and 5%w/w EC showed that the 80 % particles were retained on the sieve number 22 indicating an increase in the particle size of particles after coating which is indicative of good coating efficiency. The values of CI and angle of repose showed excellent flow properties. Particle size distribution data are presented in Figure 2.

Flow properties

To examine the flow properties, angle of repose, Carr's index, Hausner ratio and friability were measured. Angle of repose for Type 1 and Type 2 particles was 27 and 17.5°, respectively. This is indicative of good flow properties. Type 1 and Type 2 particles exhibited 14.31 and 9.52% Carr's index, respectively. Hausner ratio and friability were well within the limits of good flow behavior.

Surface morphology analysis

Type 1 particles (uncoated glipizide particles) micrograph is presented in Figure 3A. Coating imperfections were observed on particles as indicated by the rough surface. Smooth surface with minimum imperfections was observed with particles coated with the 20% HPMC and 5% EC as shown in Figure 3B. The Figure 3C is the cross sectional image of Type 2 particles showing the two distinct layer of coated polymers. Type 1 particles surface was found to be rough due to the imperfections and nature of the excipients used to prepare the particles while in case of Type 2 particles surface was found to be smooth due to coating of EC which indicates the efficient coating of the particles.

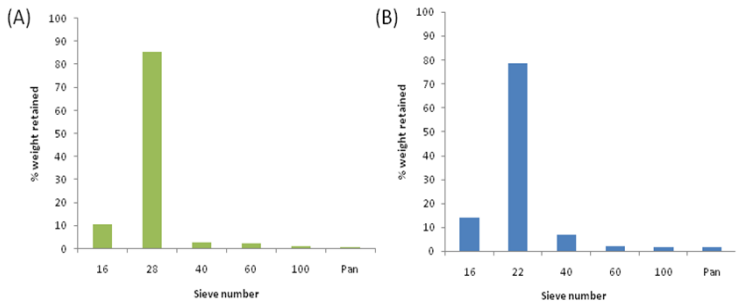

Fig. 2: Particle size distribution data

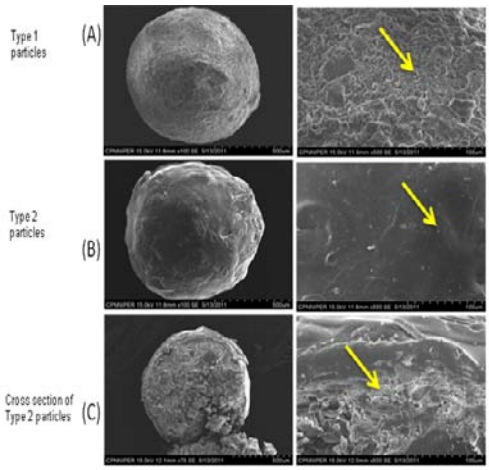

Fig. 3: SEM micrographs of (A) Type 1 particles (B) Type 2 particles (C) Cross section of Type 2 particles

Dissolution studies

To perform *in vitro* dissolution studies, phosphate buffer pH 6.8 was employed as release media. The release pattern of the final formulation showed 50% of drug released within first hour and remaining 50% drug released after a lag time of 7-8 h. Considering the cumulative release of the drug, it was observed that a release was more of delayed type. This is because of rate retarding property of the HPMC where drug release mechanism involves both diffusion through polymer and dissolution of polymer. Percent drug release vs. time profile is presented in Fig.4.

