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Research Paper

Design and development of gliclazide-loaded chitosan microparticles for oral sustained drug delivery: in-vitro/in-vivo evaluation

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Abstract

Objectives The objective of this study was to prepare gliclazide–chitosan microparticles with tripolyphosphate by ionic crosslinking.

Methods Chitosan microparticles were produced by emulsification and ionotropic gelation. The effects of process variables including chitosan concentration, pH of tripolyphosphate solution, glutaraldehyde volume and release modifier agent such as pectin added to the tripolyphosphate crosslinking solution were evaluated. The microparticles were examined with scanning electron microscopy, infrared spectroscopy and differential scanning colorimetry. The serum glucose lowering effect of gliclazide microparticles was studied in streptozotocin-diabetic rabbits compared with the effect of pure gliclazide powder and gliclazide commercial tablets.

Key findings The particle sizes of tripolyphosphate–chitosan microparticles were over the range 675–887 μm and the loading efficiency of drug was greater than 94.0%. In-vivo testing of the gliclazide–chitosan microparticles in diabetic rabbits demonstrated a significant antidiabetic effect of gliclazide–chitosan microparticles after 8 h that lasted for 18 h compared with gliclazide powder, which produced a maximum hypoglycaemic effect after 4 h.

Conclusions The results suggests that gliclazide–chitosan microparticles are a valuable system for the sustained delivery of gliclazide.

Keywords chitosan; gliclazide; microparticles; pectin; sustained release

Introduction

The use of microparticle-based therapy allows drug release to be carefully tailored to the specific treatment site through the choice and formulation of various drug–polymer combinations. The total dose of medication and the kinetics of release are variables that can be manipulated to achieve the desired result. Microparticle-based systems may increase the lifespan of active constituents and control the release of bioactive agents. Being small in size, microparticles have large surface-to-volume ratios and can be used for controlled release of insoluble drugs.

Chitosan (poly(β-(1-4)-2-amino-2-deoxy-D-glucose)), the high molecular weight cationic polysaccharide derived from chitin, has become increasingly important in the pharmaceutical field due to its good biocompatibility, biodegradability and low toxicity.[1,2] Owing to its good mucoadhesive properties,[3,4] chitosan has been employed in mucosal site-specific systems.[5–8] Moreover, chitosan has been shown to be a potential penetration enhancer for the transmucosal (intestinal, nasal, buccal and vaginal) absorption of hydrophilic drugs with a high molecular weight.[9–12] Chitosan has been proposed as a useful excipient for sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds.[13–16] Chitosan has been used in the design of different types of drug carriers for various administration routes such as oral, ocular, buccal, nasal, transdermal, parenteral and vaginal. Chitosan dosage forms can be engineered into different shapes and geometries such as nanoparticles, microparticles, hydrogels, films, fibers, sponges, inserts and rods.[17–23]

Cationic chitosan can form gels with non-ionic multivalent anionic counterions such as polyphosphate[24,25] and sodium alginate[26] by ionic crosslinking. Tripolyphosphate is a non-toxic polyanion that can interact with chitosan via electrostatic forces to form ionic

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crosslinked networks because of its quick gelling ability. This interaction could be controlled by the charge density of tripolyphosphate and chitosan, which is dependent on the pH of the solution.[27,28]

Gliclazide is a potential second generation, short-acting sulfonylurea oral hypoglycaemic agent widely used for the treatment of non-insulin-dependent diabetes mellitus.[29] In general, rapid gastrointestinal absorption is required for oral hypoglycaemic drugs in order to prevent a sudden increase in blood glucose level after food intake in patients with diabetes mellitus. However, the absorption rate of gliclazide from the gastrointestinal tract is slow and varied among subjects. 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 gastrointestinal membrane.[30] The dose of gliclazide is 80 mg and could be increased to 380 mg daily, and hence there is a need for the development of sustained release patient-compliant formulations of gliclazide.

In this study, gliclazide-loaded microparticles were prepared with chitosan using a simple, rapid technique. The formulations were characterized in an in-vitro release study. The best formulation providing sustained drug release was selected for determination of the hypoglycaemic effect in diabetic rabbits. The objectives of this study were to evaluate the effects of the following preparation processes on the release behaviour of gliclazide microparticles: (1) concentration of chitosan; (2) pH and concentration of the crosslinker solution (tripolyphosphate); (3) volume of glutaraldehyde added to the crosslinker solution; and (4) concentration of release modifier.

Materials and Methods

Materials

Chitosan, 75–85% deacetylated, intermediate viscosity (Brookfield, 1% solution in acetic acid) 200–400 mPa s, was purchased from Fluka, Switzerland. Acetic acid (99.8%) was from Sigma-Aldrich (USA). Gliclazide was a gift from Servier (Istanbul, Turkey). Sodium tripolyphosphate was obtained from Sigma (USA), pectin was obtained from BDH (UK) and glutaraldehyde was from E. Merck (Germany). A non-ionic surfactant (polyoxyethylene 20 sorbitan monooleate, Tween 80) was a gift from ICI Surfactants (Sceaux, France). Dichloromethane, high-performance liquid chromatography grade, was from Sigma Chemical Co. (USA). All other reagents used were of analytical reagent grade and were obtained from Sigma-Aldrich (USA).

