FORMULATION AND EVALUATION OF GASTRO RETENTIVE DRUG DELIVERY SYSTEM OF GLICLAZIDE

Raval Vishal*, Sailor Girish, Seth A.K. and Chauhan Sachinkumar P.

Department of Pharmacy, Sumandeep Vidyapeeth University, At & Po Pipariya, Ta- Waghodia, Dist. Vadodara-391760. (Gujarat) India.

ABSTRACT
The objective of the present investigation was to design gastro retentive ethyl cellulose loaded floating microspheres of gliclazide. To improve the bioavailability and therapeutic efficacy of the drugs used for the diseases associated with the stomach, the retention of drug delivery systems in the stomach for longer time is required. Since, the site of absorption of gliclazide is from stomach thus dosage forms that are retained in stomach by floating; would increase absorption, improve drug efficiency and decrease dose requirements. Microspheres were prepared by solvent evaporation technique. On the basis of 3² full factorial designs were employed, to study the effect of independent variable X₁ polymer-to-drug and X₂ stirring speed on dependent variables, drug entrapment efficiency, particle size and drug content. The optimized formulation a₂ exhibited a high drug entrapment efficiency of 90.54±0.34% drug content 21.55±0.56 mg/ml and the drug release was also sustained for more than 10 hours. This developed gliclazide loaded microspheres may be very much useful for prolonged systemic absorption of gliclazide for proper maintaining blood glucose level and advanced patient compliance.

Key words: gliclazide, ethyl cellulose, gastro retentive, factorial design, entrapment efficiency

INTRODUCTION
Gliclazide, 1-(3-azabicyclo (3.3.0)oct-3-yl)-3-ptolylsulphonylurea is an oral hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM).[1] Gliclazide, a second-generation sulfonylurea oral hypoglycaemic agent [2], has been shown to act directly on the pancreas, and to increase insulin secretion [3]. This effect appears to be initiated by the drug interaction with the cell surface receptors on the pancreatic b-cells [4], resulting in reduced conductance of an ATP-sensitive K+ channel. [5] However, the absorption rate of GLZ from the gastrointestinal tract is slow and variable. A slow absorption of a drug usually originates from either poor dissolution of the drug from the formulation or poor permeability of the drug across the GI membrane. [6] Gliclazide is practically insoluble in acidic media and its solubility increases as the
pH becomes more alkaline. Gliclazide belongs to the Class II of biopharmaceutical classification [7] in which the drug dissolution rate is the controlling step in drug absorption. For gliclazide, the dissolution rate depends upon the gastric emptying time. [8] For any drug or patient oral route is the most common and preferable route for the delivery of drugs. This may be due to ease of administration, patient compliance and flexibility in formulation. [9] Drugs that are easily absorbed from the gastrointestinal tract (GIT) and have a short half-life are eliminated quickly from the blood circulation, so they require frequent dosing. To avoid this drawback, the oral sustained-controlled release formulations have been developed in an attempt to release the drug slowly into the GIT and maintain an effective drug concentration in the serum for longer period of time. However, such oral drug delivery devices have a physiological limitation [10] of gastric retention time (GRT), Variable and short gastric emptying time can result in incomplete drug release from the drug delivery system (DDS) in the absorption zone (stomach or upper part of small intestine), leading to diminished efficacy of the administered dose [11, 12] .

A number of approaches have been developed to increase the residence time of drug formulation at or above the absorption window. Garg and Gupta [13] have classified the gastroretentive dosage forms into four main classes: (i) floating systems [14], (ii) expandable systems [15], (iii) bioadhesive systems [16] and (iv) high density systems [17]. Gastro retentive dosage forms prolonged gastric retention improves bioavailability reduces drug waste, is useful for drugs acting locally in the GIT, drugs which are poorly soluble, drugs which have a narrow absorption window and are unstable in intestinal fluids. [18] Apart from these advantages, these systems offer various pharmacokinetic advantages like maintenance of constant therapeutic levels over a prolonged period and thus reduction in fluctuation in therapeutic levels minimizing the risk of resistance especially in case of antibiotics. [19]

