Full Length Research Paper

Novel controlled release of antihypertensive drug: preparation, *in-vitro* and *in-vivo* evaluation

Khalid M. El-Say^{1,2}, Abdel-Rahim M. El-Helw¹, Osama A.A. Ahmed^{1,3}, Khaled M. Hosny^{1,4},
Tarek A. Ahmed^{1,2*}

¹Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, KSA.

²Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo Egypt.

³Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Minia University, Minia, Egypt.

⁴Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt.

Accepted 4 June, 2013

The objective was to control the release of diltiazem HCl. Emulsion solvent evaporation technique was applied to encapsulate diltiazem HCl using ethyl cellulose or Eudragit RS100. The prepared microspheres were characterized concerning entrapment efficiency; micromeritics properties and surface morphology using SEM and mixed with carbopol, hydroxypropyl methyl cellulose, or chitosan in different ratios. The *in-vitro* dissolution and kinetic analysis were carried out using various models. *In-vivo* studies for selected formulae were carried out compared with a marketed product to evaluate their pharmacokinetic parameters. The prepared microspheres showed entrapment efficiency of 67.45 and 76.82% for ethyl cellulose and Eudragit microspheres, respectively. Moreover, Eudragit microspheres mixed with hydroxypropyl methyl cellulose at a ratio of 1:3 showed a sustained release of diltiazem HCl over 12 h, with $t_{1/2}$ 14.8 hr. The formulated diltiazem HCl capsule can be successfully used for twice daily dosing.

Key words: Microencapsulation, chitosan, Eudragit RS 100, microspheres, bioavailability.

INTRODUCTION

Coronary heart disease (CHD), one of the major causes of death and disability, attracts much attention from biomedical research community. Calcium antagonists are considered one of the first-line medical therapies for the treatment of coronary artery disease, along with beta-blockers and long-acting nitrates (Van Kesteren and Withagen, 1998). One of the calcium channel blockers is diltiazem (DLZ) HCl which is widely used in the treatment of angina pectoris and hypertension. Furthermore, its bioavailability has been reported to be about 40% due to the fact that it undergoes extensive first-pass hepatic metabolism. Peak plasma concentrations occur about 3 to 4 h after a dose by mouth. The half-life of DLZ is

reported to be about 3 to 5 h (Sweetman, 2007). It is freely soluble in water, optimum oil-buffer partition coefficient (158 at pH7.4), and low molecular weight (450.98) make it an appropriate candidate for being incorporated into controlled-release formulations (Singh and Ahuja, 2002; Prabu et al., 2009; Dalvadi et al., 2011). During the past few decades, various types of oral controlled-release formulations have been developed to improve the clinical efficacy of drugs that have short half lives as well as to increase patient compliance. These formulations are designed to deliver drugs at a predetermined rate over a wide range of conditions and durations of therapeutic treatments (Löbenberg et al.,

*Corresponding author. E-mail dr_tarek_nour@yahoo.com; Tel.: +966 599828003.

2005; Li et al., 2007; Aamir and Ahmad, 2009; Sabitha et al., 2010; Lekshmi et al., 2012).

Microencapsulation is considered one of the most effective tools in formulating prolonged action dosage forms. It is used to modify drug release by delaying the time during which the drug is available and retard its attack by the gastrointestinal fluids. The preparation of microspheres results by the coating of the individual drug particles with polymeric inert materials, through which the drug would diffuse at a controlled and predictable rate to the surrounding medium. There are several techniques used to produce polymeric microspheres drug delivery systems, which include physicochemical processes such as solvent evaporation method (Li et al., 2007; Lekshmi et al., 2010; Jose et al., 2011) or phase separation method (Das et al., 2005; Lekshmi et al., 2012), mechanical processes such as spray drying (Davis and Illum, 1999), and a non-solvent addition process (Elbagory et al., 2007; Aamir and Ahmad 2009), or by cross-linking with glutaraldehyde using poly(vinyl alcohol)-guar gum interpenetrating network microspheres (Soppimath et al., 2000). The emulsion solvent evaporation method is commonly used for the preparation of controlled release drugs, for example, allopurinol microspheres (Arabi et al., 1996), Zidovudine sustained release (Abu-Izza et al., 1996) and nifedipine hydrochloride microspheres were prepared using acrylic polymers Eudragit RS and L, different concentration of sucrose stearate as a droplet stabilizer, and acetone-methanol mixture as a solvent (Yüksel and Baykara, 1997); and the amphiphilic block copolymers were used to prepare nifedipine microspheres by the solvent evaporation technique (Shelke and Aminabhavi, 2007). Also, the emulsion solvent evaporation method was optimized using ethyl acetate as a dispersing solvent to encapsulate nifedipine and verapamil hydrochloride with cellulose-based polymers (Soppimath et al., 2001). The processing parameters that influence the properties of DLZ microspheres were studied (Bhalerao et al., 2001). Also, the effect of plasticizers on various characteristics of Eudragit microspheres containing glipizide formulated by solvent evaporation method was also evaluated by Sahoo et al. (2011). So, the main purpose of the present work was to develop a controlled drug delivery system of DLZ for peroral administration using biocompatible ethyl cellulose and Eudragit®RS polymers in order to increase its biological half and to determine the influence of formulation and preparation variables on microsphere characteristics, such as drug incorporation and *in-vitro* release and subsequently pharmacokinetic parameters.