Preparation of gliclazide microparticles

The chitosan solution was prepared by dissolving a particular weight of chitosan in 1% v/v acetic acid with stirring for about 60 min. Tween 80 (2% w/w) was added to the solution as a surfactant. Gliclazide was dissolved in dichloromethane (oil phase) and then the drug solution was mixed with aqueous phase (chitosan solution) using a homogenizer (Yellow Line DI 25 basic; Germany) at 5000 rev/min for 2 min. The ratio of oil and aqueous phase was 1 : 10. This bubble-free o/w emulsion was dropped through a disposable plastic syringe with a 22-gauge blunt-ended needle into 100 ml gently agitated solution of the crosslinking agent (tripolyphosphate) containing 1–5 ml 25% gluraladehyde solution. The falling distance was 3 cm. The gelled beads instantaneously formed were allowed to cure in the crosslinking solution for 30 min. They were separated by filtration, washed with deionized water and dried at 37°C for 48 h in a drying room. At the same time, the pH values of tripolyphosphate aqueous solutions were adjusted from pH 9.0 (original pH value) to pH 7.0, 5.0 and 3.0, respectively, by 1 N HCl, and measured by a pH meter (Model PB11-Sartoriuos; USA). The obtained smooth, spherical and homogenous microparticles were kept for 1 h in the crosslinker fluid with stirring. Thereafter, microparticles were collected, washed with distilled water and air dried. Formulation and processing conditions of gliclazide-loaded chitosan microparticle preparations are listed in Table 1. A number of variables such as chitosan concentration, pH of the crosslinking external phase solution and addition of pectin or glutaraldehyde into the external phase were investigated for optimization of microparticle properties. Similar procedures were used to prepare placebo microparticles without gliclazide.

Drug content of microparticles

Estimation of drug content was done according to the method adopted previously.[31] Samples (25 mg) of drug-loaded microparticles were first crushed and then transferred to a 200-ml volumetric flask. A total volume of 100 ml of dichloromethane was added and the dispersion obtained was sonicated for 30 s to dissolve gliclazide. Samples were withdrawn from the undiluted solution using a syringe, then diluted and filtered (0.45 μm Millipore) before detection. The concentration of gliclazide in dichloromethane was determined using a UV/vis spectrophotometer (Ultrospec 2100 Pro; Cambridge, England) at an absorbance wavelength of 228 nm. Triplicate measurements were performed (relative standard deviation within 2%). A sample from the empty microparticles (without gliclazide) was taken as a blank. Real drug content was calculated as the detected amount of gliclazide with respect to the real amount of total solid added to the chitosan solution (polymer and gliclazide). The real drug content was expressed as a percentage (relative standard deviation within 2.8%). Encapsulation efficiency was calculated as:

$$\text{encapsulation efficiency} = \text{practical drug loading} / \text{theoretical drug loading} \times 100 \quad (1)$$

Particle size analysis

Particle size distribution of the microparticles was measured by a sieve analysis procedure. The microparticles were shaken on a mechanical shaker using a nest of standard sieves (Retsch, GmbH, Germany) for 20 min. The mean diameters of microparticles were calculated after sieving.[32]

Morphological characterization

The surface morphology of microparticles was observed by scanning electron microscopy. The microparticles were vacuum dried. Before observation, samples were mounted on metal grids using double-sided adhesive tape, coated with

Table 1 Composition, mean size, encapsulation efficiency and production yields of gliclazide-loaded chitosan microparticles

Formulations	Theoretical drug content (% w/w)	Actual drug content (% w/w)	Encapsulation efficiency (%)	Production yield (%)	Mean size (μm)
Chitosan/gliclazide ratio[a]					
1 : 1	50.0	48.4 ± 3.4	94.2 ± 3.7	92.3 ± 1.7	675
2 : 1	33.3	31.6 ± 2.9	95.6 ± 4.8	92.7 ± 2.4	764
3 : 1	25.0	24.7 ± 4.2	94.8 ± 2.8	90.5 ± 3.1	889
pH of external phase[b]					
3	33.3	34.4 ± 0.4	95.4 ± 2.8	95.3 ± 4.5	873
5	33.3	31.6 ± 2.9	96.2 ± 1.7	96.3 ± 1.5	887
7	33.3	32.4 ± 1.2	95.8 ± 3.8	93.4 ± 2.5	783
9	33.3		96.3 ± 4.2	94.7 ± 3.5	758
Volume of glutaraldehyde[c] (%)					
1	33.3	34.1 ± 3.4	93.7 ± 4.5	95.3 ± 2.6	886
3	33.3	32.6 ± 2.9	98.3 ± 3.7	97.8 ± 3.5	799
5	33.3	31.7 ± 4.2	97.9 ± 4.7	92.8 ± 1.5	764
% Pectin in the external phase[d]					
0.5	33.3	34.4 ± 3.4	94.7 ± 2.5	93.4 ± 3.5	695
1	33.3	32.6 ± 2.9	97.4 ± 2.7	92.7 ± 2.6	764
1.5	33.3	33.7 ± 4.2	96.9 ± 5.7	92.5 ± 4.6	799

$n = 3$. [a]5% w/v Tripolyphosphate pH 5 as crosslinker solution containing 1 ml glutraldehyde. [b]5% w/v Tripolyphosphate as crosslinker solution, chitosan/gliclazide ratio 2 : 1, 1 ml glutraldehyde was added in the external phase. [c]5% w/v Tripolyphosphate pH 5 as crosslinker solution, chitosan/gliclazide ratio 2 : 1. [d]5% w/v tripolyphosphate pH 5 as crosslinker solution, chitosan/gliclazide ratio 2 : 1, 1 ml glutraldehyde was added in the external phase.

gold palladium and observed microscopically (Jeol, JSM-6360 LV scanning microscope; Tokyo, Japan).

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectral data of gliclazide, chitosan and gliclazide-loaded microparticles were obtained using a Perkin-Elmer FTIR spectrophotometer instrument. FTIR spectra were recorded by a potassium bromide (KBr) disc method and scanned at the resolution of 4.0 cm^{-1} over the wavenumber region 4000–450 cm^{-1}.

