A Controlled Porosity Osmotic Pump System with Biphasic Release of Theophylline

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A controlled porosity osmotic pump system with biphasic release of theophylline was developed for the nocturnal therapy of asthma. The developed system was composed of a tablet-in-tablet (TNT) core and a controlled porosity coating membrane. Release pattern of the developed system was influenced by amount of pore former (18.2—45.5%, w/w of polymer), weight gain (16—26 mg per tablet) of the coating membrane and osmotic agents used in inner layer of the TNT core. When sodium phosphate and sodium chloride were selected as the osmotic agents in inner and outer layer of the TNT core respectively, target release profile was obtained with coating solution cellulose acetate–polyethylene glycol 400–diethyl phthalate (54.5–36.4–9.1%, w/w) at a weight gain of 16—22 mg per tablet. To examine the mechanism of drug release, release profiles of osmotic agents, micro-environmental osmotic pressure and micro-environmental pH of the formulation during dissolution were studied. Micro-environmental osmotic pressure decreased and micro-environmental pH increased continuously during the whole dissolution process, theophylline release was dominated by the successive dissolution of sodium chloride and sodium phosphate. Theophylline solubility increased as environmental pH exceeded 10.8. At the last stage of the biphasic release, micro-environmental pH in the developed formulation reached 10.9, and theophylline release was promoted by its elevated solubility despite of the decrease of micro-environmental osmotic pressure in the developed formulaiton.

Key words controlled porosity osmotic pump; biphasic release; theophylline; nocturnal asthma therapy; drug release mechanism

It is a common paradigm in clinical pharmacology that pharmacokinetic parameters are considered not to be influenced by the time of drug administration. Concerning drug concentration *vs.* time profiles, "the flatter the better" is a general aim in drug delivery.[1] However, studies on chronopharmacokinetics and chronopharmacology proved that nearly all body functions that influence pharmacokinetic parameters and the onsets or symptoms of certain disease display significant circadian rhythms.[2—5] Taking into account of the clinically relevant features of the circadian rhythms, chronopharmaceutics, which devote to the design and evaluation of drug delivery systems that release bioactive agents to proportion their serum and tissue concentrations in synchrony with known circadian rhythms in disease processes and symptoms as a means of enhancing beneficial outcomes and attenuating adverse effects, is introduced into practice as a branch of pharmaceutics.

Currently, some key technologies for oral chronopharmaceutical drug delivery systems include: 1) Multiparticulate system, which is composed of beads or microspheres coated with different controlled release coatings. Combining different beads/microspheres, various release profiles can be obtained.[6,7] 2) Erodible polymers, which can be used as matrices[8,9] or coating materials.[6] As the polymers disintegrate in gastrointestinal tract, porosity of the matrices or coatings is changed. By adjusting the proportion of different erodible polymers, diverse release profiles can be obtained. 3) Osmotic pump system, which is composed of a tablet core and a semipermeable coating membrane. By altering the composition of tablet core or coating membrane, onset of drug release can be adjusted.[10,11] 4) Pulsatile drug delivery system, which is advantageous for protecting drug from degradation and adapting drug delivery to circadian rhythms of body functions or diseases. It has been widely researched and some reviews have been made by Maroni *et al.*[12] and Bussemer *et al.*[13]

Asthma displays appreciable circadian variation in its pathogenesis.[14,15] Peak expiratory flow rate (PEF) is an objective index to evaluate ventilation functions of respiratory tract, and asthma is more severe at a lower PEF. The bathyphase of PEF is usually present between midnight and morning,[15] and asthma attacks are most severe during this period. For the nocturnal therapy of asthma, a chronopharmaceutical drug delivery system with a "slow-fast" release profile may be preferable. Drug release from the system is relatively slow at first, and at the time between midnight and morning, drug release is promoted to elevate drug blood concentration and make it synchronize with the PEF bathyphase. Thus, the biphasic drug delivery system may meet the biological requirements of asthma patients better than conventional dosage forms and provide greater patient compliance.