MATERIALS AND METHODS

Diltiazem HCl powder was supplied by Egyptian International Pharmaceutical Industries Company (E.I.P.I.Co), Egypt. Ethyl cellulose (BDH Chemicals Ltd., Poole, England), Eudragit RS 100

(Rohm Pharma, GMBH, Weiterstadt, Germany), Carbopol 934P (B.F. Goodrich, Cleveland, Ohio, USA), Hydroxypropyl Methyl cellulose, 4,000 cps (Spectrum chemical MFG. Corp., Gardena, CA, USA) and Chitosan of 70,000 molecular weight (Fluka, Chemie-AG, Buchs, Switzerland), Dichloromethane (Prolabo ; France); n-Hexane 95%, (Honil Limited, London, U.K.); Acetonitrile HPLC grade, (Merck, Darmstadt, Germany); Potassium dihydrogen orthophosphate (BDH chemicals Ltd, Pool England); Light Liquid Paraffin (WINLAB, U.K.); Span 60 (Sigma Chemical Co., Steinheim, Germany) were purchased from Sigma Aldrich (St. Louis, MO). The materials used in this study were obtained and used without any further purification.

Differential scanning calorimetry (DSC)

DSC studies were carried out using DSC instrument (DSC-60, having TA60 software, Shimadzu, Koyto, Japan). The instrument is very versatile as far interaction, and compatibility studies at pre-formulation stage were concerned and used to evaluate melting point, enthalpy changes and glass transition temperatures of drug with excipients and polymers. Approximately, 2 mg of samples were weighed into DSC aluminum pans and were crimped followed by heating under nitrogen flow (20 ml/min) at a heating rate 10°C/min from 40 - 400°C. Aluminum pan containing the same quantity of indium was used as a reference. DSC was performed on DLZ, excipients and mixtures of the final formulation.

Fourier transform infrared spectroscopy (FTIR)

FTIR studies are very helpful in the evaluation of drug-polymer interaction studies. The incompatibility between the drug and excipients can be predicted by changes in the functional peaks (characteristic wave numbers). Drug and various polymers were thoroughly mixed with 300 mg of potassium bromide, compressed to a 2 mm semitransparent disk and placed in the light path for 2 min. The FTIR spectra were recorded over the wave length range from 400 to 4000 cm⁻¹ using FTIR spectrometer (Perkin Elmer Spectrum One, Model 16 PC, Germany).

Preparation of DLZ microspheres

DLZ loaded microspheres were prepared by the emulsion solvent evaporation technique previously used by Obeidat and Price (2006) and Sahoo et al. (2011). The drug was dissolved in the polymeric solutions of ethyl cellulose (EC) or Eudragit RS 100 (ED) in dichloromethane at drug to polymer ratio of 1:3 forming the internal phase. The resultant dispersion was added drop wise to light liquid paraffin forming the external phase containing 3% Span 60 as an emulsifying agent during stirring at 800 rpm using a mechanical stirrer (Steady stir Digital, 855 Fisher Scientific, USA). The stirring was continued at room temperature until complete evaporation of the solvent. Liquid paraffin was decanted, and the formed microspheres were filtered off, washed with n-hexane several times to remove the remaining oily phase and then dried at room temperature for 24 h.

Characterization of the prepared microspheres

Determination of the entrapment efficiency

Fifty milligrams of microspheres was dissolved in dichloromethane and the drug content was determined using high performance liquid

chromatography (HPLC) (Agilent 1200 series, equipped with a UV diode array detector, and an automatic sampling system, Germany) at 236 nm. The concentration was calculated using the standard calibration curve of DLZ in dichloromethane. The actual drug content of microspheres was determined and used to calculate the entrapment efficiency of the microspheres as follows:

Entrapment efficiency = (Actual drug content / Theoretical drug content) × 100

Determination of micromeritic properties

The micromeritic properties of the prepared DLZ microspheres were studied using the following techniques:

Particle size analysis

The particle size distribution was evaluated by sieve analysis using a standard set of sieves (British Standard) with size range of 710 to 90 µm, and shaken for 10 minutes using an electric shaker. The weight of microspheres retained on each individual sieve was recorded, stored and used for further studies. The size distribution of the microspheres was determined and mean particle size of microspheres was calculated by using equation (1).

$$\text{Mean particle size} = \frac{\sum(\text{Mean particle size of the fraction} \times \text{Weight fraction})}{\sum \text{Weight fraction}} \quad (1)$$

The logarithm of the particle size was plotted against the cumulative percent frequency on a probability scale and a linear relationship was observed. From this linear plot, both the logarithm of the particle size equivalent to 50% on the probability scale, that is, the 50% size that is known as the geometric mean diameter, d_g , which is the n th root of the product of n terms and can be expressed in equation (2).

$$d_g = \sqrt[n]{dp(1) \times dp(2) \times dp(3) \times \dots \times dp(n)} \quad (2)$$

Where, dp is the mean particle diameter in µm, n is the number of fractions, and d_g is the geometric mean diameter in µm. The slope of the obtained line is known as the geometric standard deviation, σ_g , which can be calculated by equation (3). Also, lumps (> 500 µm), fines (< 100 µm), and the mean particles (500 - 100 µm) were determined (Martin et al., 1993).

$$\sigma_g = \frac{d_{50}}{d_{16}} = \frac{d_{84}}{d_{50}} \quad (3)$$

Determination of flow properties

The flow properties of DLZ microspheres were described using the following methods:

Bulk density method

The bulk density (d_B) of the microspheres was determined by pouring a sample of 50 g into a 100-ml graduated cylinder. The cylinder was then dropped from a height of 1 inch onto a hard surface three

times at 2-second intervals. The volume of the powder was then recorded and used to calculate the bulk density by dividing the weight in (g) by volume in (cm³) (Martin et al., 1993). The tapped density (d_T) of the microcapsules was determined by tapping onto a hard surface until a constant volume was achieved. This volume was taken and used to calculate the tapped density of the microspheres. Hausner ratio is the ratio between tapped density and bulk density and Carr's index (C.I.) can be estimated from equation (4). The obtained value gives an idea about the flow behavior of the microspheres

$$C.I. = 100 \times \frac{d_T - d_B}{d_T} \quad (4)$$

Angle of repose method

The flow properties were investigated by determining the angle of repose of the microspheres using the fixed-base cone method. Microspheres were allowed to fall freely through a funnel fixed 1 cm above the horizontal flat surface until the apex of the conical pile just touches the tip of the funnel. The height and diameter of the cone was measured and angle of repose was calculated by using the following formula ($\tan \theta = h / r$), where h = cone height, r = radius of a circular base formed by the microspheres on the ground (Martin et al., 1993). Each experiment was carried out in triplicate ($n = 3$).

