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Development and Evaluation of a Novel Dosage Form of Diltiazem HCl Using Ethylene Vinyl Acetate Copolymer and Sodium Starch Glycolate (*in Vitro/in Vivo* Study)

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ABSTRACT: The work aims at developing a CR formulation, with high encapsulation efficiency of diltiazem HCl, suitable for twice daily administration. Microparticles, using EVA copolymer, were prepared by coacervation-phase separation technique, subjected to controlled extraction and vacuum freeze drying processes to generate and immobilize a non uniform initial drug concentration distribution, and evaluated *in vitro* and in animals. Effects of increasing initial drug concentration, varying polymer system, increasing porosity, and decreasing tortuosity, varying the size of the microparticles and the pH of the dissolution medium on the release rate were evaluated. The results indicated that the release rate from microparticles was constant (zero-order) for an appreciable period of time but it was low for twice-daily administration. It increased with increasing initial drug concentration, varying polymer system, increasing porosity, and decreasing tortuosity, and decreasing the size of the microparticles but the duration of constant release was shorter except for formulations containing 2.00 and 2.25% sodium starch glycolate. 10-h duration of constant release was achieved and the zero-order release rate was within the required rate to achieve the desired therapeutic level. The pH of the dissolution medium did not have any effect on the release rate. The results of the *in vivo* study indicated that *in vitro* dissolution correlated well with *in vivo* $AUC_{0\text{-}10}$ and that there were no statistically significant differences in $AUC_{0\text{-}10}$ and C_{max} between the new CR formulation and Cardizem® CD. Accordingly, a new CR formulation that delivers diltiazem HCl at a constant rate, suitable for twice daily administration was developed. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: controlled release; zero order; *in vitro/in vivo* correlation; EVA copolymer; diltiazem HCl

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INTRODUCTION

Diltiazem HCl is a calcium channel blocker used in treating angina, hypertension,[1–5] and cardiac arrhythmias.[1,2,6] After oral administration, diltiazem is readily absorbed from the gastrointestinal tract[1] (90% of the orally administered dose[3,5]). Its bioavailability ranges from 30 to 60% because it undergoes extensive first pass metabolism.[1–5] Its elimination half life ($t_{1/2}$) is 3–6 h.[1–5] The usual dose of diltiazem in adults is 30 mg four times a day.[6]

Because of its low oral bioavailability, short biological half-life, multiple daily dosing, and its therapeutic use in chronic diseases, it is a good candidate for modified/controlled release oral dosage forms. These dosage forms improve clinical efficacy of the drug, reduce toxicity, and improve patient compliance and convenience.[7] They, ideally, should release the drug at a constant, or zero-order rate.[8]

Therefore, the first aim of this research was to prepare a controlled release dosage form, based on multiparticulate system that delivers the drug at a constant rate (zero-order). Multiparticulate systems are pharmaceutical formulations in which the active substance is present as a number of small independent subunits. To deliver the recommended total dose, these subunits are filled into a sachet, encapsulated or compressed into a tablet.[9] Microparticles composed of diltiazem HCl dispersed in ethylene vinyl acetate (EVA) copolymer matrix were used as the small independent subunits, multiparticulates, in this research. A dosage form based on multiparticulate system was chosen because it has many advantages. These include: (a) High degree of flexibility in the design and development of dosage forms[10,11]; (b) They can be divided into the desired dose strengths without formulation or process changes[10]; (c) Different multiparticulate systems may be blended to deliver

incompatible bioactive agents in a single dosage form, or to provide different release profiles[10]; (d) They disperse freely in the GI tract, maximize drug absorption[10]; (e) Reduction in variations in gastric emptying rates and overall transit and hence reduction in inter- and intrapatient variability[10–12]; (f) Better statistical assurance of drug release and the risk of dose dumping is equally divided[12]; (g) Lowered local concentrations and hence toxicity or irritancy[11,12]; (h) Greater safety factor in case of a burst or defective individual in subdivided dosage form[11]; and (i) They provide a suitable option for pediatric/geriatric formulations due to their swallow-ability, when mixed with food.[10] The second aim of this research was to evaluate the physical characteristics of the microparticles with respect to particle size and size distribution, bulk density and percent compressibility and to evaluate how these characteristics affect drug release. The third aim of this research was to evaluate the controlled release characteristics of the prepared dosage form versus approved marketed products by *in vitro* dissolution tests in media having different pH values and in biological models (animals).

There are several methods to prepare polymeric drug matrices. These include: addition of the drug to monomer/cross linking agent mixtures; evaporation of solutions of polymer in which the drug is dissolved or dispersed; compression of the polymer/drug mixture; by swelling of the polymer with a suitable solvent in which the drug is dissolved[13]; and coacervation-phase separation technique.[14,15] However, simple monolithic matrices fabricated by any of these methods do not yield zero-order release kinetics; instead, they yield first-order release kinetics or square-root-of-time kinetics. This is due to the increase of the diffusional length resistance with time.[16] There are many ways to overcome this limitation; one of these is having non uniform initial drug concentration distribution. The applicability of this concept and process has been demonstrated experimentally with the release of oxprenolol HCl from hydrogel beads based on 2-hydroxyethyl methacrylate polymerized with crosslinking agent. It was evident that an inflection point and a zero-order release region up to 60% of drug released were introduced by the process.[17]

In this work, coacervation-phase separation technique by adding a nonsolvent was used to prepare the microparticles with the drug dispersed in EVA copolymer matrix. The concept of having a nonuniform initial drug concentration distribution was applied to counterbalance the increase in the diffusional path length, and approach zero-order drug release from polymer matrix. Additional or further modification and control of the release rate of the drug from the formulation was attempted by increasing initial drug concentration, varying polymer system making the matrix, and varying porosity and tortuosity of the matrix, as mentioned previously by Hui and Robinson.[18]

MATERIALS AND METHODS

Preparation of Diltiazem HCl Microparticles

Diltiazem HCl was loaded into EVA copolymer by coacervation-phase separation technique. EVA copolymer pellets containing 40% vinyl acetate were dissolved in dichloromethane to give a 10% w/v solution. Totally, 6.5 g of diltiazem HCl powder were added to 25 mL of the polymer solution. The mixture was vortex-mixed for 3 min to give a uniform suspension. Using a peristaltic pump, the mixture was withdrawn and pumped drop wise into 20 mL cold unstirred absolute ethanol in a 50-mL beaker immersed in dry ice bath (−70°C). After 10–15 min, the beaker containing the microparticles was removed from the dry ice bath and allowed to warm to room temperature (20°C). After 1 h, the liquid was replaced with 10 mL fresh ethanol. The beaker containing the microparticles was set aside standing. After 20–21 h, the liquid was decanted and the microparticles were dried for 5 h in a vacuum oven.

