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Novel Two-Step Release System for the Traditional Chinese Medicine Compound Danshen

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A novel two-step release system for the traditional Chinese medicine compound Danshen was developed by combining an effervescent osmotic pump tablet (EOPT) and a pulsed-released tablet (PT) of compound Danshen into one hard capsule. The EOPT of Danshen was prepared with sodium chloride, mannitol, hydroxypropylmethylcellulose (HPMC), and sodium bicarbonate as osmotic agents. The osmotic pressure from EOPT was greatly enhanced by carbon dioxide generated from the reaction between sodium bicarbonate and acidic components from Danshen. It was shown that the tested Danshen components could be completely released from the prepared EOPT following a zero-order release for up to 12 h. The PT of compound Danshen was a three-layer coated tablet composed of organic acid and osmotic agents. Eudragit RL, HPMC and the mixture of EC and Eudragit RS, RL were the major constituents of the separation layer, swelling layer and controlling release membrane, respectively. The swelling test of the PT indicated that swelling is a prerequisite for drug release from this PT device. In addition, the swelling behavior further suggested the drug release mechanism of PT involves diffusion, the osmotic pumping effect, and organic acid-induced effect, among which the osmotic pumping effect was the most important. Moreover, there was no significant difference among the five active constituents in their release profiles from the final combined two-step release system of compound Danshen.

Key words——compound Danshen; two-step release; effervescent osmotic pump tablet; pulsed-release tablet; organic acid-induced effect; osmotic pump

INTRODUCTION

Traditional Chinese medicine compound preparations (TCMCP) are gradually becoming accepted by more people worldwide for their effectiveness, weak side effects and safety. Compound Danshen is an effective and important TCMCP in China for the treatment of cardiovascular diseases[1] and is composed of the three herbs Danshen (root of *salvia miltiorrhiza* Bge.), Sanqi (root of *Panax notoginseng*), and Bingpian (*Dryobalanops aromatica*). Clinical studies found that Danshen expands the blood vessels, increases coronary flow rate, relievesg blood stasis, improves the microcycle and changes blood viscidity, etc.[2]

The treatment of chronic cardiovascular disease requires long-term administration. Moreover, studies on chronopharmacokinetics and chronopharmacology have shown that symptoms of cardiovascular disease display significant circadian rhythms.[3–6] Clinical studies demonstrated that many cardiovascular accidents such as myocardial infarction, angina pectoris, heart rate disorder, and cardiogenic sudden death take place at around 06 : 00.[7] Equations for the frequency of the onset of myocardial infarction to plasma creatine kinase MB (CK–MB) activity suggested by Muller *et al*. also indicate that the number of myocardial infarctions per hour increases gradually and reaches peak at 09 : 00.[8,9] As it is inconvenient for patients to take medications at midnight, taking all the features of cardiovascular disease into account and designing and evaluating drug-delivery systems based on the circadian rhythms in disease processes is important.

Studies of Danshen preparation mainly include pellet and tablet, neither of which has a drug-delivery system based on circadian rhythms of disease. Therefore the current study aimed to develop a controlled drug-delivery system composed of effervescent osmotic pump tablets (EOPT) and pulsed-release tablets (PT). An EOPT usually consists of a compartment including drugs, active osmotic agents, sodium bicarbonate, and a semipermeable membrane with channeling agent. Since Danshen and most other TCMCPs consist of compounds with polyphenolic groups or carboxyl groups, such as phenolic acids, flavonoids,

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and triterpenoid saponins, they are acidic. Therefore we hypothesized that components of Danshen would react with sodium bicarbonate without the addition of any extra acids and effervesce through the microporous membrane when fluid entered the compartment. The PT consists of a core containing drugs, disintegrating agents, organic acid, and osmotic agents coated with a three-layered coating. The separation layer is usually formed by Eudragit RL, while the swelling layer is formed by hydroxypropylmethylcellulose (HPMC) and the controlled-release membrane is formed by a mixture of Eudragit RS, RL, and EC. With the combined effects of the three layers and excipents, drugs could be released from Danshen PT rapidly and completely after a time lag.

Accordingly, a two-step release system (zero order release and pulsatile release after a predetermined lag time) according with circadian rhythms of cardiovascular disease was developed by putting two tablets with different release features into one hard capsule. Such developed system is expected to be able to provide a less dosing frequency with only one dose before sleeping every night instead of three times a day in order to avoid ''cardiovascular accidents'' and to improve patients compliance.

MATERIALS AND METHODS

Materials Danshen was provided by Xi'an Hongsheng Co., P.R. China. Cellulose acetate (CA398-3, Eastman Chemical Company, USA) was selected as a coating membrane for EOPT. Eudragit RS, Eudragit RL-PO (Rohm Pharma, Germany), and EC (Colorcon, Dow Chemical, USA) were used as the controlled-release membrane for PT, and HPMC E5 and HPMC K100 M were used as swelling agents (Colorcon, Dow Chemical), Diethyl phthalate (DEP, Jinyu Fine Chemical, Tianjin, China) and triethylcitrate (TEC, Aldrich Chemical, USA) were used as plasticizers. Sodium chloride (AR, Fangzhou Chemical Agent, China), mannitol (Pearlitol 200SD, Roquette, France), and lactose (Pharmatose 90M, DMV, Netherlands) were used as osmotic agents. All other chemicals were of reagent grade. All standard solutions and dissolution media were prepared with deionized water.

Preparation of Core Tablets of EOPT and PT for Compound Danshen The EOPT formula included (w/w) 69% drugs, 10% sodium chloride, 15% mannitol, 3% HPMC K100 M, and 3% sodium bicarbonate. PT included (w/w) 35% drugs, 15% sodium carboxylmethyl starch (CMS-Na), 25% microcrystalline cellulose (MCC), 10% succinic acid, and 15% lactose. The above excipients for EOPT or PT were mixed separately for 10 min, followed by sieving through a 100-mesh screen (pore size 120 μm) and drying for 2 h. The final mixture of either EOPT or PT was directly compressed into 120 mg tablets using shallow-concave punches 5.95 mm in diameter on a single-punch tablet machine (Tianhe, Shanghai, China). The hardness of the prepared tablets was monitored using a tablet hardness tester (Model 78X-2, Shanghai, China) with a hardness of 3.0 ± 0.5 kg.

Coating of EOPT and PT Coating was carried out using the conventional pan-spray method (BY300A Pan-coater, Shanghai, China) after core tablets were warmed for 10 min. The conditions for coating EOPT and PT are listed in Table 1. For the cellulose acetate (CA) membrane of EOPT, 25.0% PEG400 (w/w) was selected as the optimal pore-forming agent concentration. For the controlled-release membrane of PT, different ratios of Eudragit RS, RL, and EC confer different lag times but the total amount of polymer was constant in the formulation. The coating percentage for EOPT and PT (w/w) was determined from the weight increase of 20 placebo tablets, which were coated at the same time.

