FORMULATION, IN VITRO AND IN VIVO EVALUATION OF GLIPIZIDE LOADED ETHYL CELLULOSE MICROPARTICLES

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ABSTRACT

Glipizide loaded ethyl cellulose microparticles prepared by emulsion solvent evaporation technique were evaluated for various physico-chemical properties viz. encapsulation efficiency, micromeritic properties like particle size, poured and tapped density, angle of repose, Carr’s index, Hausner’s ratio etc.; surface morphology, in-vitro drug release pattern and in-vivo hypoglycemic activity. The selected microparticles were spherical, discrete, free flowing and have very good entrapment efficiency (81 to 89%). Glipizide release from microparticles was slow, gradual and was dependent on polymer to drug ratio. The formulations with drug to polymer ratio of 1:1 (GEC11) and 2:1 (GEC21), released more than 75% at the end of 12 hours, with release extended up to 24 hours were selected for in vivo studies in animal model using rabbit. In vivo testing of GEC11 demonstrated significant hypoglycemic effect for 12 hours, whereas GEC21 showed for 8 hours in normal rabbits; hence GEC11 is better suitable for oral sustained release formulation.

KEY WORDS: Glipizide, ethyl cellulose, microparticles, sustained release, hypoglycemic.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia (high blood glucose concentration) caused by insulin deficiency and it is often combined with insulin resistance (Mutalik, 2006; Arunachalam and Gunasekaran, 2002). Majority of diabetic patients suffer from type 2, Non Insulin Dependent Diabetes Mellitus (NIDDM). Heterogeneous group of people, predominantly adults are affected by type 2 diabetes mellitus. (Davis and Graner, 1996; Nolte and Karam, 2001).

Current management for type 2 diabetes mellitus (NIDDM) involves frequent oral medications, 3 to 4 times a day, resulting in irregular control of the glucose level. Compliance with frequent administration of medication is often poor and can be improved with once daily formulation and combination therapy (Roger Gadsby, 2002). Convenient medication will improve patient compliance and provide better diabetic management. This can be achieved by sustained release drug delivery systems like microparticles.

Glipizide, a second generation sulphonyl urea, is an effective anti-diabetic drug and is typically prescribed to treat type 2 Diabetes Mellitus (Lebovitez, 1985; Groop, 1985; Zmeili, 1995; Schade, 1987). Predominant mechanism of action of Glipizide appears to be increasing the secretion of insulin from pancreas in both normal and diabetic patients (Jackson and Bressler, 1981; Greenfield, 1982; Nelson, 1987). Glipizide also acts by increasing endogenous insulin as well as its peripheral effectiveness (Davis and Graner, 1996; Nolte and Karam, 2001). Its short biological half life, $3.4 \pm 0.7$ hours, necessitates that it has to be administrated with doses of $2.5 \text{ to } 10$ mg twice or thrice daily (Foster and Plosker, 2000).

From the facts discussed in the previous section, it is clear that development of controlled release dosage form of Glipizide would be advantageous for the management of type 2 diabetes. There are several reports that glycemic control and insulin sensitivity can be improved by use of modified release Glipizide formulations rather than conventional formulations of Glipizide. (Sweetman, 2005; Leaf, 1999; Berelowitz, 1994). Time to time, researchers have formulated oral controlled release products of Glipizide by various techniques (Chowdary and Balatipura, 2003; Jayvadan, 2005; Thombre, 1999; Chowdary and Rao, 2003; Madhusudhan, 2009).

The microparticles require a polymeric substance as coat material or carrier. A number of different polymeric materials both biodegradable as well as non-biodegradable have been investigated for preparation...
of microspheres. (Chowdary and Rao, 2003; Vy as and Khar, 2004). Ethyl cellulose is a non toxic, biocompatible and good film forming agent (Wade and Well er, 1994), has been extensively used in macro- and micro- coating since long time (Oya and Walters, 1981; Benita and Donbrow, 1982; Chowdary , 2004) and hence has been selected as coating polymer for the present study.