Microspheres hold a unique and special position in the area of bioadhesive drug delivery systems [20]. Floating microspheres are systems used to achieve sustained or controlled drug delivery of drugs into specific or targeted sites, and thereby improve bioavailability of drugs. Microspheres also offer advantages such as limited fluctuation within a therapeutic range, reduction in side effects, decreased dosing frequency and improved
patient compliance [21, 22]. The success of microspheres for sustained or controlled delivery is due to their longer residence time at the site of absorption. [23] The objective of our study was therefore to formulate gliclazide into floating microspheres in order to increase its residence time in stomach with an objective to improve its bioavailability and in addition, extend its duration of action and characterize microspheres of gliclazide.

**MATERIALS AND METHODS**

Gliclazide was obtained as gift sample from S Kant Healthcare, vapi, India. Ethyl cellulose, Light liquid paraffin, Acetone LR, Magnesium Stearate, N-hexane and Petroleum ether was procured from Sulab laboratories, Vadodara.

**Preparation of Microspheres**

Gliclazide microspheres are prepared by solvent evaporation techniques. The $3^2$ factorial designs were employed to prepare microsphere. The drug-to-polymer ratio (1:1, 1:2, and 1:3) and stirring speed (800, 1000, and 1200) were varied in all the nine factorial design batches. Ethyl cellulose solutions were prepared in various concentrations using acetone as a solvent. The drug was dissolved in 8 ml acetone and dispersed in polymer solution. Then resulting dispersion was then poured into continuous phase consisting of liquid paraffin light (135 ml) and n-hexane (15 ml); while mixture was stirred using 3-blade propeller stirrer. Stirring was continued for up to 3 h to allow evaporation of acetone which led to the formation of solid spherical microsphere. Rigidization of microsphere was obtained by using petroleum ether. The prepared microsphere were filtered using What man no.1 filter paper and washed with 50 ml petroleum ether for 4-5 times. Microsphere were dried at room temperature for 24 hr and weighed to calculate yield of microsphere. The prepared microspheres were stored in desiccators until further investigation.

**CHARACTERIZATION OF MICROSPHERES**

**Production yield**

The yield was calculated by dividing the weight of collected Microsphere by the weight of non-volatile compound used for the preparation of microsphere and expressed in term in percentage.[9]
Percentage Yield = \frac{\text{Weight of Microsphere}}{\text{Weight (Drug+ Polymer)}} \times 100

**Particle Size analysis**

The particle size was determined using an optical microscope under regular polarized light, and mean particle size was calculated by measuring 100 particles with the help of a calibrated eyepiece micrometer.

**Drug Content**

The drug content of all the formulation was determined spectrophotometrically at 225 nm using UV-visible Spectrophotometer. About 50 mg of accurately weight of drug loaded Microsphere were added to 50 ml of phosphate buffer pH 1.2. The resulting mixture was shaken for 24 hrs in orbital shaking machine. The solution was filtered a 0.45µm pore size filter and 1 ml of this solution was appropriately diluted to 10 ml using phosphate buffer pH 1.2 and analyzed at 225 nm. Quantitative estimation of gliclazide was calculated using equation obtain by linear regression analysis of the calibration data of gliclazide in phosphate buffer pH 1.2.

**Drug Entrapment Efficiency**

50 mg of microspheres were crushed in a glass mortar and pestle and powered microspheres was suspended in 10 ml of phosphate buffer solution (Ph 1.2). After 24 hours the solution filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using following formula, [24]

\[
\text{Drug Encapsulation efficiency} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100
\]

**In Vitro Buoyancy studies**

The 50 mg of microspheres was kept in a 100 ml beaker containing simulated gastric fluid (pH 1.2), stirred on magnetic stirrer for 6 h. The layer of buoyant microsphere was pipette out and separated by filtration. Microspheres in the sinking particulate layer were collected, separated by filtration. Microspheres of both types were dried at 60^\circ C in desiccators and weighed. The buoyancy was determined formula.

\[
\text{Buoyancy (\%)} = \frac{W_f}{(W_f + W_s)} \times 100
\]

Where,\( W_f \) is the weight of floating microspheres after drying.