***In vitro* Drug Release of Danshen EOPT and PT** *In vitro* release tests of Danshen EOPT and PT were performed according to the *Chinese Pharmacopoeia* (2005) with 200 ml of water at 37 ± 0.5 °C. Samples of 5 ml were withdrawn at predetermined intervals, and equal volumes of distilled water replaced them after each sampling. The collected samples were filtered through 0.80-μm microporous membranes and the concentrations of Danshensu (DS, 3,4-dihydroxyphenyllactic acid) and protocatechuic aldehyde (PA, 3,4-dihydroxybenzaldehyde) released from the EOPT and PT were measured with an HPLC system (SPD-10A vp, Shimadzu, Japan) at 281 nm, and the concentration of ginsenoside Rg1, Rb1 (GS Rg1, Rb1) and notoginsenoside R1 (NS R1) were measured with HPLC (HP 1100 series, Agilent, USA) at 203 nm.

Dissolution tests of the EOPT containing different amounts of sodium bicarbonate (0.0%, 1.0%, 3.0%, and 5.0%, w/w) and HPMC (1.0%, 3.0%, and 5.0%, w/w) were performed to investigate the role of sodium bicarbonate and HPMC on drug release behav-

Table 1. Coating Conditions for EOPT and PT

	EOPT	PT		
	CA	Eudragit RL	HPMC	Eudragit RS, RL, and EC
Spraying rate	1 ml/min	1 ml/min	2 ml/min	1 ml/min
Product temperature	34—36℃	28—30℃	36—38℃	28—30℃
Rotating rate	40 rpm	35 rpm	30 rpm	35 rpm
Spray air pressure	0.8 kg/cm^2	0.6 kg/cm^2	0.8 kg/cm^2	0.8 kg/cm^2
Coating solution	CA/PEG400/acetone/DEP	RL/TEC/talc/water/ethanol	HPMC E5/PEG400/water/ethanol	RS, EC, RL/TEC/water/ethanol
Composition	3.57:0.89:95.18:0.36	6.85:1.37:0.34:5.72:85.72	3.38:0.34:33.86:62.42	6.88:1.38:5.72:86.02

ior. To estimate pore size on drug release, dissolution studies of the EOPT coated with CA membrane containing PEG 400 20.0%, 25.0%, and 30.0% (w/w) were carried out in water.

To study the role of the controlled-release membrane on drug release, dissolution tests of the PT coated with different ratios of Eudragit RS, RL, and EC (Eudragit RS/RL/EC : 6.0 : 5.0 : 1.0, 6.2 : 5 : 0.8, 6.6 : 5.0 : 0.4, 6.8 : 5.0 : 0.2, 6.9 : 5.0 : 0.1) were performed. To investigate the roles of the separation and swelling layer on drug release, dissolution tests of the PT with different separation (1.0%, 3.0 %, 5.0%, 7.0%) and swelling (0.0%, 5.0%, 8.0%, 12.0%) coating levels were carried out. Dissolution tests of PT with different formulations (CMS-Na/lactose/succinic acid/MCC : 2.0 : 2.0 : 0.0 : 4.0, 2.0 : 0.0 : 1.0 : 5.0, 1.0 : 2.0 : 1.0 : 4.0, 2.0 : 2.0 : 1.0 : 3.0) were performed to investigate the role of CMS-Na, lactose, and organic acid on drug release.

To examine the effects of pH on release profiles, drug release studies were conducted in different dissolution media including simulated gastric fluid (SGF, pH 1.2), simulated intestinal fluid (SIF, pH 6.8), and simulated colonic fluid (SCF, pH 7.4). In addition, release studies were also carried out in distilled water at rotation speeds of 50 rpm and 100 rpm to estimate the effects of agitation intensity on release profiles. All of the above experiments were performed in triplicate.

Scanning Electron Microscopy of EOPT and PT To elucidate the mechanism of drug release from the final prepared system, a scanning electron microscope (SEM, Hitachi S-450, Japan) was used to observe the morphology of the membrane of EOPT and PT before and after release studies.

Microenvironmental Osmotic Pressure Measurement of EOPT The EOPT osmotic tablets were withdrawn during the dissolution studies at predetermined time points (2, 4, 6, 8, 10, and 12 h). The collected tablets were cut open and 20 μl of the solution inside the tablet was sampled using a microliter syringe (Hamilton, USA) followed by further dilution to 200 μl. The osmotic pressure of the final solution was measured with an osmometer (SMC30, Tianhe Medical Instruments, China) to investigate the microenvironmental osmotic pressure of the tablet.

Swelling Study of PT During the dissolution studies of PT, the tablets were removed and placed on filter paper to remove surface water at fixed intervals. Then, the diameter and height of weighed tablets were measured using the profile projector enlargement method.[10] The tablets were first installed on a projector (1700AHDY, 3M, USA) at 3 m from a screen, and there dimensions were determined by measuring the image on the screen. The swelling ratio was calculated according to the following equation:

$$\delta = (V_t - V_0)/V_0 \times 100\% \quad (1)$$

where V_0 is the initial volume of the dry tablet, V_t is the volume of the swollen tablet at time t, and δ is the swelling ratio. After measurement of diameter, height, and weight, the tablets were again immersed in the dissolution medium.

RESULTS AND DISCUSSION

Drug Release from EOPT

Effects of Sodium Bicarbonate on Drug Release Dissolution curves of the tablets prepared with different amounts of sodium bicarbonate are shown in Fig. 1. Only approximately 60% of DS or PA was released after 12 h from tablets without sodium bicarbonate because the osmotic pressure generated by sodium chloride and mannitol was not sufficient for complete drug release. The drug release rate from EOPT increased with the increase in sodium bicarbonate. However, if the percentage was too high, drug release

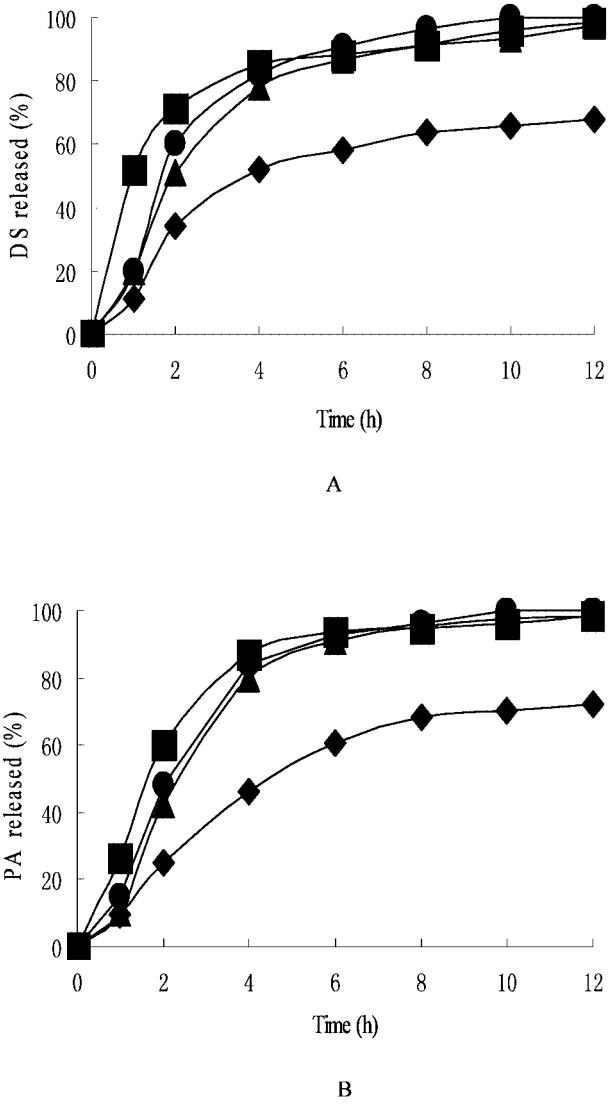


Fig. 1. Danshensu (DS) (A) and Protocatechuic Acid (PA) (B) Release Profiles of Formulations with Different Amounts of $NaHCO_3$ (Coating Level 5%)
◆: $NaHCO_3$ 0.0%, ▲: $NaHCO_3$ 1.0%, ●: $NaHCO_3$ 3.0%, ■: $NaHCO_3$ 5.0%.

