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Artigo

ENCAPSULATION AND RELEASE CHARACTERISTICS OF GLIBENCLAMIDE LOADED CALCIUM-ALGINATE BEADS

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The aims of this study were to formulate calcium-alginate beads containing glibenclamide, characterize the resulting microparticles, evaluate the release characteristics of this type of delivery system in an *in vitro* dissolution test, and compare it with two commercially available trademarks (Daonil® and Glibetab®). We obtained glibenclamide loaded calcium-alginate beads with a rough surface and a particle size between 150-200 μm. For the *in vitro* dissolution test Daonil® at 45 min showed a Q > 70%, whereas Glibetab® and glibenclamide calcium-alginate beads a Q < 70%; in spite of that glibenclamide calcium–alginate beads showed significant release properties.

Keywords: glibenclamide; calcium-alginate beads; dissolution.

INTRODUCTION

The term microspheres refer to a micro-particulate polymer based drug delivery system with an average particle size greater than 1 μm.[1] Microsphere carrier systems made from naturally occurring biodegradable polymers have attracted considerable attention for several years, although there are other methods for this purpose (air suspension and sprays, among others).[2] In the last decade, there has been considerable interest in natural polymers, such as chitosan, gelatin, polylactic acids, and alginates as drugs carriers because of their adequate biocompatibility and biodegradability.[3-6] Alginate-derived polysaccharides[7-9] are of special interest because alginates have gelling properties in the presence of divalent cations such as Ca^{2+}, Sr^{2+} or Ba^{2+}.[10] This phenomenon can be explained by the egg-box model.[11] Calcium-alginate beads have been proposed as a drug delivery system.[12]

Glibenclamide (glyburide) is a second-generation sulphonylurea that is an orally bioavailable hypoglycemic agent used in the management of type 2 diabetes. It is administered in low doses (5 mg), is quickly cleared from the body, and its active metabolites have a considerable hypoglycemic effect.[13] Different research has reported that glibenclamide has a low bioavailability, which is attributed to its poor dissolution properties.[14-16]

Different methods have been reported to determine glibenclamide levels in various biological fluids, such as plasma[17,18]and serum,[19] in pharmaceutical formulation analyses[20-22] or in simultaneous determination of anti-diabetic drugs.[23]

Our objectives were to formulate calcium-alginate beads containing glibenclamide by ionotropic gelation, characterize the resulting microparticles, evaluate the release characteristics of glibenclamide loaded calcium-alginate beads in an *in vitro* dissolution test, and compare it with two commercially available trademarks (Daonil® and Glibetab®).

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EXPERIMENTAL

Chemicals and reagents

The reference standard for glibenclamide was obtained from the United States Pharmacopeia (USP reference standard, 200 mg, Batch: 29555F2). Samples of anti-diabetic drugs were purchased from the Mexican market (Daonil® and Glibetab®, 5 mg). All solvents and chemicals were purchased from Sigma Aldrich (Mexico). Methanol and acetonitrile were HPLC grade. Hydrochloric acid, boric acid, sodium alginate, calcium chloride, potassium chloride, sodium hydroxide were analytical grade. All solvents and sample solutions were filtered through 0.45 μ filter paper.

Chromatographic conditions

The work was performed in an air-conditioned room maintained at 25 ± 2º C. HPLC was carried out with a Hewlett Packard® model 1100 (Ramsey, Minnesota, 55303, USA) with a UV-visible photodiode-array detector. Compounds were separated on a 25 cm x 4.6 mm i.d., 5 μm particle Phenomenex C_8 column under reversed-phase partition chromatographic conditions. The mobile phase was acetonitrile, potassium diphosphate and water (CH_3CN:KH_2PO_4:H_2O; 40:45:15 v/v) with pH adjusted to 6.2 ± 0.01 at a flow rate of 1 mL/min. Run time was 20 min. Mobile phase and sample solutions were degassed by sonication (Bransonic Ultrasonic Cleaner, Model 2510R-MTH) and filtered through 0.45 μ filter paper. Glibenclamide was monitored at 230 nm. All compounds were identified by comparison of retention times obtained from sample and standard solutions.

Standard solutions

A 10 mg quantity of glibenclamide was accurately weighed in an analytical balance and transferred to a 100 mL volumetric flask. The drug was dissolved in hydrochloric acid (0.1 M in methanol) and sonicated

for 15 min to prepare a standard stock solution containing 0.1 mg/mL of glibenclamide.

