Controlling of systemic absorption of gliclazide through incorporation into alginate beads

Raida S. Al-Kassas*, Omaimah M.N. Al-Gohary, Monirah M. Al-Faadhel

Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 17221, Riyadh 11484, Saudi Arabia

Received 9 March 2006; received in revised form 1 March 2007; accepted 29 March 2007

Available online 5 April 2007

Abstract

This work investigates preparation of biodegradable beads with alginate polymer by ionotropic gelation method to take the advantages of the swelling and mucoadhesive properties of alginate beads for improving the oral delivery of the antidiabetic agent gliclazide. It demonstrates that the ionic gelation of alginate molecules offers a flexible and easily controllable process for manipulating the characteristics of the beads which are important in controlling the release rate and consequently the absorption of gliclazide from the gastrointestinal tract. Variations in polymer concentration, stirring speed, internal phase volume and the type of surfactant in the external phase were examined systemically for their effects on the particle size, incorporation efficiency and flow properties of the beads. The swelling behavior was strongly dependent on the polymer concentration in the formulations and the pH of the medium. The in vitro release experiments revealed that the swelling is the main parameter controlling the release rate of gliclazide from the beads. In vivo studies on diabetic rabbits showed that the hypoglycemic effect induced by the gliclazide loaded alginate beads was significantly greater and more prolonged than that induced by the marketed conventional gliclazide tablet (Gliclazide®). The results clearly demonstrated the ability of the system to maintain tight blood glucose level and improved the patient compliance by enhancing, controlling and prolonging the systemic absorption of gliclazide.

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Keywords: Gliclazide; Alginate beads; Diabetic rabbits; Formulation parameters

1. Introduction

There has been a growing interest in the use of natural polymers as drug carriers due to their biocompatibility and biodegradability. Alginates are naturally occurring polysaccharides obtained from marine brown algae, consist of two monomeric units, β-d-mannuronic acid (M) and α-L-guluronic acid (G). These residues are arranged in homopolymeric blocks (GG and MM) and in heteropolymeric blocks (MG). Alginates show gelling properties in the presence of divalent cations such as Ca²⁺, Sr²⁺ or Br²⁺. The gelation phenomenon can be explained by the egg-box model in which divalent cation binds to two carboxyl groups on the adjacent alginate molecules (Heng et al., 2003). Gel beads of calcium alginate can be produced by extruding sodium alginate solution as droplets into calcium chloride solution. The hydrogel properties of calcium alginate beads have been proposed for controlling the release of small molecules and macromolecules (Kim and Lee, 1992; Gombotz and Wee, 1998). In addition, being mucoadhesive, these beads are likely to stick to the intestinal mucosa for prolonged period of time and have been exploited for the site specific drug delivery to mucosal tissues (Chickering et al., 1997).

Gliclazide, 1-(3-azabicyclo-[3,3,0]-oct-3-yl)-3-(p-tolyl sulfonyl)urea (Scheme 1) is an oral hypoglycemic second generation sulfonyl urea drug which is useful for a long-term treatment of non-insulin dependent diabetes mellitus (NIDDM) (Harrower, 1994). Previous studies showed that gliclazide possesses good general tolerability, low incidence of hypoglycemia (Palmer and Brogde, 1993) and low rate of secondary failure (Mailhot, 1993).

For an oral hypoglycemic drug to be effective, rapid absorption from the gastrointestinal tract is required. However, the absorption rate of gliclazide from the gastrointestinal tract is slow and varied among the subjects (Hong et al., 1998). Several studies on healthy volunteers and diabetic patients revealed that the time to reach plasma concentration (t_{max}) ranged from 2 to 8 h following a single oral administration of 80 mg of gliclazide tablet (Palmer and Brogde, 1993). The slow absorption has been suggested to be due to either poor dissolution of gliclazide owing...
to its hydrophobic nature or poor permeability of the drug across the GI membrane (Hong et al., 1998). Incorporation of gliclazid in controlled release dosage forms such as alginate beads may control its absorption from the gastrointestinal tract and overcome the variability problems. Alginate beads have been investigated for the development of oral drug delivery systems for many drugs however, reports are not available regarding the incorporation of the hypoglycemic agent gliclazid. Thus, this study was undertaken to develop controlled release formulations of gliclazide using alginate beads. The effects of processing conditions on the physical characteristics of the prepared beads and the \textit{in vitro} drug release rate were investigated. Parameters such as polymer concentration, volume of the internal dispersed phase, stirring speed and the type of surfactant in the external phase were considered. Moreover, the hypoglycemic effect of prepared gliclazide loaded alginate beads on the diabetic rabbits was investigated and compared with that of marketed conventional gliclazide tablet.