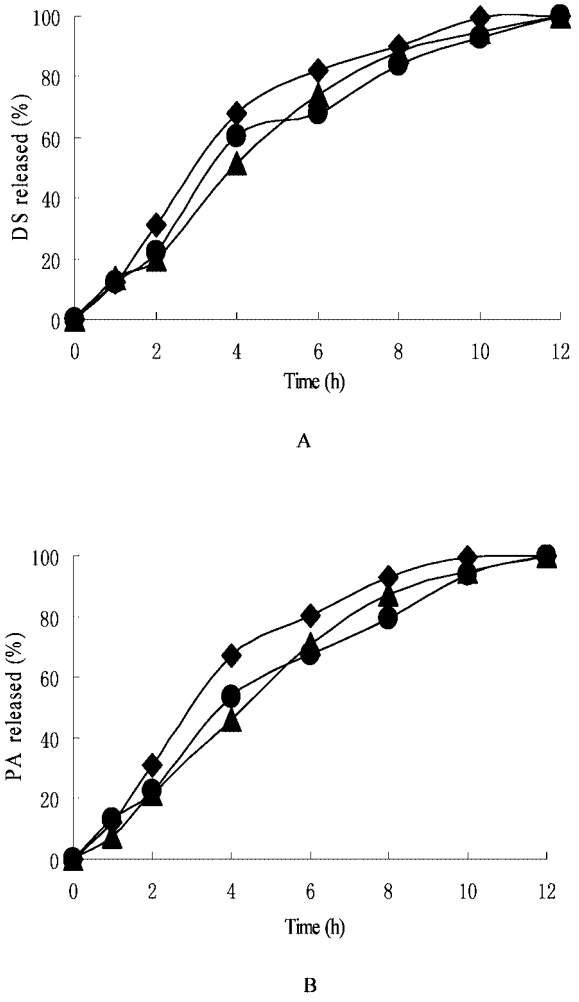
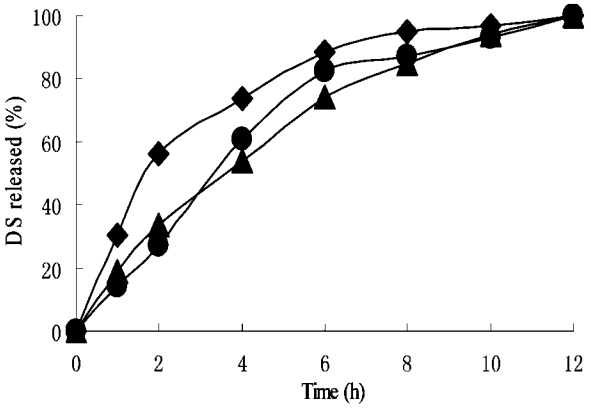


Fig. 2. DS (A) and PA (B) Release Profiles of Formulations with Different Amounts of HPMC (Coating Level 5%)
◆: HPMC 1.0%, ▲: HPMC 3.0%, ●: HPMC 5.0%.

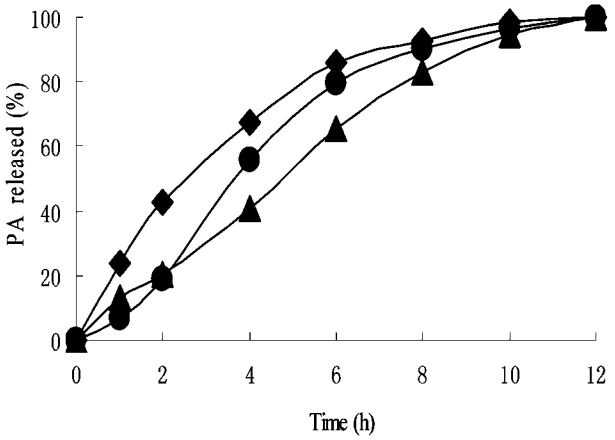
was too fast before 4 h and thus the prescription containing 3% sodium bicarbonate was the best choice for EOPT.

Effects of HPMC on Drug Release Although the drug can be released rapidly and completely from tablets containing sodium bicarbonate, the release profile shown in Fig. 1 did not follow a zero-order release during the study period of 12 h. Because the effervescent effect generated by sodium bicarbonate and acidic drugs occurred quickly and nonlinearly when fluid entering the compartment wet the contents, drugs were released too rapidly in less than 4 h. To decrease the drug release rate slightly before 4 h, retarding agents such as HPMC, polyethylene oxide (PEO), CMC–Na, and Eudragit RL were added to EOPT. HPMC as the retarder results in suitable swelling. Figure 2 shows drug release from EOPT with different amounts of HPMC. The prescription with 3% HPMC shows a tendency toward zero-order release.

Effects of Pore Former Level on Drug Release The pore-forming level is an important factor affecting the drug-release profile. EOPT cores were coated with CA membrane containing different amounts of PEG400. Weight gain of the membrane was limited to 5.0% per tablet, and the results are shown in Fig. 3. Release profiles showing a zero-order release profile were obtained when the level of PEG400 reached about 25%. When PEG400 in the membrane was more than 30%, the membrane became too porous



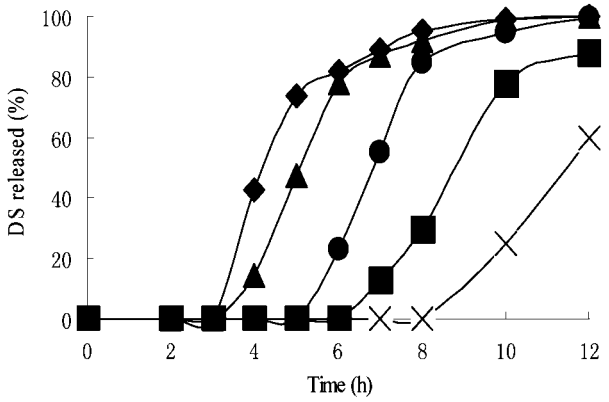
A



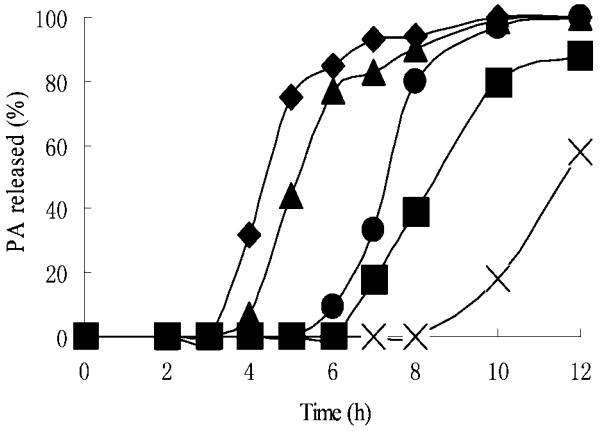
B

Fig. 3. DS (A) and PA (B) Release Profiles of Formulations with Different Amounts of PEG400 in Coating Membrane (Coating Level 5%)

◆: PEG400 20.0% (PEG400/CA, w/w); ▲: PEG400 25.0%, ●: PEG400 30.0%.



