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# Preparation and *in vitro* evaluation of a novel combined multiparticulate delayed-onset sustained-release formulation of diltiazem hydrochloride

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A combined delivery system, containing two kinds of diltiazem hydrochloride multi-layer coated pellets with different release characteristics, was developed to meet chronotherapeutic requirements. The dissolution studies *in vitro* indicated that the combined system could constantly release drug at a predetermined time in synchrony with the biologic rhythm of disease activity. These two kinds of pellets were mixed at the ratio 1 : 1. Pellet 2 could provide drug during the later phase of drug release from pellet 1, because the amount released was insufficient in the later phase (about 14–24 h after administration) for pellet 1. Addition of Tween 20 in the HPMC swelling layer was able to modify the hydration rate of HPMC layer which controlled the delayed time of the release system. It was found that the drug release kinetics followed Hixson-Crowell equation and this indicated that the main drug-transport mechanism inside the pellets was an erosion mechanism. The drug release rate was independent of the hydrodynamic conditions and pH of the external environment, and showed a decrease with increasing of osmotic pressure of the dissolution medium. These release characteristics indicated that the drug release from this system was driven by osmotic-pressure gradient.

## 1. Introduction

According to chronopharmacology, a number of physiologic phenomena, including hemodynamics, follow a pattern that is synchronous with the awake-sleep cycle. Furthermore, the blood pressure (BP) and heart rate (HR) follow a circadian pattern in the majority of patients with essential hypertension. The circadian pattern was characterized by a low span associated with sleep, rest or inactivity; and a steep rise over a 4-hour period that occurs with awakening and/or arousal; and a high span during wakefulness, increased activity, or work (White and Morganroth 1989; Bjoern 1991; Mansoor and White 1994; Youan 2004). The delivery system of antihypertensive medicines based on these pathophysiologic and epidemiologic associations should be designed to attenuate the steep increase of BP and HR in the early morning and keep the antihypertensive effect for a period of time but not excessively decrease BP during sleep (Anwar and White 1998). To optimize treatment outcomes, the pharmaceuticals were designed to release drug sustainedly for a period of time after a predetermined time. These pharmaceuticals should be dosed at bedtime, providing the greatest antihypertensive effect when the BP and HR increase steeply in the early morning and making the blood plasma drug concentration fluctuate consistent with biologic rhythm of disease activity (Smolensky and Haus 2001).

Diltiazem hydrochloride (DIL) is one of the calcium antagonists most widely used clinically, mainly as an antianginal and antihypertensive agent. However, DIL has a very short elimination half-life (Buckley et al. 1990; Chaffman and Brogden 1985) and conventional DIL capsules or tablets are administered three or four times daily. Many attempts were made to research DIL sustained-release formulations to improve the patient compliance and decrease the fluctuation of blood plasma drug concentration level (Levis and Deasy 2003; Costa et al. 2001; Li et al. 1995). However, these conventional sustained-release formulations do not meet chronotherapeutic requirements.

Therefore, extended-release formulations were developed, employing the multi-layer coating technique (Ueda et al. 1994; Qi et al. 2003; Narisawa et al. 1997; Schultz and Kleinebudde 1997). Lots of attempts were made to develop pulsatile release systems, neglecting the sustained-release characteristics of drugs after a predetermined time (Krögel and Bodmeier 1999, 1998; Fan et al. 2001; Kikuchi and Okano 2002), especially when the therapy of cardiovascular diseases was addressed. For this purpose delayed-onset sustained-release (DOSR) systems seem to be useful.

In the current study, a novel combined multiparticulate DOSR system of DIL was developed to meet the chronotherapeutic requirement of hypertension. Two kinds of pellets with different extended-release behavior, named pellet 1 and pellet 2, prepared in a fluidized bed coater, were combined to a multiparticulate system in order to obtain the required release characteristics. The predeter-

mined lag time of these two kinds of pellets was different, about $4 \sim 6$ h for pellet 1 and about 8 hours for pellet 2. Pellet 2 could provide drug during the later phase of drug release from pellet 1 to maintain a constant drug release. The combined multiple-unit system was designed to release drug constantly for about 6 h after a predetermined time of about $4 \sim 6$ h. The release kinetics and mechanism of the pellets were investigated. To elucidate the release kinetics and mechanism, the mathematical model program based on the nonlinear least square was employed to analyze the release kinetic characteristics of the DIL pellets.