2. Materials and methods

2.1. Materials

Gliclazide powder was kindly provided by Riyadh Pharma (Saudi Arabia). Sodium alginate was purchased from Hopkins & Williams Ltd. (United Kingdom). Calcium Chloride was obtained from Fluka Chemie AG (Switzerland). Pluronic® was purchased from BASF AG (Germany). Tween 80 and sodium lauryl sulfate were obtained from Sigma Chemical Co. (USA). All other chemicals were commercially available products of analytical grade.

2.2. Preparation of gliclazide loaded Ca-alginate beads

Formulation and schematic diagram of gliclazide loaded Ca-alginate beads preparations are shown in Table 1. Gliclazide loaded Ca-alginate beads were prepared using ionotropic gelation method as described by Takeshita et al. (1993) and Sugawara et al. (1994). Gliclazide powder was added to a solution of sodium alginate and dispersed homogeneously. The bubble free suspension was forced through a needle into 150 ml of a stirred (0.1 M) calcium chloride solution (CaCl$_2$) at a flow rate of 10–12 drops/min. Stirring of the mixture was continued using a mechanical stirrer at 400 rpm for 30 min. Alginate gel beads were allowed to stand in CaCl$_2$ solution for 72 h until they were fully recovered. The beads were then separated by filtration on filter paper, washed three times with 100 ml deionized water, and allowed to dry at room temperature for 24 h.

2.3. Test of drug content

An amount of beads containing a theoretical weight of 8 mg of gliclazide was accurately weighed and broken down using mortar and pestle. The ground beads were placed in 100 ml of phosphate buffer pH 7.2, and shaken for 48 h at 37°C ± 0.5. The samples were filtered through 0.45 μm filters to obtain clear solutions and analysed for the drug content spectrophotometrically at 228 nm. The Percent drug loading and incorporation efficiency were calculated using the following equations (Soppimath et al., 2001):

$$\text{Percent drug loading} = \left( \frac{\text{amount of drug in beads}}{\text{amount of beads}} \right) \times 100$$

(1)

$$\text{Percent incorporation efficiency} = \left( \frac{\% \text{drug loading}}{\% \text{theoretical loading}} \right) \times 100$$

(2)

Table 1

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Alginate concentration (%)</th>
<th>Stirring speed (rpm)</th>
<th>Volume of internal phase (ml)</th>
<th>External phase containing 1% CaCl$_2$</th>
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<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>–</td>
</tr>
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<td>1$^a$</td>
<td>66.66</td>
<td>400</td>
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<td>–</td>
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<td>2$^a$</td>
<td>40</td>
<td>400</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>3$^a$</td>
<td>33.33</td>
<td>400</td>
<td>38</td>
<td>–</td>
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<td>24.80</td>
<td>400</td>
<td>38</td>
<td>–</td>
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<tr>
<td>5$^b$</td>
<td>50</td>
<td>1000</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>6$^b$</td>
<td>50</td>
<td>1700</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>7$^c$</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>8$^c$</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>9$^c$</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>10$^d$</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>1% Pluronic</td>
</tr>
<tr>
<td>11$^d$</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>1% Tween 80</td>
</tr>
<tr>
<td>12$^d$</td>
<td>50</td>
<td>400</td>
<td>38</td>
<td>1% Na lauryl sulfate</td>
</tr>
</tbody>
</table>

$^a$ Effect of polymer concentration.

$^b$ Effect of speed of stirring.

$^c$ Effect of volume of internal phase.

$^d$ Effect of type of surfactant.
2.4. Particle size analysis and morphology

Particle size distribution and the mean diameters of the beads were determined by sieving the beads on a mechanical shaker using a nest of standard sieves [BP test sieves (Endecotts Ltd., UK)] with a shaking time of 15 min. The surface morphology was studied by scanning electron microscopy (35JEOL SEM) with gold coating.