Microparticles having two size ranges were prepared using two peristaltic pumps with different flow rates. The size of the microparticles was dependent on the flow rate at which the polymer dispersion was introduced into the nonsolvent and it decreased as the flow rate increased. The flow rate of a given peristaltic pump is affected by the inner diameter of the silicon tube used. Large microparticles were prepared using a Syva peristaltic pump (model 1500, CA) set at the maximum speed and a silicone tube with an inner diameter of 1.5 mm while small microparticles were prepared using an STA peristaltic pump (Desaga-Heideberg-Germany) with a higher flow rate and the inner diameter of the silicon tube used was 1.0 mm.

SEM

SEM images were obtained using an FEI Company-Inspect F50/FED (Eindhoven, Netherlands) after coating the microparticles with platinum using Emitech K550 X Sputter Coater (England).

Encapsulation Efficiency

Encapsulation efficiency (EE) was determined by dissolving a certain weight of the microparticles in dichloromethane. Diltiazem HCl concentration was determined spectrophotometrically by measuring the UV absorbance of the diluted portion of the resultant solution at 240 nm.[20] The corresponding concentration was calculated using a calibration curve. Encapsulation efficiency was calculated using the following formula:

$$\text{Encapsulation Efficiency} = \frac{C_{\text{in}}}{C_{\text{orig}}} \times 100\% \quad (1)$$

where C_{in} is the concentration of the drug encapsulated in the microparticles and C_{orig} is the original concentration of the drug present in the loading solution.[21,22]

Achievement of a Nonuniform Initial Drug Distribution in the Loaded Microparticles

A nonuniform initial drug distribution in the loaded microparticles was achieved by subjecting dry microparticles loaded with diltiazem HCl to a controlled-extraction process. The microparticles were suspended in 1:1 ethanol: water mixture with vigorous stirring at a rate of 250 rpm and 30°C for 15 min. The conditions of controlled extraction were optimized (data not shown). Immediately after removing the microparticles from the extracting solvent, they were vacuum freeze-dried under reduced pressure for 24 h. Microparticles subjected to the above mentioned processes were referred to as treated microparticles otherwise they were referred to as untreated microparticles.

Dissolution Tests

In vitro dissolution studies were conducted in accordance with USP 23 apparatus II procedure (Vankel VK 700) at 37°C in 900 mL water. The paddle speed was 100 rpm. Samples (5.0 mL) were withdrawn at different time intervals and analyzed or preserved refrigerated till analyzed (within 24 h). After withdrawal of a sample, an equal volume of fresh dissolution media was added to maintain a constant volume of 900 mL. Samples were assayed for diltiazem HCl by means of UV spectrophotometer at 240 nm. The amount of the drug in solution was calculated by comparing the absorbance of the sample with a predetermined standard curve. The same procedure was repeated using approved marketed products.

Processing of Data

To generate drug release rate vs. time plots, the polynomial equation that gave the best fit for the dissolution data (mean cumulative amount released, mg%, n = 2) was determined and then derivated (differentiated). Drug release rates at each time point were calculated using the first derivative of the polynomial equation.

Modification of the Release Rate from the Matrix

Drug loading by coacervation-phase separation technique was carried out as mentioned in the "Preparation of diltiazem HCl microparticles" section, with the following exceptions:

Increasing Initial Drug Concentration

Totally, 10.0 g of diltiazem HCl powder instead of 6.5 g were added to the polymer solution.

Varying Polymer System Making the Matrix

Mixtures of ethyl cellulose and EVA copolymer in different ratios (1:1, 2:1, and 3:1) instead of EVA copolymer alone were dissolved in dichloromethane to give a 10% w/v solution.

Varying Porosity and Tortuosity of the Matrix

Addition of Lactose or Sodium Starch Glycolate. Weighed amounts of lactose or sodium starch glycolate were added to the drug, mixed manually by tumbling the bottle containing the mixture for few minutes and then added to the polymer solution.

Varying Diltiazem HCl Particle Size. Diltiazem HCl having two different particle sizes (small or large) was used in the preparation of the microparticles.

To determine the particle size, size distribution studies of two batches of diltiazem HCl were carried using microscopy. Graticules or eyepieces with grids of circles and squares were used to compare the cross-sectional area of each particle in the microscopic field with one of the numbered patterns. The number of particles that best fit one of the numbered circles was recorded. The field was changed, and the procedure was repeated with another numbered circle. The procedure was repeated until the entire size range was covered. The particulate field counted was random. The total number of fields counted depended on the number of particles per field.

Physical Evaluation of Microparticles

Microparticle Size Distribution and Its Effect of on the Release Rate from the Formulation. Sizes of 20 microparticles randomly selected from a batch, were measured using a micrometer. The effect of the size of microparticles on the release rate was studied by conducting *in vitro* dissolution studies as in the "Dissolution tests" section using small or large microparticles.

Loose Bulk Density, Tapped Bulk Density, and Compressibility. To characterize the flow properties of the microparticles, loose bulk density, tapped bulk density, and compressibility were calculated as described in the United States Pharmacopeia (USP).[23] A quantity of the microparticles was carefully leveled in a cylinder without compacting, the bulk volume (unsettled apparent volume), V_o, was read. The cylinder was tapped mechanically using Jolting volumeter [(Model STAV 2003, Nottingham-UK)] until there was no change in the volume or until the difference between the two volumes was less than 2% and then the final tapped volume, V_f, was read. The loose bulk density was calculated by dividing the mass by the bulk volume (M/V_o). The Tapped bulk density was calculated by dividing the mass by the tapped volume (M/V_f). The % compressibility was calculated using the following equation $(V_o - V_f)/V_o \times 100\%$.

Effect of pH on the Release Rate from the Formulation

The effect of pH on the release rate of the drug was investigated by performing *in vitro* dissolution test as in "Dissolution tests" section, except that the dissolution medium was different. First, the prepared microparticles were stirred in simulated gastric fluid pH 1.2 for 2 h. The stirring was stopped, and the contents of the vessel were decanted or siphoned off taking maximum care that none of the microparticles were removed. A simulated intestinal fluid pH 5.0, kept at the same temperature, was added and stirring was continued for an additional 4 h. Again the stirring was stopped, and the contents of the vessel were decanted or siphoned off taking maximum care that none of the drug particles were removed. Another simulated intestinal fluid pH 6.8, kept at the same temperature, was added and stirring was continued for an additional 6 h.

HPLC Method for Diltiazem HCl

Stock solutions of diltiazem HCl (100 μg/mL) and propranolol HCl (100 μg/mL) were prepared in methanol. Working solutions for diltiazem HCl and propranolol HCl (1 μg/mL) were prepared daily by diluting aliquots of stock solutions with phosphate buffer pH 9.0. Standard solutions of diltiazem HCl were obtained from working solutions by serial dilution with phosphate buffer pH 9.0.