Microparticulate delivery systems are used for prolonged and controlled drug delivery, to improve bioavailability, to enhance stability and to target a drug to specific sites (Kondo, 1979; Gutcho, 1976; Barakat and Ahmad, 2008). Microspheres can also offer advantages like minimizing fluctuations of drug concentration within therapeutic range, maintaining steady state concentrations, reducing side effects and thereby providing better and safer therapeutic management by decreasing dosing frequency and improving patient compliance. (Vy as and Khar, 2000; Leung and Robinson, 1987; Davis and Illum, 1988; Ritschel, 1989; Haznedar and Dortunc, 2004; Esposito, 1999). It offers greater effectiveness, safety and stability than conventional dosage forms (Naha , 2008).

The objective of this study was to develop oral sustained release microparticles of Glipizide using ethyl cellulose as polymer to promote better management of NIDDM patients by improving patient compliance. This study describes the preparation and in-vitro & in-vivo evaluation of Glipizide microparticles.

2. MATERIALS AND METHODS

2.1 Materials

Glipizide was obtained as gift sample from Micro Labs, Hosur, India. Ethyl cellulose (EC having ethoxy content of 48-49.5 % by weight and viscosity of 18 - 22 cps) was obtained from Loba Chemie Pvt Ltd, Mumbai; dichloromethane AR, methanol AR, acetonitrile HPLC, tween 80 were from Qualigens, Mumbai; potassium dihydrogen orthophosphate, sodium chloride and sodium hydroxide were received from SD Fine Chemicals, Mumbai, India. All other reagents used were of analytical grade.

2.2 Preparation of Microparticles

Five different formulations of Glipizide microparticles with varying proportion of the polymer, ethyl cellulose, were made (Table 1) by emulsion solvent evaporation technique: ethyl cellulose and Glipizide were dissolved in dichloromethane, this solution was dispersed in purified water containing 0.2% tween 80 and agitated at 1000 rpm by medium duty mechanical stirrer (ROL 124, Remi Motors Ltd, Mumbai). Stirring was continued for 1 hour. The Microparticles were recovered by filtration, washed with distilled water, air dried and stored in a desiccator containing fused calcium chloride as desiccant.

2.3. Evaluation of Glipizide Microparticles

2.3.1. Entrapment efficiency

Glipizide microparticles were dissolved in acetonitrile and sodium hydroxide (0.1M) was added to precipitate the ethyl cellulose, keeping Glipizide in solution. (Madhusudhan , 2009). Then it was filtered through 0.45 µm membrane filter to remove ethyl cellulose and filtrate was used to estimate Glipizide content after suitable dilutions using UV spectrophotometer (Lambda 25, Perkin Elmer, Germany) at 276 nm. Microencapsulation efficiency (E) was calculated using the formula:

\[
E = \{\text{practical drug content (Assay)} / \text{theoretical drug content}\} \times 100.
\]

2.3.2. Drug release study

In vitro drug release from the Glipizide (USP XXVI, 2003) microparticles was carried out in USP Dissolution apparatus type I [Tablet Dissolution Tester, USP XVIII model, Electrolab, India] using 900 ml of phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C and 50 rpm. Microparticles, equivalent to 5 mg of Glipizide, were used for the study. 10 ml of the sample solution were withdrawn at predetermined time intervals (viz. 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 hours). The samples were filtered through 0.45 µm membrane filter and analyzed in a UV-VIS spectrophotometer at 276 nm. Every time the test sample was withdrawn, it was replaced with an equal amount of fresh dissolution medium, maintained at 37°C.

2.3.3. Physical characterization of the microparticles

Surface morphology of the prepared microparticles was studied using scanning electron microscope (JSM 5610 LV SEM, JEOL, Datam Ltd, Tokyo, Japan). Samples were prepared on 10 x 10 mm
brass stub and coated with gold using sputter coater (Jeol auto fine coater, Japan) at accelerating voltage of 20 KV at high vacuum mode.

Particle size analysis of Glipizide microparticles were carried out using Malvern particle size analyzer (Malvern Instruments, UK). About 10 mg of microparticles was suspended in 5 ml of purified water and analysed with an obscuration index of about 10% (measure of amount of light lost due to introduction of sample against light path). Particle size distribution curves (Volume % vs size) were recorded.