2.5. Density measurements

The density of the 50 ml of 0.1 M HCl solution was determined in a tarred 50 ml density bottle. Approximately 1 g of the beads was weighed accurately and placed in a previously tarred density bottle and filled with 0.1 M HCl. The bottle was then stoppered to exclude any air bubbles by gentle agitation. From the weight of the beads and the weight of the displaced HCl solution, the densities of the beads were calculated. The determinations were carried out three times to establish the reproducibility of the results.

2.6. Swelling measurement

Swelling rate of the beads was measured as a function of pH. The beads were incubated in phosphate buffer (pHs 5.8 and 7.2) and 0.1 M HCl (pH 1.2) solutions at 37 °C. At different time intervals the beads were removed and weighed after drying the excess water using filter papers. The extent of swelling was determined by suspending the beads in 0.1 M HCl solution at 37 °C until equilibrium was reached. The beads were then removed and weighed after drying the excess water. The swelling rate and the extent of swelling were determined by calculating the water uptake using the following equation:

\[ \% \text{Water uptake} = 100 \times \left( \frac{\text{weight of wet microspheres}}{\text{weight of dry microspheres}} - 1 \right) \]

The weight measurements of the swollen beads were determined using a single pan balance (Mettler AE240S, Switzerland) having an accuracy up to fifth decimal.

2.7. Determination of the flow property of gliclazide loaded Ca-alginate beads

The flow properties of the beads were evaluated from the changes in the volume due to rearrangement and packing occurring during tapping in a graduated measuring cylinder and was expressed as

1. Carr’s compressibility index (Carr, 1965):

\[ \text{CC} \% = \left( \frac{\rho t - \rho b}{\rho t} \right) \times 100 \]

2. Hausner ratio (HR) (Hausner, 1967):

\[ \text{HR} = \frac{\rho t}{\rho b} \]

2.8. In vitro dissolution studies

Weighed quantities of beads equivalent to 80 mg of gliclazide were placed in basket which was lowered into 900 ml of test solutions [phosphate buffer (pHs 5.8 and 7.2) and 0.1 M HCl (pH 1.2)]. The solutions were previously warmed and maintained at 37 °C ± 0.5 and the baskets were rotated at 100 rpm. At appropriate time intervals, 5 ml samples were collected, filtered through 0.45 μm millipore filter unit and analysed for drug content spectrophotometrically at 228 nm. An equal volume of fresh medium was added to the test solution to maintain constant volumes.

2.9. In vivo studies

2.9.1. Induction of diabetes into a rabbit model

Male New Zealand white rabbits weighing 3.0–4.0 kg were used for the study. After fasting for 24 h prior to treatment, 140–150 mg kg⁻¹ of alloxan solution (3% alloxan prepared with normal saline solution and sterilized by filtration) was injected into the marginal ear vein of the rabbits, which were then kept in different cages and supplied food and drink ad libitum. Seventy-two hours later, rabbits had stabilized with a blood glucose concentration above 300–380 mg dl⁻¹ were used for the study. The 18 diabetic rabbits were randomly divided into three groups, and each group (n = 6) was kept in one cage and treated orally with one of the following formulations: (1) control (normal saline solution), (2) marketed conventional gliclazide tablets (Gliclazide®) received from Alphapharma, UK (gliclazide dose 80 mg) which were delivered with distilled water to the esophagus of the rabbits through a cut-end plastic syringe placed into the mouth (Tunçel et al., 1996) and (3) gliclazide loaded Ca-alginate beads (formulation code 1³) (gliclazide dose 80 mg). The beads were suspended in distilled water before oral administration.