\( W_s \) are the weight of settled microspheres. [25]
**In vitro drug release study**

The drug release study was performed using USP I basket apparatus at 37\(^{0}\)C±1\(^{0}\)C and 100 rpm using 900 ml of is phosphate buffer (pH 1.2) containing 1% SLS as a dissolution medium. Microsphere equivalent to 10 mg of gliclazide were used for the test, filled in hard gelatin capsule. Sample of 5 ml were withdrawn at predetermined time interval and filter through 0.45 micron membrane filter, diluted suitable and analyzed at 225nm. Percentage drug dissolved at different time intervals was calculated using Beers Lambert’s law equation. [23]

**Surface morphology (SEM)**

The morphology of optimized batch was determined using scanning electron microscopy (SEM). Prior to examination, the samples were mounted on to metal stubs using a double sided adhesive tape under vacuum. The scanning electron microscope was operated at an acceleration voltage of 20 kV.

**Release Kinetics and Mechanism**

To know the release mechanism and kinetics of gliclazide, optimized formulation was attempted to fit in to mathematical models and \( n \), \( r^2 \) values for zero order, first order, higuchi and peppas models.

**Stability Studies**

The optimized formulation (a2) of microspheres was tested for stability studies. The sample was stored at 4±1°C, 25±2°C and 60±5% RH and 40±2°C and 75±5% RH over period of one month. Sample was evaluated at 0, 15, 30, 45 and 60 days, for their percentage entrapment efficiency, any change in their physical appearance and drug release.

**RESULT AND DISCUSSION**

**FORMULATION OF MICROSPHERES**

The floating microsphere of gliclazide were prepared by solvent evaporation method by using \( 3^2 \) factorial designs by taking different drug polymer ratio i.e. 1:1,1:2 & 1:3 prepared at different RPM i.e. 800,1000 & 1200. The results depicted clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among nine batches.
Table 1. Formulation of gliclazide microsphere by $3^2$ factorial design layout

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Formulation code</th>
<th>Drug: Polymer ($X_1$)</th>
<th>Stirring speed ($X_2$) rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
</tr>
<tr>
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<td>a3</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
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<td>a4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
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<td>a6</td>
<td>0</td>
<td>+1</td>
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<td>7</td>
<td>a7</td>
<td>+1</td>
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<tr>
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<tr>
<td>9</td>
<td>a9</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

Translation code levels in actual unit

<table>
<thead>
<tr>
<th>Variables level</th>
<th>Low (-1)</th>
<th>Medium (0)</th>
<th>High (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug: Polymer ($X_1$)</td>
<td>1:1</td>
<td>1:2</td>
<td>1:3</td>
</tr>
<tr>
<td>Stirring speed ($X_2$) rpm</td>
<td>800</td>
<td>1000</td>
<td>1200</td>
</tr>
</tbody>
</table>

CHARACTERIZATION OF MICROSPHERES:

The prepared formulations were optimized on the basis of particle size, EE, drug content, % buoyancy & in vitro release studies.

Particle Size

The mean particles size of microspheres was found to be in a range of 106.36±2.29 to 139.22±2.32 µm (Table 7.5). Particle size is decrease with increase stirring speed and polymer ratio. Particle size of optimized batch was found to be 118.75±2.12 µm.

Drug Content

Drug content of all batches was in the range 21.55±0.56 to 14.2±0.48 .The optimum batch a2 has high drug content 21.55±0.56.