Theophylline is effective for the treatment of asthma, and it has been used for the chronotherapy of nocturnal asthma. Freichel and Lippold[9] described an oral erosion controlled drug delivery system which could release theophylline with a late burst. Since the burst is observed after 18 h on the release profile, such a system seems not applicable for nocturnal asthma treatment. Santus and Baker[16] introduced an OROS® system with biphasic salbutamol release. Because the system exploits the unusual solubility properties of salbutamol, it is not suitable for theophylline. Thus, few drug delivery systems are available to realize a "slow-fast" theophylline release today.

The purpose of this work is to develop a controlled poros-

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ity osmotic pump system for the biphasic (slow-fast) delivery of theophylline. The system is composed of a tablet-in-tablet (TNT) core and a rate-controlling coating membrane. Osmotic agents in inner layer and outer layer of the TNT core are sodium phosphate and sodium chloride respectively. Theophylline release from the system follows a "slow-fast" pattern, 8 h after dissolution, micro-environment pH in the formulation is elevated to nearly 11 because of the dissolution of sodium phosphate, and the solubility of theophylline is promoted in the alkaline environment. Thus, theophylline release is accelerated and a biphasic drug is obtained.

Experimental

Materials Theophylline (99.63% purity) was purchased from Zhong'an Pharmaceutical, China. Sodium chloride (AR, Fangzhou chemical agent, China), sodium phosphate (AR, Tianhua technology, China), monobasic sodium phosphate (AR, Tianhua technology, China), mannitol (200SD, Roquette, France) and lactose (90M, DMV, Netherlands) were used as osmotic agents. Polyvidone K30 (Juyuan biological technology, China) and magnesium stearate (Mallinckrodt, U.S.A.) were applied as adhesive agent and lubricant agent respectively. Cellulose acetate (CA398-3, Eastman Chemical Company, U.S.A.) was selected as coating membrane. Polyethylene glycol 400, 600, 1500 (BASF, Germany) were employed as channeling agent for controlling membrane porosity. Diethyl phthalate (DEP, Jinyu fine chemical, Tianjin, China) was selected as plasticizer. All other chemicals were of reagent grade. All standard solutions and dissolution media were prepared with deionized water.

Effect of pH on the Solubility of Theophylline To investigate the effect of pH on theophylline solubility, excess amount of theophylline was added to 10 ml buffers with different pH. The solutions were shaken on a shaking water bath (Luhe biochemical instruments, China) at 37±0.5 °C for 12 h. The samples were filtered through 0.45 μm microporous membranes and properly diluted with deionized water. Solubility of theophylline in the solutions was measured by UV-absorption measurement (UV2450, Shimadzu, Japan) at a wave length of 272 nm.

Tablet-in-Tablet (TNT) Core Preparation Composition of the TNT cores was listed in Table 1. PolyvidoneK30 was diluted to 20% (w/v) with ethanol. Theophylline and osmotic agents were blended evenly and sieved through 80 mesh sieve (180 μm). PolyvidoneK30 solution was added gradually and mixed uniformly with the mixture. The wet mess was dried at 50—60 °C for 4 h. The dried mess was powdered with mortars and passed through 80 mesh sieve (180 μm) .The obtained powder was lubricated with magnesium stearate. Inner layer was compressed into 250 mg tablets (TDP single station tablet machine, Tianhe, China, 8 mm round, concave punches). To prepare the TNT core, volume of the die cavity (11 mm round, concave punches) was adjusted equivalent to the weight of the TNT core (650 mg).

Table 1. Composition (%, w/w) of Tablet-in-Tablet Core

Materials	Composition (%, w/w)	
	Inner layer	Outer layer
Theophylline	20.0	12.5
Sodium chloride	—	81.5
Sodium phosphate	74.0	—
Polyvidone K30	5.0	5.0
Magnesium stearate	1.0	1.0

Table 2. Composition (%, w/w) of Coating Solutions

Materials	CA	PEG400	PEG600	PEG1500	DEP
Solution 1	72.7	18.2			9.1
Solution 2	63.6	27.3			9.1
Solution 3	54.5	36.4			9.1
Solution 4	45.5	45.5			9.1
Solution 5	54.5		36.4		9.1
Solution 6	54.5			36.4	9.1

Pre-weighed amount of outer layer powder (160 mg) was first placed in the die cavity as bottom layer; next, the inner layer was placed manually at the central of bottom layer; and last, the remaining volume of the die cavity was filled with 240 mg outer layer powder and compressed with a maximum compressing force of the tablet machine to obtain the TNT core. Average hardness of the TNT cores was found to be 13±0.5 kg/cm^2 (Tablet hardness tester, Huangpu analytical instrument, Shanghai, China). Drug content of the TNT cores was within the limits of 95—105%.