2.9.2. Measurement of blood glucose level

Blood samples were collected from the marginal ear vein of the rabbits and the blood glucose levels were determined by placing one drop of the fresh blood on Glucoscan Test Strip (Lifescan Inc., CA, USA) and reading by a Glucoscan 3000 Meter (Lifescan Inc.). Blood glucose concentrations were measured at −1 (1 h before oral administration), 0, 2, 4, 6, 8, 10, 12 and 24 h after dosing, respectively. Results were shown as mean values of plasma glucose level (±S.D.) of six animals. The mean blood glucose levels determined in samples collected before gliclazide administration were taken as the baseline levels. Using these data, the percentage of glucose reduction at each time after dosing was calculated and plotted against time.
3. Results and discussion

3.1. Effect of processing conditions on the micromeretic properties of gliclazide loaded Ca-alginate beads

Gliclazide loaded Ca-alginate beads were prepared by varying the concentration of Na alginate in the formulations to investigate the effect of this parameter on the micromeretic (particle size, yield and flowability) properties of the beads (Table 2). It is evident that decreasing the polymer concentration has resulted in a decrease in the percentage yield. This effect can be explained by the fact that as the concentration of Na alginate decreases the quantity of the polymer becomes insufficient to cover gliclazide particles completely and form beads. Measurement analysis of the particle size indicated that the average mean diameter increases significantly ($p < 0.05$) with increasing the polymer concentration. Plot of the average mean diameter of the beads against the alginate concentration exhibited close to linearity with $r^2$ value = 0.9899 (Fig. 1). Jefferey et al. (1991) have explained that the reason for this finding is the higher the concentration of the polymer in the sample may lead to an increase in the frequency of collision, resulting in the fusion of semi formed particles and production of an overall increase in the size of the beads. Other researchers have reported a similar relationship between the polymer concentration and the mean size (Arica et al., 2005).

Increasing the speed of stirring from 400 to 1700 rpm was accompanied by a marked decrease in the bead’s yield and the average mean diameter (Table 2). The diameter of particles prepared at speed of 400 rpm was about 1.5 time larger than those prepared at speed of 1700 rpm. The tendency of the droplets to coalesce and aggregate at the slower stirring speed appeared to be correspondingly high, resulting larger mean diameter. At high stirring the increased shearing stresses generated in the emulsified system tended to divide the emulsion droplets and finally induced a decrease in the mean particle size. Arica et al. (2005) suggested that the size of the droplets formed during microencapsulation using high stirring speed, might be closely related to the size of the final beads, which increased significantly by decreasing the stirring speed. The shearing forces also resulted in a breakdown of the primary emulsion droplets and reduction in the total percentage yield.

Table 2 shows that the size of the alginate matrices was controlled by varying the volume of the internal aqueous phase. Increasing the volume from 38 to 100 ml, have decreased significantly ($p < 0.05$) the bead size. This maybe due to a reduction in the viscosity of the internal phase which led to an increase in the efficiency of stirring and reduction in the size of the emulsion droplets and consequently formulation of smaller particles and low percentage yield.

The effect of presence of different types of surfactants in the external phase on the particle size of the alginate beads is recorded in Table 2. The mean bead diameters decreased significantly ($p < 0.05$) in the presence of surfactant. Some investigators (Heng et al., 2003) reported that this effect was considered to be caused by a decrease in the interfacial ten-

### Table 2

Effect of formulation conditions on the micromeretic properties of gliclazide loaded Ca-alginate beads