A



B

Fig. 4. DS (A) and PA (B) Release Profiles in Water from PT Coated with Compound Membrane in Different Ratios (Controlling Coating Level 3.0%)

◆: Eudragit RS/RL/EC (6.0 : 5.0 : 1.0), ▲: Eudragit RS/RL/EC (6.2 : 5 : 0.8), ●: Eudragit RS/RL/EC (6.6 : 5.0 : 0.4), ■: Eudragit RS/RL/EC (6.8 : 5.0 : 0.2), ×: Eudragit RS/RL/EC (6.9 : 5.0 : 0.1).

and drugs were released very rapidly in 6 h.

Dissolution Release of Drugs from PT

Effects of Controlled-Release Membrane on Drug Release EC and Eudragit RS and RL as water-insolube, pH-independent coating materials are used widely in the controlled drug-delivery systems. The membrane formed by EC has high friability and weak permeability and viscosity. In contrast, the membrane formed by Eudragit RS and RL is characterized by weak friability, hyperviscosity, and adjustable permeability. Therefore, because of the opposite properties of the two types of polymers, the compound membrane formed by them became more fragile and coating solution containing them became less viscous which made coating easy. Furthermore, different lag times can be achieved by coating the tablets with different ratios of Eudragit RS, RL, and EC.

Figure 4 shows the drug-release profiles in water from PT coated with compounding membrane in different ratios. The typical pulsatile-release pattern characterized by different lag times from about 3.0 to 8.0 h are seen in each profile, and because the permeability of Eudragit RL is 2-fold higher than that of RS, the lower the proportion of Eudragit RL in the compound membrane, the longer the lag time before drug release. Moreover, there was a slight decrease in the cumulative release rate over 12 h as the lag time increased.

Effects of Swelling Layer on Drug Release The HPMC layer can gel and swell in contact with fluid penetrating through the controlled-release membrane. This function can make the controlling membrane porous and crackled and consequently extend the lag time. The release profiles of tablets coated with different amounts of HPMC are shown in Fig. 5. The profiles show that the HPMC layer plays an important role in the pulsatile release of PT. It can be concluded that with the increase in coating, the lag time increases, whereas the cumulative release percentage decreases slightly.

Effects of the Separation Layer on Drug Release The separation layer formed by the permeable Eudragit RL polymer can increase the cumulative release and result in more rapid release after the lag time by avoiding the gelled HPMC layer enveloping the disintegrated core. Figure 6 shows the the release profiles of tablets with different separation coating levels. The greatest cumulative release rate and the most rapid release after the lag time occurrd in tablets with 5.0% coating.

Effects of Excipents on Drug Release Behavior The excipents of PT include CMS-Na as a disintegrating agent, lactose as an osmotic agent and organic acid as a film permeation enhancer. The use of organic acid was reported to involve an electrostatic interaction between quaternary ammonium groups of Eudragit polymer and organic acid.[11] With the combined effects of excipents, drugs are released from PT rapidly and completely after the lag time. Dissolution curves for tablets with different formulations are shown in Fig. 7. It is obvious that each excipient contributes to the pulsatile release of the tablets to some

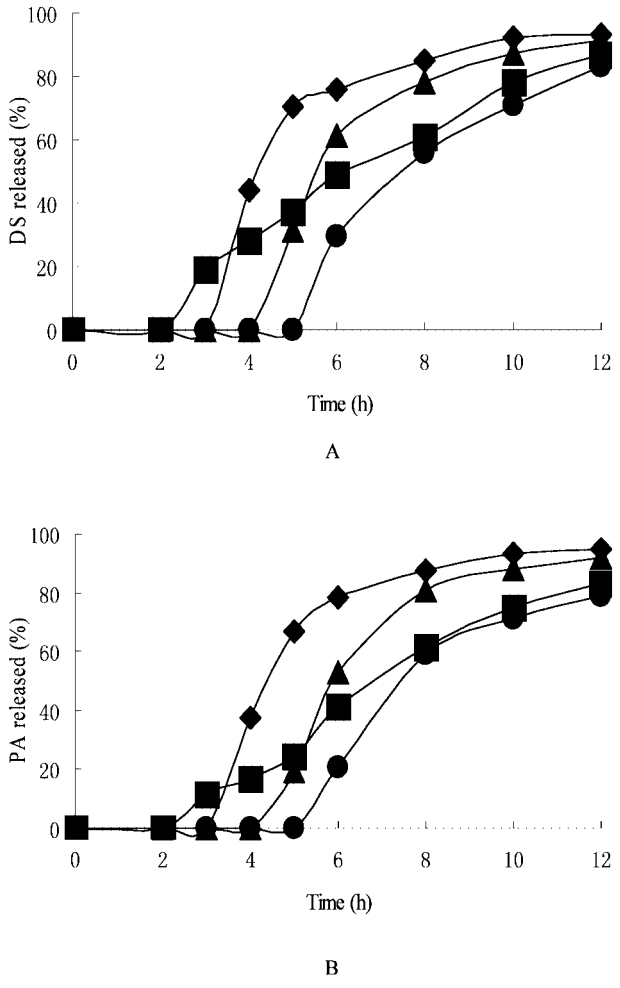


Fig. 5. DS (A) and PA (B) Release Profiles in Water from PT with Different HPMC Coating Levels (Contoll Coating Level 3%)
HPMC coating level: ◆: 5.0%, ▲: 8.0%, ●: 12.0%, ■: 0.0%.

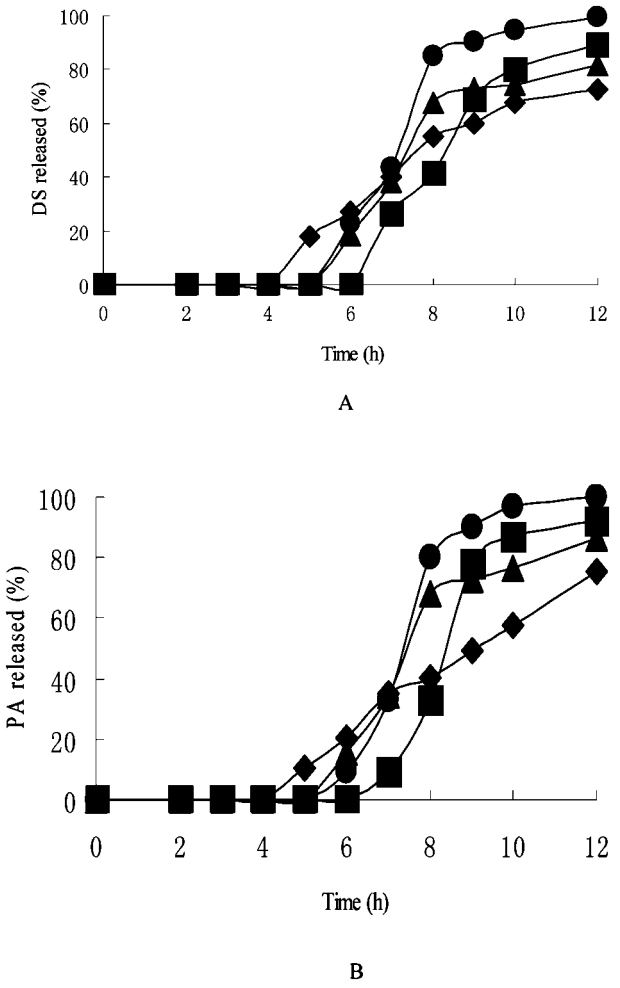


Fig. 6. DS (A) and PA (B) Release Profiles in Water from PT with Different Separation Coating Levels (Controlling Coating Level 3%, HPMC Coating Level 8%)
Separation coating level: ◆: 1.0%, ▲: 3.0%, ●: 5.0%, ■: 7.0%.