Coating Different coating solutions were prepared and components of the solutions were given in Table 2. 36.4% (w/w) PEG400 was selected as the optimal pore forming agent concentration, other coating solutions were used to study the effects of amount and type of pore forming agents on theophylline release. Coating was carried out by spray pan coating machine with hot air blower (BY300A pan-coater, Shanghai, China). Rotation speed of the pan was set at 50 rpm, spray rate was fixed at 1 ml·min^{-1}. Average weight gain after coating was controlled at different amounts to study the influence of coating thickness on theophylline release.

***In Vitro* Drug Release** *In vitro* release of theophylline from coated tablets was studied using paddle type apparatus with 900 ml different dissolution medium at a temperature of 37±0.5 °C.

To study the effect of pH on release profile, release studies were conducted in dissolution medium with different pH (simulated gastric fluid pH 1.2, phosphate buffer pH 6.8, deionized water pH 7.0, and phosphate buffer pH 7.4) at a rotation speed of 100 rpm.

In order to estimate the effect of agitation intensity on release profile, release studies were carried out in deionized water at the rotation speeds of 50, 100 and 150 rpm.

Release studies were carried out in a defined medium during the entire dissolution. All experiments were repeated three times. Samples of 5 ml were withdrawn at specified time points (0, 1, 2, 4, 6, 8, 10, 12 h) and replaced with fresh dissolution medium. Obtained samples were properly diluted and analyzed by UV-absorption measurement.

***In Vitro* Osmotic Agents Release** To explore the mechanism of the biphasic theophylline release, release of the osmotic agents in TNT cores during dissolution were studied using paddle type apparatus with 900 ml deionized water at 37±0.5 °C, and the rotation speed was set at 100 rpm. At predetermined time points (0, 2, 4, 6, 8, 10, 12 h), one tablet was withdrawn from vessel, cut open and the remained content was dissolved in 50 ml deionized water. Sodium chloride in the content was analyzed by titrimetric method using $AgNO_3$ (0.1 mol/l) as volumetric solution. To determine sodium phosphate in the remained TNT core, excess amount of ammonia–ammonium chloride buffer (pH 10.0) and magnesium sulfate (0.1 mol/l) were added to the solution of the remained content, according to the following chemical reaction, sediment containing phosphate anion and magnesium cation was formed. The sediment was filtered through a filter paper and the remained magnesium in filtrate was titrated with EDTA (0.1 mol/l). By subtracting the remained magnesium from the added gross, sodium phosphate in the remained TNT core could be obtained. All experiments were repeated three times using different tablets.

$$MG^{2+}+PO_4^{3-}+NH_4^+ \rightarrow MgNH_4PO_4\downarrow$$

Micro-Environmental Osmotic Pressure Measurement To measure the micro-environmental osmotic pressure in the formulation during dissolution, one tablet was withdrawn from vessel at predetermined time points (2, 4, 6, 8, 10, 12 h), cut open and 10 μl solution in the formulation was sampled carefully by a microliter syringe (Hamilton, U.S.A.) and diluted to 100 μl. Osmotic pressure of the diluted solution was measured by osmometer (SMC30, Tianhe medical instrucment, China) and the micro-environmental osmotic pressure in the formulation was 10 times of that of the diluted solution. Before measurement, osmometer was calibrated using calibration standards of double distilled water and 1000 mmol·l^{-1} sodium chloride water. All experiments were repeated three times.