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (μm) ± S.D.</th>
<th>%Yield</th>
<th>Carr’s compressibility index</th>
<th>Hausner ratio (HR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>–</td>
<td>–</td>
<td>40.8</td>
<td>1.69 ± 0.017</td>
</tr>
<tr>
<td>Control</td>
<td>1398 ± 20</td>
<td>92.88 ± 0.51</td>
<td>5.3</td>
<td>1.05 ± 0.01</td>
</tr>
<tr>
<td>1</td>
<td>1572 ± 20</td>
<td>94.07 ± 0.13</td>
<td>3.8</td>
<td>1.03 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>1182 ± 10</td>
<td>91.86 ± 0.61</td>
<td>5.8</td>
<td>1.06 ± 0.000</td>
</tr>
<tr>
<td>3</td>
<td>1126 ± 30</td>
<td>84.01 ± 0.46</td>
<td>5.3</td>
<td>1.03 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>966 ± 10</td>
<td>82.33 ± 0.29</td>
<td>5.2</td>
<td>1.05 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>1262 ± 40.0</td>
<td>85.11 ± 1.35</td>
<td>5.4</td>
<td>1.055 ± 0.001</td>
</tr>
<tr>
<td>6</td>
<td>855 ± 1.00</td>
<td>80.00 ± 1.00</td>
<td>7.9</td>
<td>1.085 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>949 ± 10.00</td>
<td>86.66 ± 0.34</td>
<td>6.6</td>
<td>1.07 ± 0.002</td>
</tr>
<tr>
<td>8</td>
<td>881 ± 30.00</td>
<td>85.33 ± 1.46</td>
<td>6.9</td>
<td>1.071 ± 0.015</td>
</tr>
<tr>
<td>9</td>
<td>731 ± 2.00</td>
<td>83.33 ± 2.08</td>
<td>7.6</td>
<td>1.081 ± 0.019</td>
</tr>
<tr>
<td>10</td>
<td>1190 ± 16</td>
<td>93.33 ± 0.47</td>
<td>3.5</td>
<td>1.036 ± 0.001</td>
</tr>
<tr>
<td>11</td>
<td>1227 ± 38</td>
<td>92.00 ± 0.20</td>
<td>4</td>
<td>1.040 ± 0.015</td>
</tr>
<tr>
<td>12</td>
<td>1147 ± 44</td>
<td>91.33 ± 0.47</td>
<td>3.5</td>
<td>1.035 ± 0.001</td>
</tr>
</tbody>
</table>

* Effect of polymer concentration.
* Effect of speed of stirring.
* Effect of volume of internal phase.
* Effect of type of surfactant.
sion developed at the surface of the globules formed in the emulsifying medium. The surfactant migrated to the surface of the gliclazide loaded Ca-alginate globules upon addition to the external phase. This provided stability to the globules and formation of small beads. However, Table 2 shows that change in the surfactant type was devoid of the effect on the mean particle size. Moreover, neither the type of the surfactant nor its presence had effect on the yield of the beads.

Table 2 summarizes the flowability of gliclazide loaded Ca-alginate beads exemplified by Carr’s compressibility index and HR. The flowability of gliclazide powder was poor but the flowability of alginate beads was excellent. All beads possessed Carr’s compressibility indices in the range of 5–10 (Carr, 1965) and HR of less than 1.25 (Hausner, 1967). However, a slight increase in Carr’s indices and HR of the beads was noticed by increasing the stirring speed, internal phase volume and decreasing the polymer concentration in the formulation owing to the small particle size that was produced from preparing the beads at these formulation parameters. As the particle size decreases, the cohesivity of the particles increases and the inter-particulate forces between the beads become stronger. Scanning electron micrograph of a dried bead loaded with gliclazide showed that the bead exhibited very rough surface (Fig. 2). The drug crystals observed on the surface were probably formed as a result of their migration along with water to the surface during drying. A similar result was found by Fathy et al. (1998) for alginate beads loaded with Tiaramide.

### 3.2. Incorporation efficiency

Table 3 demonstrates the effect of processing conditions on the incorporation efficiency of gliclazide loaded Ca-alginate beads. The loading capacity of the majority of the systems investigated were very high (>90%) owing to the low solubility of gliclazide in water and the minimum loss of the drug during preparation of the beads or the subsequent washing stages. However, a slight reduction in incorporation efficiency was observed by increasing the internal phase volume due to a reduction in the viscosity which resulted in an increase in the partitioning of the drug into the external phase and produced a reduction in the drug content. The incorporation efficiency was also related to the particle size of the beads. The incorporation efficiencies appeared to decrease by decreasing the particle size. Variation of the polymer concentration was devoid of a significant change in the incorporation efficiency.

### 3.3. Swelling behavior and in vitro release studies

The swelling behavior of alginate polymer is the major factor controlling the release of the drugs from the bead systems. Lin and Ayres (1992) have reported that alginate beads can swell when hydrated. For this reason, the swelling behavior of gliclazide loaded Ca-alginate beads was determined in 0.1 M HCl (pH 1.2) and phosphate buffers (pHs 5.8 and 7.2). As shown in Fig. 3, the beads exhibited highest swelling rate in pH 7.2, while the lowest swelling rate was noticed in pH 1.2. The swelling rate at pH 5.8 being intermediate between the two pHs. Maximum

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**Fig. 2.** Scanning electron micrograph of gliclazide loaded Ca-alginate bead.