Sample preparation

For analysis of the tablet dosage form, twenty tablets were weighed individually and their average weight determined. After that, tablets were crushed to a fine powder and an amount of powder equivalent to the weight of one tablet was transferred to a 100 mL volumetric flask. The drug was dissolved in hydrochloric acid (0.1 M in methanol) and sonicated for 15 min. Samples were then filtered through 0.45 µ filter paper.

Validation of the HPLC method

Specificity

Specificity was determined by analyzing placebos: matrix samples without analyte. The system response was examined for interference or overlap in glibenclamide responses.

Linearity

A calibration curve was obtained at five concentration levels of a glibenclamide standard solution (0.06 - 0.14 mg/mL). Linearity was analyzed using the least square regression method in triplicate at each concentration level.

Accuracy and precision

Method precision was determined by intra-day and inter-day repeatability, which was evaluated by analyzing the 6 standard solutions (0.1 mg/mL). In the intra-day study, concentrations were calculated three times on the same day at intervals of 2 h. In the inter-day study, concentrations were measured on three different days. Precision was expressed as a coefficient of variation (CV%). The accuracy of the method was confirmed by studying recovery at 3 different concentrations: 80, 100, and 120%. Accuracy was expressed as a coefficient of variation and a standard deviation (CV < 2.0% and SD < 1.0%).

Limit of quantitation and limit of detection

The limit of detection was expressed as the analyte concentration corresponding to a sample blank value plus 3 standard deviations. The limit of quantitation was expressed as the analyte concentration corresponding to sample blank value plus 5 standard deviations.

Glibenclamide loaded calcium-alginate beads

Preparation of glibenclamide loaded calcium-alginate beads

Glibenclamide loaded calcium-alginate beads were prepared using ionotropic gelation. Glibenclamide (5 g) was initially dissolved in ethanol (10 mL) and later added to a solution of sodium alginate (2%) and dispersed homogeneously at 60 ºC. An air-bubble free suspension was forced through a needle into 150 mL of a calcium chloride solution (0.1 M) at a flow rate of 10-20 drops/min. The mixture were stirred using a mechanical stirrer at 400 rpm for 30 min. Alginate gel beads were allowed to stand in calcium chloride solution for 72 h until they were fully recovered. The beads were then separated by filtration, washed three times with 100 mL deionized water, and allowed to dry at room temperature for 24 h.

Particle size analysis and morphology

Particle size distribution and mean diameters of the beads were determinated by optical microscopy. Surface morphology was studied by scanning electron microscopy with gold coating.

Test of content of glibenclamide in loaded calcium-alginate beads

One hundred milligrams of loaded calcium-alginate beads containing a theoretical weight of 9 mg of glibenclamide were accurately weighed. Ground beads were placed in 50 mL hydrochloric acid 0.1 M and sonicated for 15 min. Samples were filtered through 0.45 µ filter paper to obtain clear solutions and analyzed for drug content by HPLC at 230 nm. The percentage of drug loading and incorporation efficiency were calculated using the Equations 1 and 2:

Percent drug loading = amount of drug in beads / amount of beads x 100 (1)

Percent incorporation efficiency = % drug loading / % theoretical loading x 100 (2)

Stability of content of glibenclamide in loaded calcium-alginate beads

Content of drug (glibenclamide) in loaded calcium-alginate beads was carried out by HPLC method at 0, 3, 6, 9 and 12 months.

Quality control of two trademark formulations

Daonil® and Glibetab® were analyzed according to the Mexican Pharmacopea (Farmacopea de los Estados Unidos Mexicanos, FEUM, 8th edition, MGA, 0299)[24] determinating physical description, identity (Perkin Elmer FT-Infrared, Paragon 1000), weight, weight variation, disintegration (Vankel Disintegration Equipment, Model 35-1000), friability (Vankel Friability Tester), hardness (Vankel Strong-Cobb, Model VK200 Hardness Tester) and thickness, uniformity of content and content of active ingredient.