Results and Discussion

Effect of pH on Drug Solubility Theophylline solubility at different pH was determined and the results were showed in Table 3. From Table 3, it can be concluded that the solubility of theophylline does not change before pH 10.8 is reached, after which the solubility increases rapidly. Taking pH as independent variable and theophylline solubility as dependent variable, theophylline solubility–pH curve can be made and expressed by multiple linear regression as the fol-

Table 3. Theophylline Solubility at Different pH (n=3)

pH	4.5	6.0	7.0	7.9	9.0	10.0
Solubility (mg·ml^{-1})	7.4±0.4	6.9±0.5	6.4±0.4	6.9±0.5	6.3±0.7	7.0±0.7
pH	10.4	10.8	11.0	11.4	11.8	
Solubility (mg·ml^{-1})	7.2±0.5	8.8±0.7	10.3±0.9	11.4±0.7	14.9±1.2	

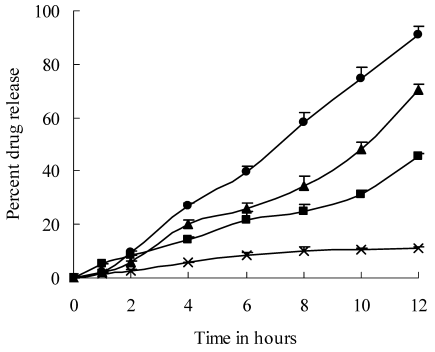

Fig. 1. Theophylline Release Profiles of Formulations with Different Amount of PEG400 in Coating Membrane (n=3, in Water at 100 rpm)

Composition of coating membrane: ×, CA–PEG400–DEP (72.7%–18.2%–9.1%); ■, CA–PEG400–DEP (63.6%–27.3%–9.1%); ▲, CA–PEG400–DEP (54.5%–36.4%–9.1%); ●, CA–PEG400–DEP (45.5%–45.5%–9.1%).

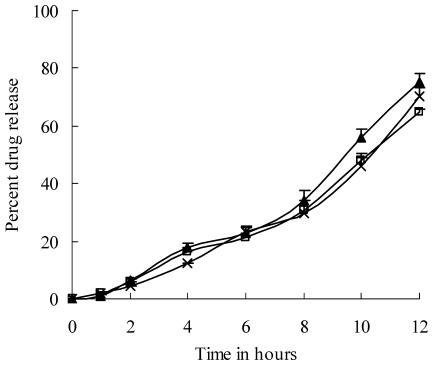

Fig. 2. Theophylline Release Profiles of Formulations with Different PEG in Coating Membrane (n=3, in Water at 100 rpm)

Pore former type: ×, PEG400; □, PEG600; ▲, PEG1500.

lowing:

$$C_S = 0.0209k^4 - 0.5864k^3 + 6.0873k^2 - 27.791k + 54.183 \quad (3 \le k \le 12,\ r = 0.9881) \qquad (1)$$

Where C_S is the solubility of theophylline and k is pH. Theophylline dissolution from formulations may be promoted by elevating micro-environmental pH.

Formulation Development The purpose of this work is to develop a controlled porosity osmotic pump system for the biphasic delivery of theophylline. The developed system is composed of a tablet-in-tablet (TNT) core and a rate controlling coating membrane. Inner layer and outer layer of the TNT core are consisted of theophylline and osmotic agents (sodium phosphate and sodium chloride, respectively). Composition of the TNT core is showed in Table 1. The TNT core is coated with a membrane consisting cellulose acetate (CA) as semi-permeable membrane forming polymer, polyethylene glycol (PEG) as leachable pore-forming material and diethyl phthalate (DEP) as plasticizer. CA membrane is permeable to aqueous fluids but impermeable to the components of the TNT core. During dissolution, water is imbibed by the TNT core from dissolution medium across the membrane, and pores are formed in the membrane gradually following the dissolution of PEG. DEP is a water-insoluble plasticizer remaining in the membrane to maintain its flexibility.

Effect of the Amount of Pore Former To study effect of the amount of pore former on theophylline release, TNT cores were coated with controlling membrane containing different amount of PEG400 (composition of coating solutions: solution 1—4, Table 2). Weight gain of the membrane was controlled between 15—17 mg per tablet, and the results were showed in Fig. 1.

Various release patterns were obtained when different amount of PEG400 was contained in the coating membranes. Release profiles showing biphasic pattern were obtained as the amount of PEG400 was between 27.3% and 36.4%. When PEG400 in the membrane was equal to 45.5%, the membrane became too porous after coming in contact with dissolution media to control theophylline release, and a zero order release profile was observed. When the PEG400 amount in the membrane was equal to 18.2%, numbers of pores formed in the membrane were not sufficient to contribute to significant theophylline release during the whole dissolution process.