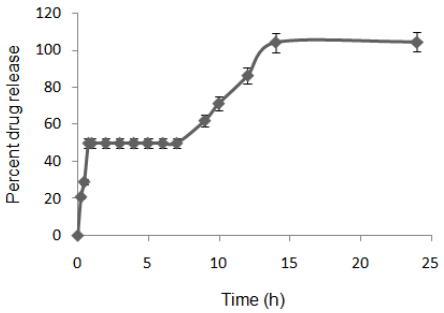

Fig. 4: Percent drug release vs. time profile

In vivo pharmacokinetic studies

As in the case of diabetes mellitus there is increase in blood glucose level after taking the meal, to lower down the glucose level we need such a delivery system which can release the drug at a desired time point. To fulfill this requirement, pulsatile delivery is better option to release the drug immediately and after a delayed time period. In the market, immediate and sustained release formulations are available but these are having problems like pancreatic dysfunctioning and patient noncompliance due to higher dosing frequency. Hence to assure *in vivo* performance of formulation, pharmacokinetic study was carried out to study the release pattern of final formulation. Plasma concentration of glipizide loaded particles (5 mg/kg of each of Type 1 and Type 2 particles) after oral administration was compared with plasma concentration of glipizide suspension (5 mg/kg). The plasma concentration-time profile of glipizide loaded particles, and glipizide suspension is shown in Figure 6. The area under the curve (AUC) was calculated by trapezoidal method from Kinetica 5.0 software. The relevant pharmacokinetic parameters such as C_{max}, t_{max} and AUC_{total} are represented in Table 5.

Pharmacokinetic study showed that 50% of drug released within 1 h and second dose released after a lag time of 6 h which confirms the pulsatile release pattern of the formulation. On the basis of pharmacokinetic studies results it is concluded that this pulsatile formulation can reduce the multidosing and hence, avoid the pancreatic dysfunctioning.

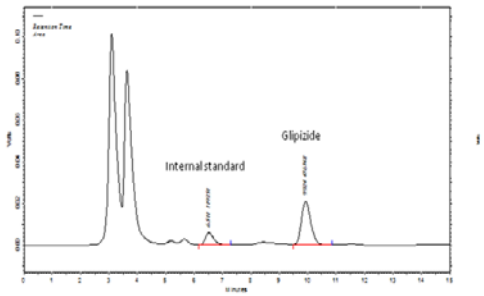

Fig. 5: HPLC chromatograph of internal standard and glipizide

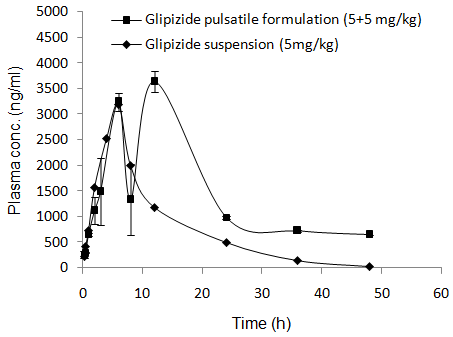

Fig. 6: Comparative in vivo plasma concentration- time profile of a) Glipizide loaded formulation and b) Glipizide suspension after oral administration (all values are reported in mean ±SD, n=4

Table 5: Pharmacokinetic parameters for glipizide suspension and formulation

Formulation studied	Dose (mg/kg)	C_{max} (µg/ml)	t_{max1} and t_{max2} (min)	AUC_{total} (µg min/mL)
Glipizide suspension	5	3300	240	36610.7
Glipizide loaded particles	5+5	3300 and 3600	240 and 720	70743.6

CONCLUSION

Present study is focused on the multiunit pulsatile delivery system for glipizide for treatment of diabetic patients. Type 1 particles were prepared by extrusion- spheronization process and characterized for particles size distribution, flow properties and surface morphology. Particles size analysis showed maximum retention at sieve no. 28 and 22 for Type 1 and Type 2 particles, respectively. Particles were of free flowing in nature as evidenced by flow properties outcomes. Surface morphology showed a rough surface for Type 1 particles and smooth surface for Type 2 particles after coating. *In vitro* release and *in vivo* pharmacokinetic studies of final formulation exhibited biphasic release pattern. Hence, it is concluded that the multiunit pulsatile delivery system could be one of the best option for the treatment of diabetes.

CONFLICT OF INTERESTS

Declared None

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