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Research Article

Preparation and Characterization of Chitosan Microparticles for Oral Sustained Delivery of Gliclazide: In Vitro/In Vivo Evaluation

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Strategy, Management and Health Policy				
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ABSTRACT Chitosan microparticles were prepared with tripolyphosphate (TPP) by ionic cross-linking with gliclazide (GLZ) as a model drug. The particle sizes of TPP-chitosan microparticles ranged from 675–887 μm with loading efficiencies of greater than 94%. Chitosan concentration, TPP solution pH, and glutaraldehyde volume solution added to the TPP cross-linking solution affected drug release characteristics. Pectin interactions with cationic chitosan on the surface of TPP/chitosan microparticles led to the formation of polyelectrolyte complex films that improved drug sustained release performance. In vivo testing of the GLZ-chitosan microparticles in diabetic albino rabbits demonstrated a significant antidiabetic effect of GLZ/chitosan microparticles after 8 h that lasts for 18 h compared with GLZ powder that produced a maximal hypoglycemic effect at 4 h, suggesting that GLZ/chitosan microparticles represent an improved system for the long-term delivery of GLZ. Drug Dev Res 72:235–246, 2011.
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Key words: chitosan; gliclazide; microparticles; sustained release; pectin

INTRODUCTION

The use of microparticles-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 the variables that can be manipulated to achieve the desired result. Microparticle-based systems may increase the half-life 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 recently gained increasing importance in the pharmaceutical field due to its good biocompatibility, biodegradability. and low toxicity [Alves and Mano, 2008; Paños et al., 2008]. Due to its good mucoadhesive properties [Ferrari et al., 1997; Luana Perioli et al., 2008], chitosan has been employed in mucosal site–specific systems [Witschi and Mrsny, 1999; Senel et al., 2000a; Portero et al., 2007; Tao et al., 2009]. Moreover, it has been shown to be a potential

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penetration enhancer for the transmucosal (intestinal, nasal, buccal, and vaginal) absorption of hydrophilic drugs with high molecular weight [Bernkop-Schnürch, 2000; Tengamnuay et al., 2000; Senel et al., 2000b; Rossi et al., 2003a]. Chitosan has also been proposed as a useful excipient for sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds [Sahoo et al., 2010; Zerrouka et al., 2004; Mutalik et al., 2008; Rokhade et al., 2007]. Chitosan has been used in the design of different types of drug carriers for various administration routes, e.g., oral, ocular, buccal, nasal, transdermal, parenteral, and vaginal. Chitosan dosage forms can be engineered into different shapes and geometries including nanoparticles, microparticless, hydrogels, films, fibers, sponges, and rods [Gupta and Ravi Kumar, 2000; Sun et al., 2008; Shu and Zhu, 2002; Barat et al., 2007].

Cationic chitosan can form gels with non-ionic multivalent anionic counter ions such as polyphosphate [Mi et al., 1999a,c] and sodium alginate [Anal et al., 2003] by ionic cross-linking. Tripolyphosphate (TPP) is a non-toxic polyanion that can interact with chitosan via electrostatic forces to form ionic cross-linked networks because of its quick gelling ability. This interaction could be controlled by the charge density of TPP and chitosan, which is dependent on the pH of solution [Mi et al., 1999b, 2001].

Gliclazide is a short-acting sulfonylurea oral hypoglycemic agent widely used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) [Schernthaner, 2003]. In general, rapid gastrointestinal (GI) absorption is required for oral hypoglycemic drugs, to prevent a sudden increase in blood glucose level after food intake in patients with NIDDM. However, the absorption rate of gliclazide 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 [Campbell et al., 1991]. The dose of gliclazide is 80 mg and could be increased to 380 mg daily, highlighting a need for the development of a sustained-release, patient-compliant formulation of gliclazide.

In this study, GLZ-loaded microparticles were prepared with chitosan using a simple, rapid technique. Formulations were characterized by in vitro release studies. The best formulation providing sustained drug release was selected for determination of the hypoglycemic effect on the diabetic rabbit. The objective of this study was to evaluate the effect of the preparation process on the release behavior of GLZ microparticles such as (1) concentration of chitosan, (2) pH and concentration of cross-linker (TPP) solution, (3) volume of glutaraldehyde added to the cross-linker 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, Buchs, Switzerland. Acetic acid (99.8%, Sigma-Aldrich, St. Louis, MO), Gliclazide (GLZ) was provided as a gift from Servier (Istanbul, Turkey). Other materials were sodium tripolyphosphate, TPP (Sigma), Pectin (BDH, London, UK), Glutaraldehyde (GL) (E. Merck, Darmstadt, Germany). A nonionic surfactant (polyoxyethylene 20 sorbitan monooleate, Tween® 80) was a gift from ICI Surfactant (Sceaux, France). All other reagents were all analytical reagents grade from Sigma-Aldrich Chemical.