## 2. Investigations, results and discussion

### *2.1. Preparation and dissolution profiles of the DOSR system*

Both pellet 1 and pellet 2 were multi-layer coating pellets of DIL (Fig. 1). Nonpareil beads were coated in a fluidized bed coater with three different functional layers, namely, the drug deposition layer, the HPMC swelling layer (or diffusion layer) forming the diffusion barrier, and the outer controlling layer (or retention layer) preventing the dissolution and erosion of the swelling layer. A surfactant agent (Tween 20) was added in the swelling layer and this was an important factor for controlling the drug release rate of pellets. The formulations of pellet 1 and pellet 2 are shown in Table 1.

The proportion of the two kinds of pellets was determined by comparing the fluctuation level of the beginning phase (about 6–12 h after administration) of drug release after the predetermined time (about 4–6 h after administration) with different ratio of pellet 1 and pellet 2. Zero-order release model (Q%-t) was used to simulate the drug release data in the period of about 6–12 h after administration for preparations (A), (B), (C), (D), and (E) containing 1 : 1, 2 : 1, 3 : 2, 4 : 5, 2 : 3 of DIL amount ratios of pellet 1 and pellet 2, respectively, as follows. (Q% was the cumulative percentage of drug release):

(A) $Q\% = 9.4000\,t - 6.6902 \ldots\ldots 6 \leq t \leq 12,\ r = 0.9976$
(B) $Q\% = 10.0060\,t - 5.7226 \ldots\ldots 6 \leq t \leq 12,\ r = 0.9912$
(C) $Q\% = 9.7735\,t - 4.9895 \ldots\ldots 6 \leq t \leq 12,\ r = 0.9937$
(D) $Q\% = 9.1925\,t - 7.6351 \ldots\ldots 6 \leq t \leq 12,\ r = 0.9936$
(E) $Q\% = 9.0266\,t - 8.3910 \ldots\ldots 6 \leq t \leq 12,\ r = 0.9950$

Preparation (A) was selected for its best correlation coefficient (r) (Li and Zhu 2004) and therefore, pellet 1 and pellet 2 were combined to the desired multiple-unit DOSR system according to the ratio 1 : 1 of DIL amount containing in the two kinds of pellets. The drug release rates of DIL from these two kinds of pellets increased steeply after the predetermined time about 4–6 h for pellet 1 and about 8 h for pellet 2, respectively. The pulsatile release behavior of each kind of pellets was disadvantageous for DIL to hold the mild therapeutic effect. However, while pellet 1 was combined with pellet 2 at the ratio 1 : 1 of drug, DIL could release gently from the combined multiple-unit formulation after the predetermined time of about 4–6 h and maintained the constant release characteristics over a period of about 6 h. The amount of drug release from pellet **1** was insufficient in the later phase (about 14–24 h after administration), but the amount of drug release from pellet **2** could supply the drug release from pellet **1** during the later release phase to maintain a constant rate of drug release for several hours. Therefore, the significant delayed-onset sustained-release characteristics of the DOSR system was obtained by combining pellet 1 and pellet 2 at the ratio 1 : 1 of DIL.

**Table 1: Composition of pellet formulations**

| Tween 20 added Formulations | drug layer* | swelling layer** | in the swelling layer*** | controlling layer*** |
|---|---|---|---|---|
| Pellet 1 | 43% | 20% | 1.0% | 20.5% |
| Pellet 2 | 43% | 20% | 0.5% | 32.0% |

* the content of drug in the drug-loaded spheres before coating; **: the coating level of swelling layer based on the drug-loaded spheres; ***: the concentration of Tween 20 in 4% w/v HPMC solution; ****: the coating level of controlling layer based on the weight of pellets after coating the swelling layer.

### *2.2. Effect of the addition of Tween 20 in the HPMC swelling layer on the release profiles*

In the multi-layer coated pellets of DIL, nonpareil beads were coated with three different functional layers, namely, drug-loaded HPMC deposition layer, HPMC diffusion layer forming the diffusion barrier, and Surelease® controlling layer preventing the dissolution and erosion of the diffusion layer. Addition of the proper amount of Tween 20 in the swelling layer could adjust the hydrated rate of HPMC layer. The HPMC swelling layer and the Tween 20 added into the swelling layer were used to control the beginning time of the drug release.