Differential scanning calorimetry

Temperature and enthalpy values were measured with a Mettler Star system equipped with a DSC-912 Module on 3–5-mg samples in crimped sealed aluminium pans under a static air atmosphere. An empty pan was used as reference. The heating rate was 10°C/min over 30–300°C. Measurements were carried out in triplicate.

In-vitro gliclazide release

The in-vitro release of gliclazide–chitosan microparticles was measured using a USP rotating basket apparatus (Model DT-6; Erweka, Germany). Microparticles equivalent to 80 mg gliclazide, were added to each basket, rotated at 100 rev/min. The volume of dissolution medium was 900 ml and was maintained at 37 ± 0.2°C. Different dissolution medium (0.1 N HCl, pH 1.2, for the first 2 h, pH 7.4 phosphate-buffered solution for further 6 h) was used for gliclazide release test. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals (15, 30, 60, 90, 120, 150, 180, 210, 240, 300 and 360 min) and immediately replaced by 5 ml of fresh dissolution medium. The samples were analysed using a UV/vis spectrophotometer (Ultrospec-2100 Pro) at 228 nm after filtration through a 0.45-μm membrane filter. Another amount of ground microparticles equivalent to 80 mg gliclazide was dispersed in 150 ml of dissolution medium and sonicated for 2 h. The suspension was filtrated through a 0.45-μm membrane filter. The absorbance of the filtrate was also measured at 228 nm for the total amount of gliclazide released. The sample absorption degree was detected by using non-loaded gliclazide microparticles as correction. None of the ingredients used in the microparticle formulations interfered with the assay. The time for 50% drug released (T50%) was determined for the different formulae. All dissolution tests were performed in triplicate.

The data obtained from the in-vitro release studies were analysed by various models such as zero order (Equation 2), first order (Equation 3), Higuchi (Equation 4) and Korsmeyer-Peppas (Equation 5) models. The equations were as follows:

$$Q_t = k_0 t \quad (2)$$

$$\mathrm{Ln}(Q_0 - Q_t) = k_1 t \quad (3)$$

$$Q_t = k_H t^{1/2} \quad (4)$$

$$Q_t = k_P t^n \quad (5)$$

where Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug in the microparticles, k_0, k_1, k_H and k_P are release rate constants, and n is the release exponent indicative of mechanism of release. In spherical matrices, if $n < 0.5$, a Fickian diffusion mediated drug release occurs; if $0.5 < n < 0.85$, non-Fickian transport occurs; erosion mediated release occurs if $n > 0.85$.[33]

Hypoglycaemic activity in diabetic rabbits

Male New Zealand white rabbits, 2.5–3 kg, were used as the animal model. All investigations were performed according to European Community guidelines for animal experimentation. Experimental design and treatment of animals were approved by the Animal Care Committee of King Saud University, School of Medicine. The animals were housed in polypropylene cages, six animals per cage with free access to standard laboratory diet and water. They were kept at 25 ± 1°C and 55% relative humidity with a 12-h light/dark cycle. Diabetes was induced in overnight-fasted rabbit by injecting streptozotocin (100 mg/kg i.p.) dissolved in citrate buffer (3 mM; pH 4.5).[34] Three days later, rabbits with blood glucose levels between 300 and 400 mg/dL were selected for the study.[35] The animals were fasted overnight before starting the experiment. The rabbits were assigned to four different groups of six rabbits each and treated as follows: group 1 (diabetic control) was treated with 1% CMC suspension in normal saline; group 2 was treated with commercial gliclazide tablet at a dose of 10 mg/kg orally; group 3 received a suspension of gliclazide in 1% CMC at a dose of 10 mg/kg orally; and group 4 received gliclazide-loaded chitosan microparticles at a dose of 10 mg/kg. The microparticles were suspended in distilled water before oral administration. The drug solution or vehicle was administered orally by gastric intubation using an oral feeding needle. The dose of gliclazide was selected by conducting the hypoglycaemic experiments with doses of 1–10 mg/kg. Blood was collected from the marginal ear vein of the rabbits at time intervals between 2 and 24 h after treatment and before oral administration. Blood serum was separated by centrifugation at 1850*g* for 15 min. Serum glucose levels were determined using Glucoscan Test Strips (Lifescan Inc., CA, USA) and reading by a Glucoscan 3000 meter (Lifescan Inc). The mean serum glucose levels determined in samples collected before gliclazide determination were taken as the baseline levels and plotted against time.

Statistical analysis

Results were analysed and expressed as mean ± SD. Effects of various factors on chitosan gliclazide microparticles were assessed using the Kruskal-Wallis test. Individual differences between the different formulations of microparticles were determined using a non-parametric post-hoc test (Dunn's test) using GraphPad InStat Software (version 1.13; GraphPad Software, San Diego, CA, USA). A value of $P < 0.05$ was considered statistically significant. Results are presented as mean values ± SD

Results and Discussion

Chitosan microparticles composed of different negatively and positively charged polymers represent a drug delivery system that can be prepared without a tedious process. Chitosan–tripolyphosphate matrices have been used in the pharmaceutical industry for many years and the interaction between tripolyphosphate and chitosan has been reported.[36] Sufficient charge numbers (or density) are necessary for anions to crosslink chitosan by electrostatic force. Tripolyphosphate is a multivalent anion and carries a maximum of five negative charges. On the other hand, chitosan is a weak polybase with a maximum of thousands of positive charges. However, the charge number of the anions and chitosan are all mainly controlled by solution pH.