The accumulative theophylline release after 12 h was increased from 10 to 90% with the amount of PEG400 in the membrane increased from 18.2 to 45.5%. In this study, only the formulation coated with coating solution 3 showed a biphasic release profile and a relatively high accumulative drug release (more than 70%) after 12 h, so 36.4% (w/w) PEG400 was selected as the optimal amount of pore former.

From the results, it could be concluded that the release profiles of theophylline were related to the amount of pore former in coating membrane. To realize biphasic theophylline release, PEG400 amount in the coating membrane should be controlled at proper degree to prevent too fast drug release at the first stage, and ensure adequate accumulative drug release during the entire dissolution process.

Effect of Pore Former Type To study the effect of pore former type on theophylline release, TNT cores were coated with controlling membrane containing 36.4% (w/w) different types of PEG (composition of coating solutions: solution 3, 5 and 6, Table 2). Weight gain of the membrane was controlled between 15—17 mg per tablet. Obtained release profiles were shown in Fig. 2, and each profile showed a typical biphasic pattern.

Similarity factor (f_2) and difference factor (f_1, or f_1 fit factor)[17] were applied to evaluate the release profiles of theophylline when different pore formers were used in coating membrane. Similarity factor (f_2) can be defined as follows:

$$f_2 = 50 \log_{10}\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \times 100\right\} \qquad (2)$$

where n is the number of sample points, W_t is an optional

weight factor, R_t and T_t are the reference assay and test assay at time point t respectively. f_2 value of 100 suggests that test and reference profiles are identical, and as the value becomes smaller, the dissimilarity between the profiles increases. f_2 value between 50 and 100 suggests that test and the reference profiles are similar. In this study, all the three formulations were evaluated under same conditions, so W_t was set at 1. PEG400 was set as reference, f_2 value of PEG600 and PEG1500 were 77.5 and 66.2 respectively.

Difference factor (f_1) can be described as follows:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100\% \quad (3)$$

where f_1 describes the relative error between test and reference profiles, f_1 value increases proportionally with the dissimilarity between the two profiles, and f_1 value is zero when the two profiles are identical. PEG400 was set as reference, f_1 value of PEG600 and PEG1500 were 8.4% and 15.0% respectively. Similarity factors (f_2) and difference factors (f_1) suggested that the three different PEG type do not influence theophylline release from the formulations obviously.

Effect of Weight Gain Strength of mechanical destructive forces in the gastrointestinal tract of humans and dogs are 1.9 N (approximately 190 g) and 3.2 N (approximately 320 g) respectively.[18,19] To maintain the integrity of osmotic pump tablets, coating membrane of the tablets should be firm enough to resist the mechanical destructive forces. It is reported that the burst strength of exhausted coating membranes are directly proportional to the weight gain of the membranes.[20,21] To develop a formulation which is durable to the destructive forces in gastrointestinal tract, a higher weight gain may be preferable.

To investigate the effect of weight gain on theophylline release, the TNT cores were coated with coating solution 3 (Table 2) to get formulations with different weight gain, and the release profiles of the formulations were shown in Fig. 3.

As Fig. 3 showed, theophylline release decreased with an increase in weight gain of the coating membrane, and when the weight gain was 22 mg per tablet (22 mg/T) at the most, the accumulative theophylline release after 12 h was more than 70%. Taking the release profile of 16 mg/T weight gain as reference, similarity factor (f_2) and difference factor (f_1) of the release profile of 22 mg/T weight gain were 61 and 18 respectively. From the results, it could be concluded that when weight gain of the coating membrane was controlled at 16—22 mg/T, release profiles of the coated tablets had no evident difference, and 16—22 mg/T was the optimal weight gain of the coating membrane.