Surface morphology of microspheres

The surface morphology of the microspheres was examined by scanning electron microscope (Philips XL30, Netherlands) operated at 4-25 kV on samples gold-sputtered for 120 s at 10 mA, under argon at low pressure.

Formulations of DLZ controlled release capsule

Microspheres equivalent to 10 mg DLZ according to its DLZ content were mixed with Carbopol (CRB), hydroxypropyl methyl cellulose (HPMC) or chitosan (CS) at different ratios of 1:1, 1:2, and 1:3. The obtained mixtures were further mixed with the calculated amount of Avicel and magnesium stearate for 10 min and then filled into hard gelatin capsules (size 0). Twenty formulae were formulated along with the formulation codes as shown in Tables 1 and 2.

In- vitro dissolution studies

Dissolution studies were performed in a USP Dissolution Tester Apparatus II (ERWEKA DT 700, Germany) at $37 \pm 0.5^\circ\text{C}$. The paddles were rotated at a speed of 100 rpm. Each of the formulated capsules was placed in the cylinder with 900 ml distilled water as a dissolution medium. An aliquot of samples were withdrawn and replaced with fresh dissolution media at time intervals of 0.25, 0.5, 1, 2, 3, 6, 9, and 12 h. The drug content in the filtered samples was analyzed using HPLC (Agilent 1200 series equipped with UV, diode array, detector, and an automatic sampling system, Germany). Reversed-phase column 25 cm × 4.6 mm (i.d.) C18, 5 µm – Intersil® ODS-3 (GL Sciences Inc, Japan). The mobile phase consists of a mixture of (28:72) acetonitrile/0.05M potassium dihydrogen orthophosphate (pH 3.5), and the flow rate was 1 ml/min and DLZ content was determined at 236 nm. An injection

Table 1. Composition of the formulated capsules containing DLZ loaded-EC microspheres.

Ingredient (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
DLZ loaded-EC microspheres	59.3	59.3	59.3	59.3	59.3	59.3	59.3	59.3	59.3	59.3
Carbopol	-	59.3	118.6	177.9	-	-	-	-	-	-
HPMC	-	-	-	-	59.3	118.6	177.9	-	-	-
Chitosan	-	-	-	-	-	-	-	59.3	118.6	177.9
Avicel	188.2	128.9	69.6	10.3	128.9	69.6	10.3	128.9	69.6	10.3
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total weight	250	250	250	250	250	250	250	250	250	250

Table 2. Composition of the formulated capsules containing DLZ loaded-ED microspheres.

Ingredient (mg)	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20
DLZ loaded-ED microspheres	52.8	52.8	52.8	52.8	52.8	52.8	52.8	52.8	52.8	52.8
Carbopol	-	52.8	105.6	158.4	-	-	-	-	-	-
HPMC	-	-	-	-	52.8	105.6	158.4	-	-	-
Chitosan	-	-	-	-	-	-	-	52.8	105.6	158.4
Avicel	194.7	141.9	89.1	36.3	141.9	89.1	36.3	141.9	89.1	36.3
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total weight	250	250	250	250	250	250	250	250	250	250

volume of 10 μ l and DLZ could be detected at a retention time of 3.5 min. Assay performance was evaluated through determination of specificity, recovery, linearity, the limit of quantification (LOQ), the limit of detection (LOD), precision, accuracy as reported in the International Conference on Harmonization guidelines. The release experiments were done in triplicate. This drug release profile was fitted into several mathematical models to explain the release mechanism.

Kinetic analysis of release data

The amount of DLZ released was calculated from the regression line of the standard curve. The release data obtained were fitted to different kinetic models; zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The best fitness of the model was chosen to determine the corresponding drug release mechanism for the formulated capsules.

In- vivo evaluation

Formulated DLZ capsules showed the desired release characteristics were chosen for the *in-vivo* studies in comparison to the commercial sustained release capsules.

Study design

Single dose one period parallel design was chosen. The dose was 3.6 mg and the sampling points were determined as per USFDA guidance "the sampling times should extend to at least 3 multiples of the drug's apparent terminal elimination half-life, beyond the time when maximum blood concentrations are achieved". It was stated

that the $t_{1/2}$ for DLZ is 3-5 h and t_{max} is 3-4 h (Sweetman 2007). Wistar rats with average weight 300 g were used for the *in-vivo* studies. The rats were acclimatized for one week before the study in a temperature controlled room. The rats were kept with free access to food and water. During this week, the animals were maintained with a 12 h light/darkness cycles. The study was started during the light cycle. Animals were divided into three groups ($n = 3$); group I administered F7, group II administered F17 and group III administered the commercial DLZ capsule as control. The dose was administered to the rat in the form of oral suspension using gastric tube.

Sample collection

Blood samples were collected in glass tubes before administration of the dosage form, and at 0.25, 0.5, 1, 3, 6, 9, 12, 16, 20 and 24 h after drug administration. All samples were collected through the orbital plexus. Plasma was immediately separated from the blood cells by centrifugation at 6000 rpm for 10 min and stored frozen at -20°C till HPLC analysis.