Poured density and tapped density of microparticles were measured. Carr’s Index (%) and Hausner’s Ratio were calculated (James Wells, 2002) by the following formula:

Carr’s Index (%) = [(Tapped density - Poured density)/Tapped density] x 100

Hausner’s Ratio = Tapped density / Poured density

The angle of repose was determined by funnel method and was calculated using the following formula:

θ = tan⁻¹(h/r)

where, θ = angle of repose, h = height of heap and r = radius of base of the heap.

2.3.4. In vivo evaluation

In vivo studies were carried out on selected formulations of Glipizide loaded ethyl cellulose microparticles (GEC21 and GEC11) in young healthy New Zealand white rabbits of either sex, weighing between 1.5 and 2 kg. The animals were fed with standard diet and housed under standard temperature and humidity controlled environment. The animal experiments were carried out at central animal house (Regn. No.: 160/1999/ CPCSEA) of Annamalai University with prior approval from IAEC (Approval No.: 572, dated 11.09.2008). The animals were kept under fasting condition with water ad libitum before commencement of the experiment.

The animals were divided into three groups of four animals each (n = 4) and was administered orally with 800 µg/kg body weight of standard Glipizide (Choudary and Rao, 2003) or Glipizide equivalent microparticles as detailed below:

Group I (Standard) : Standard Glipizide.
Group II (Test1) : Formulation GEC11
Group III (Test 2) : Formulation GEC21

Blood glucose levels were estimated, using ‘one touch ultra-2 glucometer’ (blood glucose monitoring system of Lifescan Inc., Johnson and Johnson, USA), at 0 hr, 1 to 12 hrs at every hour interval and thereafter every 2 hours upto 18 hrs and finally at 24 hrs following drug administration. Blood glucose levels of individual animals, expressed as percentage reduction in the glucose level vs. time were determined. Statistical analysis was done by one way analysis of variance (ANOVA) followed by Tukey test (all pairs wise multiple comparison test).

3. RESULTS AND DISCUSSION

Ethyl cellulose microparticles of Glipizide could be prepared by emulsion solvent evaporation technique by using drug and polymer in various ratios. The percentage yields of microparticles were good and ranged from 79% to 91% w/w (Table 3). Ethyl cellulose was selected as polymer because it is non-toxic, biocompatible, and having good film forming and release retarding properties (Dahl, 2006).

3.1 Physico-chemical characterization of the microparticles

SEM images revealed that microparticles were discrete and spherical with smooth surface and complete coverage (Figure 2). The mean particle size of microparticles ranged from 25 to 445 µm. The size distribution of microparticles (Malvern) exhibited varying distribution patterns for batches made with different drug-polymer ratios (Figure 1). In particles with polymer ratio up to 50% it was normal distribution, while with around 66.6% of polymer the peak was very broad and flattened and with 75% and higher it was negatively skewed. The sizes of microparticles increased with increased proportion of polymer. As the polymer ratio increases the polymer solution becomes more viscous and the energy applied may not be adequate to reduce the globule size, leading to formation of bigger size microparticles. This corroborates with earlier findings (Haznedar and Dortunc, 2004; Pongaibul, 1984 and 1988). The particle size analysis data are furnished in Table 3.

The entrapment efficiency was in the range of 56 to 89 %, drug content varied from 13 to 40 % (Table 3). Low values of standard deviation confirm uniformity of drug content in each batch of microparticles. The encapsulation efficiency was found to be directly proportional to polymer proportion in all the formulations except the one with 80% polymer.
From experimental results it was observed that % yield of microparticles depends on the scale up quantities i.e. increases to some extent when respective weights of drug and polymer are increased without change in their ratio, e.g. in ratio of drug to polymer, 1:2, when, 100 mg of Glipizide and 200 mg of ethyl cellulose were taken, the yield was 63.7%, while with 500 mg of Glipizide and 1000 mg of ethyl cellulose, the yield increased to 82.61%.