Microparticle characterization

Chitosan microparticles containing gliclazide were evaluated for particle size, yield and encapsulation efficiency (Table 1). The mean diameter of the microparticles was 4–5 mm before drying. After drying, water content dropped from 90% to 7–15% and microparticle size decreased sharply. The viscosity of the chitosan sample has importance in the formation of microparticles. Chitosan microparticles could not be prepared from samples with less than 1% w/v chitosan. Very high viscosity chitosan samples (>3% w/v) did not form smooth round microparticles because of dropping difficulty. Microparticles were ranged mainly between 750 and 860 μm in diameter. A narrow range of weight distribution of microparticles was noticed for all batches prepared (see Table 1). Particle size revealed an increase with increasing amount of chitosan. This could be due to the higher amount of chitosan present, leading to an increase in viscosity in the polymer solution, thereby producing bigger droplets during emulsification that were later hardened in the presence of tripolyphosphate and glutaraldehyde. Similar findings were observed for other formulations containing 0.5–1.5% w/v pectin, but the change in size was not significant ($P > 0.05$). Another interesting observation was that particle size decreased with an increase in crosslinking extent (1–5% glutaraldehyde). It was observed that the particle size with 1 ml of glutaraldehyde added was greater than that with 5 ml glutaraldehyde added. This could be due to the formation of more rigid network structures with the greater degree of crosslinking.[16] Since gliclazide is not soluble in water, it was not dissolved in solution during the crosslinking and hardening process. Therefore, the loss of gliclazide from microparticles was minimal during the hardening and washing process. The microparticles showed good encapsulation efficiency, greater than 94.5% in all cases, and the efficiency was not affected by the chitosan concentration or the concentration or pH of the crosslinking agent. The addition of pectin to 5% tripolyphosphate solution having pH 7 did not significantly improve the encapsulation efficiency ($P > 0.05$). Moreover, no significant differences were found between the gliclazide encapsulation efficiency of microparticles prepared with pectin combined with 5% tripolyphosphate solution having pH 7 because of the low water solubility of gliclazide. The results are in agreement with previous results of Shu and Zhu[37] upon preparing chitosan beads loaded with water-soluble and water-insoluble model drugs. In all cases, high loading efficiency was obtained due to poor water solubility of the drug. The yield and encapsulation efficiency of gliclazide–chitosan microparticles are summarized in Table 1.

Figure 1 shows the surface morphology of gliclazide microparticles. The procedure developed provided spherical particles of homogenous surface with no tendency to aggregate (Figure 1a). After crosslinking, the colour of the microparticles changed from white to dark brown with the variation