***In vitro* dissolution test**

All dissolution tests were performed using a Vankel VK 7000 dissolution tester (six vessels) in accordance with the USP 29 general method.[25] Dissolution studies of glibenclamide loaded calcium-alginate beads and two commercially available products (tablets) of glibenclamide were conducted using USP apparatus 2 (paddle method). Weighed quantities of beads equivalent to 5 mg of glibenclamide were placed in vessels, which were lowered into 500 mL of phosphate buffer 0.05 M, pH 7.4 ± 0.05, previously deaerated. Solutions were pre-treated and maintained at 37 ± 0.5 ºC and the paddles were set at 75 rpm. At appropriate time intervals (10, 20, 30, 45 and 60 min), 5 mL samples were collected, filtered through 0.45 µ filter paper and analyzed for drug content by HPLC at 230 nm. An equal volume of fresh medium was added to the test solution to maintain constant volumes.

RESULTS AND DISCUSSION

Validation of the HPLC method

Analytical performance parameters, such as specificity, linearity, range, precision, and accuracy, limit of quantification and limit of detection were validated according to International Conference Harmonization ICH Q2B guidelines.[26] A symmetrical peak was observed for glibenclamide with a retention time of 17.84 min. Specificity was indicated by the absence of any endogenous interference at retention times of the peak. Linearity of the method used was evaluated on a standard curve (0.06-0.14 mg/mL) of the peak area versus the concentration of the analyte. A five-point calibration curve was constructed using working standards and was found linear ($y = 56697x - 723.54$, $r^2 > 0.9999$). Results of the precision assays are presented in Table 1.

Table 1. Intra-day and inter-day precision test results

Dose	Intra-day precision (CV %)	Inter-day precision (CV %)		
		Day 1	Day 2	Day 3
0.1 mg/mL	0.24	0.56	0.61	0.62

Six replicate sample solutions were prepared from the stock solution. The accuracy of the method was confirmed by measuring recovery at 3 different concentrations: 80, 100, and 120% (Table 2).

Table 2. Recovery study results

Dose %	Amount added	Amount recovery	Recovery (%, ± SD)	CV %
80	4.05 mg	4.03	99.5 ± 0.53	1.2
100	5.05 mg	5.07	100.3 ± 0.34	1.1
120	6.05 mg	6.3	104.1 ± 0.65	1.0
Mean			101.3 ± 0.5	1.1

The results demonstrated that the method is a very accurate quantitative estimation of glibenclamide because these were within acceptable limits (CV < 2.0% and SD < 1.0%). The determined limit of detection and quantitation were 0.005344 and 0.00663 mg/mL, respectively.

The first stage of this project was the validation of an HPLC-based analytical method in order to quantify glibenclamide in polymeric calcium-alginate beads. We found that the HPLC analytical method developed was linear in the range proposed (0.06-0.14 mg/mL); the correlation coefficient and determination were > 0.998. The accuracy of the method showed acceptable recovery percentages of glibenclamide with a CV < 2%, this was considered a condition in validation studies. According to the FEUM 8th ed. and the USP 29th ed., the quantification of glibenclamide is determined by the UV-Vis and HPLC methods, respectively. So, with these results we suggest that the determination of glibenclamide can now be done by HPLC.

Glibenclamide loaded calcium-alginate beads

Glibenclamide loaded calcium-alginate beads were prepared using ionotropic gelation, a technique that allows control of the particle size obtained. In previous work of our research group (unpublished data), we established the optimal conditions to prepare glibenclamide-loaded calcium-alginate beads and determined that the concentration of sodium alginate in the formulations is a critical parameter that decreases the percentage yield as has also been reported by other research groups.[27] Analysis of particle size indicated that the average mean diameter was 150-200 µm. Scanning electron micrograph of a dried bead loaded with glibenclamide showed that the bead exhibited a very rough surface (Figure 1). Particle size of the microparticles is a very important parameter, since it affects drug release and pharmacokinetics. The particle size obtained in this method (150-200 µm) is below the average (300 µm) reported by Rodriguez-Llimos *et al*.[28] and Ramesh *et al*.,[29] which could also be caused by the process variables.