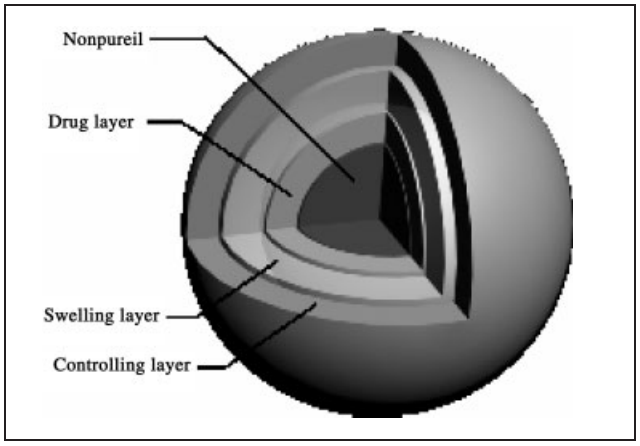


Fig. 1: The structure of pellets

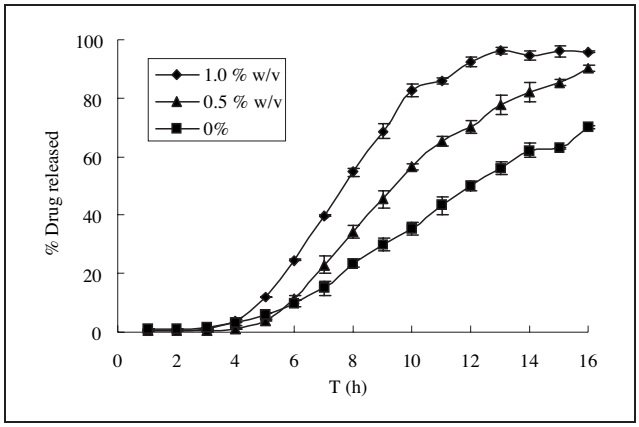


Fig. 2: Effect of the amount of Tween 20 (0%, 0.5% w/v, 1.0% w/v added in 4%w/v HPMC solution) in the HPMC swelling layer on the drug release from pellet 1. (n = 6)

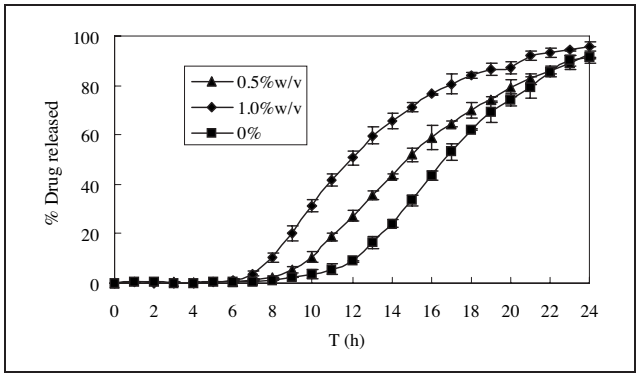


Fig. 3: Effect of the amount of Tween 20 (0%, 0.5% w/v, 1.0% w/v added in 4% w/v HPMC solution) in the HPMC swelling layer on the drug release from pellet 2. (n = 6)

Three levels of Tween 20, including 0%, 0.5% and 1.0% (w/v, the weight based on the volume of 4% w/v HPMC solution), were incorporated in the diffusion layer at 20% (w/w, based on the drug-loaded beads) coating level, respectively. Fig. 2 and Fig. 3 show the release profiles of DIL from the multi-layer coated pellets with different amount of Tween 20 in the diffusion layer for pellet 1 and pellet 2, respectively. For pellet 1, the dissolution half-life time ($t_{50\%}$) were 12.0, 9.4 and 7.6 h when the amount of Tween 20 added was 0%, 0.5% and 1.0%, respectively, while for pellet 2, the dissolution half-lifes ($t_{50\%}$) were 16.7, 14.8 and 10.4 h, respectively. The results demonstrated that the drug release rate significantly increased with increasing amounts of Tween 20 in the HPMC solutions.

At the initial stage of dissolution, water molecules diffused through the film coating because of the activity gradient of water, hydrating the swelling layer. Tween 20 dissolved very fast, and as a surfactant agent lowered the surface tension between HPMC and water to facilitate the hydration of the swelling layer. Further water diffused into the core to give rise to the solution of the drug deposition layer and the sucrose core. The solution of the core content created the high osmotic pressure in the core. This, the weakness of the external environment and the strong tensile force generated by the expansion of the HPMC swelling layer caused the rupture of the pellet coat. The drug began to release from the gap of the outer layer, with the action of the hydrostatic pressure of the swelling layer and the osmotic-pressure gradient between the core contents and the external dissolution medium. After the drug had been delivered, the hollow shell of the pellet remained, see scanning electron micrographs of pellet 1, as shown in Fig. 4. The SEM photographs showed that the pellets had smooth surfaces prior to the dissolution test (Fig. 4a) and each coating layer could be distinguished obviously from the section photograph (Fig. 4b). After the dissolution test, a gap appeared on the outer ethylcellulose layer, as Fig. 4c and Fig. 4d showed. At the final stage of dissolution, the inner components of the pellets released completely and the hollow pellet shell remained, as Fig. 4e showed. The hydration of the swelling layer created the lag time of the drug release. Therefore, Tween 20 exerted its influence on the lag time mainly by affecting the hydration time of the swelling layer. The proposed mechanism of drug release from the DIL pellets was as follows.