Methods

Preparation of GLZ microparticles

The chitosan solution was prepared by dissolving the desired weight of chitosan in 1% (v/v) acetic acid and stirring for approximately 60 min. Tween 80 (2% w/w) was added into the solution as a surfactant. GLZ was dissolved in dichloromethane (oil phase) and then the drug solution was mixed with aqueous phase (chitosan solution) by homogenization (Yellow Line DI 25 basic, Germany) at 5,000 rpm 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-g blunt-ended needle into 50 ml of the gently agitated solution of the cross-linking agent (TPP) containing 1–5 ml 25% gluraladehyde (GL) solution. The falling distance was 3 cm. The gelled beads, instantaneously formed, were allowed to cure in the cross-linking solution for 30 min, and were then 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 TPP aqueous solutions were adjusted from pH 9.0 (original pH value) to pH 7.0, 5.0, and 3.0, respectively, using HCl. The smooth, spherical, and homogenous microparticles obtained were stirred for an hour in the cross-linker fluid. Microparticles were then collected, washed with distilled water, and air dried. Formulation and processing conditions of GLZ-loaded chitosan microparticles preparations are listed in Table 1. A number of variables such as chitosan concentration, pH of the cross-linking external phase solution, and addition of pectin or glutaraldehde into the external phase were investigated for optimization of microparticle properties. Similar procedures were used to prepare placebo

TABLE 1. Composition, Mean Size, Encapsulation Efficiency, and Production Yields of GLZ Loaded Chitosan Microparticles ($n = 3$)

Formulations	Theoretical drug content (%w/w)	Actual drug content (%w/w)	Encapsulation efficiency (%)	Production yield (%)	Mean size (μm)
Chitosan:G LZ ratio					
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					
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, GL (ml)					
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					
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

microparticles without GLZ entrapped. All batches were prepared in triplicate.

Drug Content of Microparticles

Estimation of drug content was as described [Rokhade et al., 2006]. Samples (25 mg) of drug-loaded microparticles were crushed and then transferred to a 200-ml volumetric flask. A total volume of 100 ml of dichloromethane was added and the dispersion obtained sonicated for 30 s to dissolve GLZ. Samples were withdrawn from the undiluted solution using a syringe, then diluted and filtered (Millipore, Billerica, MA, 0.45 μm) before detection. The concentration of GLZ in dichloromethane was determined using a UV-Vis Spectrophotometer (Ultrospec 2100 Pro, Buckinghamshire, UK) at an absorbance wavelength of 228 nm. Triplicate measurements were performed (relative standard deviation, R.S.D., within 2%). Samples from the empty microparticles (without GLZ) were used as blank. Real drug content was calculated as the detected amount of GLZ with respect to the real amount of total solid added to the chitosan solution (polymer and GLZ). The real drug content was expressed in percent (R.S.D. within 2.8%). Encapsulation efficiency was calculated as:

$$\text{Encapsulation efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100$$

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, Haan, Germany) for 20 min. The mean microparticle diameters were calculated after sieving [Allen, 1975].

Morphological Characterization

The surface morphology of microparticles was observed by scanning electron microscopy (SEM). The microparticles were vacuum dried. Prior to observation, samples were mounted on metal grids, using double-sided adhesive tape, coated with gold palladium, and observed microscopically (Jeol, JSM-6360 LV scanning microscope, Tokyo, Japan).

Fourier Transform Infrared (FT-IR) Spectral Studies

FT-IR spectral data of GLZ, chitosan, and GLZ-loaded microparticles were taken using a Perkin-Elmer Fourier transformed infrared (FT-IR) spectrophotometer instrument. FT-IR spectra were recorded using a potassium bromide (KBr) disc method and scanned at the resolution of 4.0 cm^{-1}over the wave number region 4,000–450 cm^{-1}.

Differential Scanning Calorimetric (DSC) Study

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 aluminum pans under a static air atmosphere. An empty pan was used as reference. The heating rate was 10°C min^{-1} over the 30–300°C. Measurements were conducted in triplicate.

In Vitro GLZ Release Studies

In vitro release of GLZ chitosan microparticles was measured using a USP rotating basket apparatus (Model DT-6 Erweka, Heusenstamm, Germany). An amount of microparticles equivalent to 80 mg

GLZ was added to each basket, rotating at 100 rpm. The volume of dissolution medium was 900 mL and maintained at 37 ± 0.2°C. A different dissolution medium (pH 1.2 HCl solution for the first 2 h, pH 7.4 phosphate buffered solution for a further 6 h) was used for GLZ 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 replaced by 5 mL of fresh dissolution medium immediately. Samples were assayed via UV-Vis Spectrophotometry (Ultrospec-2100 Pro) at 228 nm after filtration through a 0.45-μm membrane filter. Another sample of ground microparticles equivalent to 80 mg GLZ was dispersed in 150 mL of dissolution medium and sonicated for 2 h. The suspension was filtrated through a 0.45-μm membrane filter and the absorbance of the filtrate measured at 228 nm for the total amount of GLZ released. The sample absorption degree was detected by using non-loaded GLZ microparticles as correction. None of the ingredients used in the microparticle formulations interfered with the assay. $T_{50\%}$ was determined for the different formulae. All dissolution tests were performed in triplicate.

Analysis of Release Profiles

The data obtained from the in vitro release studies were analyzed by zero order, first order, Higuchi, and Korsmeyer–Peppas models. The equations were as follows:

$$\text{Zeroorder: } Q_t = k_0 t \tag{1}$$

$$\text{Firstorder: } \mathrm{Ln}(Q_0 - Q_t) = k_1 t \tag{2}$$

$$\text{Higuchi: } Q_t = k_{\mathrm{H}} t^{1/2}, \tag{3}$$

$$\text{Korsmeyer–Peppas: } Q_t = k_{\mathrm{P}} t^n \tag{4}$$

where Q_t is the amount of drug released in time t and Q_0 is the initial amount of drug in the microparticless, k_0, k_1, k_{H}, and k_{P} are release rate constants, 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; and erosion-mediated release occurs if $n > 0.85$ [Ritger and Peppas, 1987].