HPLC determination of DLZ in rat plasma

The concentrations of DLZ in rat plasma samples were determined using a modified HPLC method (Yeung et al., 1996). A volume of 100 μ l acetonitrile, 100 μ l of (0.02 mg/ml) of cetrizine HCl dissolved in acetonitrile and 30 μ l of 0.1N HCl were added to 200 μ l of each rat plasma sample in a clean glass test tube. The mixture was vortexed for 10 s and centrifuged at 3000 rpm for 10 min. An aliquot (30 μ l) of the clear supernatant was injected directly onto the HPLC system (Agilent 1200 series equipped with UV, diode array, detector, and an automatic sampling system, Germany). Reversed-

phase column 25 cm $\times$ 4.6 mm (i.d.) C18, 5 μ m – Intersil® ODS-3 (GL Sciences Inc, Japan). The column effluent was monitored by UV detector at 236 nm and the eluent flow was 1.3 ml/min. The mobile phase consisted of 35 % acetonitrile and 65 % of 0.05M ammonium acetate buffer. The retention time for cetirizine HCl and DLZ were 3.1 and 6.1 min, respectively. Blank rat plasma was spiked with known amounts of DLZ to produce standard samples with concentrations in the range of 1–50 μ g/ml. Calibration curves were constructed from the concentration of DLZ in each standard sample and the resulting peak area. The concentrations of DLZ in the unknown samples were determined from the calibration curves. Linearity, selectivity, precision and accuracy were determined to validate the HPLC method of analysis. The calibration curves were linear over the entire range, with correlation coefficient always greater than 0.999. There was no interference between the endogenous peaks in the rat plasma and the peaks for DLZ. Within-day precision was determined from the analysis of three calibration curves on the same day and the coefficient of variation (CV) ranged from 0.058% to 1.18%. Between-day precision was determined from the analysis of three different calibration curves on three different days during the study period, and the CV was in the range 0.068% to 1.16%. The accuracy of the assay, determined from the predicted DLZ concentration in each standard, was in the range 98.86–103% within-day and 82.6–119.15% between-day.

Pharmacokinetic parameters

The following pharmacokinetic parameters were determined; maximum plasma concentration (C_{max}), time point of maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from zero h to the last measurable concentration ($AUC_{0-\infty}$), and area under the plasma concentration-time curve from zero h to infinity ($AUC_{0-\infty}$). Similarly, the elimination half life ($t_{1/2}$), the mean residence time (MRT), and the total renal clearance (TRC) were determined using pharmacokinetic software Kinetica™ (version 4, Thermo Scientific, MA, USA).

Statistical analysis

The pharmacokinetic parameters obtained from the three groups of rats were analyzed by oneway ANOVA using SPSS version 18 software.

RESULTS AND DISCUSSION

Pre-formulation studies are known to be an essential prerequisite to develop a successful formulation. DSC and FTIR spectroscopic analysis were used for the evaluation of physicochemical compatibility and interactions. They helped in the prediction of possible interaction of DLZ with the polymers used in microsphere formulations. It was recommended that the ratio of the drug to polymers used in this study was 1:1 to maximize the possibility of interaction and helps in easier detection of incompatibilities as investigated previously (Sultana et al., 2009).

Thermal analysis of DLZ and polymers using DSC

DSC thermograms of DLZ, EC, DLZ-loaded EC micro-

spheres, ED and DLZ-loaded ED microspheres are presented in Figures 1 and 2. The DSC thermogram of DLZ showed an endothermic peak indicating the melting point 226°C which is very close to the literature value of drug (Kulkarni et al., 2011). The DSC thermograms of DLZ with EC or ED mixtures exhibited the same type of endothermic peaks of DLZ. From this observation, it is concluded that no possible interaction detected for the drug with the used polymers.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectrum of DLZ showed two characteristic peaks around 1681 and 1743 cm^{-1} due to the presence of lactam carbonyl group and ester carbonyl group, respectively. In addition to the peaks at 2966 cm^{-1} (C-H stretching), 2387 cm^{-1} (C-S), and 1475 cm^{-1} (O-CH₃) are observed (Figures 3 and 4). Similar peaks were also seen in DLZ loaded EC and ED microspheres spectra indicating the stable nature of DLZ during microencapsulation process.

Evaluation of DLZ microspheres

Entrapment efficiency of DLZ microspheres

The percentage of drug entrapment in EC microspheres was found to be 67.45 % while that of ED microspheres were 75.83 %. These results revealed acceptable entrapment efficiency for DLZ in the prepared microspheres, regardless of DLZ water solubility.

Micromeretic evaluation of DLZ microspheres

Particle size analysis

The particle size analysis curves of both types of the prepared microspheres are presented in Figures 5 and 6. The arithmetic mean diameter is 425 and 158 μ m for DLZ-loaded EC and DLZ-loaded ED microspheres, respectively. Figure 5 shows that mean microsphere size represents 74.93 and 95.52% for EC and ED microspheres, respectively. The size distribution can be specified by two values; the geometric mean diameter (d_g) and the geometric standard deviation (σ_g). From the observed data, d_g of EC microspheres was 330 μ m, and its σ_g was 0.67 which is in agreement with the previous work (Agnihotri and Aminabhavi, 2005). On the contrary, the frequency distribution data of ED microspheres revealed that, d_g is 72 μ m and σ_g is 0.51. The obtained particle size analysis data illustrated in Figure 5 indicates a wide particle size distribution of EC microspheres and a narrow size distribution of ED microspheres while Figure 6 presented the percentage of fines, means, and lumps

Figure 1. DSC thermograms of DLZ, EC, and DLZ-loaded EC microspheres.