Sample preparation involved adding 750 μL of 0.1*M* potassium dihydrogen phosphate solution (pH adjusted to 7.5) to 1.0 mL plasma spiked with 200 μL of propranolol HCl (100 ng/mL). Then, the drug and the internal standard were extracted with 5 mL diethyl ether and then back-extracted into an aqueous 0.075% phosphoric acid solution. Finally, 100 μL of the aqueous solution of each sample was injected into the equilibrated HPLC-UV system.[24]

The analysis was performed using an HPLC system with a UV-Visible detector (SPD-10A vp) set at 239 nm, an auto injector (SIL-10AD vp), a liquid chromatograph (LC-10AD vp), a degasser (DGU-12A), and a system controller (SCL-10A vp), Shimadzu, Japan. It was operated using isocratic conditions on C-18 RP (125 mm × 4.6 mm ID) 5 μm at ambient temperature. The mobile phase consisted of a mixture of acetonitrile and 0.05*M* potassium dihydrogen phosphate (pH adjusted to 3.5 using orthophosphoric acid) (26:74, v/v) at a flow rate of 1.75 mL/min.

Calibration curves in the concentration range of 25–200 ng/mL were constructed using 1.0 mL plasma samples and quantitation

was achieved by measuring of the peak-area ratio of the drug to the internal standard.

The HPLC method was validated in terms of linearity, accuracy and precision, recovery, and stability (data not shown).

Bioevaluation of the New Controlled Release Diltiazem HCl Formulation in Rabbits (*In Vivo* Study)

The study design was single dose, randomized, two treatments, two periods, two sequences cross over under fasting conditions with an interval of one weak between dosing.[25]

One capsule of the reference product (Cardizem® CD containing a dose equivalent to 30 mg of diltiazem HCl/kg of body weight of rabbit) against one capsule of the test product (New controlled release formulation containing a dose equivalent to 30 mg of diltiazem HCl/kg of body weight of rabbit).

Eight male rabbits weighing 2.1–2.7 kg were used in the study. One rabbit died at the end of period I. Each formulation was administered orally at 8.00 am by compulsive swallowing with 10 mL of tap water, following an overnight fast of 12 h. The animals were provided water and standard diet after 6 h of drug administration. Food in both periods was identical. Venous blood samples (at least 0.5 mL) were taken from the ear marginal vein at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 10.0 h after drug administration. Heparinized eppendorfs were used for sample collection. All blood samples were centrifuged directly after blood sampling for 5 min at high speed using an eppendorf centrifuge. The separated plasma portions were stored frozen at −20°C until analysis.

The determination of diltiazem HCl plasma concentrations was carried out in the laboratories of the faculty of pharmacy at Jordan University of Science and Technology (J.U.S.T.) by the newly developed and validated HPLC assay.

The area under the plasma concentration vs. time curve from time zero to the time of the last sample withdrawal ($AUC_{0\text{-}t}$) was calculated using the trapezoidal rule.[8,26] The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were recorded directly from the plasma concentration-time profile of the individual rabbits.

A statistical analysis using ANOVA was performed for comparing the pharmacokinetic parameters ($AUC_{0\text{-}t}$ and C_{max}) of the new controlled release formulation and Cardizem® CD.[25] The software Kinetica, version 4.0 was used in the statistical analysis of data.

RESULTS AND DISCUSSION

Preparation of Diltiazem HCl Microparticles

EVA copolymer was selected for this work because it possible to tailor the release rate of a device to a desired value with small changes in the membrane composition.[27] The incorporation of vinyl acetate comonomer units (between 9 and 40%) into a polyethylene (semicrystalline) backbone chain induces differences in crystallinity and crystalline structure, melting point, solubility, permeability, density, and glass transition temperature, affecting the flexibility and thermoplastic characteristics of the copolymer.[27,28] As the permeability of the copolymer changes substantially with vinyl acetate content, the release rate of a device changes.[27] In addition to that, it is biocompatible, nonbiodegradable, hydrophobic copolymer[29] that has been specifically used, in the pharmaceutical field, for the development of films, stent coatings, implantable devices, and vaginal rings.[19]

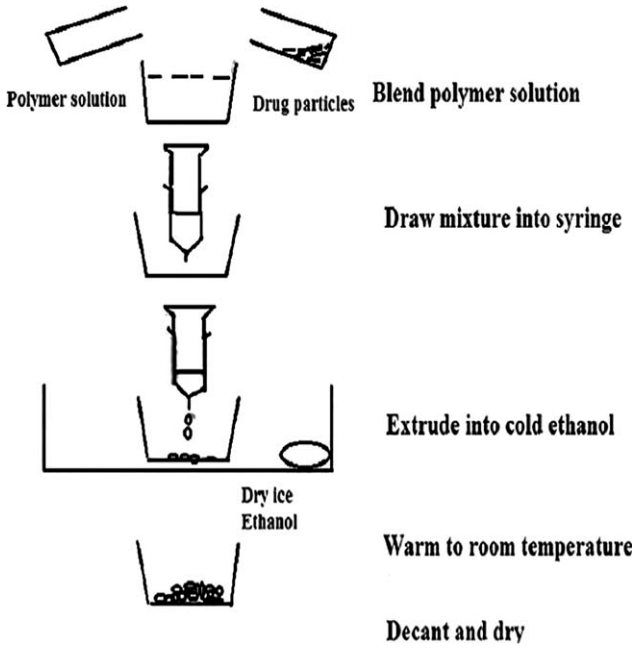

Figure 1. Schematic illustration of microsphere preparation.[19]

Coacervation-phase separation technique that was used to prepare the microparticles involved four steps.[14,15] Initially, a 10% solution of EVA copolymer in dichloromethane was prepared, and then diltiazem HCl was suspended in the solution. The polymer was then caused to slowly precipitate by the addition of a nonsolvent (absolute ethanol) kept at −75°C. Under these conditions, EVA initially precipitated as highly swollen liquid polymer phase. During the precipitation process, the liquid phase coated the dispersed active agent droplets driven by the tendency to minimize its surface free energy. In the final stage of the process, the microcapsule shell was desolvated and hardened. A schematic representation of microsphere preparation is shown in Figure 1.[19]

The resultant microparticles were nearly spherical in shape, nontacky, and nonsticky as shown in Figure 2. The encapsulation efficiency of this technique was high and 67% of the original drug concentration added was encapsulated in the polymeric matrices.

Achievement of Zero Order Kinetics by Having a Nonuniform Initial Drug Distribution in the Loaded Microparticles