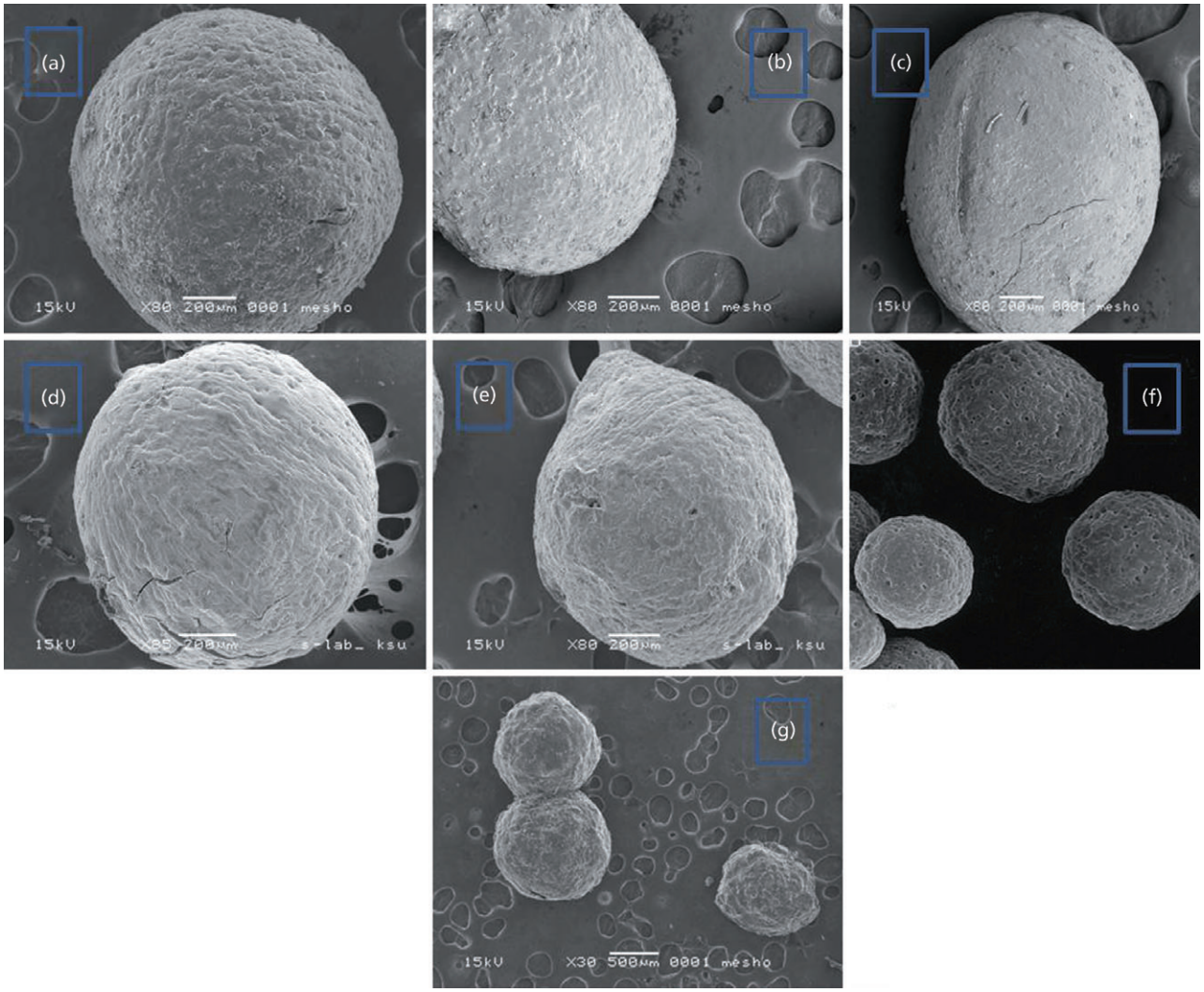

Figure 1 (a) Scanning electron microscopy of gliclazide–chitosan microparticles. Without glutaraldehyde (A); 1 ml glutaraldehyde (B); crosslinking solution had pH 3 (C); pH 5 (D); pH 7 (E); pH 9 (F); crosslinking solution contained 1% pectin (G). (b) Scanning electron microscopy and surface morphology of gliclazide–chitosan microparticles. Crosslinking solution had pH 3 (A); pH 5 (B); pH 7 (C); pH 9 (D); crosslinking solution contained 5 ml glutaraldehyde (E); 1% pectin (F).

of pH from 3.0 to 9.0. The microparticles prepared in crosslinker with a higher pH value were porous and brittle with large wrinkles compared with the microparticles prepared in acidic conditions (Figure 1b). All chitosan gel microparticles prepared by the crosslinking method had good sphericity. In chitosan microparticles the polyelctrolyte complex occurs between chitosan and tripolyphosphate, and also between chitosan and pectin, and it protects the gel matrix from environmental conditions. Coating pectin on the surfaces of these microparticles improved the surface morphology, yielding white microparticles; pectin can form a polyelectrolyte complex film on the microparticles surface with cationic chitosan.

Surface morphology revealed the presence of cracks and pores when the tripolyphosphate crosslinking solution had pH 7 and 9. Microparticles in this study also showed surface-adhered drug particles.

Fourier transform infrared spectroscopy

Infrared spectroscopy is a very useful tool for the determination of functional groups. FTIR of gliclazide showed peaks of –NH stretching (3274 cm^{-1}), =CH stretching (3113 cm^{-1}), O = C (1705 cm^{-1}), C = C aromatic (1596 cm^{-1}) C-H deformation (1467–1430 cm^{-1}) and SO_2-NH (1352 cm^{-1}). Similar peaks were seen in gliclazide-loaded chitosan microparticles. The IR spectrum of chitosan microparticles showed peaks of assigned saccharide structure at around 905 and 1153 cm^{-1}, and a protonated amino characteristic peak at around 1570 cm^{-1}. There was a stronger absorption band at 1650 cm^{-1} of the assigned amide groups. The broad and strong band ranging from 3200 to 3600 cm^{-1} may be due to the overlapping of –OH and –NH stretching vibration, which is consistent with the peak at 1155 cm^{-1} assigned to C-N stretching vibration.[38] The appearance of a characteristic peak at 1150 cm^{-1} assigned to $P = O$ groups of