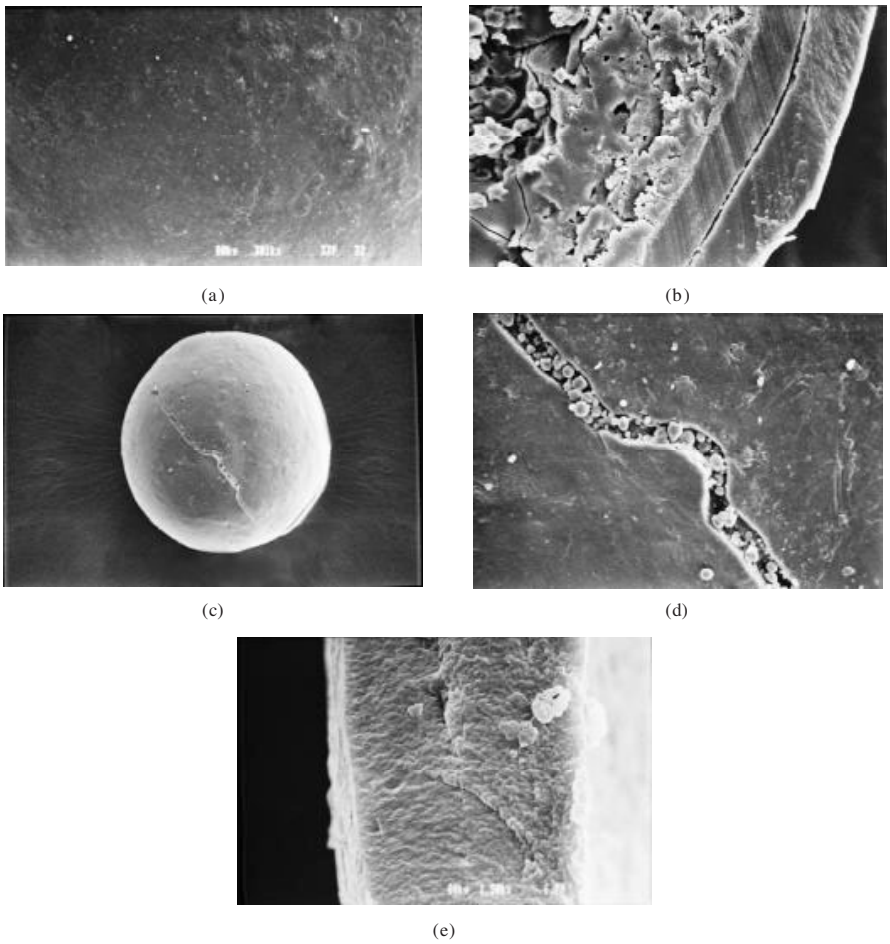


Fig. 4:
Scanning electron micrographs showing the morphology of pellet 1 prior to and after the dissolution test. (a) Surface of the pellets prior to the dissolution test (Mag 300 ×); (b) Section of the pellets prior to the dissolution test (Mag 500 ×); (c) Surface of the pellets after the dissolution test (Mag 300 ×); (d) Total surface of the pellets after the dissolution test (Mag 50 ×); (e) Section of the pellets after the dissolution test (Mag 1500 ×).

**Table 2: *In vitro* dissolution release models**

| Models | Linear models | Nonlinear models |
|---|---|---|
| Higuchi | $Q_t = b_1 + k_1 (t - L)^{0.5}$ | $Q_t = B_1 + K_1 (t - L)^{0.5}$ |
| Hixson-Crowell | $(100 - Q_t)^{1/3} = b_2 + k_2 (t - L)$ | $Q_t = 100 - [- K_2(t - L) + B_2]^3$ |

$Q_t$, cumulative percentage of release at t sampling time (%); L, lag time; k1, k2, K1, K2, release rate constant; b1, b2, B1, B2, constant.

**Table 3: Results simulated with linear kinetic models**

| Formulations | Models | Mathematical Equation | $R^2$ |
|---|---|---|---|
| Pellet 1 | Higuchi | $Q_t = 6.8277 (t\text{-}5)^{1/2} + 11.927$ | 0.8446 |
| | Hixson-Crowell | $(100 - Q_t)^{1/3} = -0.3134 (t\text{-}5) + 5.1016$ | 0.9887 |
| Pellet 2 | Higuchi | $Q_t = 6.1378 (t\text{-}8)^{1/2} + 11.797$ | 0.9583 |
| | Hixson-Crowell | $(100 - Q_t)^{1/3} = -0.2207 (t\text{-}8) + 5.2209$ | 0.9805 |
| Combination | Higuchi | $Q_t = 5.7915 (t\text{-}5)^{1/2} + 0.9125$ | 0.9363 |
| | Hixson-Crowell | $(100 - Q_t)^{1/3} = -0.1869 (t\text{-}5) + 5.2654$ | 0.9673 |

### *2.3. Release kinetic characteristics of pellets*

To analyze the release kinetic characteristics and describe the drug transport mechanism in the pattern of diffusion or erosion, several release models (Table 2), such as Higuchi (Higuchi 1961; Higuchi 1963) and Hixson-Crowell (Hixson and Crowell 1931), were used to simulate the determined data of drug release.