Figure 2. DSC thermograms of DLZ, ED, and DLZ-loaded ED microspheres.

of both types of DLZ microspheres

Flow properties

The flow properties of the DLZ microspheres are shown in Table 3. The ED microspheres were found to be denser than EC microspheres which improved its flowability. This finding was confirmed by the values of Hausner ratio, Carr's index, and angle of repose. Hausner ratio was 1.3, Carr's index was 23.12 %, and the angle of repose was 43.8° for EC microspheres. According to

USP 29-NF24, passable to flow materials have Hausner ratio ranged from 1.26 – 1.34; Carr's index from 21 – 25 and angle of repose from 41 – 45°. On the other hand, the flow parameters of ED microspheres were 1.15 for Hausner ratio, 13 % for Carr's index, and 34.6° for the angle of repose. These results indicate good flowability of ED microspheres.

Surface morphology by SEM

Figure 7 shows SEM pictures taken from dried microspheres prepared by emulsion solvent evaporation

Figure 3. FTIR spectra of DLZ (A), EC (B), and DLZ-loaded EC microspheres (C).

Figure 4. FTIR spectra of DLZ (A), ED (B), and DLZ-loaded ED microspheres (C).

Figure 5. Frequency distribution curve of both types of DLZ microspheres.

Figure 6. Percentage of fines, means, and lumps of both types of DLZ microspheres.

Table 3. Flow properties of DLZ-loaded microspheres.

Parameter	EC microspheres	ED microspheres
Bulk density (g/cm ³)	0.256	0.435
Tapped density (g/cm ³)	0.333	0.5
Hausner ratio	1.3	1.15
Carr's index	23.12	13
Bulkiness (cm ³ /g)	3.9	2.3
Angle of repose (°)	43.8	34.6

technique. The surface of the microspheres prepared with ethyl cellulose was rather smooth and porous as can be seen in the photomicrograph; there are many pores and cavities in the microspheres (Figure 7A and B). Figure 7B shows the surface morphology of microsphere at a higher magnification which displays the porous structure of the microspheres. This could explain the low bulk density of EC microspheres. Similar observations were made for microspheres prepared with ED (Figure 7C and D) with the exception that these microspheres appeared rough with less porous surface but were mostly spherical in appearance. The pictures also show that ED microspheres are not aggregates.

***In-vitro* dissolution**

The dissolution profiles of DLZ-loaded EC microspheres (F1), DLZ-loaded ED microspheres (F11), F7, F17, and the marketed DLZ sustained release capsules are presented in Figure 8. The release from DLZ-loaded EC microspheres was found to be faster in comparison to DLZ-loaded ED microspheres; this could be related to the porous nature of EC microspheres which allow the release of DLZ more easily than less porous ED

microspheres. By mixing the microspheres with different polymers CRB, HPMC, and CS, the release rate was remarkably extended particularly at higher polymer concentrations. This could be attributed to the increase in the length of the diffusional pathway developed by the increase in polymer concentration (Prasanth et al., 2011). On the other hand, by increasing the polymer concentration, large amount of drug got bind in the polymer matrix that leads to decrease in the rate of release (Chinna et al., 2010). The results shown in Figure 9 revealed that both CRB and CS, even at higher ratios, did not control the release of DLZ from formulated capsules to meet the desired criteria of sustained release formulation which is clear from the dissolution profiles of (F4, F14) and (F10, F20), for CRB and CS, respectively.

On the basis of sustained release criteria, the drug should reveal less than 30% release in the first hour, that is, no dose dumping, about 60% release in 5-7 h, and more than 85% release of drug in more than 12 h, while maintaining smooth release for in between time points (Carrico, 1996). Similar criteria were specified for bioequivalence studies of modified oral dosage formulations during approval (Cartwright and Matthews, 1994). Formulae (F2-4, F8-10, F12-16 and F18-20) were rejected because 30% of the drug was released during

Figure 7. Scanning electron photomicrographs of (A) DLZ-loaded EC microspheres (X600); (B) DLZ-loaded EC microspheres (X3,500); (C) DLZ-loaded ED microspheres (X100); DLZ-loaded ED microspheres (X5,000).

Figure 8. Dissolution profiles of F1, F7, F11, F17, and marketed DLZ capsules.

Figure 9. Dissolution profiles of formulated capsules containing CRB, HPMC, and CS at (1:3) microspheres: polymer ratio.

the first hour which is considered to be not satisfactory for the desired criteria for sustained release formulations. This could be attributed to the low polymer concentrations in those capsules in addition to the high solubility of DLZ. On the other hand, F7 and F17 which contain HPMC, at a ratio of 1:3 microspheres: HPMC fulfilled the desired criteria required in a controlled release product. HPMC hydrate upon contact with water forms a rate controlling gel layer around the solid microspheres. This layer acts as a physical and diffusion barrier to the rate of water-entrance and the diffusional/erosional release of drug (Colombo et al., 1996). Moreover, the presence of HPMC in the formulae at higher concentration increased the viscosity in the gel layer which led to further controlling of DLZ release. This finding was in a good agreement with the previous work confirmed that, the drug release is controlled by the rate of diffusion through such a gel layer formed by HPMC (Katzhendler et al., 1997; Mitchell et al., 1993; Gao et al., 1995). According to these results, F7 and F17 were selected for further *in-vivo* study.