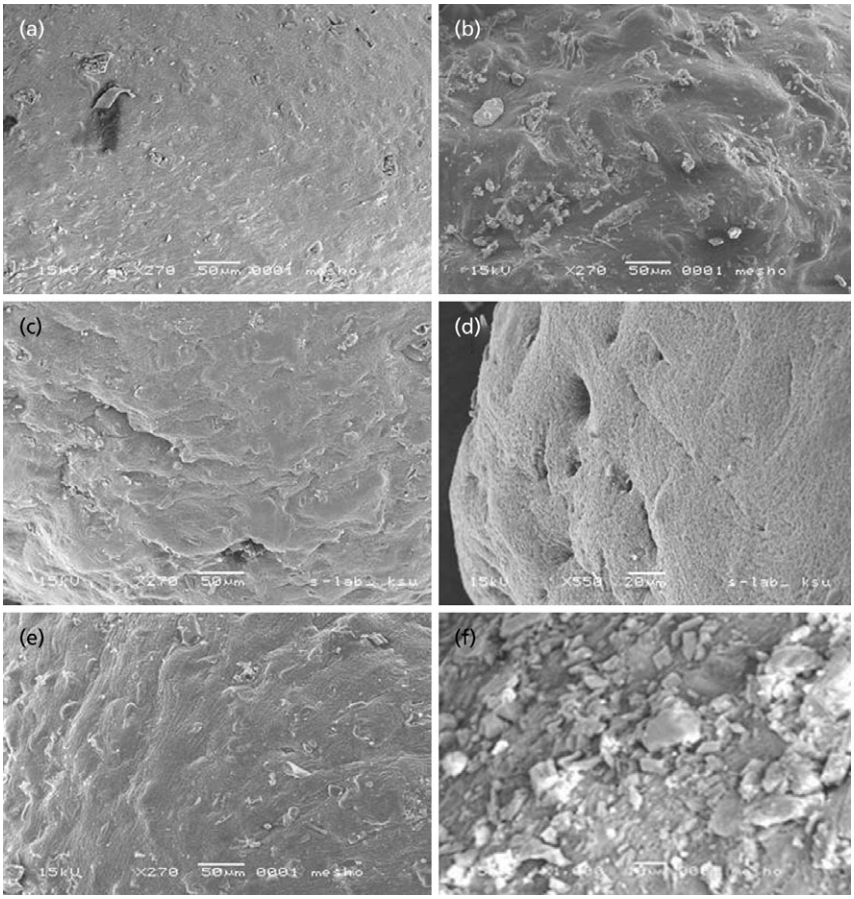

Figure 1 *Continued*

tripolyphosphate is evidence of the ionic crosslinking of chitosan. It was found that the intensity of $P = O$ absorbance at 1150 cm^{-1} of crosslinked chitosan gel microparticles increased with a decrease in pH, suggesting that chitosan can bind with tripolyphosphate ions more easily at lower pH values. These results are in agreement with Alsarra *et al*.[39] A percentage of tripolyphosphate is dissociated into $P_3O_{10}^{5-}$ at low pH. Moreover, chitosan is a weak polybase, and as the pH of the solution decreased, the ionization of the amine group of chitosan increased. Ionic crosslinking dominated in the chitosan microparticles prepared in acidic tripolyphosphate solution. It is known that the p*K*a of chitosan is about 6.3;[40,41] chitosan has slight ionic crosslinking at high pH values, as tripolyphosphate is dissociated into OH^-, $HP_3O_{10}^{-4}$ and $P_3O_{10}^{-5}$, and chitosan microparticles are formulated by deprotonation which is characterized by low crosslinking density. This means that the charge density of chitosan and crosslinker must be sufficiently high at the pH value to allow optimum interaction and ensure a high crosslinker density.[42] FTIR spectral data were also used to confirm the chemical stability of gliclazide in gel microparticles. In the case of drug-loaded microparticles, all the bands that were observed in gliclazide also appeared, indicating the chemical stability of gliclazide after encapsulation into the polymer matrix.

Differential scanning calorimetry

Under the experimental conditions, the DSC thermogram of pure chitosan had no characteristic endotherm, but it had a large exothermic decomposition peak at about 250°C (Figure 2), while that of the crosslinking complex was a bit smaller and shifted to about 230°C, further confirming that chitosan is not present in the free form. DSC was also performed on pure gliclazide and gliclazide-loaded microparticles (Figure 2). The DSC thermogram revealed an sharp endothermic peak at 185°C of melting gliclazide that did not appear in the chitosan-gliclazide loaded microparticles, indicating the amorphous dispersion of gliclazide into the chitosan matrix.

Gliclazide release studies

Drug release behaviour of the formulations based on chitosan crosslinking with 5% w/v tripolyphosphate were evaluated by performing the in-vitro release experiments in simulated gastric and intestinal pH conditions. Results of percentage cumulative release against time for drug-loaded microspheres for the different formulations were compared in Figures 3–6.

The effect of tripolyphosphate concentration (5%, 10% w/v) at a fixed pH of the gelling medium at 5.0 was also studied in preliminary experiments. A lower concentration of

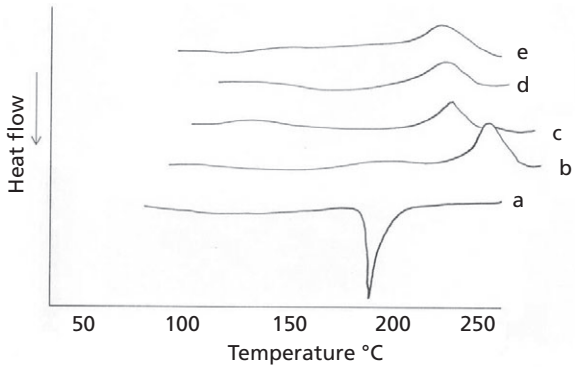

Figure 2 Differential scanning calorimetry spectra. a, Pure gliclazide; b, chitosan; c, gliclazide-loaded chitosan microparticles prepared at pH 3; d, gliclazide-loaded chitosan microparticles prepared at pH 9; e, gliclazide-loaded chitosan–1% pectin microparticles.

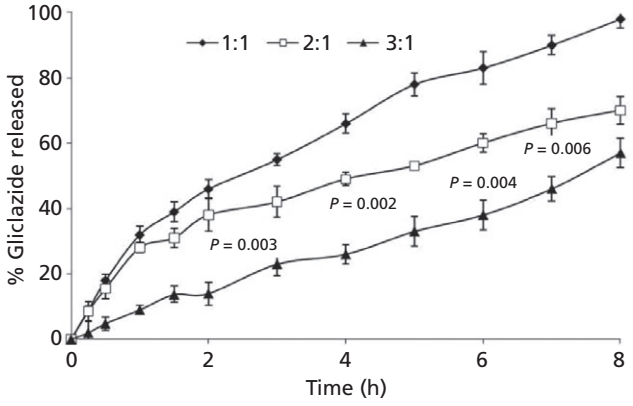

Figure 3 Influence of the chitosan/drug ratios on the gliclazide release from microparticles (5% w/v tripolyphosphate pH 5).

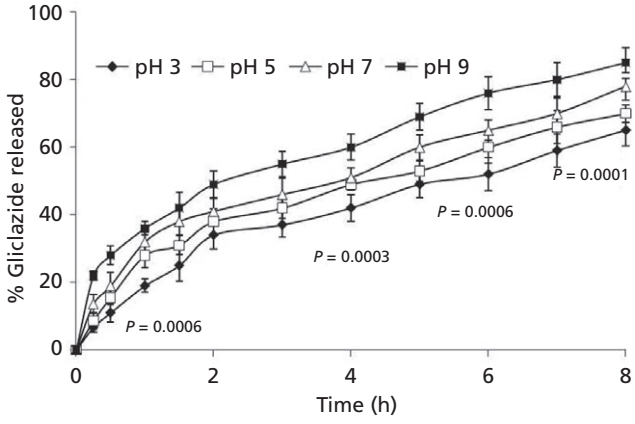

Figure 4 Influence of pH of 5% w/v tripolyphosphate solution on the gliclazide release from microparticles (chitosan/gliclazide ratio 2 : 1).

tripolyphosphate 1% w/v resulted in an uncrosslinked chitosan film that spontaneously dissolved in 0.1 N HCl. (data not shown). At higher concentrations, 5–10% w/v, gliclazide release from microparticles was found to be independent of tripolyphosphate concentration ($P > 0.05$). On the other hand,

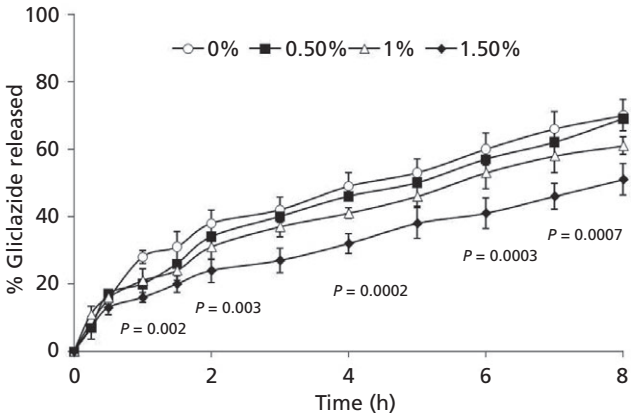

Figure 5 Influence of pectin concentration on the gliclazide release from microparticles (5% w/v tripolyphosphate pH 5; chitosan/gliclazide ratio 2 : 1).