An important step in designing a controlled release dosage form is to estimate the zero-order rate constant (K_r^o) which must be equal to the elimination rate (R) in order to provide a constant blood level of the drug. Since in controlled drug delivery systems the drug liberation is usually over a longer period of time than the duration of the distribution phase, a one-compartment open model can be selected[30] and the elimination rate (R) is given by the following equation:

$$R = C_P \, Cl_T \tag{2}$$

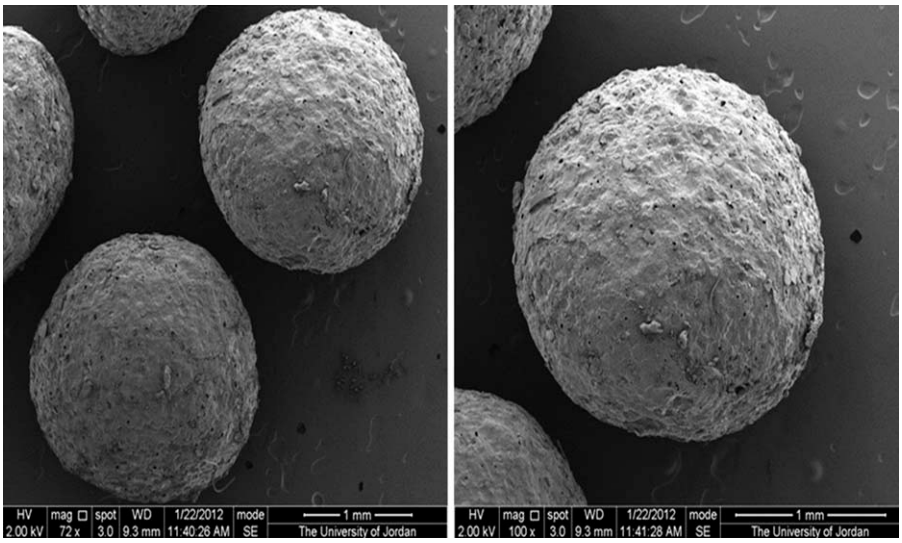

Figure 2. SEM images of treated diltiazem HCl microparticles with an average diameter of 1.8 mm containing 2.00 % w/w sodium starch glycolate, at two magnifications.

where C_p is the drug therapeutic level and Cl_T is the clearance of the drug.

Mean peak plasma concentrations of 117 ± 20 ng/mL were reached on average 3.0–4.5 h after ingestion of a therapeutically effective oral dose of diltiazem HCl of 90 mg. Given that the minimum clearance equals 900–1015 mL/min,[31] a constant release rate of 5.24–8.34 mg/h is required in the formulation.

Cumulative amounts of diltiazem HCl released from untreated microparticles, treated microparticles and Cardizem® SR into 900 mL water at 37°C and 100 rpm were processed and presented as release rates vs. time plots in Figure 3. As expected, simple monolithic matrices fabricated by coacervation phase-separation technique and having uniform initial drug concentration distribution (untreated microparticles) did not yield zero-order release kinetics; instead, they yielded an initially high release rate followed by a rapid decline which is characteristic of Fickian diffusion. In contrast, treated microparticles with a nonuniform initial drug distribution, resulted in 10-h duration of constant-rate release region which is characteristic of non-Fickian diffusion. This constant-rate release region is a consequence of the nonuniform drug concentration distribution compensating for the increased diffusional distance with time.

Unfortunately, the constant release rate from treated microparticles was much lower than that from the brand name product, Cardizem® SR capsules, containing the same amount of drug. Many attempts were done to increase the zero order release rate from treated microparticles and make it comparable with that from the commercial product. The results of these attempts are discussed in the proceeding sections.

Modification of the Release Rate from the Matrix

Increasing Initial Drug Concentration. The first attempt was to increase the initial drug concentration in the microparticles. This was done by increasing the amount of drug dispersed in the polymer solution. The encapsulation efficiency increased from 67% to 75%. This resulted in an initially high release rate followed by a rapid decline (data not shown); probably because the porosity of the matrix increased upon drug depletion and counter balanced the effect of having nonuniform initial drug concentration distribution.

Varying Polymer System Making the Matrix. The second attempt was to vary the polymer system making the matrix by using different ratios of ethyl cellulose polymer to ethylene vinyl acetate copolymer (1 : 1, 2 : 1, and 3 : 1). The results showed that increasing the ratio of ethyl cellulose to EVA copolymer increased the release rate proportionally (data not shown). The

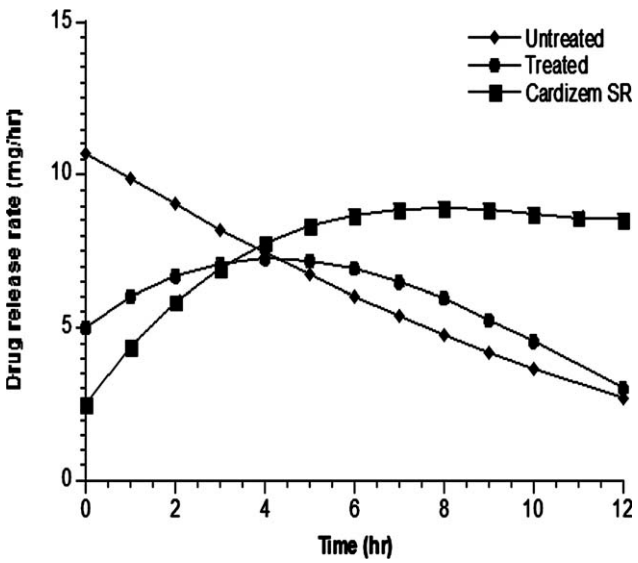

Figure 3. Diltiazem HCl release rates from untreated microparticles, treated microparticles, and Cardizem® SR into 900 mL water at 37°C and 100 rpm.

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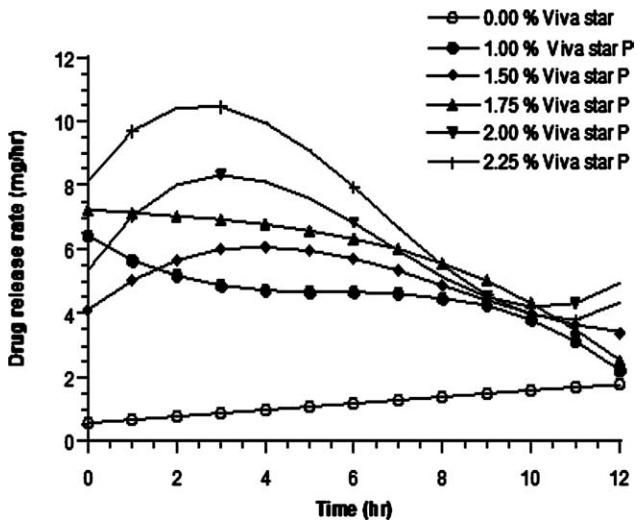

Figure 4. Diltiazem HCl release rates from untreated microparticles containing 0.00% sodium starch glycolate, treated microparticles containing 1.00, 1.50, 1.75, 2.00, and 2.25% w/w sodium starch glycolate into 900 mL water at 37°C and 100 rpm.

release rate from microparticles prepared using ethyl cellulose: EVA copolymer in a 1 : 1 ratio was found to be higher than that from microparticles prepared using EVA copolymer alone and almost comparable with that from Cardizem® SR 90 mg but with a shorter duration of constant release. This is probably because ethyl cellulose is more permeable to diltiazem HCl than EVA copolymer and counter balanced the effect of having non-uniform initial drug concentration distribution.

Varying Porosity and Tortuosity of the Matrix. The third attempt was to increase the porosity and decrease the tortuosity by the addition of either lactose or sodium starch glycolate or by using a drug with a larger particle size.