The determined data of pellet **1,** pellet **2** and their combination were simulated with the linear mathematical models as listed in Table 2. Table 3 summarizes the fitting equations and the correlative coefficients $R^2$ by the mathematical release models. The lag time was roughly estimated as 5 h, 8 h and 5hr for pellet 1, pellet 2, and their combination, respectively. The error between the determined data and simulated curves was significant which could be observed from the deviation sum of square (SS) indicating the difference between the determined data and simulated curves (Table 4). However, the results demonstrated that the best-fit equations were obtained by fitting with Hixson-Crowell model for pellet 1, pellet 2 and their combination according to the better correlative coefficient $R^2$ and smaller SS value.

The same conclusions were obtained by simulating with the nonlinear kinetic models (listed in Table 2) based on the nonlinear least square criterion, as shown in Table 5 and Fig. 5. According to the minimum deviation sum of square (SS) and the minimum Akaikes informatic criterion (AIC), the best-fit Hixson-Crowell equations were obtained. However, comparing the SS values which were obtained from the linear models (summarized in Table 3) with those obtained from the nonlinear least square models (shown in Table 4), it was found that the SS values calculated from the nonlinear models, which reflected the difference between the determined data and the simulated data, were much smaller than those calculated from the linear models. The results indicated that the equations simulated with the nonlinear models were more accurate than those simulated with the linear models. It implied that the simulated profiles deduced by the nonlinear least square model program was much approximate to the determined values (Fig. 5).

The simulated results, as Table 2, Table 4 and Fig. 5 show, indicated that Hixson-Crowell equation denoting the erosion mechanism was a more proper model to describe the DIL dissolution kinetics simulated with either the linear models or the nonlinear models than the Higuchi equation denoting the diffusion mechanism. Therefore, the main release mechanism of pellet 1, pellet 2 and their combination was not diffusion mechanism but erosion mechanism (Yan et al. 2000). And this conclusion was further proved by the model fitting result of Ritger-Peppas equation $Q(t) = k (t-L)^n$, where Q (t) was the percentage of drug released after time t, k was a constant, L was the lag time and n was the time exponent that characterized the drug transport mechanism (Ritger and Peppas, 1987a, b; Langer and Peppas 1981). The results of Peppas equation simulated by nonlinear models, i.e. the cumulative percentage released was plotted against the time after the lag time, were shown in Table 6. The time exponent n was over 0.89 and indicated that the erosion was the major mechanism and the diffusion was the minor mechanism

**Table 4: SS Values obtained by linear models**

| Formulations | Higuchi | Hixson-Crowell |
|---|---|---|
| Pellet 1 | 40062.90 | 876.53 |
| Pellet 2 | 31338.08 | 7624.26 |
| Combination | 9027.23 | 4611.50 |

**Table 5: Results simulated with nonlinear kinetic models**

| Formulations | Models | k | L | b | SS | AIC |
|---|---|---|---|---|---|---|
| Pellet 1 | Higuchi | 29.69 | 6.661 | 5.521 | 829.121 | 126.967 |
| | Hixson-Crowell | 0.337 | 2.001 | 5.952 | 82.499 | 85.430 |
| Pellet 2 | Higuchi | 26.63 | 10.27 | 4.801 | 159.997 | 117.653 |
| | Hixson-Crowell | 0.215 | 2.724 | 5.929 | 98.596 | 107.003 |
| Combination | Higuchi | 25.96 | 7.835 | 2.563 | 293.507 | 131.002 |
| | Hixson-Crowell | 0.211 | 5.493 | 4.912 | 73.485 | 98.536 |

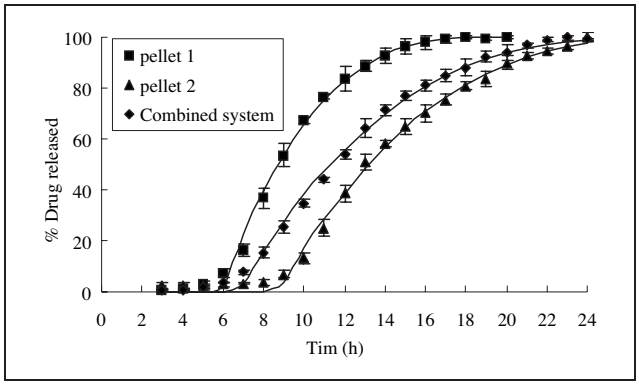


Fig. 5: Cumulative release percentage-time curves fitting by nonlinear Hixson-Crowell equation. Points (■ pellet 1; ▲ pellet 2; ◆ combined system) were the determined values. Smooth curve was the simulated results of nonlinear Hixson-Crowell (n = 6)