Kinetic treatment of the *in-vitro* release data

The release behaviors of DLZ from the different formulae were fitted to various kinetic orders and models for prediction of their release behavior. Zero-order (Wagner, 1969) is expressed in equation (5) as:

$$Q_t = Q_0 - k_0 t \quad (5)$$

While the first-order (Desai et al., 1966) is expressed in

the equation (6):

$$\ln Q_t = \ln Q_0 - k_1 t \quad (6)$$

According to Higuchi relationship (Higuchi, 1963), the amount of drug released were plotted as cumulative drug release versus square-root time as in the equation (7). The equation explains diffusional release rate as indicated below:

$$Q_t = k_H t^{1/2} \quad (7)$$

Where, Q_t is the amount of drug released or dissolved at time t , Q_0 is amount of drug released or dissolved at a time $t = 0$, and k_0 , k_1 , and k_H are the release rate constant for zero, first, and Higuchi's equations, respectively. Two factors diminish the applicability of Higuchi's equation to matrix systems. This model fails to allow the influence of swelling of the matrix (upon hydration) and gradual erosion of the matrix. Therefore, the dissolution data were also fitted according to the well-known exponential equation (8) of Ritger and Peppas (Korsmeyer et al., 1983; Ritger and Peppas, 1987), which is often used to describe the drug release behavior from polymeric systems.

$$\frac{M_t}{M_\infty} = k t^n \quad (8)$$

Where, M_t is the amount of drug released at time t , M_∞

Table 4. Release kinetics of DLZ from different controlled release formulations.

Formulation code	Zero order	First order	Higuchi	Korsmeyer
	r	r	r	n
F1	0.6688	0.8877	0.7764	0.1232
F7	0.9875	0.9977	0.9988	0.6977
F11	0.9342	0.9720	0.9723	0.0796
F17	0.9003	0.9733	0.9660	0.4980

the quantity of drug released at infinite time, k the kinetic constant and (n) is an exponent. The value of (n) is related to the geometrical shape of the formulation and determines the release mechanism. Thus, when the value of (n) is $\leq 0.5/0.45/0.43$ (thin films/cylinders/spheres, respectively) indicates pure diffusion, Fickian or case I release. When the value of (n) is $>0.5/0.45/0.43$ but $<1.0/0.89/0.85$, pure case II, non-Fickian or anomalous release is operating. Case II generally refers to erosion of the polymeric chain. Anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled-drug release (Shato et al., 1997). When (n) equals 1, it indicates zero-order release; where the drug release rate is independent of time and involves polymer relaxation and chain disentanglement. While, $(n) >1.0/0.89/0.85$ indicates super case II type of release.

The model that best fits the release data was evaluated by the correlation coefficient (r) except in Peppas model which was evaluated by (n) value. The (r) value was used as criteria to select the best model that describe drug release from the controlled release capsules. The calculated (r) and the exponent (n) values for the *in-vitro* release of DLZ from capsules based on different kinetic orders or systems are shown in Table 4. F1 and F11 were the DLZ-loaded microspheres with EC and ED, respectively, without incorporation of polymers. F7 and F17 were the same microspheres incorporated with HPMC in ratio 1: 3 that met the desired criteria of controlled release among all formulations.

Kinetic treatment results of the prepared microspheres revealed that the (n) values were 0.123 and 0.079 for DLZ-loaded EC (F1) and DLZ-loaded ED microspheres (F11), respectively. These results indicated that the release followed Fickian diffusion mechanism. On the other hand, incorporation of HPMC to the prepared microspheres increased the (n) value to 0.698 and 0.498 for F7 and F17, respectively, which subsequently changed the mechanism to non-fickian one. The change in mechanism could be related to the nature of HPMC as a swellable polymer. So, in addition to diffusion mechanism, other factors are included in the release mechanism attributed to relaxation of HPMC chains, imbibitions of water causing HPMC to swell and changing it from initial glassy to rubbery state (Siepmann and Peppas, 2001). The increase in volume as a result of

HPMC swelling leads to diffusion boundaries shift that necessitate further calculation in addition to Fick's second law of diffusion (Siepmann and Peppas, 2001). So, data were further treated by equation (8) that revealed the non-fickian (anomalous) mechanism.

In-vivo study

The *in-vivo* studies were carried out using two formulae F7, and F17 as it showed the optimum controlled release behavior among all formulations. The study was carried out in comparison with the marketed DLZ capsule. The mean plasma concentration-time data of DLZ following the oral administration of the aforementioned formulae is presented in Table 5 and shown in Figure 10. The mean peak plasma concentrations (C_{max}) were 0.236, 0.269, and 0.272 $\mu\text{g/ml}$ for F7, F17, and the commercial DLZ, respectively, with the same t_{max} . The mean AUC₍₀₋₂₄₎ was found to be 18.48, 13.07, and 14.93 $\mu\text{g}\cdot\text{h/ml}$ for F7, F17, and the marketed, respectively. Similarly, the $t_{1/2}$ was found to be 5.35, 14.80, and 5.83 h, while, the mean residence time (MRT) was found to be 7.72, 12.33, and 7.78 h for F7, F17, and the marketed capsules, respectively. From the obtained results, we can conclude that capsules containing ED microspheres mixed with HPMC in ratio 1: 3 (F17) offer the good criteria for controlled DLZ effect. The statistical results between the studied three groups indicated that there was no significant difference between the prepared microspheres and the commercial sustained release product (P -value = 0.767) which indicates the usefulness of the technique used for sustaining the release of diltiazem.

Conclusions

The results of this study revealed that the incorporation of retardant polymers, HPMC, with DLZ-loaded ED microspheres leads to controlling the release of DLZ. In addition to, the pharmacokinetic parameters of the developed formulation will provide a longer period of residence time required for achieving once or twice dosing daily of DLZ in the management of coronary heart diseases especially in treatment of angina and hypertension.

Table 5. Mean bioavailability and pharmacokinetic parameters of DLZ following the administration of a single dose of F7, F17 and marketed sustained release DLZ capsule.