Drug Entrapment Efficiency
The drug entrapment efficiency was an important variable for assessing the drug loading capacity of microspheres and their drug release profile. However stirring speed has negative effect on drug entrapment efficiency, hence the stirring speed increased, the drug entrapment efficiency decreased. The drug entrapment efficiency was in the range of 59.66±0.32 % to 90.54±0.34 % indicates that as drug-to-polymer ratio increase there was decrease in entrapment efficiency. This may be due to the permeation characteristic of each polymer used that may have facilitated the diffusion of drug to surrounding medium during the preparation of floating microspheres.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug content(mg/ml)</th>
<th>Mean particle size (µm)</th>
<th>Drug entrapment efficiency (%)</th>
<th>Buoyancy%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>21.13±0.23</td>
<td>139.22±2.32</td>
<td>88.78±0.23</td>
<td>95.21±0.61</td>
</tr>
<tr>
<td>a2</td>
<td>21.55±0.56</td>
<td>118.75±2.12</td>
<td>90.54±0.34</td>
<td>96.54±0.68</td>
</tr>
<tr>
<td>a3</td>
<td>19.00±0.60</td>
<td>106.36±2.29</td>
<td>79.83±0.13</td>
<td>94.89±0.41</td>
</tr>
<tr>
<td>a4</td>
<td>16.9±0.48</td>
<td>125.44±1.72</td>
<td>71±0.45</td>
<td>93.84±0.54</td>
</tr>
<tr>
<td>a5</td>
<td>19.30±0.68</td>
<td>118.01±2.21</td>
<td>81.09±0.56</td>
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<tr>
<td>a6</td>
<td>16.59±0.45</td>
<td>111.59±4.42</td>
<td>69.70±0.24</td>
<td>94.75±0.57</td>
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<tr>
<td>a7</td>
<td>12.95±0.62</td>
<td>138.48±2.25</td>
<td>54.41±0.36</td>
<td>95.65±0.48</td>
</tr>
<tr>
<td>a8</td>
<td>14.5±0.58</td>
<td>124.43±2.34</td>
<td>60.92±0.52</td>
<td>94.25±0.65</td>
</tr>
<tr>
<td>a9</td>
<td>14.2±0.48</td>
<td>118.23±2.43</td>
<td>59.66±0.32</td>
<td>93.58±0.24</td>
</tr>
</tbody>
</table>

In vitro buoyancy studies
The percentage buoyancies of the microspheres were found to be decrease with increase in drug-to-polymer ratio. This may be due to the rapid penetration of dissolution medium
in the microsphere. The maximum decrease in buoyancy with increase in concentration polymer was found with ethyl cellulose (1:3); this may be due to the rapid ingress of dissolution medium in microsphere due to rapid dissolution of ethyl cellulose creating more porous structure. The percentage buoyancy of the floating microspheres was in the range of 93.58±0.24 % to 96.54±0.68 %. The a2 formulation shows 96.54±0.68 % that is good floating ability then the others.

**In vitro drug release studies**

The cumulative release of gliclazide microspheres significantly decreased with increasing in polymer concentration the increased density of the polymer matrix at higher concentration resulted in a increased diffusion path length .this may decreased the overall drug release from polymer matrix .In vitro drug release of 9 batch were in the range 69.65±0.034 to 83.57±0.033 % CDR .

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>a1</th>
<th>a2</th>
<th>a3</th>
<th>a4</th>
<th>a5</th>
<th>a6</th>
<th>a7</th>
<th>a8</th>
<th>a9</th>
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<td>0</td>
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<tr>
<td>1</td>
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<td>6.16</td>
<td>5.42</td>
<td>6.94</td>
<td>6.48</td>
<td>5.47</td>
<td>6.56</td>
<td>4.98</td>
<td>4.89</td>
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<td>9.05</td>
<td>9.78</td>
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<td>9.05</td>
<td>7.32</td>
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<td>3</td>
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<td>20.50</td>
<td>21.49</td>
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<td>74.35</td>
<td>71.61</td>
<td>69.65</td>
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</table>
Fig 1. Dissolution profile of formulation a1 to a3

Fig 2. Dissolution profile of formulation a4 to a6
Selection of Ideal Batch:
Among the different gliclazide containing microspheres formulations, the formulation a2 was selected as the ideal formulation, after considering its optimum mean Particle size, better drug content, good percentage buoyancy and also drug release at sustained manner up to 24 hr.