Burst strength of the exhausted coating membrane was estimated by a tablet hardness tester (Huangpu analytical instrument, Shanghai, China). After dissolution, the remained tablets were put on the hardness tester, and burst strength of the exhausted coating membrane was estimated by the pressure needed to break the coating membrane. At the weight gain of 16 mg/T and 22 mg/T, pressure needed to rupture the exhausted coating membranes were 0.30 kg/cm^2 and 0.42 kg/cm^2 respectively. Because surface area of the exhausted coating membranes were about 0.95 cm^2, burst strength of the exhausted coating membranes were about 300 g and 420 g. Both of them were above the mechanical destructive forces in the gastrointestinal tract of humans and dogs. Satisfactory theophylline release profile and coating membrane firmness were obtained as the tablets were coated with coating solution 3 to a weight gain of 16—22 mg/T. The TNT core coated with coating solution 3 to a weight gain of 16—22 mg/T was took as the optimized formulation.

Effect of pH In order to study the effect of pH on theophylline release, release studies of the optimized formulation were conducted in simulated gastric fluid (SGF, pH 1.2), phosphate buffer (pH 6.8), deionized water (pH 7.0), and phosphate buffer (pH 7.4). Figure 4 showed the release profiles of theophylline in the different dissolution medium. Taking the release profile in deionized water as reference, difference factor (f_1) of the release profiles in SGF, pH 6.8 PBS and pH 7.4 PBS were 51, 23 and 17; and similarity factor (f_2) of the same profiles were 35, 56 and 63 respectively. From the f_1 and f_2 value, it can be concluded that the release profiles in water, pH 6.8 PBS and pH 7.4 PBS were similar and the release properties of the formulation may not be changed in intestinal tract.

In SGF, the accumulative theophylline release after dissolution was about 20—30%. Theophylline release was prevented by the acidic dissolution media 6 h after dissolution. The similarity of part of the release profiles (from 1 to 6 h) was also studied. Taking the release profile in deionized water as reference, difference factors (f_1) of the release profiles in SGF, pH 6.8 PBS and pH 7.4 PBS were 30, 20 and

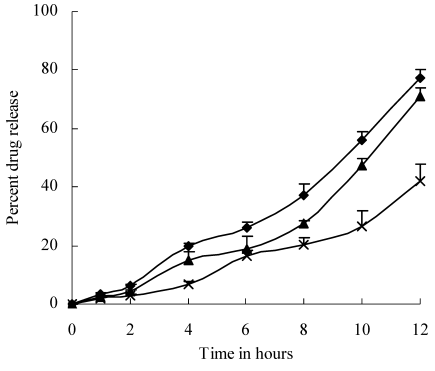

Fig. 3. Effect of Weight Gain on Theophylline Release from Formulations (n=3, in Water at 100 rpm)

Weight gain: ◆, 16 mg/T; ▲, 22 mg/T; ×, 26 mg/T.

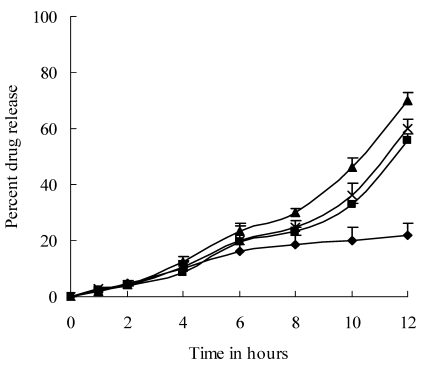

Fig. 4. Effect of pH on Theophylline Release from the Optimized Formulations (n=3, at 100 rpm)

pH of the dissolution medium: ◆, 1.2; ■, 6.8; ▲, 7.0; ×, 7.4.

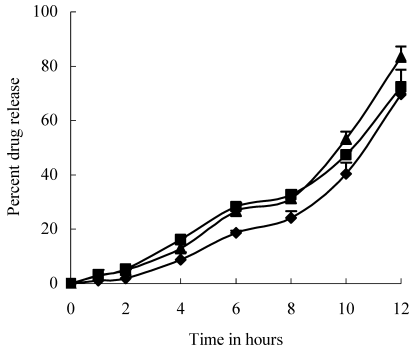

Fig. 5. Effect of Agitation Intensity on Theophylline Release from the Optimized Formulations ($n=3$, in Water at 100 rpm)

Rotation speed: ◆, 50 rpm; ■, 100 rpm; ▲, 150 rpm.