Parameter	F7	F17	Marketed DLZ capsule
Dose, mg	2.4	2.4	2.4
C _{max} , µg/l	2357	2688	2715
(t _{max}), hr	1	1	1
k _{el} , hr ⁻¹	0.129	0.047	0.119
t _{1/2 el} , hr	5.346	14.797	5.831
TCR, ml/min	3.025	1.847	2.280
AUC ₍₀₋₂₄₎ , µg.hr/l	18479	13073	14932
AUC _(0-∞) , µg.hr/l	19483	18411	15302
MRT, hr	7.716	12.329	7.779

Figure 10. Means of plasma concentrations-time profiles of DLZ from F7, F17 and the marketed DLZ capsule.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Mohamed Ayman El-Zahabi, Department of chemistry and medicinal chemistry, Faculty of Pharmacy, King Abdulaziz University, for his valuable collaboration in the interpretation of FTIR spectra results of the formulations. The authors would also like to thank Osama A. Fahmy and Majed Alsawahli, Lecturers in pharmaceuticals and industrial pharmacy department, Faculty of Pharmacy, King Abdulaziz University, for their valuable technical support in the experimental work.

ABBREVIATIONS

DSC, Differential scanning calorimetry; **DLZ**, diltiazem; **FTIR**, Fourier transform infrared spectroscopy; **EC**, ethyl cellulose; **ED**, Eudragit RS 100; **HPMC**, hydroxypropyl

methyl cellulose; **CS**, chitosan; **LOQ**, limit of quantification; **LOD**, limit of detection; **CRB**, carbopol.

REFERENCES

- Aamir MN, Ahmad M (2009). Development of Tramadol Microparticles by Non-solvent Addition Method and their In Vitro Characterization. Iranian Polymer J. 18(12):937–46.
- Abu-Izza KA, Garcia-Contreras L, Lu DR (1996). Preparation and evaluation of sustained release AZT-loaded microspheres: optimization of the release characteristics using response surface methodology. J. Pharmaceut. Sci. 85(2):144–9.
- Agnihotri SA, Aminabhavi TM (2005). Development of novel interpenetrating network gellan gum-poly(vinyl alcohol) hydrogel microspheres for the controlled release of carvedilol. Drug .Dev. Ind Pharm. 31(6):491-503.
- Arabi H, Hashemi SA, Fooladi M (1996). Microencapsulation of allopurinol by solvent evaporation and controlled release investigation of drugs. J. Microencapsulat. 13(5):527–35.
- Bhalerao SS, Lalla JK, Rane MS (2001). Study of processing parameters influencing the properties of diltiazem hydrochloride microspheres. J. Microencapsulat. 18(3):299–307.