Addition of Lactose or Sodium Starch Glycolate. The results showed that increasing the amount of lactose added increased the release rate proportionally. The release rate from microparticles prepared using 1.0% lactose was higher than that from microparticles prepared using EVA copolymer alone and almost comparable to that from Cardizem® SR 90 mg but with a shorter duration of constant release (data not shown). Lactose, a water soluble diluent, diffused outwards increasing the porosity and decreasing the tortuosity of the diffusion path of the drug, stimulated water penetration into the inner part of matrix, increased the hydrophilicity of the system and caused marked increase in drug release rate. This probably counter balanced the effect of having non-uniform initial drug concentration distribution and shifted the release mechanism towards Fickian diffusion.

Sodium starch glycolate, a swelling agent, was incorporated in order to increase the release rate and to approach zero-order release kinetics. The addition of a hydrophilic excipient to ethylene vinyl acetate copolymer was previously demonstrated by the Follonier et al.[32] Sodium starch glycolate is a sodium salt of carboxymethyl ether of starch that has been widely used in oral pharmaceuticals as a disintegrant in capsule and tablet formulations. Disintegration occurs by rapid uptake of water followed by rapid and enormous swelling. In water, sodium starch glycolate swells up to 300 times its volume.[33] It was incorporated in a concentration of 1.00, 1.50, 1.75, 2.00, and 2.25% w/w in the microparticles. Cumulative amounts of diltiazem HCl released from untreated microparticles containing 0.00% sodium starch glycolate, treated microparticles containing 1.00, 1.50, 1.75, 2.00, and 2.25% w/w sodium starch glycolate, into 900 mL water at 37°C and 100 rpm were processed and presented as release rates vs. time plots in Figure 4. The results showed that increasing the amount of sodium starch glycolate added increased the release rate proportionally. Swelling of sodium starch glycolate caused the microparticles to swell. This was possible because of the rubbery state of the polymer which is advantageous in this case. This swelling, in addition to having non uniform initial drug concentration distribution, compensated for the lengthening of the diffusion pathway by an increase in the surface area available for diffusion[31] and zero-order release kinetics were achieved with most of the formulations.

To confirm the exact release mechanism, the dissolution data (up to 60% of the total released drug) were fitted according to Korsmeyer-Peppas equation [eq. (3)] and the results are shown in Figure 5.

$$\frac{M_t}{M_\infty} = k.t^n \tag{3}$$

where, M_t is the amount of drug released at time t and M_∞ is the amount released at time $= \infty$, thus M_t/M_∞ is the fraction of drug released at time t, k is kinetic constant, t is release time and "n" is the diffusional exponent for drug release. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of "n" gives an

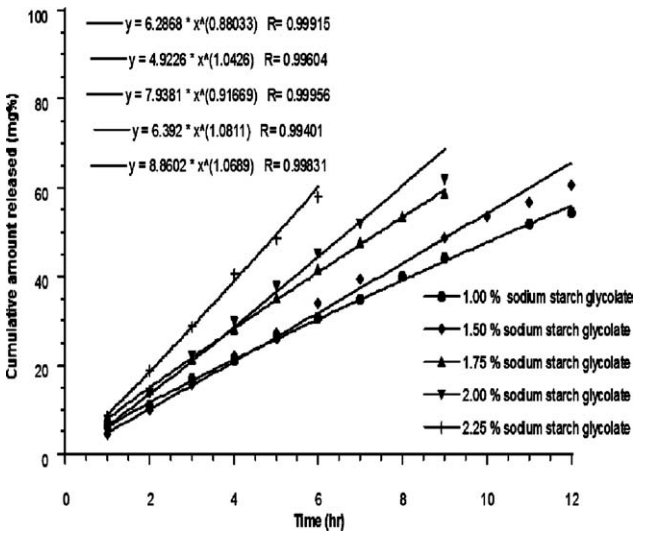

Figure 5. Analysis of cumulative amounts of diltiazem HCl released (up to 60% of the total released drug) from microparticles containing 1.00, 1.50, 1.75, 2.00, and 2.25% w/w sodium starch glycolate according to power law.

Table I. Various Diameters for Two Batches of Diltiazem HCl Particles Measured by Means of an Optical Microscope

Size-group μm	Mean of size-group, d	Number in each size-group, n	
		Batch 1	Batch 2
0.32–0.46	0.39	158	102
0.46–0.64	0.55	111	86
0.64–0.91	0.78	54	70
0.91–1.29	1.10	5	34
1.29–1.82	1.56	-	35
1.82–2.58	2.20	-	22
2.58–3.64	3.11	-	10
SUM		328	359
Mean length, d_{ln} (μm)		0.52	0.87
Mean surface, d_{sn} (μm)		0.54	1.07
Mean volume, d_{vn} (μm)		0.57	1.29
Mean surface-length, d_{sl} (μm)		0.57	1.31
Mean volume-surface, d_{vs} (μm)		0.62	1.87
Mean weight-moment, d_{wm} (μm)		0.68	2.27

$d_{ln} = \frac{\sum nd}{\sum n}$, $d_{sn} = \sqrt{\frac{\sum nd^2}{\sum n}}$, $d_{vn} = \sqrt[3]{\frac{\sum nd^3}{\sum n}}$, $d_{sl} = \frac{\sum nd^2}{\sum nd}$, $d_{vs} = \frac{\sum nd^3}{\sum nd^2}$, $d_{wm} = \frac{\sum nd^4}{\sum nd^3}$.

indication of the release mechanism; when $n = 1$, the release rate is independent of time (zero-order) (case II transport), $n = 0.5$ for Fickian diffusion and when $0.5 < n < 1.0$, diffusion and non-Fickian transport are implicated. Last, when $n > 1.0$ super case II transport is apparent.[34]

The values of n for large microparticles containing fine diltiazem HCl and 2.00% or 2.25% w/w sodium starch glycolate were equal to 1 and indicated that the mechanism for both solvent penetration and drug release from these formulations achieved zero-order release for an appreciable period of time (10 h for microparticles containing 2.00 w/w sodium starch glycolate and 6 h for microparticles containing 2.25% sodium starch glycolate). More complex kinetics set in at a later time probably due to the complex structure of the microparticles.

Varying Diltiazem HCl Particle Size. The porosity and tortuosity of a matrix are affected by the drug particle size. Diltiazem HCl particles sizes in two batches were measured by microscopy and the results are presented in Table I. Microscopy is the most direct method for size distribution measurement.[35] The results indicated that the mean diameter of diltiazem HCl particles in batch 1 was much smaller than the mean diameter of diltiazem HCl particles in batch 2. Diltaizem HCl from batch 1 was referred to as fine diltiazem HCl where as diltaizem HCl from batch 2 was referred to as coarse diltiazem HCl. Release rates from microparticles containing drug with different particles sizes and 2.25% w/w sodium starch glycolate into 900 mL water at 37°C and 100 rpm were compared and as shown in Figure 6. The release rate from microparticles containing coarse diltiazem HCl was initially high followed by a rapid decline as compared with that from microparticles containing fine diltiazem HCl and