Hypoglycemic Activity in Diabetic Rabbit

Male New Zealand white rabbits weighing 2.5–3 kg were used for the animal model. All investigations were performed according to the 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, 6 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 rabbits by injecting streptozotocin (100 mg/kg; i.p.) dissolved in citrate buffer (3 mM; pH 4.5) [Grover et al., 2002]. Three days later, rabbits with blood glucose levels between 300–400 mg/dL were selected for the study [Satyanarayana and Kilari, 2006]. The animals were fasted overnight before starting the experiment. Rabbits were assigned to four groups of 6 rabbits each and treated as following: group I (diabetic control) treated with 1% CMC suspension in normal saline; group II treated with commercial GLZ tablet, at a dose of 5 mg/kg, orally; group III received suspension of GLZ in 1% CMC (5 mg/kg), orally; and group IV received GLZ-loaded chitosan microparticles (5 mg/kg). Microparticles suspended in distilled water or vehicle was administered orally by gastric intubation. The dose of gliclazide was selected by conducting the hypoglycemic experiments with doses of 1–10 mg/kg. Blood was collected from the marginal ear vein of the rabbits at time intervals between 2–24 h after treatment and before oral administration. Blood serum was separated by centrifugation at 4,000 rpm for 15 min. Serum glucose levels were determined using Glucoscan test Strip (Lifescan Inc., Milpitas, CA) and reading by a glucoscan 3000 Meter (Lifescan Inc). The mean serum glucose levels determined in samples collected before GLZ determination were taken as the baseline levels and plotted against time.

Statistical Analysis

Results were analyzed and expressed as mean $\pm$ SD. Effects of various factors on chitosan GLZ microparticles were statistically analyzed by Student's t-test using graph Pad InStat Software-1.13 version (Graph Pad Software, San Diego, CA). The differences were considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSION

Chitosan microparticles composed of different negatively and positively charged polymers represent a type of drug delivery system that can be prepared in a facile manner. Chitosan/TPP matrices have been used in the pharmaceutical industry for many years. Sufficient charge numbers (or density) are necessary for anions to cross-link chitosan by electrostatic force. TPP is a multivalent anion that carries a maximum of 5 negative charges. On the other hand, chitosan is a weak polybase with a maximum of thousands of positive charges (Fig. 1). However, the charge number of the

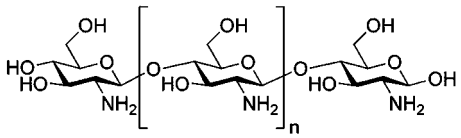

Fig. 1. Chitosan: β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

anions and chitosan are all mainly controlled by solution pH.

Microparticle Characterization

Particle size, encapsulation efficiency and surface morphology

Chitosan microparticles containing GLZ were evaluated for particle size, yield, and encapsulation efficiency (Table 1). The mean diameters of the microparticles were 4–5 mm before drying. After drying, water content dropped from 90% to 7–15% and microparticle sizes sharply decreased. The viscosity of chitosan sample has importance in the formation of microparticles. Chitosan microparticles could not be prepared from samples less than 1% w/v chitosan. Extra high-viscosity chitosan samples (> 3%w/v) did not form smooth round microparticles because of dropping difficulty. Microparticles ranged mainly between 675–887 μm in diameter with a arrow range of weight distribution of microparticles occurring for all batches prepared (see Table 1). Particle size revealed an increase with increasing amounts of chitosan, It was found that particle size using 3%, w/w, chitosan was higher than that of 1% w/w chitosan. This could be due to the higher amount of chitosan present, leading to a viscosity increase in polymer solution, producing bigger droplets during emulsification that later hardened in the presence of TPP and GL. 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 is that particle size decreased with an increase in cross-linking extent. The particle size of 1 mL GL added was higher than that of 5 mL GL added possibly due to the formation of more rigid network structures at higher cross-linking [Rokhade et al., 2007]. Since GLZ is insoluble in water, it was not dissolved in solution during the cross-linking and hardening process. Therefore, the loss of GLZ from microparticles was minimal during the hardening and washing process. The microparticles showed good encapsulation efficiency, greater than 94.5% in all cases, with efficiency not affected by the chitosan concentration or the concentration and pH of the cross-linking agent. Addition of pectin to 5% TPP solution (pH = 7) had no effect on encapsulation efficiency ($P > 0.05$). Additionally no