It was observed that both poured density and tapped density increased with increase of polymer ratio which is justified with relatively higher bulk density of the polymer. Flowability determinants of the microparticles viz. poured density, tapped density, Carr’s Compressibility Index and Hausner’s Ratio are given in Table 2. According to Carr’s compressibility scale, for excellent flowability, the Carr’s index should be between 5 and 15. The Carr’s Index for all the formulations in the present investigation is within 11, indicating excellent flowability (Carr 1965). The Hausner’s Ratios for the formulations are less than 1.2, indicating free flow properties (Hausner, 1967; James Wells, 2002). Angle of repose of the microparticles ranged between 23° and 30°, indicating good flow properties and corroborates well with Carr’s Index and Hausner’s Ratio.

3.2. In vitro dissolution studies

Glipizide release from microparticles was studied in phosphate buffer (pH 7.4) for 24 hr. All the formulations showed burst release during 1st hour and the release ranged from 13% to 35%. In vitro drug release rate of the microparticulate formulations were in the following order: GEC21 > GEC11 > GEC12 > GEC13 > GEC14 (Figure 3). This clearly indicates that release rate decreases as ratio of polymer to drug increases. Thus it may be concluded that the higher ratio of hydrophobic polymer (ethyl cellulose) had more retarding effect on drug release. It was observed that the drug release from the microparticles was slow and extended over 12 hours and the release rate depended on the proportion (ratio) and thickness (load) of coat polymer. The percentage drug release of the formulations varied from 44 to 83% at the end of 12 hours, it was highest in the formulation with drug to polymer ratio 2:1 and lowest with 1:4. In vitro drug release of formulations with drug to polymer ratios 2:1 (GEC21) and 1:1 (GEC11) above 75% at the end of 12 hrs and Tₜ₉ more than 4 hours makes them suitable for 12 hours oral sustained release. Hence, the formulations, GEC21 and GEC11 were selected for in vivo studies in animal model.

3.3. In vivo hypoglycemic activity of Glipizide microparticles

In vivo evaluation of the microparticles of GEC21 and GEC11 were carried out in healthy normal rabbits by measuring the hypoglycemic effect produced after oral administration of Glipizide microparticles and pure standard drug Glipizide at a dose equivalent to 800 μg/kg of body weight. When standard drug Glipizide was administered rapid reduction in blood glucose levels were observed: maximum decrease of 55.75 ± 1.5% was observed at 2 hours after administration and normal level were reverted back within 6 hours (Figure 4). In case of formulations GEC21 and GEC11, the lowering of glucose level was gradual and maximum reduction in glucose level of 44.25 ± 0.97 and 36.75 ± 1.71 reached at 4th and 5th hour respectively after oral administration. The hypoglycemic effect was slow and sustained for more than 8 hours and 12 hours for GEC21 and GEC11 respectively. A 25% reduction in glucose levels is considered significant for hypoglycemic effect (Kahn and Shechter, 1991). From the results of ANOVA (One way analysis of variance) significant difference was observed between the hypoglycemic activity of Standard Glipizide with that of GEC21 and GEC11, (P < 0.05). The result of in vivo hypoglycemic effect of Glipizide ethyl cellulose microparticles in rabbits, (Figure 4) shows that GEC11 has more prolonged effect than GEC21 and both have more sustained hypoglycemic effect compared to that of standard drug Glipizide.

4. CONCLUSION

Ethyl Cellulose microparticles of Glipizide were prepared successfully by solvent evaporation technique with various drug to polymer ratios. The microencapsulation efficiency increased with increasing polymer to drug ratio and varied from 56 to 89% and were reasonably good (>80%) when drug to polymer ratio was between 1:1 and 1:3. All the Glipizide microparticles showed good flow properties as evidenced by Carr’s Index, Hausner’s Ratio and Angle of Repose.