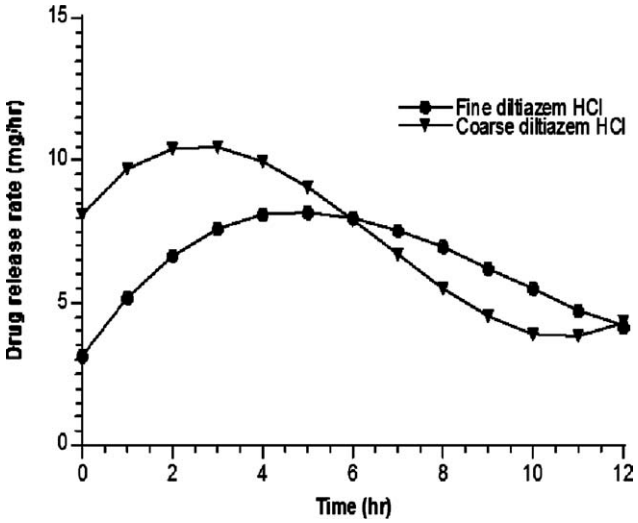

Figure 6. Diltiazem HCl release rates from treated microparticles containing drug with different particle size and 2.25% w/w sodium starch glycolate into 900 mL water at 37°C and 100 rpm.

the same percentage of sodium starch glycolate (2.25% w/w). This was expected since the use of coarse drug would result in the formation of a highly porous matrix. Larger water-filled pores were formed as water was imbibed from the surface of the microparticles to replace the coarse active agent that leached out. The increase in the porosity probably counter balanced the effect of having non uniform initial drug concentration distribution.

Physical Evaluation of Microparticles

Microparticle Size Distribution and Its Effect of on the Release Rate from the Formulation. Control of the particle size is essential in achieving the necessary drug release properties, flow properties and proper mixing of granules and powders. Therefore, sizes of 20 microparticles randomly selected from a batch having either small or large microparticles were measured using a micrometer and are represented in Tables II and III, respectively. Sizes of microparticles from the batch having small size ranged between 0.9 and 1.2 mm with a median of 1.05 mm and no particles were in the size group of 0.8–0.9 mm. Sizes of microparticles from the batch having large size ranged between 1.5 and 2.1 mm with a median of 1.8 mm and no particles were in the size group of 1.4–1.5 mm. Thus, the results indicated that the distribution profiles of both batches were narrow. With a narrow distribution profile, there is no large acceleration of the drug release at short times and a great retardation of release at long times as evident with a flat distribution profile.[36]

Even though both size distributions were narrow, the mean diameter of the size range was different. Therefore, the effect of the size of the microparticles on the release rate of diltiazem HCl was studied. Release rates from treated microparticles having different diameters and containing fine drug and 2.00% w/w sodium starch glycolate into 900 mL water at 37°C and 100 rpm are shown in Figure 7. The increase in the specific surface area of the

Table II. Summation for the Determination of the Median Diameter of 20 Untreated Small Microparticles Containing 0.00 % w/w Sodium Starch Glycolate Measured by a Micrometer

Size-group (mm)	Number in each size-group, *n*	Number less than maximum of size-group	Percentage of particles in each size-group	Percentage of particles less than maximum size of group
0.9–1.0	4	4	20	20
1.0–1.1	10	14	50	70
1.1–1.2	6	20	30	100

microparticles resulting from reducing the size (median diameter = 1.05 mm) induced an initially higher release rate followed by a rapid decline, as compared with that from large microparticles (median diameter = 1.80 mm) with smaller specific surface area. This might be explained by the rapid release of the drug easily accessible at the surface of the microparticles followed by a slow release where diffusion prevails. This probably counter balanced the effect of having non uniform initial drug concentration distribution. Thus the possibility of achieving a nearly zero-order release is higher when using microparticles with a large size than when using microparticles with a small size.

Bulk Density and Compressibility. To characterize the flow properties of the granules, bulk density and compressibility were determined and the results are given in Table IV. The percentage of compressibility was 8.00%. Powders having a percentage of compressibility less than 20–21 have good flowability.[6] Since the prepared microparticles were free flowing, regular in shape and size they would result in uniform filling and a capsule dosage form is suitable. Thus no glidants and lubricants, that can have an effect on drug release, would be required to improve the filling properties of the mix.

Effect of pH on the Release Rate from the Formulation

The effect of pH on the release of diltiazem HCl from microparticles was studied by performing *in vitro* dissolution in media having different pH values. The employed pH values (1.2, 5.0, and 6.8) were physiologically meaningful in that they reflected the pH of fasted stomach, duodenum, and proximal jejunum.[37] By considering the residence time of the drug in different regions of the GI tract (different pH values) and since it has a considerable effect on the bioavailability,[38] release studies were carried out in simulated gastric fluid pH 1.2 for 2 h followed by simulated intestinal fluid pH 5.0 for 4 h and another simulated intestinal fluid pH 6.8 for 6 h. The dissolution was performed in this way (subjecting the dosage form consecutively to media with increasing pH values after time intervals rather than subjecting the dosage form to separate media with different pH values) to simulate the passage of the dosage form from the stomach to the different segments of the intestine in terms of pH and residence time.

The cumulative amount released from microparticles containing 2.00% w/w sodium starch glycolate into 900 mL of media having different pH values at 37°C and 100 rpm is shown in Figure 8. The formulation did not exhibit any significant difference in the release rate as a function of pH. This result was expected since the solubility of diltiazem HCl was found to be fairly independent on the pH of the media.[39] The employed pH values were below the pKa of diltiazem HCl which is 7.7.[40] In addition, the concentration achieved in the dissolution media after 100% release (~100 μg/mL, i.e. 0.01–0.02 of saturation solubility) was so low that very small saturation solubility differences could be excluded as a reason for a pH-dependent drug release from this formulation. Results also indicated the pH-independence and the well-behaved nature of the polymer in the pH range studied.

Selection of the Proper Formulation for the Bioevaluation Study

Based on the above given results, release rates from treated microparticles with a mean diameter of 1.80 mm containing fine diltiazem HCl and 2.00% and 2.25% w/w sodium starch glycolate that achieved zero-order release, were compared to those from Cardizem® SR capsules and Bi-Tildiem® tablets. The

Table III. Summation for the Determination of the Median Diameter of 20 Untreated Large Microparticles Containing 2.25 % w/w Sodium Starch Glycolate Measured by a Micrometer

Size-group (mm)	Number in each size-group, *n*	Number less than maximum of size-group	Percentage of particles in each size-group	Percentage of particles less than maximum size of group
1.5–1.6	1	1	5	5
1.6–1.7	3	4	15	20
1.7–1.8	6	10	30	50
1.8–1.9	6	16	30	80
1.9–2.0	2	18	10	90
2.0–2.1	2	20	10	100

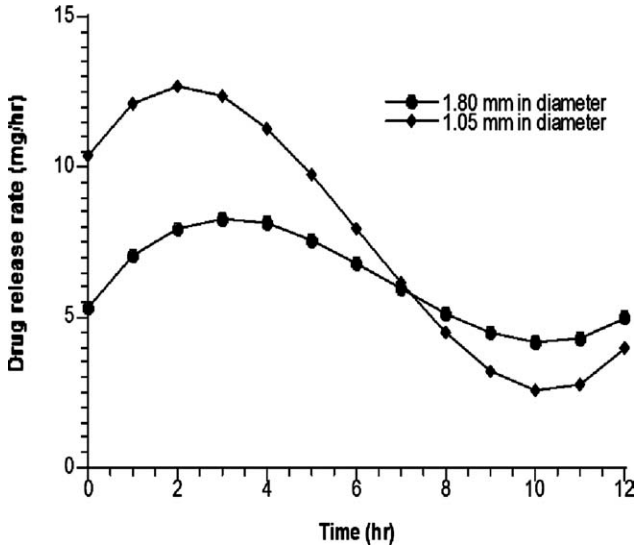

Figure 7. Diltiazem HCl release rates from treated microparticles having different diameters and containing fine drug and 2.00% w/w sodium starch glycolate into 900 mL of water at 37°C and 100 rpm.