- Carrico C (1996). AAPS/USP Workshop on Dissolution Calibration and Testing. *Pharmaceut. Res.* 13(1):6–9.
- Cartwright AC, Matthews B (1994). *International Pharmaceutical Product Registration*. Second Edi. London: Taylor, Francis; p. 307–63.
- Chinna GB, Shyam SR, Vimal KVM, Sleeva RM, Sai K (2010). Formulation and Evaluation of Indomethacin Microspheres using natural and synthetic polymers as Controlled Release Dosage Forms. *Int. J. Drug Discovery*, 2(1):8–16.
- Colombo P, Bettini R, Santi P, De Ascentiis A, Peppas N (1996). Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport. *J. Controlled Release*, 39(2):231–7.
- Dalvadi HP, Patel JK, Rajput GC, Muruganantham V, Jayakar B (2011). Development and characterization of controlled release mucoadhesive tablets of captopril. *Ars Pharmaceut.* 52(2):31–7.
- Das S, Banerjee R, Bellare J (2005). Aspirin loaded albumin nanoparticles by coacervation: implications in drug delivery. *Trends, Biomaterials, Artificial Organs*, 18(2):203–12.
- Davis SS, Illum L (1999). Sustained release chitosan microspheres prepared by novel spray drying methods. *J. Microencapsulat.* 16(3):343–55.
- Desai SJ, Singh P, Simonelli AP, Higuchi W (1966). Investigation of factors influencing release of solid drug dispersed in inert matrices III. Quantitative studies involving the polyethylene plastic matrix. *J. Pharm. Sci.* 55(11):1230–4.
- El-bagory IM, Hosny EA, Al-suwayeh SA, Mahrous GM, Al-jenoobi F (2007). Effects of sphere size, polymer to drug ratio and plasticizer concentration on the release of theophylline from ethylcellulose microspheres. *Saudi Pharm. J.* 15(10):1–5.
- Gao P, Nixon PR, Skoug J (1995). Diffusion in HPMC gels. II. Prediction of drug release rates from hydrophilic matrix extended-release dosage forms. *Pharma.* 12(7):965–71.
- Higuchi T (1963). Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J. Pharm. Sci.* 52(12):1145–9.
- Jose S, Prema MT, Chacko AJ, Thomas AC, Souto E (2011). Colon specific chitosan microspheres for chronotherapy of chronic stable angina. *Colloids and surfaces B: Biointerfaces*, 83(2):277–83.
- Katzhendler I, Hoffman A, Goldberger A, Friedman M (1997). Modeling of drug release from erodible tablets. *J. Pharma Sci.* 86(1):110–5.
- Van Kesteren HA, Withagen AJ (1998). A Comparative Study of Once-Daily Amlodipine versus Twice-Daily Diltiazem Controlled Release (CR) in the Treatment of Stable Angina Pectoris. *Cardiovasc Drugs Ther.* 12:233–7.
- Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas N (1983). Mechanisms of potassium chloride release from compressed, hydrophilic, polymeric matrices: effect of entrapped air. *J. Pharm. Sci.* 72(10):1189–91.
- Kulkarni RV, Mangond BS, Mutalik S, Sa B (2011). Interpenetrating polymer network microcapsules of gellan gum and egg albumin entrapped with diltiazem–resin complex for controlled release application. *Carbohydrate Polymers*, 83(2):1001–7.
- Lekshmi UMD, Poovi G, Kishore N, Reddy P (2010). In vitro characterization and in vivo toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles. *Int. J. Pharm.* 396(1-2):194–203.
- Lekshmi UMD, Poovi G, Reddy PN (2012). In-vitro observation of repaglinide engineered polymeric nanoparticles. *Digest J. Nanomaterials, Biostructures*, 7(1):1–18.
- Löbénberg R, Kim JS, Amidon GL (2005). Pharmacokinetics of an immediate release, a controlled release and a two pulse dosage form in dogs. *Euro. J. Pharm. Biopharmaceut.* 60(1):17–23.
- Li M-G, Lu W-L, Wang J-C, Zhang X, Wang X-Q, Zheng A-P, Zhang Q (2007). Release profile comparison and stability of diltiazem-resin microcapsules in sustained release suspensions. *Int. J. Pharm.* 352(1-2):81–91.
- Martin A, Bustamante P, Chun AHC (1993). *Micromeritics*. Mundorff GH, editor. *Physical Pharmacy; Physical Chemical Principles in the Pharmaceutical Sciences*. Fourth Edi. Philadelphia, Pennsylvania, USA: Lippincott Williams & Wilkins, Baltimore, Maryland, USA; p. 423–52.
- Mitchell K, Ford JL, Armstrong DJ, Elliott PNC, Hogan JE, Rostron C (1993). The influence of drugs on the properties of gels and swelling characteristics of matrices containing methylcellulose or hydroxypropylmethylcellulose. *Int. J. Pharmaceut.* 100(1-3):165–73.
- Obeidat WM, Price JC (2006). Preparation and evaluation of Eudragit S 100 microspheres as pH-sensitive release preparations for piroxicam and theophylline using the emulsion-solvent evaporation method. *J. Microencapsulat.* 23(2):195–202.
- Prabu SL, Shirwaikar AA, Shirwaikar A, Ravikumar G, Kumar A, Jacob A (2009). Formulation and evaluation of oral sustained release of Diltiazem Hydrochloride using rosin as matrix forming material. *Ars Pharmaceutica*, 50:32–42.
- Prasanth VV, Chakraborty A, Mathew ST, Mathappan R, Kamalakkannan V (2011). Formulation and evaluation of Salbutamol sulphate microspheres by solvent evaporation method. *J. Appl. Pharm. Sci.* 01(05):133–7.
- Ritger P, Peppas N (1987). A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. *J. Controlled Release*, 5:37–42.
- Sabitha P, Ratna JV, Reddy KR (2010). Design and evaluation of controlled release chitosan-calcium alginate microcapsules of antitubercular drugs for oral use. *Int. J. Chem. Tech. Res.* 2(1):88–98.
- Sahoo SK, Behera AL, Mallik B, Patil S (2011). Effect of Plasticizers on Various Characteristics of Eudragit Microspheres formulated By Solvent Evaporation Method. *Int. J. Drug Develop. Res.* 3(3):285–90.
- Shato H, Miyagawa Y, Okabe TMM, Sunada H (1997). Dissolution mechanism of diclofenac sodium from wax matrix granules. *J. Pharmaceut. Sci.* 86:929–34.
- Shelke NB, Aminabhavi TM (2007). Synthesis and characterization of novel poly(sebacic anhydride-co-Pluronic F68/F127) biopolymeric microspheres for the controlled release of nifedipine. *Int. J. Pharm.* Dec 10;345(1-2):51–8.
- Siepmann J, Peppas N (2001). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adva. Drug Delivery Rev.* 48(2-3):139–57.
- Singh B, Ahuja N (2002). Development of controlled-release buccoadhesive hydrophilic matrices of diltiazem hydrochloride: optimization of bioadhesion, dissolution, and diffusion parameters. *Drug Dev. Indust. Pharm.* 8(4):431–42.
- Soppimath KS, Kulkarni AR, Aminabhavi TM (2000). Controlled Release of Antihypertensive Drug From the Interpenetrating Network Poly (Vinyl Alcohol) Guar Gum Hydrogel Microspheres. *J. Biomater. Sci. Polym. Ed.*; 11(1):27–43.
- Soppimath KS, Kulkarni AR, Aminabhavi TM (2001). Encapsulation of antihypertensive drugs in cellulose-based matrix microspheres: characterization and release kinetics of microspheres and tableted microspheres. *J. Microencapsulat.* 18(3): 397–409.
- Sultana Y, Mall S, Maurya DP, Kumar D, Das M (2009). Preparation and in vitro characterization of diltiazem hydrochloride loaded alginate microspheres. *Pharmaceut. Develop. Technol.* 14(3):321–31.
- Sweetman S (2007). *Martindale: The Complete Drug Reference*. 35th ed. London: Pharmaceutical Press;
- Wagner J (1969). Interpretation of percent dissolved-time plots derived from *in vitro* testing of conventional tablets and capsules. *J. Pharm. Sci.*, 58:1253–7.
- Yeung PKF, Buckley SJ, Hung OR, Pollak PT, Barclay KD, Feng JDZ, Farmer PS, Klassen GA (1996). Steady State Plasma Concentrations of Diltiazem and Its Metabolites in Pat. *Therapeutic Drug Monitor.* 18(1):40–5.
- Yüksel N, Baykara T (1997). Preparation of polymeric microspheres by the solvent evaporation method using sucrose stearate as a droplet stabilizer. *J. Microencapsulat.* 1(6):725–33.