Glibenclamide loaded calcium-alginate beads HPLC retention time was 17.84 min (Figure 2) and 9.01 mg of glibenclamide in 100 mg of microparticles were detected. Determination of percent drug loadings and percent incorporation efficiency were 9.01 and 70%, respectively, according to the Equations 1 and 2. Encapsulation efficiency was 70%, which could be considered satisfactory; however, this result suggests analyzing the variables that may be reducing the effectiveness of the beads (time and agitation speed, contact time with calcium chloride, and the concentration of solutions) because

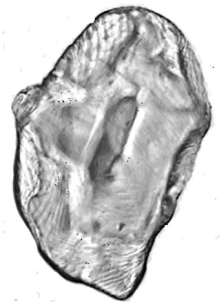

Figure 1. *Scanning electron micrograph of glibenclamide loaded calcium-alginate beads*

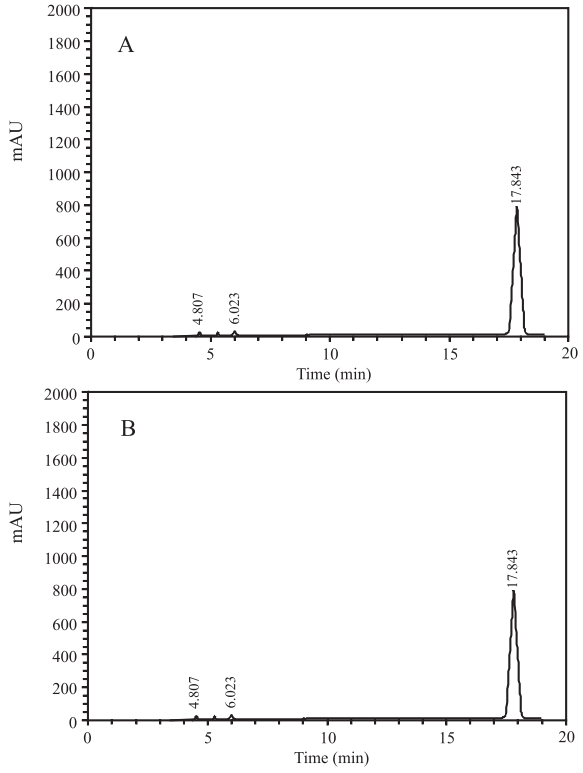

Figure 2. *Retention time in the HPLC method A) Standard of glibenclamide, B) Glibenclamide loaded calcium-alginate beads*

Arica *et al.* reported an efficiency of 80-90%.[30] Additionally, stability of glibenclamide in loaded calcium-alginate beads was evaluated by determining content of drug by HPLC method at 3, 6, 9 and 12 months (Table 3). The data show a stable formulation.

Table 3. Stability of glibenclamide in loaded calcium-alginate bead

Time (months)	Mean content of glibenclamide/ 100 mg of microparticles	% HPLC	SD
0	9.01 mg	100.11	± 0.21
3	9.15 mg	101.16	± 0.33
6	9.07 mg	100.77	± 0.53
9	8.97 mg	99.66	± 0.15
12	8.96 mg	99.55	± 0.18

Trademark formulations

The quality control tests of Daonil® and Glibetab® were assays carried out according to the FEUM 8th ed.; results from each formulation are shown in Table 4.

Table 4. Results from quality control tests of Daonil® and Glibetab® tablets

Tests	Specifications	Daonil	Glibetab
Identity	Same as standard	Approved	Approved
Weight average	160 mg ± 7.5%	Approved	Approved
Weight variation	CV < 5%	Approved	Approved
Disintegration	≤ 15 min	Approved	Approved
Friability	≤ 1%	Approved	Approved
Hardness	≤ 3 Kps, CV < 10%	Approved	Approved
Content of active ingredient	90.0 -110.0%, SD ≤ 2.0%	Approved	Approved
Uniformity of content	85.0-115.0%, SD ≤ 6.0%	Approved	No approved

Quality control was applied to the tablets in order to determine if they met FEUM specifications. Physical description, shape, size, and markings form a part of manufacturer´s own product specification. Both formulations approved the mechanical characteristics tests (hardness, disintegration and friability). The two formulations in this study satisfied the specified requirements for the quality control test. However, Glibetab® did not approve uniformity of content (SD > 6%) and these results may have a negative effect on the *in vitro* dissolution test as well as bioavailability.