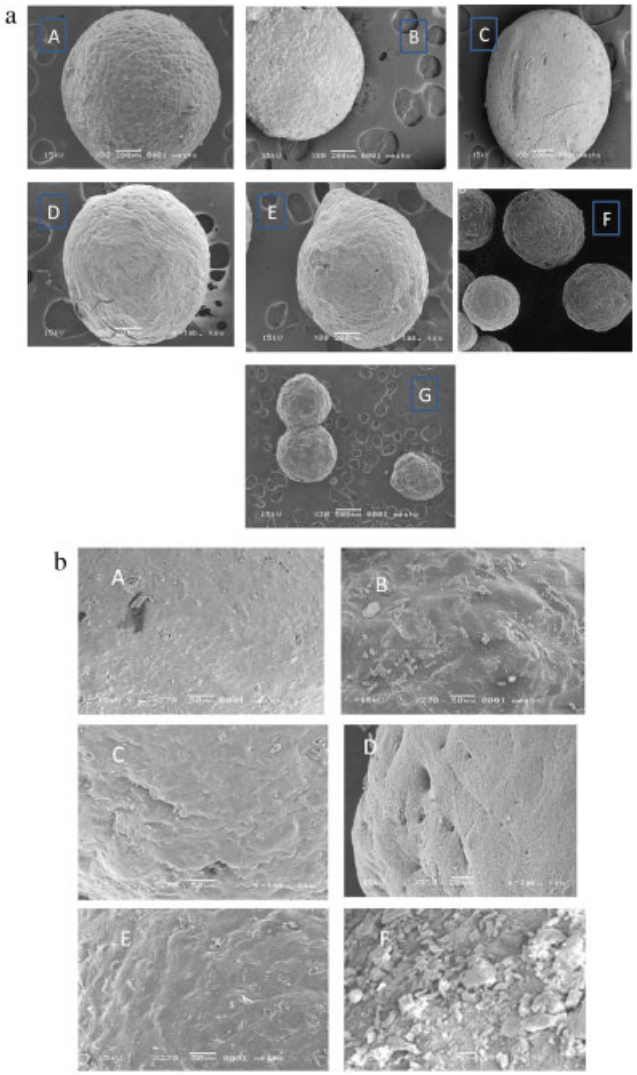

Fig. 2. **a:** SEM of GLZ-chitosan microparticles: (A) without Glutaraldehyde; (B) 1 ml Glutaraldehyde; crosslinking solution has (C) pH 3; (D) pH 5; (E) pH 7; (F) pH 9; crosslinking solution contain (G) 1% pectin. **b:** SEM and surface morphology of GLZ-chitosan microparticles: crosslinking solution has (A) pH 3; (B) pH 5; (C) pH 7; (D) pH 9; crosslinking solution contains (E) 5 ml glutaraldehyde; (F) 1% pectin.

difference occurred between the GLZ encapsulation efficiencies of microparticles prepared with pectin combined with 5% TPP solution (pH = 7), because of the low water-solubility of GLZ. The results are in agreement with the previous results of Shu and Zhu [2000] using chitosan beads loaded with water-soluble and water-insoluble model drugs. In all cases, high loading efficiency was obtained due to the poor water solubility of the drug. Gliclazide-chitosan microparticles' yield and encapsulation efficiencies are summarized in Table 1.

Figure 2 shows the surface morphology of GLZ microparticles. The procedure developed provided

spherical particles of homogenous surface with no tendency to aggregate (Fig. 2A). After cross-linking, the color of the microparticles changed from white to dark brown with a variation in pH from 3.0 to 9.0. The microparticles prepared in higher pH value of cross-linker were porous and brittle with larger wrinkles than the microparticles prepared under acidic conditions (Fig. 2B). All chitosan gel microparticles prepared by the cross-linking method had good sphericity. In chitosan microparticles, the polyelctrolyte complex