In vitro drug release of the formulations depends on drug to polymer ratios and formulations with drug to polymer ratio 2:1 (GEC21) and 1:1 (GEC11) released
Glipizide above 75% at the end of 12 hrs and $T_{50}$ were more than 4 hours, making them suitable for oral sustained release product, hence the above two formulations were selected for in vivo studies. The release from above formulations was slow, gradual, sustained and expected to exhibit prolonged hypoglycemic activity in vivo. In vivo studies of microparticles in rabbits demonstrated significant and prolonged hypoglycemic effect beyond 12 hours, in case of GEC11 (Glipizide ethyl cellulose microparticles 1:1). This conforms to in vitro drug release profile. Since the microparticles prepared with Glipizide to ethyl cellulose in ratio of 1:1 (GEC11) showed desired release and good hypoglycemic activity in animal model, it may be further exploited as a successful sustained release product.

### Table 1: Formulation of Glipizide loaded ethyl cellulose microparticles

<table>
<thead>
<tr>
<th>S.No</th>
<th>Formulation Code</th>
<th>Glipizide: Ethyl Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GEC21</td>
<td>2:1</td>
</tr>
<tr>
<td>2</td>
<td>GEC11</td>
<td>1:1</td>
</tr>
<tr>
<td>3</td>
<td>GEC12</td>
<td>1:2</td>
</tr>
<tr>
<td>4</td>
<td>GEC13</td>
<td>1:3</td>
</tr>
<tr>
<td>5</td>
<td>GEC14</td>
<td>1:4</td>
</tr>
</tbody>
</table>

### Table 2: Physical Characteristics of Glipizide loaded Ethyl Cellulose Microparticles

<table>
<thead>
<tr>
<th>S.No</th>
<th>Form. Code</th>
<th>Poured Density (g/cm³)</th>
<th>Tapped Density (g/cm³)</th>
<th>Angle of Repose (degrees)</th>
<th>Carr’s Index (%)</th>
<th>Hausner’s Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GEC21</td>
<td>0.177</td>
<td>0.187</td>
<td>28.61</td>
<td>5.35</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>GEC11</td>
<td>0.185</td>
<td>0.192</td>
<td>26.56</td>
<td>3.65</td>
<td>1.04</td>
</tr>
<tr>
<td>3</td>
<td>GEC12</td>
<td>0.217</td>
<td>0.227</td>
<td>23.45</td>
<td>4.40</td>
<td>1.05</td>
</tr>
<tr>
<td>4</td>
<td>GEC13</td>
<td>0.332</td>
<td>0.356</td>
<td>24.71</td>
<td>6.75</td>
<td>1.07</td>
</tr>
<tr>
<td>5</td>
<td>GEC14</td>
<td>0.375</td>
<td>0.419</td>
<td>29.74</td>
<td>10.50</td>
<td>1.12</td>
</tr>
</tbody>
</table>

### Table 3: Physicochemical properties of Glipizide loaded ethyl cellulose microparticles

<table>
<thead>
<tr>
<th>S.No</th>
<th>Formulation Code</th>
<th>Entrapment Efficiency (%)</th>
<th>Drug Content (%)</th>
<th>Particle size (μm) (Malvern)</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GEC21</td>
<td>56.17 ± 1.47</td>
<td>37.67 ± 1.21</td>
<td>25.11</td>
<td>79.43</td>
</tr>
<tr>
<td>2</td>
<td>GEC11</td>
<td>81.17 ± 1.17</td>
<td>40.17 ± 1.17</td>
<td>26.67</td>
<td>83.67</td>
</tr>
<tr>
<td>3</td>
<td>GEC12</td>
<td>81.83 ± 1.33</td>
<td>19.83 ± 1.47</td>
<td>37.04</td>
<td>87.23</td>
</tr>
<tr>
<td>4</td>
<td>GEC13</td>
<td>88.83 ± 1.94</td>
<td>22.67 ± 1.17</td>
<td>95.02</td>
<td>90.85</td>
</tr>
<tr>
<td>5</td>
<td>GEC14</td>
<td>65.17 ± 1.72</td>
<td>13.17 ± 1.16</td>
<td>445.38</td>
<td>82.15</td>
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</table>

* The values are expressed as Mean ± SD; n= 6.
REFERENCES


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