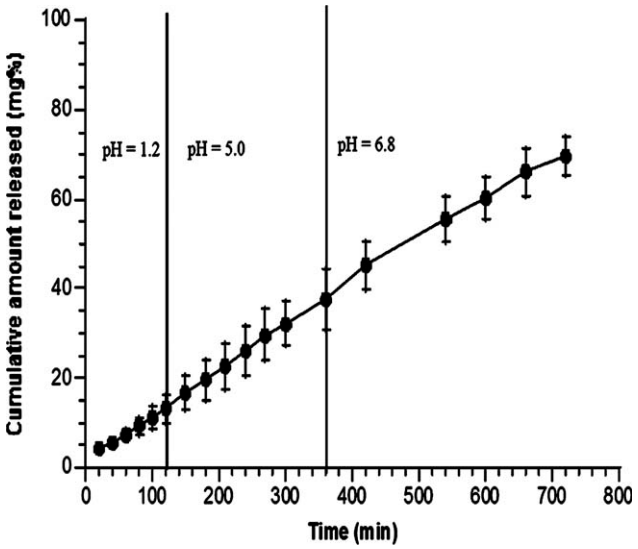

Figure 8. Cumulative amount of diltiazem HCl released (mean ± SD) from treated microparticles containing 2.00% w/w sodium starch glycolate into 900 mL simulated gastric fluid pH 1.2 (0–120 min), simulated intestinal fluid pH 5.0 (120–360 min) and simulated intestinal fluid pH 6.8 (360–720 min), at 37°C and 100 rpm ($n = 2$).

results are shown in Table V. Plasma drug levels from the four controlled release formulations were simulated using eq. (2) and are shown in Figure 9. Therapeutic blood levels of diltiazem HCl are in the range of 50–200 ng/mL. Toxic levels are unknown, but they appear to be in excess of at least 1200 ng/mL.[41] It is evident that release rates from those microparticles were within the required range (5.24–8.34 mg/h) for an appreciable period of time, were close to release rates from Cardizem® SR and Bi-Tildiem®, and achieved drug concentrations within the therapeutic level. Accordingly, treated microparticles with a mean diameter of 1.8 mm containing fine diltiazem HCl and 2.00% w/w sodium starch glycolate that achieved zero-order release for a longer period of time (10 h) were selected for the bioevaluation study.

Bioevaluation of the New Controlled Release Diltiazem HCl Formulation in Rabbits (*In Vivo* Study)

With a proper choice of each one of the parameters studied, a controlled release formulation of diltiazem HCl based on multiparticulate system with the desired release profile (zero-order) was achieved. A weight of selected formulation equivalent to 30 mg drug was encapsulated in hard gelatin capsules (size 3) and used for the bioevaluation.

Rabbits were chosen as the animal model for the study since they are suitable to investigate the kinetics and metabolism of diltiazem.[42] The study was carried out in the animal house at J.U.S.T.

Table IV. Physical Evaluation of Treated Microparticles

Loose bulk density (g/mL)	Tapped bulk density (g/mL)	Percent compressibility (%)
0.3665	0.3984	8.00

Since all dosage strengths of twice-daily Cardizem® SR capsules (60, 90, and 120 mg) were discontinued and withdrawn from the market due to manufacturing issues and since twice-daily Bi-Tildiem® tablets (90 mg) were coated tablets and could not be divided in order to adjust the dose for rabbits, using once-daily formulations such as Cardizem® CD 120 mg (the lowest dosage strength) was considered. Cumulative amounts of diltiazem HCl released from Cardizem® CD and the new controlled release formulation into 900 mL of water at 37°C and 100 rpm were further processed and presented as release rates vs. time plots in Figure 10. In Cardizem® CD capsules, 40% of the beads (surrounded by the thinner copolymer membrane) release the drug within 12 h of oral administration and 60% of the beads (surrounded by the thicker copolymer membrane) release the drug throughout the last 12 h of a 24-h period following oral administration. The manufacturer states that Cardizem® CD capsules provide therapeutic plasma concentrations of diltiazem over a 24-h period.[5] Consequently, a once-daily controlled release product of diltiazem cannot serve as a reference for the evaluation of bioequivalence (BE) of a twice-daily product.

The objective of the *in vivo* study was to construct an *in vitro/in vivo* correlation between the amount of *in vitro* dissolution and *in vivo* AUC rather than determining the BE between a single dose of the new controlled release formulation as the test product against Cardizem® CD capsules as the reference product.

Diltiazem HCl plasma concentrations (mean ± SD) after oral administration of single doses of 30 mg/kg of the new controlled release formulation and Cardizem® CD to rabbits are shown in Figure 11. The determined pharmacokinetic parameters ($AUC_{0\text{-}10}$, C_{max} and T_{max}) are represented in Table VI.

Table V. Diltiazem HCl Release Rates form Treated Microparticles Containing 2.00% and 2.25% w/w Sodium Starch Glycolate, Cardizem®SR and Bi-Tildiem®

	Release rate (mg/h)			
Time (h)	2.00 % w/w sodium starch glycolate	2.25 % w/w sodium starch glycolate	Cardizem®SR	Bi-Tildiem®
0	5.33	8.10	2.49	6.60
1	7.03	9.69	4.34	9.14
2	7.98	10.43	5.81	10.77
3	8.29	10.47	6.93	11.59
4	8.12	9.97	7.76	11.73
5	7.58	9.07	8.33	11.29
6	6.82	7.93	8.68	10.37
7	5.97	6.69	8.85	9.10
8	5.15	5.51	8.89	7.59
9	4.51	4.54	8.83	5.95
10	4.19	3.93	8.72	4.29
11	4.30	3.82	8.60	2.72
12	4.99	4.37	8.51	1.36

The results indicated that *in vitro* dissolution ($AUC_{0\text{-}10}$ for the new controlled release formulation/$AUC_{0\text{-}10}$ for Cardizem® CD = 1.3) correlated well with the *in vivo* AUC ($AUC_{0\text{-}10}$ for the new controlled release formulation/$AUC_{0\text{-}10}$ for Cardizem® CD = 1.2). Statistical analysis (ANOVA) of the determined pharmacokinetic parameters demonstrated that there were no significant differences in C_{max} and $AUC_{0\text{-}10}$ between the two formulations. Also the analysis indicated that there were greater intersubject differences in pharmacokinetic parameters ($AUC_{0\text{-}10}$ and C_{max}) for Cardizem® CD as reflected by the % CVs (Table VI). Thus clinical efficacy of the drug could be improved, toxicity could be reduced and patient compliance and convenience could be enhanced by administering a twice-daily formulation.