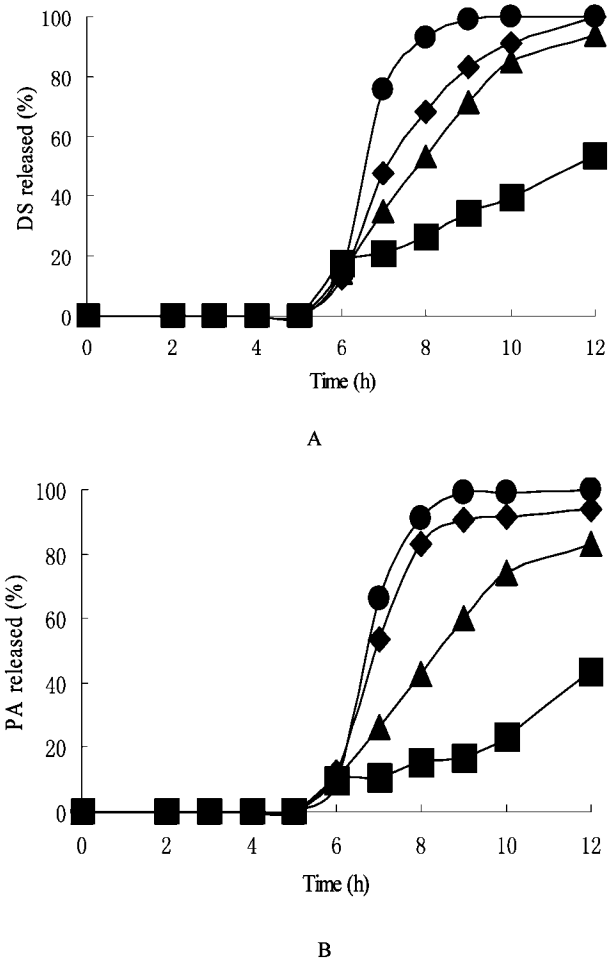


Fig. 7. DS (A) and PA (B) Dissolution Profiles of PT with Different Formulations (Controll Coating Level 3%, HPMC Coating Level 8%, Separation Coating Level 5%)

◆: CMS-Na/lactose/succinic acid/MCC (2.0 : 2.0 : 0.0 : 4.0), ▲: CMS-Na/lactose/succinic acid/MCC (2.0 : 0.0 : 1.0 : 5.0), ■: CMS-Na/mantiol/succinic acid/MCC (1.0 : 2.0 : 1.0 : 4.0), ●: CMS-Na/lactose/succinic acid/MCC (2.0 : 2.0 : 1.0 : 3.0).

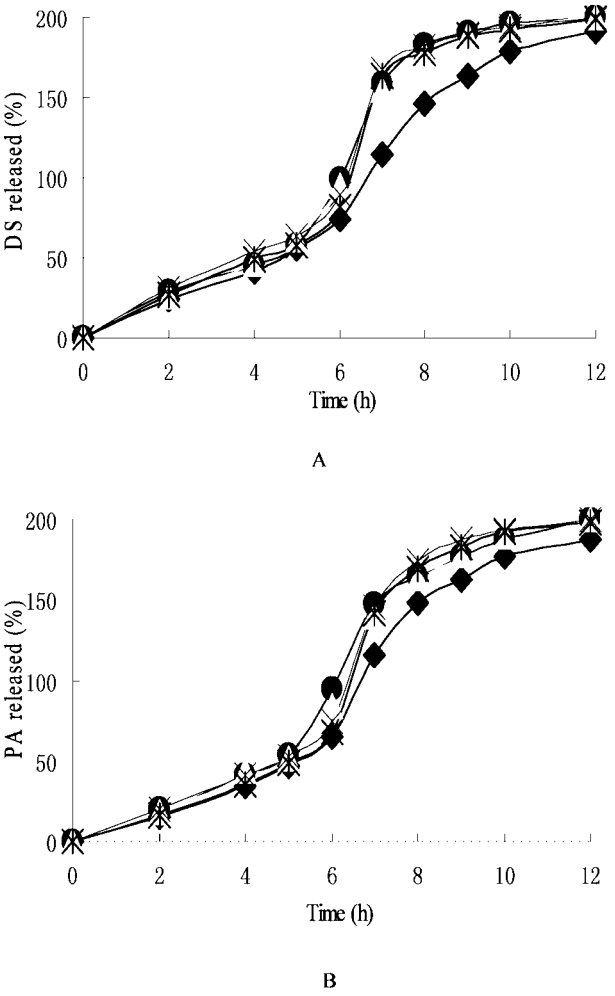


Fig. 8. DS (A) and PA (B) Release Profiles from the Two-step Release System at Different pH Values and Different Rotation Speeds

◆: In SGF at 100 rpm, ●: in SIF at 100 rpm, ▲: in SCF at 100 rpm, ×: in water at 100 rpm, *: in water at 50 rpm

extent.

Evaluation of the Final Two-Step Release System

Effects of pH and Agitation Intensity on Drug Release from the Final Two-Step Release System

As seen in the profiles in Fig. 8, we found no obvious difference in SIF, SCF, and water or different rotation speeds, but the release rate in SGF from the system decreased slightly after 6 h. The reason may be that most constituents of Danshen are acidic compounds of which the release is inhibited by acidic dissolution media. Since the gastric emptying time after normal food intake is between 4 and 6 h[12] and the tablets have already reached the small intestine after 6 h, the *in vivo* release of the two-step release system does not change with the change in pH in the gastrointestinal tract.