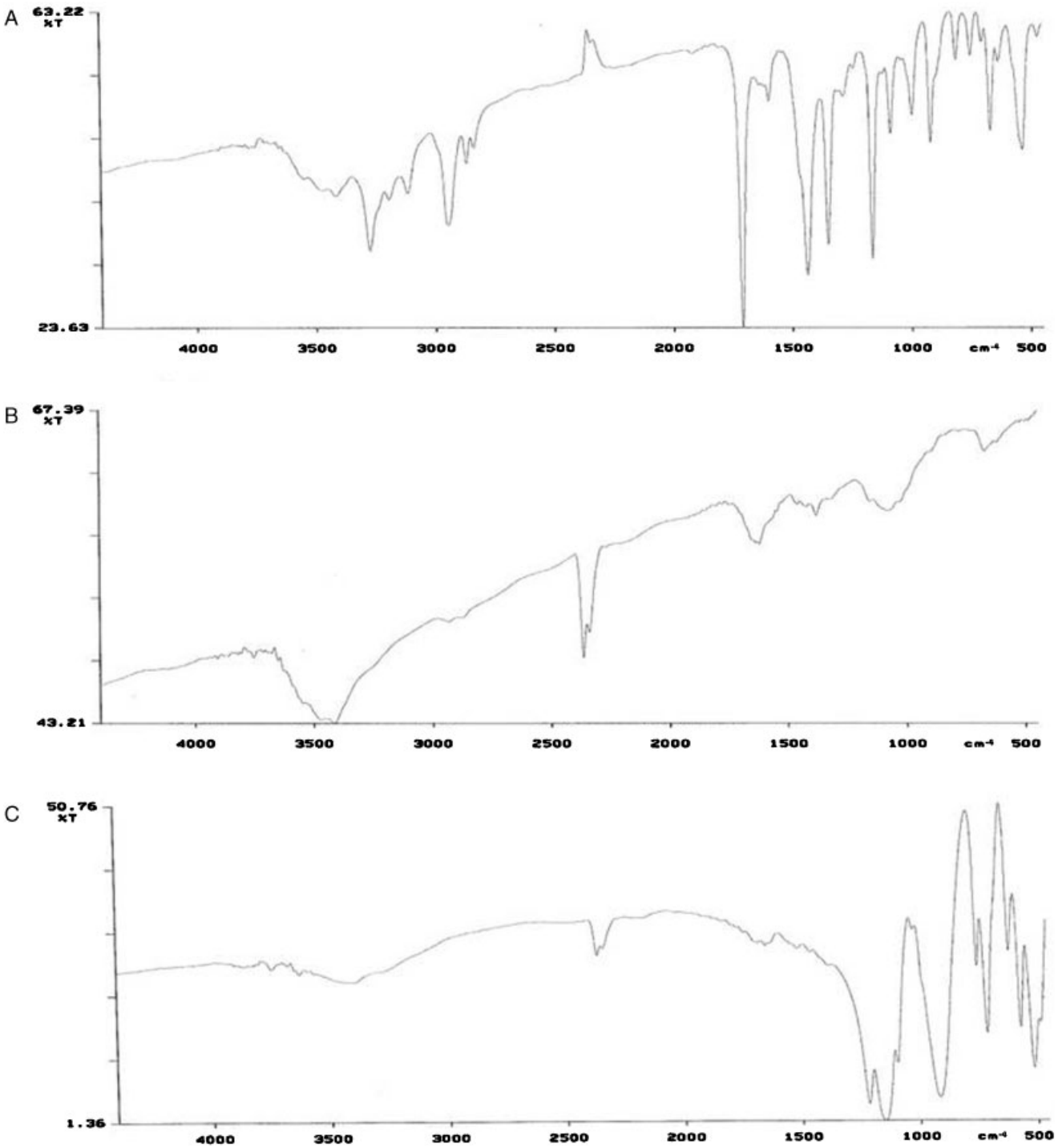

Fig. 3. FT-IR spectra of: (**A**) pure GLZ; (**B**) plain chitosan; (**C**) plain TPP.

occurs between chitosan and TPP, and also between chitosan and pectin, and protects the gel matrix from environmental conditions. Coating pectin on these microparticle surfaces improved surface morphology, yielding white-colored microparticles due to pectin forming a polyelectrolyte complex film on the microparticles surface with cationic chitosan. Surface morphology revealed the presence of cracks and pores at pH 7 and 9. Microparticles in this study also showed surface-adhered drug particles.

Fourier transform infrared spectroscopy

The IR spectrum of the resulting GLZ microparticles is shown in Figures 3 and 4. The FT-IR of gliclazide showed peaks of −NH stretching (3,274 cm^{-1}), = CH stretching (3,113 cm^{-1}), O = C (1,705 cm^{-1}), C = C aromatic (1,596 cm^{-1}) C–H deformation (1,467–1,430 cm^{-1}) SO_2-NH (1,352 cm^{-1}). Similar peaks were seen in GLZ-loaded chitosan microparticles. The IR spectrum of chitosan microparticles showed peaks of assigned saccharide structure around 905 and 1,153 cm^{-1} and a protonated amino characteristic peak at around 1,570 cm^{-1}. There was a stronger absorption band at 1,650 cm^{-1} of assigned amide groups. The broad and strong band ranging from 3,200–3,600 cm^{-1} may be due to the overlapping of −OH and −NH stretching vibration, consistent with the peak at 1,155 cm^{-1} assigned to C–N stretching vibration [Nalva, 1997]. The appearance of a characteristic peak at 1,150 cm^{-1} assigned to P = O groups of TPP was evidence of ionic cross-linking of chitosan. The intensity of P = O absorbance at 1,150 cm^{-1} of cross-linked chitosan gel microparticles increased with a decrease in pH suggesting that chitosan can bind with TPP ions more easily under lower pH conditions. These results are in agreement with Alsarra et al. [2004]. The percentage of TPP was 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 in chitosan increased. Chitosan microparticles prepared in acidic TPP solution were completely ionic cross-linking dominated. The p*K*a of chitosan is about 6.3 [Shu et al., 2001; Li et al., 2004]; at high pH, chitosan is slightly ionic cross-linking, as TPP dissociates into OH^- and $HP_3O_{10}^{-4}$ and $P_3O_{10}^{-5}$ and chitosan microparticles are dominated by deprotonation, which may lead to lower cross-linking density. Thus, the charge density of chitosan and cross-linker must be sufficiently high at the pH value to allow optimal interactions and ensure a high cross-linking density [Chen et al., 2008]. FTIR spectral data were also used to confirm the chemical stability of GLZ in gel microparticles. In the case of drug-loaded microparticles, all the bands that were observed in GLZ have also appeared, indicating the chemical stability of GLZ after encapsulation into the polymer matrix.

Differential Scanning Calorimetry

Under the experimental conditions, the DSC thermogram of pure chitosan had no characteristic endotherm while it had a large exothermic decomposition peak at about 250°C (Fig. 5b), where that of the cross-linking complex was somewhat smaller and shifted to about 230°C, further confirming that chitosan is not present in its free form. DSC performed on pure GLZ and GLZ-loaded microparticles (Fig. 5a,c–e) revealed an endothermic sharp peak at 185°C of melting GLZ that was not apparent in the chitosan-GLZ loaded microparticles indicating the amorphous dispersion of GLZ into the chitosan matrix.

GLZ Release Studies

Drug release behavior of the formulations based on chitosan cross-linking with 5% w/v TPP were evaluated in vitro in simulated gastric and intestinal pH conditions. Results of the percentage cumulative release versus time for drug-loaded microspheres for the different formulations are shown in Figures 6–9. The effect of TPP concentration (5, 10% w/v) at a fixed pH of the gelling medium at 5.0 was also studied in preliminary experiments. Lower concentrations of TPP 1% w/v resulted in non-crosslinked chitosan film that spontaneously dissolved in 0.1 N HCl (data not shown). At higher concentrations, 5–10% w/v, GLZ release from microparticles was independent of TPP concentration ($P > 0.05$). On the other hand, Remuňán-Lŏpez and Bodmeier [1997] reported that the diffusion of chlorphenaramine maleate from chitosan films decreased with an increase in the concentrations of the TPP solution. In addition, they showed that the swelling and permeability characteristics of chitosan films were dependent on the concentration of the cross-linking agent.