**Table 6: Simulating results of modified Ritger-Peppas equation by nonlinear method**

| Formulations | n | k | L | SS | AIC |
|---|---|---|---|---|---|
| Pellet 1 | 0.904 | 46.078 | 6.962 | 396.199 | 113.675 |
| Pellet 2 | 1.006 | 26.355 | 9.891 | 98.919 | 107.075 |
| Combination | 0.982 | 26.572 | 7.849 | 292.253 | 132.908 |

(Ritger and Peppas 1987a, b; Langer and Peppas 1981). This conclusion was consistent with the drug transport mechanism described above.

### *2.4. Release mechanism of drug*

The structure and release kinetics of pellet 1 was similar to pellet 2, as described above, therefore pellet 1 was taken as an example to explain release mechanism of pellet 1 or pellet 2 or their combination.

#### *2.4.1. Effect of dissolution media pH on release profiles*

The results of DIL release profiles from pellet 1 in three kinds of dissolution media are shown in Fig. 6. The $f_2$ similarity factor among the dissolution media of distilled water – PBS of pH 6.8, distilled water – hydrochloric acid aqueous solution of pH 1.2 and PBS of pH 6.8 – hydrochloric acid aqueous solution of pH 1.2 were 80.22, 74.88 and 66.59, respectively and therefore the dissolution profiles determined in different medium could be considered similar ($f_2 > 50$). Consequently, the drug release profiles from the pellets were independent of environment pH values.

Generally, the key distinguishing feather of osmotic drug delivery systems (compared with other technologies used in controlled-release formulations) is that they release drug at a rate which is independent of the pH and hydrodynamics of the external dissolution medium (Ramakrishna and Mishra 2002; Thombre et al. 2004; Razaghi and Schwartz 2002). Therefore, to further explain the release characteristics, the effect of the stirring speed on the drug release profiles of the pellets was investigated.

#### *2.4.2. Effect of the stirring speed on the release profiles*

The release profiles of pellet 1 as an example are shown in Fig. 7. The $f_2$ similarity factor among the stirring speeds of 50–100 rpm, 50–150 rpm, and 100–150 rpm were 70.82, 72.12, and 66.09, respectively. The results indicated that the differences among the stirring speeds of 50, 100 and 150 rpm were small, and the DIL dissolution profiles under different agitation conditions could be considered similar ($f_2 > 50$). The drug release rates were independent of the hydrodynamic conditions.

Release rate independent of the hydrodynamic conditions and the pH of the environment suggested that the drug release mechanism was osmotic-driven release. To further verify that the drug release from the pellets was governed by osmotic-pressure action, the dissolution tests in vitro were performed in sodium chloride solution with different osmotic pressure as followed.

#### *2.4.3. Effect of the dissolution medium with different osmotic pressure on the drug release profiles*

Dissolution medium with different osmotic pressures gave rise to the different osmotic-pressure gradients between the outer layer and the inner environment, which would influence on the drug release rate. The release profiles of the pellets in different dissolution media were compared in Fig. 8. With increasing of the osmotic pressure of the dissolution media, a decrease of the drug release rate was observed. This phenomenon could be explained that the increase of osmotic pressure of the dissolution media caused the osmotic-pressure difference between the external dissolution medium and the core contents to decrease, resulting in the slowing of drug release rate. Therefore, the results indicated that the osmotic-pressure difference played an important role in the drug release process. Consequently, these results demonstrated that the mechanism

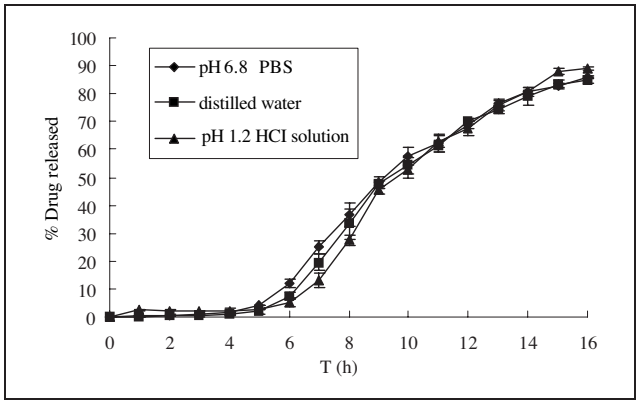


Fig. 6: Effect of pH of dissolution medium on the release profiles of pellet 1. (n = 6)