The results of the statistical analysis showed no significant difference in the studied pharmacokinetic parameters (data not shown).

CONCLUSIONS

Coacervation-phase separation technique appears to be particularly suitable for the manufacture of a controlled release dosage form, based on EVA copolymer multiparticulate system. The encapsulation efficiency was high (67–75%). The microparticles being spherical in shape, uniform in size, with a percent

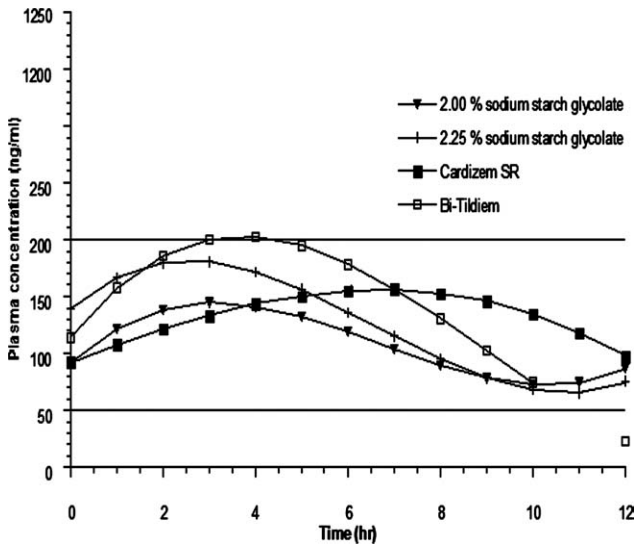

Figure 9. Simulated plasma levels of diltiazem HCl released from treated microparticles containing 2.00 and 2.25% w/w sodium starch glycolate, Cardizem® SR, and Bi-Tildiem®.

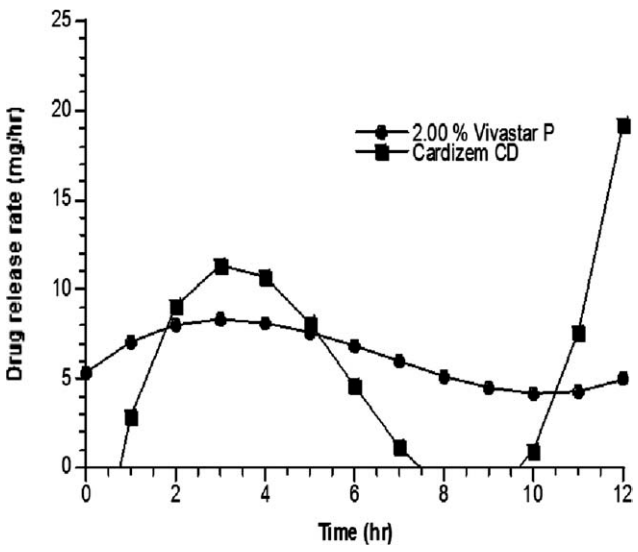

Figure 10. Diltiazem HCl release rates from the new controlled release formulation and Cardizem® CD into 900 mL water at 37°C and 100 rpm.

compressibility of 8%, were suitable for filling into hard gelatin capsules.

Zero-order delivery of diltiazem HCl at the desired rate from a simple matrix was achieved for an appreciable period of time by having a non uniform initial drug concentration distribution generated and immobilized via controlled extraction and high vacuum freeze drying processes; the incorporation of a swelling agent (sodium starch glycolate) in a concentration of 2.00% w/w; and by the proper choice of each one of the parameters studied (the drug particle size and the size of the microparticles).

Comparative dissolution studies proved that our dosage form provides release comparable with commercial products. Furthermore, our formulation offers a number of advantages over existing systems, including ease of manufacture and of release modulation, as well as reproducibility of release profile under well-defined conditions. Our delivery system has the potential to fully deliver its drug content in a controlled manner over a long time-period (10 h).

Whether the dissolution behavior of a drug in a dosage form is affected by the pH of the dissolution medium depends on the properties of the drug itself (pKa) and on the properties of the polymer used. Diltiazem HCl, with a pKa of 7.7, was not affected by the pH of the GI tract because of its pH-independent solubility. The pH values encountered along the GI tract are below the pKa of diltiazem HCl. EVA copolymer showed well-behaved nature and pH-independence in the pH range from 1.2 to 6.8.

Therapeutic blood levels of diltiazem HCl following oral administration of the new controlled release formulation to rabbits were maintained for 10 h with insignificant intersubject variation compared with Cardizem® CD, as reflected by the CV%. Thus, it is reasonable to expect from this work that the new controlled release formulation would improve clinical efficacy of the drug, reduce toxicity, and improve patient compliance and convenience.

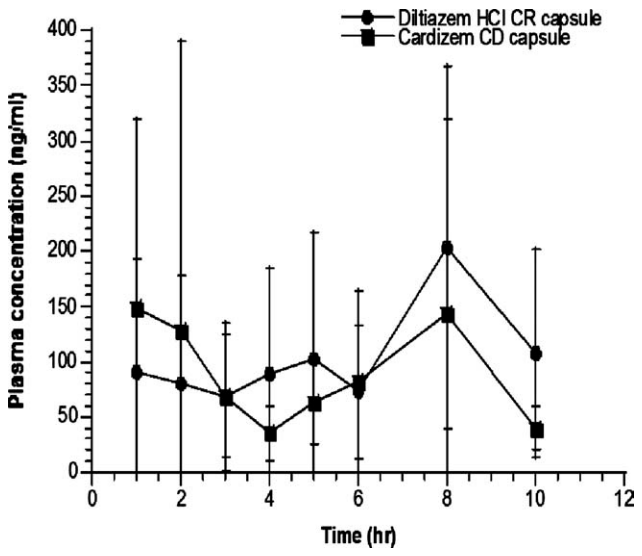

Figure 11. Diltiazem HCl plasma concentrations (mean ± SD) after oral administration of single doses of 30 mg/kg of two controlled release formulations to rabbits ($n = 7$).

Table VI. Pharmacokinetic Parameters of Diltiazem HCl (mean ±SD) After Oral Administration of Single Doses of 30 mg/kg of Two Controlled Release Formulations to Rabbits

Pk parameter	The new controlled release capsules	CV%	Cardizem® CD	CV%
$AUC_{0\text{-}10}$ (ng.h/mL)	1104.7 ± 243.1	22.0	1034.8 ± 458.5	44.3
C_{max} (ng/mL)	327.8 ± 81.6	24.9	356.1 ± 231.9	65.1
T_{max} (h)	5.2 ± 2.8	55.1	5.2 ± 2.7	52.1

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