Effect of Cross-Linking Solution pH

Figure 6 shows the release behavior of GLZ from chitosan microparticles prepared with 5% w/v TPP solutions at various pH levels. As the pH of TPP solution increased, the release profiles of GLZ from TPP-chitosan microparticles increased, confirming that the degree of ionization TPP is pH-dependent with ionization of the amine groups decreasing with increasing pH that decreases opportunities for ionic interactions with TPP. The loss of charge density in these polyionic species reduced the extent of cross-linking and the strength of ionic attraction, allowing formation of an open porous structure when chitosan microparticles were prepared in TPP solution of

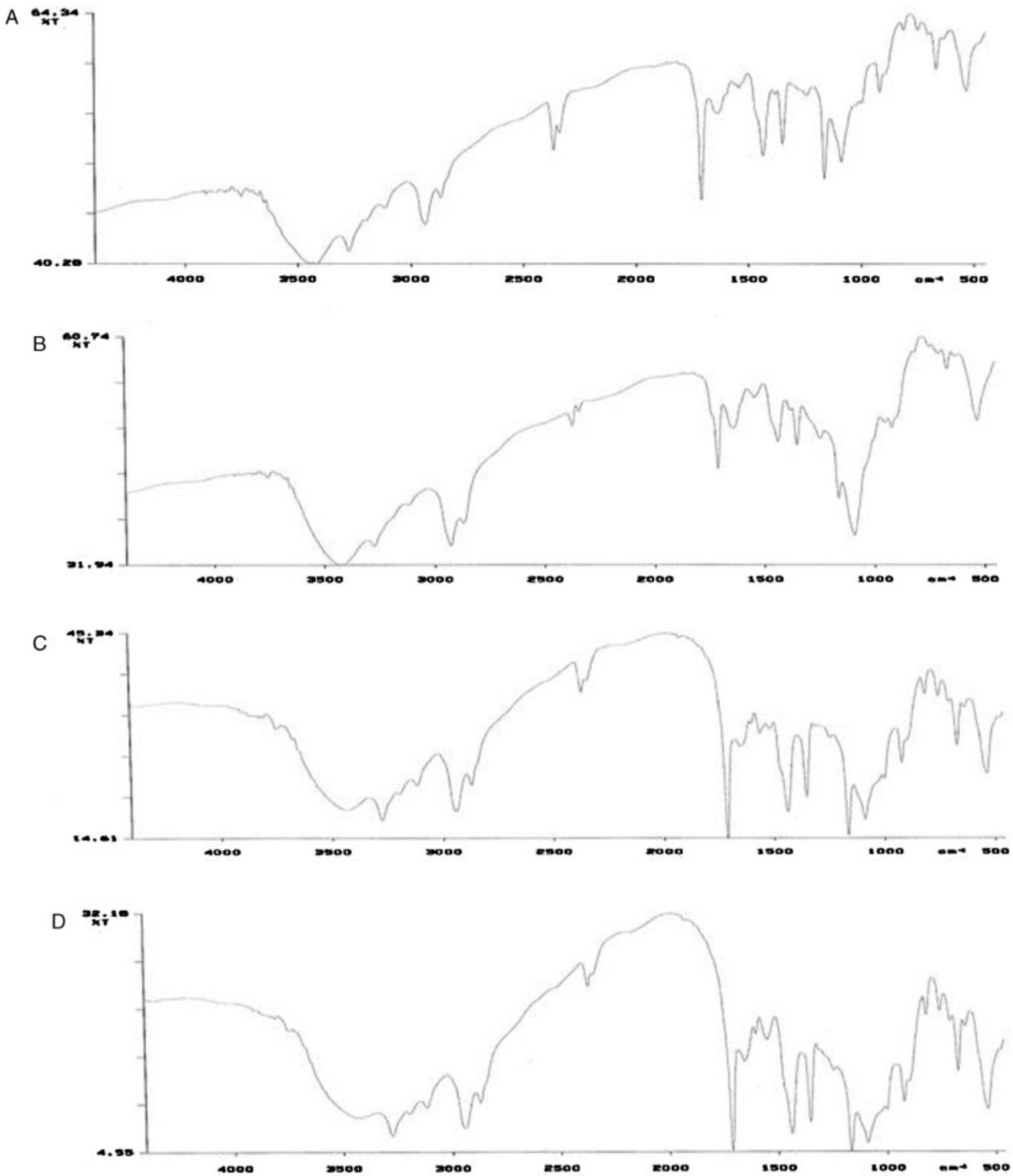

Fig. 4. FT-IR spectra of: (**A**) GLZ-loaded chitosan microparticles prepared without added glutaraldehyde; (**B**) GLZ-loaded chitosan microparticles prepared at pH 3; (**C**) GLZ-loaded chitosan microparticles prepared at pH 9; (**D**) GLZ-loaded microparticles using 1% pectin.

higher pH. This porous structure is more degradable than higher density structures. The release behavior of GLZ from microparticles prepared in TPP solution of higher pH was faster than microparticles prepared at pH 3 and 5 in agreement with the findings of Shu and Zhu [2000].