Drug Release from the Two-Step Release System

Figure 9 shows the dissolution profiles of the major active components of Danshen from the two-step release system in water at 100 rpm. To determine whether there was any difference in release behavior between the active constituents, the similarity factor (f_2)[13] was applied:

$$f_2 = 50 \log_{10} \left\{ \left[1 + (1/N) \sum_{i=1}^{N} (R_t - T_t) \right]^{-0.5} \times 100 \right\} \quad (2)$$

where R_t and T_t represent the reference assay and test assay at the ith time point, respectively, and N is the number of time points tested. An f_2 value greater than 50 suggests that there is no statistical difference between the test and reference profiles. If DS was used as the reference, the f_2 value of PA, NS R1, GS Rg1, and GS Rb1 were 57.9, 51.3, 59.0, and 60.5, respec-

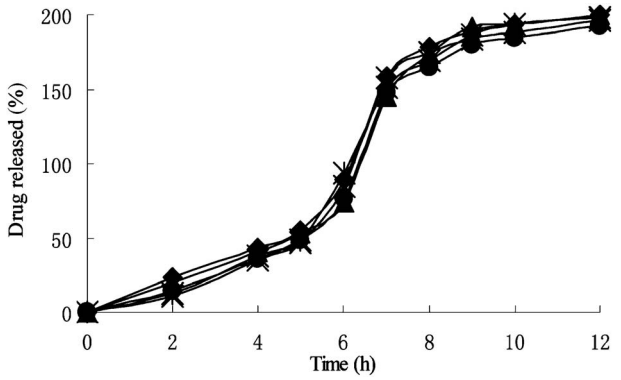


Fig. 9. DS, PA, GS Rg1, GS Rb1, and NS R1 Release Profiles from the Two-step Release System
◆: DS, ▲: PA, ●: NS R1, ×: GS Rg1, *: Gs Rb1

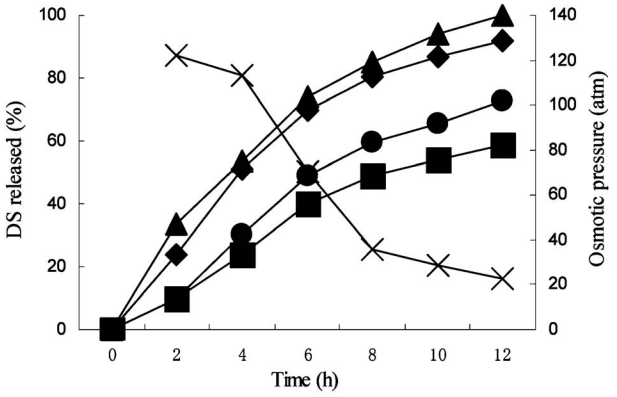


Fig. 10. DS Release in Water and Sodium Chloride Solutions of Different Osmotic Pressure and Microenvironmental Osmotic Pressure of EOPT during Dissolution (Coating Level 5%)
▲: in water, ◆: osmotic pressure 12.2 atm, ●: osmotic pressure 22.4 atm, ■: osmotic pressure 44.8 atm, ×: microenvironmental osmotic pressure.

tively.

As expected from the design of the system, the five active constituents were released from the system following a similar order consistent with the circadian rhythms of cardiovascular disease. Therefore only the release behavior of DS from the two-step release system was investigated in subsequent experiments to represent that of the other four active constituents.

Mechanism of Drug Release from EOPT Osmotic pressure is the driving force for drug release from an elementary osmotic pump tablet. In the EOPT form the current study, osmotic pressure was generated by osmotic agents, sodium bicarbonate, and HPMC. To study the effects of osmotic pressure, release studies of EOPT were conducted in water and sodium chloride solutions with different concentrations. Cumulative DS release and microenvironmental osmotic pressure of EOPT during dissolution are shown in Fig. 10. It can be concluded that drug release from EOPT is dependent on the osmotic pressure of the release media. As the osmotic pressure of the media increases, drug release from the tablet decreases.

There have been reports on the osmotic pumping mechanism for drug release from coated devices.[14] Based on the general expression described by Eq. (3), the mechanism of drug release from EOPT is.

$$d_M/d_t = (A/h)K\Delta\pi \cdot C \qquad (3)$$

where A and h are the membrane area and membrane thickness, respectively; K is the cross-product of mechanical permeability and the reflection coefficient; $\Delta\pi$ is osmotic pressure; and C is the concentration of drug in the dispensed fluid. Since in EOPT $\Delta\pi$ is generated by three types of material, Eq. (3) was modified by substituting Δs, Δg, and Δp as shown in Eq. (4):

$$d_M/d_t = (A/h)K(\Delta s+\Delta g+\Delta p) \cdot C \qquad (4)$$

where Δs is the pressure generated by osmotic agents; Δg is the pressure of carbon dioxide generated from the reaction between acidic drugs and sodium bicarbonate and Δp is swelling pressure generated by polymer HPMC. These results combined with Fig. 10 suggest that the osmotic pressure difference across the film is the key factor affecting drug release.

To examine the changes before and after dissolution studies in the membrane structure, the surface of EOPT was studied using SEM. As shown in Figs. 11 (A) and (B), the surface of coated tablets was smooth before contact with the dissolution media. However, unlike general osmotic tablets, some minor cracks were found in the membrane in addition to channels or pores made by the pore-forming agent after dissolution studies, which may have been due to gas and swelling pressure generated by the effervescent reaction and the polymer HPMC. In summary, it can be concluded that the generation of gas, swelling of HPMC, and leaching of the pore-forming agent from the membrane resulted in a porous membrane with cracks through which the drug was released with the help of pressure in the tablet compartment.

Mechanism of Drug Release from PT The PT were prepared with a core coated with three different polymers that can be observed with SEM (Fig. 11 (C)), which clearly demonstrated the core, separation layer, swelling layer, and controlled-release

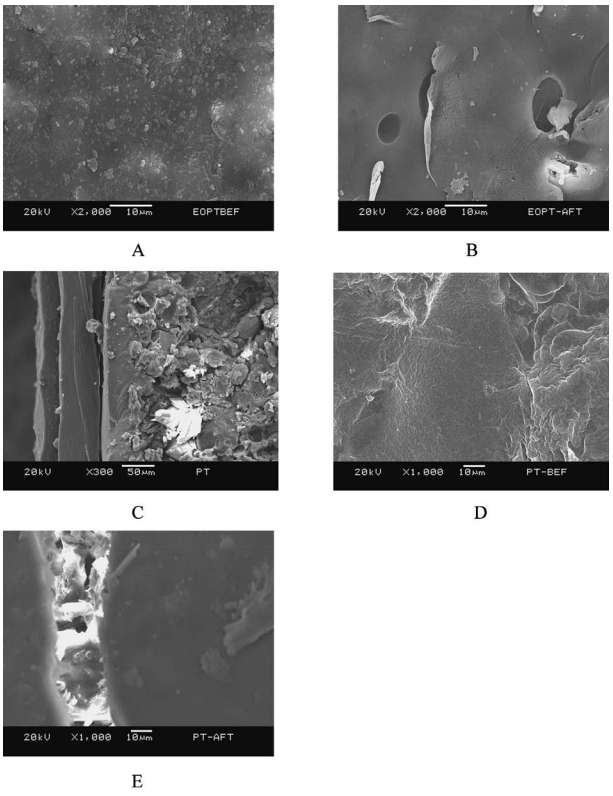


Fig. 11. SEM Micrograph of the Surface Membrane and Cross-section

A: Membrane structure of EOPT before dissolution studies, B: Membrane structure of EOPT after dissolution studies, C: Cross-section of PT, D: Surface of the outer membrane of PT before dissolution studies, E: Surface of the outer membrane of PT after dissolution studies.

membrane. After PT came into contact with solution media, water penetrated into the inner layer by diffusion through the controlled release membrane. The influx rate of water is controlled by the amount of Eudragit RL in the compound membrane and the coating. The HPMC layer gelled and swelled to make the compound membrane more porous when in contact with water, what accelerated the penetration rate of water. Because of the high water permeability of the separation layer, water can reach the core quickly and wet it. Then, CMS–Na in the core swells to disintegrate the core, and drugs, succinic acid, and lactose are dissolved. Finally, drugs are released quickly through the controlled-release membrane and separation layer, the permeability of which was increased by the reaction between the succinic acid and Eudragit polymer under the osmotic pressure generated by lactose. The separation layer is important to release the drug quickly after the lag time by preventing the gelled HPMC layer from tightly enveloping the disintegrated core.