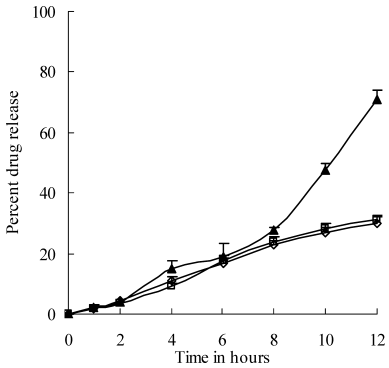

Fig. 6. Theophylline Release Profiles of Formulations with Different Osmotic Agents in Inner Layer of the TNT Cores ($n=3$, in Water at 100 rpm)

Osmotic agents in inner layer: ◇, monobasic sodium phosphate; □, mannitol; ▲, sodium phosphate.

19; and similarity factors (f_2) of the same profiles were 77, 83 and 88 respectively. The f_1 and f_2 values suggested that during the first 6 h of dissolution, theophylline release in media with different pH were similar. Gastric emptying time at normal foodintake conditions is between 4 and 6 h.[22] Six hours after administration, the optimized formulation should have been transported to intestinal tract. So, combining the properties of theophylline release with the rules of gastric emptying, it could be supposed that *in vivo* release properties of the optimized formulation would not change due to the change of pH along gastrointestinal tract.

Effect of Agitation Intensity To study the effect of agitation intensity on theophylline release, experiments were carried out using paddle type apparatus in 900 ml deionized water at rotation speed of 50, 100 and 150 rpm. Figure 5 showed that the release profiles of theophylline from the optimized formulations. Taking the release profile at 50 rpm as reference, difference factors (f_1) of the release profiles at 100 rpm and 150 rpm were 25 and 31; and similarity factors (f_2) of the same profiles were 61 and 55 respectively. The f_1 and f_2 values suggested that the release of theophylline was not influenced obviously by the agitation intensity, and the optimized formulation could be expected to show a release profile independent of the *in vivo* hydrodynamic conditions.

Effect of Osmotic Agents The biphasic theophylline release was supposed to be caused by the dissolution of sodium phosphate in inner layer of the TNT core. To approve the hypothesis, TNT cores with mannitol, monobasic sodium phosphate (MSP) and sodium phosphate (SP) as osmotic agent of the inner layers were prepared respectively according to the procedure mentioned in 2.3 (TNT core preparation). The proportion and quantity of all other components in the TNT cores kept unchanged. The obtained cores were coated with coating solution 3 to a weight gain of 20 mg per tablet. Figure 6 showed the release profiles of obtained formulations. Only the formulation with sodium phosphate in inner layer of the TNT cores exhibited biphasic release and others showed a zero-order pattern. pH value of the saturated solution of mannitol, MSP and sodium phosphate were 7, 4 and 12 respectively. Only sodium phosphate can elevate micro-environmental pH of the formulation obviously after dissolution and promote the solubility of theophylline. So sodium phosphate was selected as the osmotic agent of the inner layer. The promoted theophylline solubility may contribute to the elevated theophylline release at the second stage of the biphasic release.

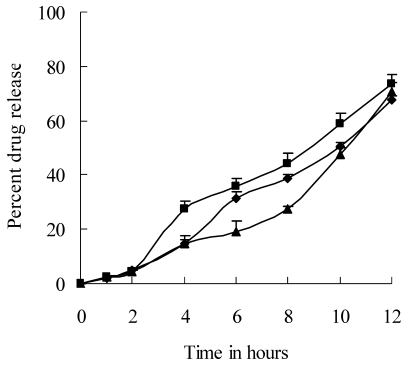

Fig. 7. Theophylline Release Profiles of Formulations with Different Osmotic Agents in Outer Layer of the TNT Cores ($n=3$, in Water at 100 rpm)

Osmotic agents in outer layer: ◆, lactose; ■, mannitol; ▲, sodium chloride.

To study the effect of osmotic agents in outer layer of the TNT cores, mannitol, lactose and sodium chloride were chosen as osmotic agents in outer layer of the cores, and formulations were prepared respectively according to the above mentioned procedure and coated with coating solution 3 to a weight gain of 20 mg per tablet. Figure 7 showed the release profiles of formulations with different osmotic agents in outer layer of the TNT cores. All obtained release profiles displayed biphasic patterns, but shapes of the release profiles were affected by the osmotic agents used in outer layer. When sodium chloride is used in outer layer, release profile of the obtained formulation showed an obvious "slow-fast" pattern, so it was selected as the osmotic agent of the outer layer.