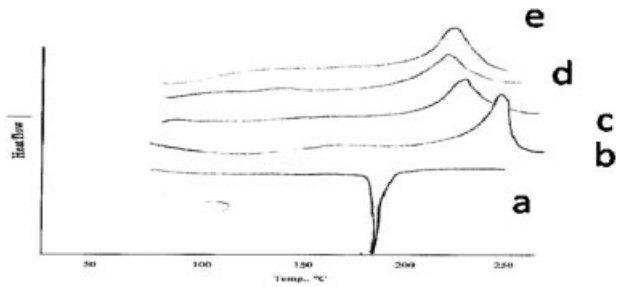

Fig. 5. DSC spectra of (**a**) pure GLZ; (**b**) chitosan; (**c**) GLZ-loaded chitosan microparticles prepared at pH 3; (**d**) GLZ-loaded chitosan microparticle prepared at pH 9; (**e**) GLZ-loaded chitosan/1% pectin microparticle.

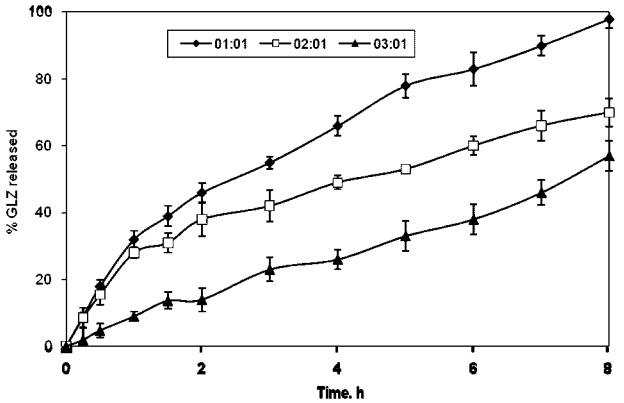

Fig. 6. The influence of the drug: chitosan ratios on GLZ release from microparticles (5% w/v TPP pH 5).

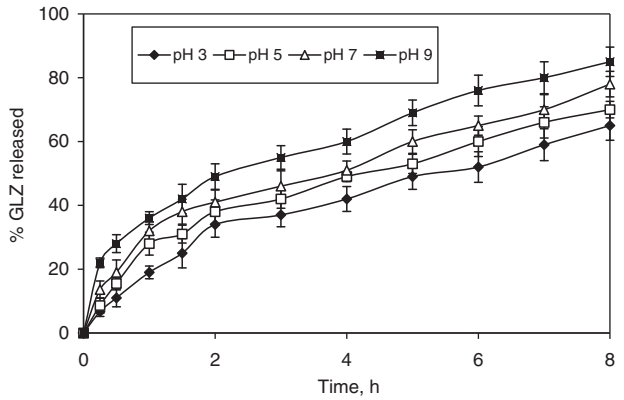

Fig. 7. The influence of pH of 5% w/v TPP solution on GLZ release from microparticles (chitosan:GLZ ratio 2:1).

Effect of Chitosan Concentration

Drug release behavior of chitosan matrices can be modulated by its swelling-erosion rate. In ionic cross-linked hydrogels, gel swelling and the erosion of network structure can be prevented by inter-ionic

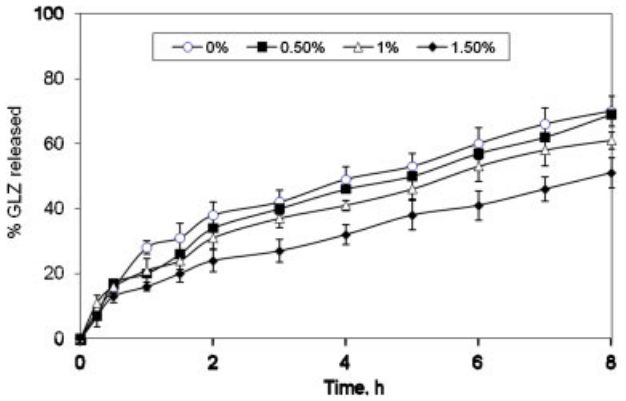

Fig. 8. The influence of pectin concentration on the GLZ release from microparticles (5% w/v TPP pH 5; chitosan:GLZ ratio 2:1).

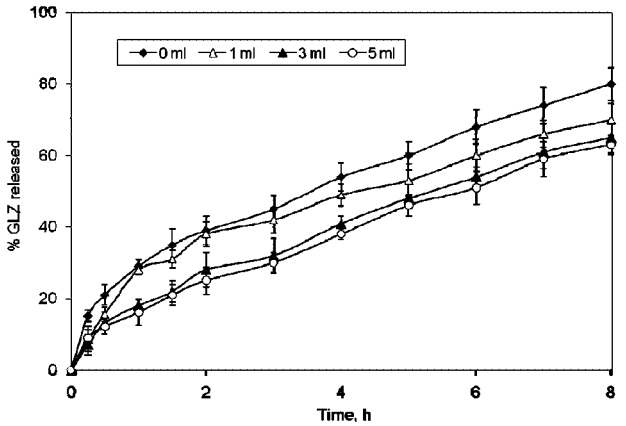

Fig. 9. The influence of glutaraldehyde volume on the GLZ release behavior from microparticles (5% w/v TPP pH 5; chitosan:GLZ ratio 2:1).

interactions, which are related to the chitosan concentration used for preparing the drug-loaded microparticles. The drug release behavior of chitosan microparticles prepared at fixed pH of the gelling medium (pH = 5) with different chitosan concentrations is shown in Figure 7. With increased chitosan concentrations, GLZ release decreased indicating that the release behavior of the drug is related to the viscosity of chitosan solution. This result is consistent with the literature showing that increasing chitosan concentrations decreased the percent release [González-Rodrĭguez et al., 2002; Yassin et al., 2006].