As shown in Eq. (5), a simple semiempirical equation called the power law can be used to predict the mechanism of drug release from this pulsed-release device[15]:

$$M_t/M_\infty = k(t-T_{lag})^n \quad (5)$$

where, M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively; k is a constant incorporating structural and geometric characteristics of the device; T_{lag} is lag time; and n is the release exponent, indicating the mechanism of drug release. The power law can be seen as a generalization of the observation that is the superposition of two apparently independent mechanisms of drug transport, diffusion and swelling. For cylinders such as tablets, $n=0.45$ indicates diffusion-controlled drug release and $n=0.89$ indicates swelling-controlled drug release. Values of n between 0.45 and 0.89 can be regarded as the drug indicator for the superposition of both mechanisms of drug transport. Adapting the data from the dissolution test of PT to Eq. (5), n and k can be obtained. Then, Eq. (5) can be described as:

$$M_t/M_\infty = 0.3993(t-T_{lag})^{0.7051} \quad (6)$$

$n=0.7051$ indicates that both diffusion and swelling are included in the mechanism of drug transport in this case.

Fragility of the Controlled-release Membrane

The fragility of the controlled-release membrane which can be changed by different ratios between EC and Eudragit polymer is a key factor influencing drug release. The more fragile the membrane, the easier it can rupture and the faster drug is released from the tablet. To elucidate the relationship between the membrane fragility and drug release rate, dissolution studies of PT coated with controlled-release membrane containing different amounts of EC were performed (Fig. 12). The relationship between the amount of EC and lag time and the relationship between the amount of EC and drug release rate were obtained (Fig. 13). From the results shown in Figs. 12 and 13, the membrane containing 41.7% EC was more fragile than the other formulae. The lag time of tablets coated with this type of membrane was the shortest and the drug release rate was the highest.

The surfaces of the outer membrane before and after drug release were monitored by SEM and are shown in Figs. 11 (D) and (E). Since there are obvious differences between the EC and Eudragit polymer, wrinkles in the surface of membrane formed by

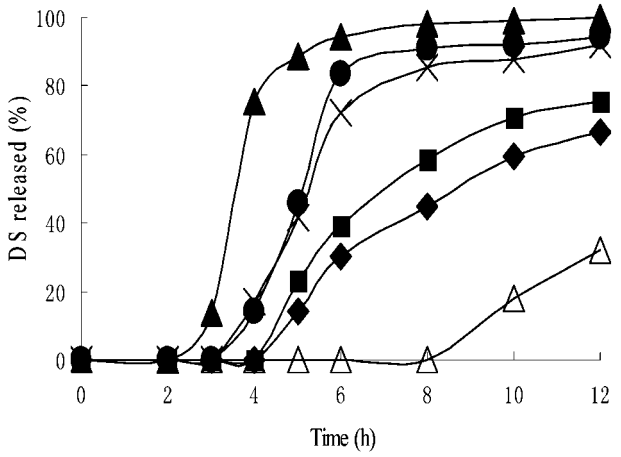


Fig. 12. DS Release Profiles from PT Coated with Controlle-release Membrane Containing Different Amounts of EC
Amount of EC in membrane (w/w, controll coating level 5%, Eudragit RL 8.3%): ◆: 0.0%, ■: 8.3%, ●: 25%, ▲: 41.7%, ×: 66.7%, △: 83.3%.

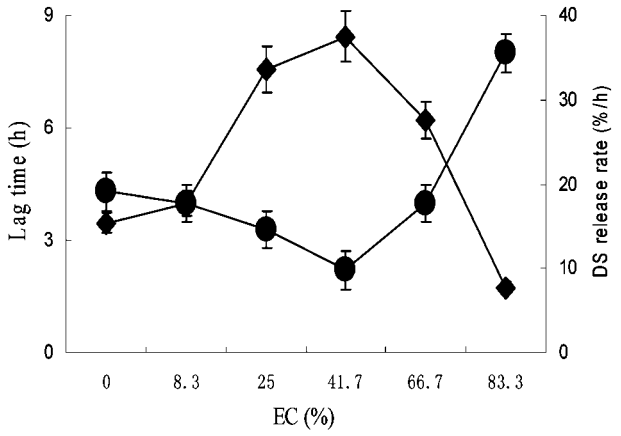


Fig. 13. Relationship between Percentage of EC and Lag time, and Percentage of EC and Drug Release Rate
●: lag time, ◆: release rate.

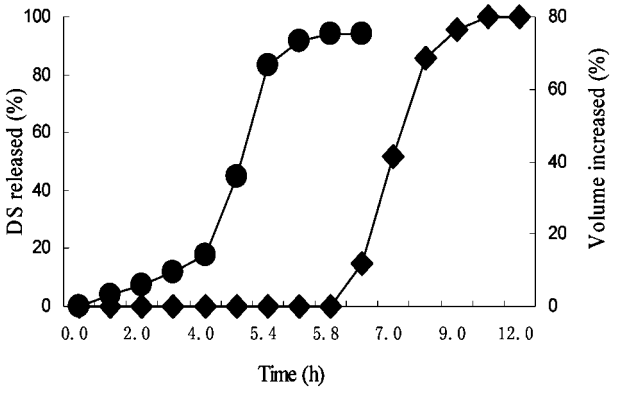


Fig. 14. Profiles of Drug Release and the Increase in Tablet Volume during Dissolution Process
●: Volume increased, ◆: DS released.

them can be clearly observed. When the swelling layer of HPMC and the core begin to swell in contact with water, the membrane can be easily disrupted starting from the wrinkle and form many cracks. Then the penetration rate of water into the core accelerates and the core continues to swell until the membrane is completely ruptured.

Swelling Behavior From the dissolution test of PT, we observed that drug release was always accompanied by swelling. To elucidate the relationship between them, swelling studies were performed using a profile projector. The DS release profile of the pulsed-release tablets is shown along with its volume increase during the drug release process in Fig. 14. Drug release always began after the tablet swelled completely. No obvious volume increase was observed in the first 4 h because little water reached the swelling layer, and this long period without expansion was followed by a rapid volume increase. During this period, the swelling HPMC made the outer membrane form many cracks and accelerated the penetration rate of water. When the core swelled and disintegrated after contact with water, the volume of tablet reached its maximum at 5.8 h, which was approximately coincident with the lag time of tablets observed from the DS release profile.

Organic Acid-Induced Effect In Fig. 9, the increase in the drug release rate is observed in PT containing succinic acid as a film permeation enhancer. To examine the permeability enhancement of succinic acid, dissolution tests of PT without succinic acid in water and with various concentrations of succinic acid solution were performed. As shown in Fig. 15, the lag time decreased and release rate was enhanced with increasing succinic acid concentration. It was concluded that the drug release rate can be improved with a permeability enhance such as succinic acid.