In vitro dissolution test

A huge number of controlled drug delivery schemes have been developed in recent years to target drugs to a specific site in a suitable rate. Polymeric delivery systems have long been used in the delivery of both water-soluble and insoluble drugs. This affords less recurrent administrations, thereby increasing the patient compliance and reducing the treatment failure. The encapsulation is also capable of protecting the therapeutic compound within the body, potentially optimizes therapeutic responses, extended efficacy, and also avoid peak-related side-effects by maintaining more constant blood levels of the drug. Additionally, drug dissolution tests are a fundamental part of drug development and commercially available products. In this context we present results (Table 5) of the *in vitro* dissolution tests of glibencamide loaded calcium-alginate beads and the two trademark drugs using the USP 2 paddle apparatus. The Food and Drug Administration (FDA) initially recommended a borate buffer pH 9.0 for the dissolution test of glibenclamide, but later, El-Massik *et al.* suggested a phosphate buffer pH 7.4 as a better discriminatory dissolution medium,[31] so we used a phosphate buffer pH 7.4 ± 0.05. On the other hand, it was confirmed that drug dissolution testing is a highly variable technique[32] and it was later suggested that some form of modification to the current dissolution system is needed to address this variability.[33] *In vitro* dissolution testing of Daonil® and Glibetab® showed that both formulations release similar amounts of glibenclamide during the first 3 sampling times (10, 20 and 30 min), but during the fourth time (45 min) Daonil® (71.2%) showed better release than Glibetab® (34%). So Glibetab® did not meet the FDA recommended values (Q > 75%, 45 min).

Table 5. Concentration average (mg) and percentage of dissolved drug (%) *in vitro* dissolution test of glibenclamide loaded calcium-alginate bead, Daonil® and Glibetab®. Each data point represent mean ± SD, n = 3

	Concentration average and percentage		
Time (min)	Glibenclamide loaded calcium-alginate bead	Daonil®	Glibetab®
10	0.0 mg 0.0% ± 0.0	0.106 mg 2.132% ± 0.9	0.116 mg 2.32% ± 1.3
20	0.0 mg 0.0% ± 0.0	0.121 mg 2.42% ± 0.7	0.162 mg 3.24% ± 1.4
30	0.267 mg 5.34% ± 0.5	0.353 mg 7.07% ± 0.7	0.425 mg 8.5% ±1.8
45	2.33 mg 46.78% ± 0.7	3.56 mg 71.2% ± 0.8	1.70 mg 34.0% ± 1.2
60	2.77 mg 55.4% ± 0.7	4.08 mg 81.7% ± 0.9	3.96 mg 79.2% ± 1.3

In contrast, the *in vitro* dissolution test for glibenclamide loaded calcium-alginate beads showed 0.0% dissolution at 10 and 20 min sample time. During the fourth and fifth samples times, release was 46.78 and 55.4%, respectively; meaning that drug release of this formulation is biphasic. These results differed from those reported by others researches. Galal *et al.* reported that the initial amount of glibenclamide released was 67% after 15 min[34] and Amaechi *et al.*, in microspheres produced with a crosslinking time of 1 h had the highest delayed release of the incorporated drug;[1] however, they used semi-solid and matrix 10% mucuna gum, respectively. The swelling behavior of alginate polymer is the major factor controlling the release of the drug from the bead system and different researchers have reported that alginate beads could swell when hydrated. Also, in phosphate buffer, the rapid swelling and erosion of the beads have greatly contributed in facilitating drug release, but according to the obtained results of the *in vitro* dissolution, this behavior was not observed in glibenclamide formulation. This behavior is atypical, although we used a low concentration of polymer (2%). If the alginate concentration increased, the release rate of the beads decreased because the density of the beads increases as the concentration of the polymer increases. Consequently, it is necessary to study additional factors, such as the effect of formulation conditions on microparticle properties, the effect of polymer concentration in the formulation on the extent of swelling, and the effect of polymer concentration on the release rate, which could affect our drug release system. However, we suggest that glibenclamide calcium-alginate beads can be used since the objective is potentially optimizes therapeutic responses, extended efficacy, and also avoid peak-related side-effects by maintaining more constant blood levels of the drug. *In vivo* pharmacokinetic studies are necessary, although, the study by Al-Kassas *et al.* clearly demonstrated the ability of loaded calcium alginate beads in enhancing, prolonging and controlling the absorption of the drug.[35]

CONCLUSION

In summary, we obtained glibenclamide calcium-alginate beads by ionotropic gelation with a particle size of 150-200 μm and a rough surface. An analytical HPLC method was validated to quantify glibenclamide, according to ICH. Commercial products containing glibenclamide satisfied the specified requirements for quality control testing according to the FEUM, except for Glibetab® tablets, which did not approve uniformity of content test. In the *in vitro* dissolution test, Daonil® showed a Q > 70% at 45 min. Glibetab® and glibenclamide calcium-alginate beads did not approve test with a Q < 70%; in spite of that glibenclamide calcium–alginate beads showed significant release properties.

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