Effect of Added Pectin to the Cross-Linking Solution

Addition of pectin in the 5% w/v TPP solution with a pH value of 5, resulted in decreased GLZ release ($P < 0.05$). At 8 h, the percent GLZ release from microparticles was 71, 62, and 50% following addition of 0.5, 1, and 1.5% of pectin in the cross-linking TPP

solution, respectively (Fig. 8). Encapsulation efficiency was not significantly affected by the addition of pectin to the cross-linking solution. In contrast, Aral and Akbuğa [1998] found that the encapsulation efficiency of bovine serum albumin (BSA)/chitosan beads was significantly affected by the addition of sodium alginate and glutraldehyde to the TPP solution. Ishak et al. [2007] found that the external phase composition significantly affected loading efficiency and buoyancy of metronidazole/chitosan beads.

GLZ/chitosan microparticles containing 1% pectin were selected for in vivo studies.

Effect of Glutaraldehyde Volume

GLZ microparticles did not disintegrate in 0.1 N HCl or in phosphate buffer pH 7.4 during the release study. This may be dependent on the hardening of chitosan microparticles with glutaraldehyde during preparation [Sezer and Akbuğa, 1995]. These authors found that the surface of the beads became seamless after hardening. The release of GLZ from chitosan microparticles was significantly affected by the glutaraldehyde volume added to the 5% w/v TPP solution at pH 5, increasing up to 3 ml ($P<0.05$; Fig. 9). This was also reflected in GLZ release where the $T_{50\%}$ of the GLZ increased from 4 h for 0% glutaraldehyde to 6 h for microparticles prepared with the addition of 3 ml glutaraldehyde into the cross-linking TPP solution. Further increases in glutaraldehyde volume up to 5 ml did not affect GLZ release.

The kinetics of drug release were determined with respect to zero order, first order, the Higuchi model, and the Korsmeyer and Peppas models. Correlation coefficient values and $T_{50\%}$ are shown in Table 2. The Higuchi model was the best kinetic fit for all GLZ microparticles formulations, except those composed of 3:1 chitosan:GLZ where zero order kinetics were predominant. The values of n within the range of 0.372–0.503 indicate that the Fickian diffusion dominated and as a result no degradation of the particles in the medium at the end of the dissolution, as the Higuchi kinetic represents the drug release by diffusion from an inert matrix [Desai et al., 1965].

Serum Glucose Levels of the Streptozotocin-Induced Diabetic Rabbit Model

The diabetic control group did not show any significant change in serum glucose level (SGL) over the experimental. As the time progressed, however, a light decrease in SGL was observed in the control group due to the fasting effect on blood glucose level. Figure 10 shows the decrease in serum glucose levels after oral administration of optimized GLZ- microparticles containing 1% pectin, pure GLZ powder, and the commercial tablet to diabetic rabbits. The results reveal that the decrease in SGL of the basal level of the group treated with the optimized GLZ microparticle formulation or GLZ powder was significantly lower than controls ($P<0.05$). GLZ powder-treated rabbits showed a rapid reduction in SGL within 2 h, and a maximum hypoglycemic response after 4 h with $46.7\pm5.7\%$ decreases in SGL from the basal level. For GLZ-microparticles, the hypoglycemic response

TABLE 2. Correlation Coefficient Values (r), Release Exponent (n), Release Constant (k), and $T_{50\%}$ of GLZ Release From Chitosan Microparticles

Formulations	Zero order	First order	Higuchi model	Korsmeyer–Peppas model	n	K (% h^{-n})	$T_{50\%}$ (h)
Chitosan:GLZ 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, GL (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

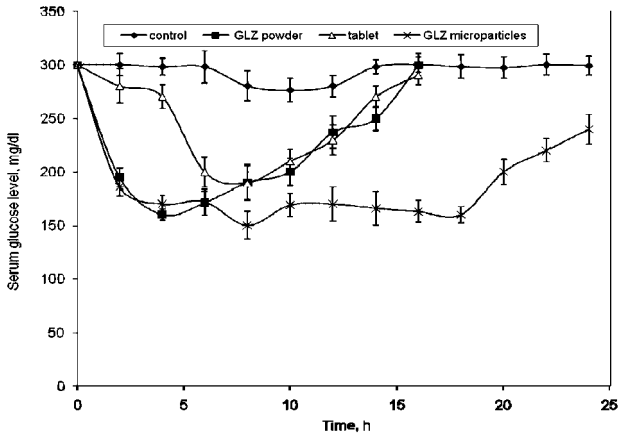

Fig. 10. Serum glucose levels in diabetic rabbits ($n = 6$). Control group treated orally with CMC 1% suspension. Test animals: GLZ powder and GLZ microparticles in a dose of 10 mg/kg, commercial tablet in a 10-mg/kg dose orally.

was gradual. A maximum hypoglycemic effect was observed after 8 h (52.7 ± 6.8% decrease in SGL) and remained stable up to 18 h. The GLZ-marketed tablet showed a maximum hypoglycemic response after 8 h with a 36.6% decrease in SGL of the basal level compared to plain GLZ powder ($P < 0.05$). The data presented here show that TPP/chitosan microparticle-loading gliclazide had a homogenous structure with high drug loading. Pectin coated the surface of TPP/chitosan to form a complex film, which prolonged the release period. In vivo assessment of the developed GLZ microparticles in a diabetic rabbit model revealed better activity. These sustained-release microparticles may be considered for further human evaluation as a promising controlled release dosage form for gliclazide.

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