Osmotic Pumping Effects To determine whether the osmotic pressure can influence drug release, a dissolution study was performed in solutions with different osmotic pressure. In this case, the inorganic salts sodium chloride and potassium chloride may affect the permeability of the Eudragit polymer-based membrane through ion exchange, and therefore glucose was selected as an osmotic pressure-adjusting agent.[16] Figure 16 shows the DS release profiles from PT in water and glucose solutions with various concentrations. From the results, it can be concluded that with the increase in osmotic pressure, the lag time was

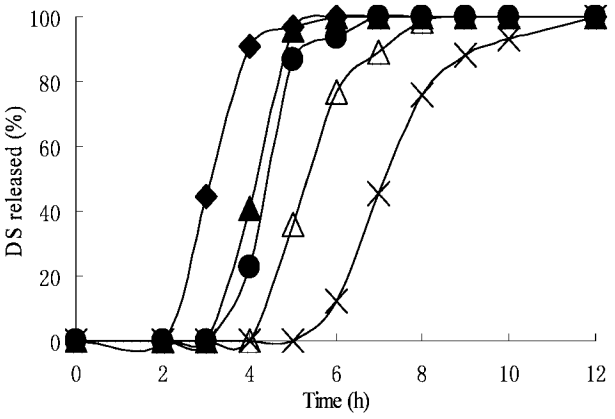


Fig. 15. DS Release Profiles of PT in Water and Succinic Acid Solution at Various Concentrations
Succinic acid concentration: ◆: 0.5 M, ▲: 0.2 M, ●: 0.1 M, △: 0.05 M, ×: in water.

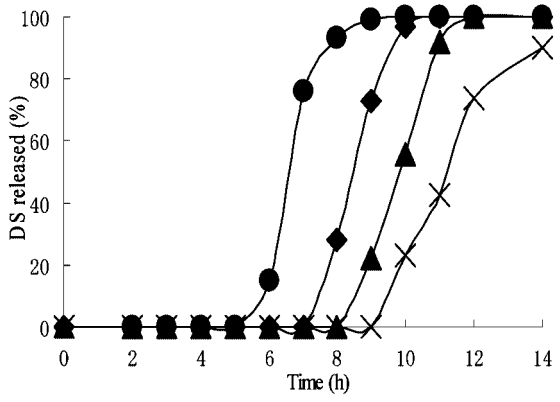


Fig. 16. DS Release Profiles from PT in Water and Glucose Aqueous Solutions at Various Concentrations
Osmotic pressure: ◆: 13.7 atm, ▲: 27.3 atm, ×: 41.0 atm, ●: in water.

prolonged

From the fitting results of Eq. (5), both diffusion and swelling are included in the mechanism of drug transport. Some previous studies reported that in addition to simple diffusion, the osmotic pump mechanism also contributes to drug release from film-coated devices, in which the drug release rate can be represented by Eq. (7) under the condition of zero hydrostatic pressure:[16,17]

$$dM/dt = (A/h)\,k\Delta\pi C_s + (A/h)\,PC_s \qquad (7)$$

where dM/dt is the steady-state release rate; A is membrane surface area; C_s is drug solubility; h is membrane thickness; $\Delta\pi$ is osmotic pressure difference across the membrane; k is the water permeability coefficient; and P is the permeability coefficient of the drug through the coating film. In this case, considering the organic acid-induced mechanism, Eq. (7) can

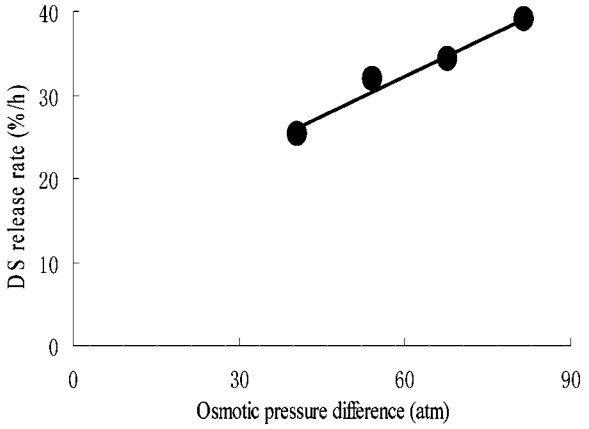


Fig. 17. Relationship between DS Release Rate and Osmptic Pressure Difference

be further modified to:

$$dM/dt = (A/h)\,k\Delta\pi C_s + (A/h)\,PC_s + \Delta\,(dM/dt)_i \qquad (8)$$

where $\Delta\ (dM/dt)_i$ is the increase in the drug release rate caused by the organic acid-induced mechanism.

As shown in Fig. 14, the device always began to release drugs after the tablet had swelled sufficiently which indicates that swelling is the basis and prerequisite for drug release in this case. Thus we can estimate the role of diffusion, osmotic pumping, and organic acid-induced mechanism in the drug release behavior according to Eq. (8).

From the results of dissolution tests in solutions with different osmotic pressure, the relationship between the drug release rate and osmotic pressure difference (microenvironmental osmotic pressure of PT determined at the time when the tablet swelled sufficiently is 81.5 atm) can be observed (Fig. 17). When $\Delta\pi$ is equal to zero, only diffusion and the organic acid-induced mechanism contribute to drug release from the device. Moreover, the contribution of the organic acid-induced mechanism to drug release can be approximately obtained from the drug release rate from PT without organic acid, as shown in Fig. 7. Finally, the Fo value that represents the percentage that osmotic pumping contributes to the drug release rate in water can be described as:

$$F_o = \frac{(dM/dt)_o}{(dM/dt)_w} = \frac{(dM/dt)w - (dM/dt)_d - (dM/dt)_i}{(dM/dt)_w} \times 100\% \qquad (9)$$

where $(dM/dt)_w$ is the drug release rate in water; $(dM/dt)_d$, $(dM/dt)_i$ and $(dM/dt)_o$ represent the

drug release rate contributed by the diffusion, organic acid-induced, and osmotic pumping mechanism, respectively. According to Figs. 7 and 17, (dM/dt) d, $(dM/dt)_i$ and $(dM/dt)_o$ were about 5.9%, 7.3% and 25.8%, respectively. The F_o value was approximately 66.2%, and F_d and F_i were 15.1% and 18.7%, respectively, suggesting that after sufficient swelling of the tablet, the osmotic pumping mechanism is the major factor in drug release from the PT of Danshen.

CONCLUSIONS

A two-step release system for Danshen, in which drug release is in accordance with the circadian rhythms of cardiovascular disease, was developed by EOPT and PT into one hard capsule. EOPT of compound Danshen was successfully prepared with the appropriate proportions of sodium chloride, mannitol, sodium bicarbonate, and HPMC. The drug was completely released from the prepared EOPT device with a zero-order release rate over 12 h. Drug release from the developed formulations of EOPT was inversely proportional to the osmotic pressure of the release media, confirming that osmotic pressure is the key factor affecting the drug release rate. Accordingly, PT of Compound Danshen was achieved by coating the core with a separation layer, swelling layer, and controlled-release membrane. As discussed above, each contributed to the pulsatile release of drug from the device. The drug release mechanism of the final prapared pulsed-release system involved diffusion, osmotic pumping effects, and organic acid-induced effect, among which osmotic pumping was the most important. Moreover, there was no significant difference among the five active constituents in their release profiles from the final two-step release system in compound Danshen, which indicated that the pharmacodynamic action is not changed by this device and the two-step release system can be useful to improve pharmacotherapy based on the concept of chronotherapy.

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