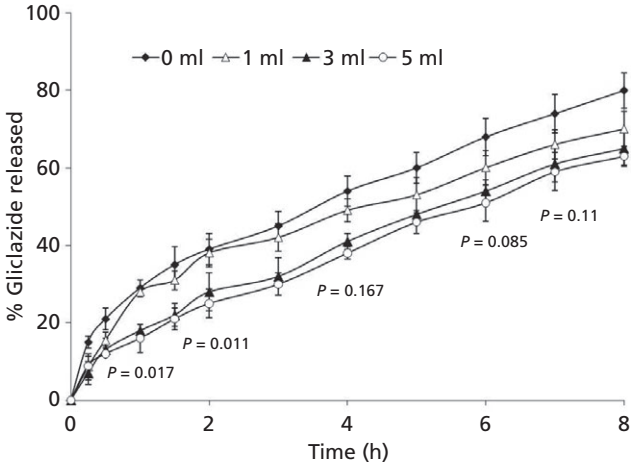

Figure 6 Influence of glutaraldehyde volume on the gliclazide release behaviour from microparticles (5% w/v tripolyphosphate pH 5; chitosan/gliclazide ratio 2 : 1).

Remunán-Lopez and Bodmeier[43] reported that the diffusion of chlorphenaramine maleate from chitosan films decreased as the concentration of the tripolyphosphate solution increased. In addition, they showed that the swelling and permeability characteristics of chitosan films were dependent on concentration of crosslinking agent.

Effect of pH of the crosslinking solution

Figure 3 shows the release behaviour of gliclazide from chitosan microparticles prepared with 5% w/v tripolyphosphate solutions at various pH levels. As the pH of tripolyphosphate solution increased, the release of gliclazide from tripolyphosphate–chitosan microparticles increased. This result confirmed that the ionization degree of tripolyphosphate is dependent on the pH of the solution; the ionization of the amine groups would decrease with the increase in pH and hence decrease the opportunities for ionic interactions with

tripolyphosphate. The loss of charge density in these polyionic species reduced the extent of crosslinking and the strength of the ionic attraction, allowing the formation of open porous structures when the chitosan microparticles were prepared in tripolyphosphate solution of higher pH. This porous structure is more degradable than a high density structure and therefore the release behaviour of gliclazide from microparticles prepared in tripolyphosphate solution of higher pH was much faster than from microparticles prepared in pH 3 and 5. This result is in agreement with that of Shu and Zhu.[37]

Effect of chitosan concentration

Usually, drug release behaviour for a chitosan matrix can be modulated by swelling–erosion rate. In ionic-crosslinked hydrogels, swelling of gel and erosion of network structure are prevented by inter-ionic interaction, which is related to the chitosan concentration used for preparing the drug-loaded microparticles. The drug release behaviour of chitosan microparticles prepared at fixed pH of the gelling medium (pH 5) with different chitosan concentrations is shown in Figure 4. When the chitosan concentration increased, gliclazide release decreased. This result indicates that the release behaviour of drug is relative to the viscosity of the chitosan solution. This was expected since with increasing chitosan amount in the formulations, a closer network, which should decrease the diffusion of drug outwards of the microparticles, was formed. This result agrees with the literature reports that increasing chitosan concentrations decreases the percentage drug release.[44,45] The studies showed that the low concentration of chitosan formed weak microparticles upon interaction with tripolyphosphate, resulting in high swelling ability, and therefore the release of drug increased.

Effect of pectin added to the crosslinking solution

The addition of pectin to the 5% w/v tripolyphosphate solution (pH 5) resulted in a decreased gliclazide release rate ($P < 0.05$). The release of gliclazide from the microparticles was 71, 62 and 50% after 8 h upon addition of 0.5, 1 and 1.5% of pectin in the crosslinking tripolyphosphate solution, respectively (Figure 5). In addition, encapsulation efficiency was not significantly affected by the addition of pectin to the crosslinking solution. On the other hand, Aral and Akbuğa[46] found that the encapsulation efficiency of bovine serum albumin (a water-soluble protein)–chitosan beads was significantly affected by the addition of sodium alginate and glutraldehyde in the tripolyphosphate solution. Also, Ishak *et al*.[47] found that the external phase composition had a significant effect on loading efficiency and buoyancy of metronidazole–chitosan beads. The gliclazide–chitosan microparticles containing 1% pectin were selected for the in-vivo studies.

Effect of glutaraldehyde volume

Gliclazide microparticles did not disintegrate in 0.1 N HCl or in phosphate buffer pH 7.4 during the release study. This may be due to the hardening of chitosan microparticles with glutaraldehyde during preparation, as previously noted by Sezer and Akbuga,[48] who found that the surface of the beads become seamless after hardening. The release of gliclazide from chitosan microparticles was significantly affected by increasing the glutaraldehyde volume from 0 to 5 ml added to the 5% w/v tripolyphosphate solution (pH 5) ($P < 0.05$) Figure 6. This was also reflected in gliclazide release, where the T50% of gliclazide increased from 4 h for 0% glutaraldehyde to 6 h for microparticles prepared with the addition of 3 ml glutaraldehyde into the crosslinking tripolyphosphate solution. Further increases in glutaraldehyde volume up to 5 ml did not significantly affect gliclazide release ($P > 0.05$).