**Fig. 3.** Swelling behavior of gliclazide loaded Ca-alginate beads in 0.1 M HCl and phosphate buffer (pHs 5.8 and 7.2).
swelling for the beads was reached at 2 h in phosphate buffer pH 7.2 after which erosion and breakdown took place.

The extent of swelling of the beads prepared using different concentrations of alginate polymer was followed in 0.1 M HCl (pH 1.2) and the results showed that the swelling was related to the polymer concentration with swelling being more significant for beads containing high polymer content (Fig. 4). These results are in agreement with findings for Gaudio et al. (2005) who studied the rehydration kinetics for beads containing different concentrations of alginate in a simulated gastric fluid (pH 1.2) without enzyme. They reported an increase in the beads volume as the concentration of sodium alginate increased.

Fig. 5 shows the release profiles of gliclazide in 0.1 M HCl (pH 1.2) and phosphate buffers (pHs 5.8 and 7.2). Hong et al. (1998) have constructed a pH-solubility profile for gliclazide and found that the drug possessed higher solubility at pHs 1.2 and 7.2 than at pHs 2.6–5.6. Despite the increased solubility, the release rate of gliclazide at pH 1.2 was slow and sustained which maybe due to the stability of alginate at lower pHs (Yotsuyanagi et al., 1987) and the conversion of Ca-alginate to the insoluble but swelling alginic acid (Hodson et al., 1995). In phosphate buffer pH 7.2, the rapid swelling and erosion of the beads (as shown previously in the swelling study) have greatly contributed in facilitating the drug release rate. It has been reported that the swelling of alginate beads in presence of Ca\(^{2+}\) capturing agent depends on the progressive displacement of Ca\(^{2+}\) ions within the beads (Gaudio et al., 2005). It has also been reported that the swelling can be enhanced by the presence of phosphate ions which act as calcium sequestrant (Ostberg and Graffner, 1994). The swelling behavior of gliclazide loaded Ca-alginate beads at pH 7.2 could be explained by the ionotropy that occurs between Ca\(^{2+}\) ion of the alginate and Na\(^{+}\) ions present in phosphate buffer and consequently, capturing of the Ca\(^{2+}\) by phosphate ions. This results in dis-aggregation in the bead structure leading to erosion and dissolution of the swollen beads. The solution of the polymer with the trend of volume expansion (Martinse et al., 1989) is called “liquification”. The ion exchange with phosphate buffer which resulted in swelling and erosion of the beads (Turkoglu et al., 1997) and formation of the solute Ca phosphate all have led to increasing the drug release rate.

Changing the pH of the phosphate buffer from 7.2 to 5.8 caused a delay in gliclazide release rate owing to the lower number of Na\(^{+}\) ions present in the buffer of pH 5.8 and consequently slower rate of ion exchange and swelling and erosion of the polymer at this pH. These results indicate that swelling is the main parameter controlling the release rate of gliclazide from alginate beads and confirm the findings of Tønnesen and Karlsen (2002) who reported that release rate from alginate matrices is modulated by a swelling–dissolution–erosion process.

The differences between the release profiles of alginate beads with increasing the polymer concentration are shown in Fig. 6. As the alginate concentration increased, the release rate of gliclazide from the beads decreased. The decrease in the release rate can be explained by the increase in the extent of swelling and the gel layer thickness that acted as a barrier for the penetration medium thereby retarding the diffusion of gliclazide from the swollen alginate matrix.
Table 4
Density measurements of gliclazide loaded Ca-alginate beads containing different concentrations of the polymer

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Density (gm cm(^{-3})) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.456 ± 0.030</td>
</tr>
<tr>
<td>1(^a)</td>
<td>1.614 ± 0.102</td>
</tr>
<tr>
<td>2(^a)</td>
<td>1.380 ± 0.046</td>
</tr>
<tr>
<td>3(^a)</td>
<td>1.132 ± 0.130</td>
</tr>
<tr>
<td>4(^a)</td>
<td>1.074 ± 0.011</td>
</tr>
</tbody>
</table>

\(^a\) Effect of polymer concentration.