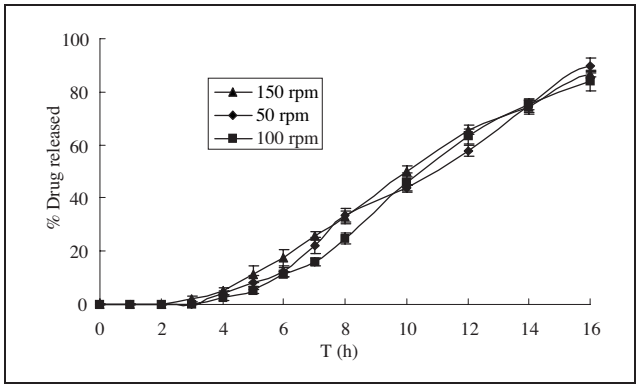


Fig. 7: Effect of the stirring speed on the release profiles of pellet 1. (n = 6)

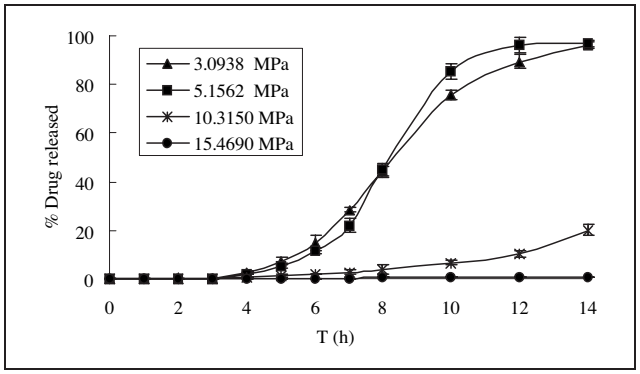


Fig. 8: Effect of the osmotic pressure of dissolution medium on the release profiles of the diltiazem hydrochloride pellet 1. (n = 6)

of the drug release was osmotic-driven. This mechanism was consistent with the performance data on the pellets discussed in this paper and also in agreement with the mechanism of drug release from osmotic systems described in the literature (Theeuwes 1975; Zentner et al. 1985; Herbig et al. 1995; Thombre et al. 1999, 2004; Eckenhoff et al. 1981).

## 3. Experimental

### *3.1. Materials*

Diltiazem hydrochloride was purchased from Shanghai Huaihai Pharmacy, Shanghai, China. Methocel® E3-LV, E5-LV and Surelease® E-7-19010 were kindly supplied by Shanghai Colorcon® Coating Technique Co., Ltd., China. Nonpareil beads were purchased from Shanghai Huagao Nonpareil Company, Shanghai, China. PEG6000 and Tween 20 were purchased from Shanghai Chemical Regent Company, Shanghai, China. All other reagents used in the study were of analytical grade.

#### *3.2.1. Preparation of drug-loaded spheres*

A solution of diltiazem hydrochloride (800 g) in 700 ml water was mixed with 700 ml of an aqueous 4% w/v HPMC E3 (3 mPa · s) solution as blinder agent. DIL-loaded spheres (drug containing, 43%w/w) were prepared by spraying the drug-binder solution onto the nonpareil beads (0.6-0.8 mm, 1kg) using a bottom-spray fluidized bed coater (Glatt® GCPG 1.1, Germany). The drug-layering conditions shown in Table 7. After loading the drug, the spheres were dried in the coating chamber for a further 15 min at 40 °C. The process efficiency was above 98%.

#### *3.2.2. Coating of drug-loaded spheres*

The drug-loaded spheres (0.8–1.0 mm) were sequentially coated with the middle swelling layer and the outer controlling layer in a fluidized bed coater (Glatt® GCPG 1.1, Germany). An aqueous 4% w/v HPMC E5-LV (5 mPa · s) solution containing different amount of Tween 20, and 10% (w/w, based on the dry weight of polymer) PEG6000 as a plasticizing agent, was fed through inlet onto drug-loaded pellets. The spray coating conditions are listed in Table 2. The coating level of the outer layer was expressed as the percentage weight of dry polymer applied with respect to the weight of beads used. The coating level of the swelling layer was 20% (w/w, based on the drug-loaded spheres) for both two kinds of pellets. And the amount of Tween 20 added into the swelling layer was 1.0% and 0.5% (w/v, based on the volume of the coating solution) for pellet 1 and pellet 2, respectively.
The pellets were further coated with Surelease® polymer aqueous dispersion. The coating conditions are shown in Table 8. Finally, the outer controlling layer comprised the dry weight of Surelease® at coating level of 20.5% and 32.0% (w/w, based on the swelling layer coated pellets) for pellet 1 and pellet 2, respectively. All the efficiencies of aforementioned process were above 98%.