The kinetics of drug release were determined with respect to zero order, first order, Higuchi and Korsmeyer and Peppas models. Correlation coefficients and T50% values are given in Table 2. It was found that the Higuchi model was the best fit for all the gliclazide microparticle formulations, except gliclazide microparticles composed of 3 : 1 chitosan/gliclazide, where zero order kinetics were predominant. The values of n were over the range of 0.372–0.503, indicating that Fickian diffusion dominated. It was seen that there was no degradation of the particles in the medium at the end of the dissolution, this confirm that the drug released by diffusion from an inert matrix according to Higuchi kinetics.[49]

Serum glucose levels of the streptozotocin-induced diabetic rabbit model

The diabetic control group did not show any significant change in serum glucose level throughout the experiment, although, as time progressed, a slight decrease in serum glucose level was observed in the control group due to the fasting effect on blood glucose level. Figure 7 shows the decrease in serum glucose levels after oral administration of optimized gliclazide microparticles containing 1% pectin, pure gliclazide powder and a commercial tablet to diabetic rabbits. The results showed that the decreases in serum glucose level from the basal level of the group treated with the optimized gliclazide microparticles or gliclazide powder were significantly lower than in the control group ($P < 0.05$). Rabbits treated with gliclazide plain powder showed a rapid reduction in serum glucose level within 2 h, and a maximum hypoglycaemic response after 4 h, with a $46.65 \pm 5.73\%$ decrease in serum glucose levels. In the case of gliclazide microparticles, the hypoglycaemic response was gradual. A maximum hypoglycaemic effect was observed after 8 h ($52.65 \pm 6.81\%$ decrease in serum glucose levels) and thereafter remained stable up to 18 h. Rabbits treated with the marketed gliclazide tablet showed a maximum hypoglycaemic response after 8 h and a 36.6% decrease in serum glucose levels compared with plain gliclazide powder ($P < 0.05$).

Conclusions

The data presented here show that the gliclazide-loaded tripolyphosphate–chitosan microparticles had a homogenous structure with high drug loading. Pectin can coat the surface of tripolyphosphate/chitosan to form a complex film, which prolonged the drug release period. The in-vivo study of the gliclazide microparticles in a diabetic rabbit model revealed

Table 2 Correlation coefficients, release exponents, release constant and the time for 50% drug released (T50%) of gliclazide released from chitosan microparticles

Formulations	Zero order (*r*)	First order (*r*)	Higuchi model (*r*)	Korsmeyer-Peppas model (*r*)	Release exponent (*n* value)	Release constant (*k*) (% h^{-n})	T50% (h)
Chitosan/gliclazide ratio							
1 : 1	0.9720	0.8864	0.9656	0.9971	0.497	24.632	2.5
2 : 1	0.9568	0.8717	0.9961	0.9846	0.484	22.356	4.0
3 : 1	0.9949	0.9433	0.9655	0.9967	0.914	7.826	7.5
pH of external phase							
3	0.9692	0.8915	0.9951	0.9967	0.456	21.799	5.0
5	0.9568	0.9317	0.9962	0.9974	0.432	23.673	4.5
7	0.9534	0.8798	0.9949	0.9964	0.495	26.756	3.8
9	0.9451	0.8573	0.9955	0.9935	0.406	35.895	2.2
Volume of glutaraldehyde (ml)							
0	0.9693	0.8917	0.9983	0.9880	0.387	26.844	3.5
1	0.9568	0.8717	0.9961	0.9892	0.372	22.368	4.5
3	0.9865	0.9103	0.9934	0.9964	0.421	19.711	5.5
5	0.9905	0.9199	0.9898	0.9885	0.457	17.675	5.8
% Pectin in the external phase							
0.5	0.9711	0.8932	0.9964	0.9889	0.501	21.778	5.0
1	0.9761	0.9076	0.9965	0.9968	0.468	19.132	5.5
1.5	0.9770	0.9089	0.9959	0.9951	0.503	16.097	7.5

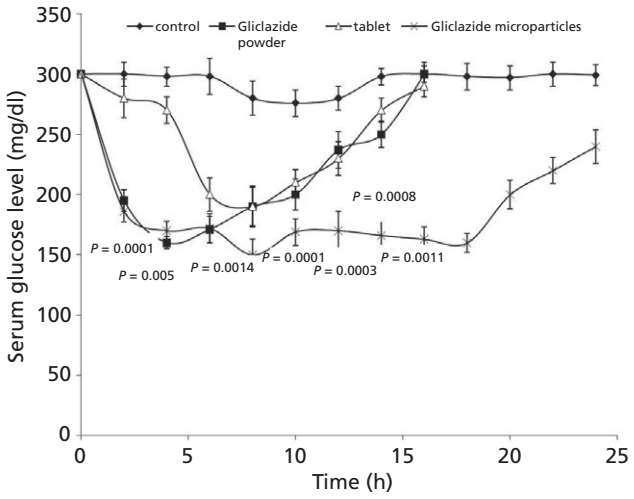

Figure 7 Serum glucose levels in a diabetic rabbits ($n = 6$). Control group were treated orally with CMC 1% suspension; gliclazide powder and gliclazide microparticles in doses up to 10 mg/kg, commercial tablet in a 10 mg/kg dose orally.

significant antidiabetic activity. These sustained release microparticles may be considered for further evaluation in humans as a promising controlled release dosage form for gliclazide.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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