The density of the beads containing different concentration of the alginate polymer was determined and the results are shown in Table 4. The density of the beads increases as the concentration of the polymer increases suggesting that the beads formed at high polymer concentration are more compact and less porous than those prepared at low polymer content. These results are confirmed by the scanning electron micrographs (Fig. 7a and b) which show that the surface of the beads prepared using low polymer concentration (formula 4\(^a\)) is rougher and more wrinkled and loosely bound in comparison with the surface of beads prepared using high polymer concentration (formula 1\(^a\)).

3.4. In vivo studies

The diabetic rabbit was chosen as an animal model due to its low cost of maintenance and its ease of handling. All tested groups showed no statistical difference in blood glucose levels before treatment (\(p < 0.05\)).

Fig. 8 shows the behavior of gliclazide loaded Ca-alginate beads (80 mg) and the marketed conventional gliclazide tablets (Gliclazide\(^®\)) (80 mg) administered orally to diabetic rabbits. The efficacy of the formulations was estimated by measuring the plasma glucose concentration. The results reveal that the blood glucose levels of the group treated with gliclazide loaded Ca-alginate beads and the group treated with the marketed gliclazide tablet were lower than that of the group treated with saline. Alloxan, a β-cytotoxin, was demonstrated to produce a massive destruction of β-cells of the islets of langerhans resulting in reduced synthesis and release of insulin (Rerup, 1970). It has been reported that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan whereas, they are in active in intense alloxan diabetes (nearly all β-cells have been destroyed) (Grodsky et al., 1971). However, the results of the study showed that gliclazide reduced blood glucose levels in the hyperglycemic rabbits, indicating that the state of the diabetes was mild and that not all the β-cell of the islets of langerhans were destroyed by alloxan during induction of diabetes. Nammi et al. (2003) found that glibenclamide reduced blood levels of the diabetic rabbits induced by alloxan.

Analysis of variance of the data 2 h after treatment with gliclazide formulations showed that there was a significant difference (\(p < 0.05\)) in the blood glucose level between groups treated with saline and those treated with gliclazide alginate beads however, the marketed gliclazide tablets did not produce a significant effect on the blood glucose level of the diabetic rabbits. Four hours after administration of the formulations, the blood glucose levels of gliclazide loaded Ca-alginate beads and the gliclazide marketed tablets groups were significantly lower than that of the saline group. Moreover, the hypoglycemic effect induced by gliclazide-alginate beads was significantly greater than that induced by the marketed gliclazide tablets and this effect was lasted for 24 h. Alginate is a mucoadhesive polymer.

Fig. 7. (a) Scanning electron micrograph of gliclazide loaded Ca-alginate beads containing high polymer concentration. (b) Scanning electron micrograph of gliclazide loaded Ca-alginate beads containing low polymer concentration.

Fig. 8. Hypoglycemic effect of oral administration of isotonic saline solution, gliclazide marketed tablet and gliclazide loaded Ca-alginate beads.
(Gombotz and Wee, 1998). It has been reported that adhesion of alginate beads to the stomach is great (Gaserod et al., 1998). The above studies suggest that alginate beads swell slowly in stomach after they are hydrated and consequently adhere to the stomach mucosa allowing more gliclazide to be absorbed by minimizing the diffusion barriers and increasing the period of absorption by prolonging the residence time of the drug in the stomach. The beads are subsequently transfer to the upper part of the intestine where they swell more and release the drug through the gel layer that was formed at the matrices periphery. However, their erosion will take place in the lower intestine. The results of the study clearly demonstrated the ability of gliclazide loaded Ca-alginate beads to enhance, prolong and control the gastrointestinal absorption of gliclazide.

4. Conclusion

Entrapment and controlling the release of the antidiabetic agent gliclazide has been studied. The gel matrix consists of calcium alginate beads obtained by ionotropic gelation method. The ability of the system to incorporate and control the release of gliclazide has been investigated through variation in the processing conditions such as polymer concentration, stirring speed, internal phase volume and type of surfactant in the external phase. Alginate beads can control, improve and prolong the systemic absorption of the gliclazide through their mucoadhesive properties. This effect results in maintaining tight blood glucose level and improved patient compliance.

References