**Table 7: Spray parameters applied during the process of preparation of drug-loaded spheres**

| | |
|---|---|
| Inlet air temperature | 55 °C |
| Product air temperature | 40 °C |
| Outlet air temperature | 35 °C |
| Atomizing air pressure | 2.0 bar |
| Spray rate | 8 − 10 mLmin$^{-1}$ |
| Air flow rate | 80–90 m$^3$ hr$^{-1}$ |

**Table 8: Spray coating conditions of the process of film coating of the pellets**

| | Swelling layer | Outer layer |
|---|---|---|
| Inlet air temperature | 63 °C | 60 °C |
| Product air temperature | 38–40 °C | 36–38 °C |
| Outlet air temperature | 34–36 °C | 33–35 °C |
| Atomizing air pressure | 2.0 bar | 2.0 bar |
| Spray rate | 8–10 mLmin$^{-1}$ | 8–10 mLmin$^{-1}$ |
| Air flow rate | 80–90 m$^3$/h | 80–90 m$^3$/h |

#### *3.2.3. Combined of two kinds of pellets*

Two kinds of pellets with different release behavior were mixed in a plastic bag according to the combined proportion of 1 : 1 (the ratio of DIL amount, containing in pellet 1 and pellet 2) and then were enclosed into the hard capsule to obtain a combined delayed-onset sustained-release pellets system.

### *3.3. In vitro dissolution testing*

Drug release *in vitro* was determined in a rotating basket apparatus (ZRS-4 dissolution tester; Tianjing University Radio Factory, China). The medium was 900 ml distilled water at 37 ± 0.5° and the rotating speed was 100 rpm. At predetermined time intervals, 5 ml samples were withdrawn, filtered, and assayed by a UV spectrophotometer (Model 752C; Shanghai Analytical Instrument Factory, Shanghai, China) at 236 nm, while an equal volume of fresh dissolution medium added into the apparatus. The content of DIL ($C_n$) at a certain time was determined by standard curve and the actual concentration (c) was calculated from

$$c = C_n \sum_{i=1}^{n-1} C_i \times 5/900 \quad (1)$$

(n is the time of sampling corresponding to $C_n$).
The similarity factor was evaluated to compare DIL release profiles:

$$f_2 = 50 \log \left\{ \left[ 1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

This similarity factor is a logarithmic reciprocal square root transformation of one plus the average mean squared differences in percentage dissolved between the test $T_t$ and reference $R_t$ products over all time points n. The FDA suggests that two dissolution profiles are declared similar if $f_2$ is between 50 and 100 (Moore and Flanner, 1996; Peh and Wong, 2000).

### *3.4. Release mechanism of pellets*

The structure and release kinetics of pellet 1 was similar to pellet 2, as described above, therefore pellet 1 was taken as an example to explain release mechanism of pellet 1 or pellet 2 or their combination.

#### *3.4.1. Effect of dissolution media pH on release profiles*

DIL release profiles from pellet 1 in hydrochloric acid aqueous solution of pH 1.2, in phosphate buffer solution (PBS) of pH 6.8 and in distilled water were determined in a rotating basket apparatus at 100 rpm. The dissolution profiles of pellets were determined to investigate the influence of dissolution media pH values on the DIL release profiles.

#### *3.4.2. Effect of the stirring speed on the release profiles*

To study the effect of agitation intensity on the drug release profiles, the drug release studies of pellet 1 were performed with a relatively low (50 rpm), middle (100 rpm), and high (150 rpm) stirring speed using the rotating basket apparatus in 900 ml of dissolution fluid (distilled water), as described above.

#### *3.4.3. Effect of the dissolution medium with different osmotic pressure on the drug release profiles*

To further explain the release mechanism, the dissolution studies of pellet 1 were performed in different osmotic pressure of sodium chloride (NaCl) solution at 37 ± 0.5°C and the rotating speed was 100 rpm. The concentrations of NaCl in the dissolution medium were 0.03901, 0.06502, 0.1301, and 0.1951 g/ml corresponding to the osmotic pressure of 3.0938, 5.1562, 10.3150, 15.4690 MPa calculated according to the Van't Hoff equation.

The method of collection and assay of the samples and calculation of the drug concentrations were the same to the above.

### *3.5. Scanning Electron Microscopy*

Microphotographs were obtained from the pellets before and after dissolution, which were coated with a thin gold-palladium layer, using a scanning electron microscope (SEM) (ISI-SX-40, Japan Akashi).

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