Surface Morphology (SEM):
The SEM photograph of a2 showed that the prepared floating microspheres were spherical with a smooth surface.
Fig4. SEM photograph of microspheres of optimize batch (a2)
Released Kinetics:
The *in vitro* release data obtained from formulation a2 was fitted to various kinetic models. In all the cases, R value of Higuchi model close to 1 so it was concluded that diffusion was the main mechanism of drug release from floating microspheres. Diffusion coefficient (n) for korsmeyer-Peppas model was 0.843. Where n = slope (n ≤ 0.5 - fickian diffusion; 0.5 < n <1 – non fickian diffusion; 1- case-II transport; > 1-super case- II transport). Further, observed diffusion coefficient values are indicative of the fact that the drug release from formulation follows non fickian transport mechanism.

<table>
<thead>
<tr>
<th>%cdr</th>
<th>log%cdr</th>
<th>t</th>
<th>Log t</th>
<th>√t</th>
<th>3√t</th>
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<td>0</td>
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<td>1</td>
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<tr>
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<td>0.301</td>
<td>1.4142</td>
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<td>1.732</td>
<td>1.4422</td>
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<tr>
<td>83.57</td>
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<td>24</td>
<td>1.3802</td>
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<td>2.8844</td>
</tr>
</tbody>
</table>

**Fig 5. Release profile of gliclazide-loaded microspheres**
(a) Zero order kinetic

(b) First order kinetic

Kors meyer peppas model

www.pharmasm.com Impact factor – 0.3317/ ICV – 4.01
(c) Kors meyer peppas model

![Higuchi equation graph]

\[ y = 20.38x - 17.07 \]
\[ R^2 = 0.995 \]

square root of time

(d) Higuchi equation

![Hixson crowel model graph]

\[ y = 42.22x - 42.31 \]
\[ R^2 = 0.980 \]

cube root of time

(e) Hixson crowel model

Table 5. Model fitting for the release profile of optimized microspheres

<table>
<thead>
<tr>
<th>Zero order</th>
<th>First order</th>
<th>Hixson crowel model</th>
<th>Peppas model</th>
<th>Higuchi model</th>
<th>N value of peppas model</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.955</td>
<td>0.498</td>
<td>0.980</td>
<td>0.984</td>
<td>0.995</td>
<td>0.843</td>
</tr>
</tbody>
</table>
Therefore the n value obtained from Korsmeyer-Peppas model confirmed non-fickian type drug release from the microspheres. R value of higuchi model indicates that the drug follows the diffusion mechanism for drug release.

**Stability Studies:**

Stability studies of gliclazide loaded microspheres for optimized batch (a2) were conducted over a period of 2 months. Drug stability in the microspheres formulations was assessed by calculating the % drug remaining of stored formulation. There was no effective change in the gliclazide content in the formulation stored at 25°C±2°C/60%±5% RH and 5°C±2°C at the end of 60 days of stability studies. However, the samples kept at 40°C±2°C/75%±5% RH, significant reduction in amount of gliclazide was detected at the end 60 days. This might be due to the degradation of both drug and polymer.

**CONCLUSION**

The results of a $3^2$ full factorial design revealed that the independent variables like polymer-to-drug ratio and stirring speed significantly affected the dependent variables like drug entrapment efficiency, particle size and in vitro buoyancy. The floating microspheres of gliclazide may enhance the absorption and bioavailability by increasing gastric residence time. It could reduce dose frequency, decrease side effects, and improve patient compliance.

**ACKNOWLEDGEMENTS**

The authors are highly thankful to Department of Pharmacy, Sumandeep Vidyapeeth, Pipariya, Vadodara for providing all facilities to carry out the work.

**REFERENCES**


For Correspondence:
Raval Vishal
Email: vishalraval.raaval5@gmail.com