Mechanism of Drug Release Drug in controlled porosity osmotic pump system is released by micro-environmental osmotic pressure and diffusion through pores created by the dissolution of pore formers incorporated in the coating membrane. The micro-environmental osmotic pressure is created mainly by the dissolution of osmotic agents after water is imbibed across the coating membrane. Thus, drug release from the system is directly related to the dissolution and release of osmotic agents. There are two kinds of osmotic agents incorporated in the TNT core of the optimized formulation. To investigate the mechanism of the biphasic theophylline release, it's necessary to study the relation between the release of theophylline and that of the osmotic agents. Figure 8 showed

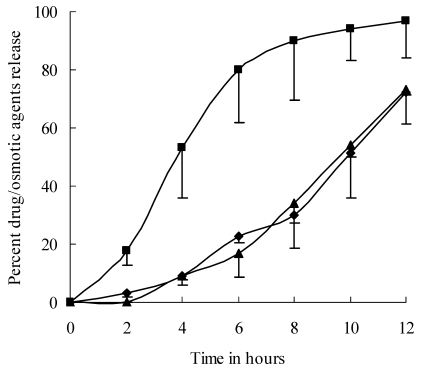

Fig. 8. Release Profiles of Theophylline, Sodium Chloride and Sodium Phosphate from the Optimized Formulations (n=3, in Water at 100 rpm)

◆, theophylline; ■, sodium chloride; ▲, sodium phosphate.

the release profiles of theophylline, sodium chloride and sodium phosphate from the optimized formulation. During the first 6 h of dissolution, the accumulative release of sodium chloride, theophylline and sodium phosphate were about 80%, 20% and 15% respectively. The osmotic pressure in the formulation was mainly created and the release of theophylline was possibly dominated by the dissolution of sodium chloride. However, at the last 6 h of dissolution, 20% sodium chloride, 60% sodium phosphate and 50% theophylline were released. It indicated that the micro-environmental osmotic pressure in the formulation and the release of theophylline at this stage were dominated by the dissolution of sodium phosphate.

The accelerated theophylline release at the end of the biphasic release was supposed to be caused by elevated micro-environmental pH after the dissolution of sodium phosphate. Released theophylline, sodium chloride and sodium phosphate during the intervals between two consecutive sampling time points were calculated according to the following equation:

$$M=T_t-T_{t-1} \tag{4}$$

Where M is the released drug or osmotic agents from time point $t-1$ to t, T_t and T_{t-1} are the accumulative theophylline or osmotic agents release at time point t and $t-1$ respectively. The saturated solution of the released theophylline and osmotic agents from time point $t-1$ to t were taken as simulated micro-environmental solution of the optimized formulation at time point t. pH value of the simulated micro-environmental solutions was determined with a pH meter (PHS-25, Leici, China). Before use, the pH meter was calibrated with standard buffers of pH 4.00, 6.86, 7.41 and 9.18 according to its operating instruction.

Accumulatively theophylline release and pH value of the simulated micro-environmental solutions during dissolution were showed in Table 4 and Fig. 9. Micro-environmental pH increased continuously during the dissolution. Taking time as independent variable and micro-environmental pH as dependent variable, micro-environmental pH–time curve could be made and expressed by multiple linear regression as the following:

$$k=-0.0039t^4+0.1246t^3-1.4305t^2+7.1065t-2.4167 \quad (2\le t\le 12,\ r=0.9964) \tag{5}$$

Table 4. Percent Drug Release, pH of Simulated Micro-Environmental Solutions and Micro-Environmental Osmotic Pressure of the Optimized Formulation during Dissolution (n=3)

Time point	Micro-environmental pH	Micro-environmental osmotic pressure (mmol·l^{-1})	Percent drug release (%)
2	7.0±0.3	11020±560	3.2±1.2
4	10.2±0.5	10960±632	8.9±1.2
6	10.4±0.5	10340±340	22.9±2.4
8	10.9±0.4	7780±368	30.1±2.6
10	11.1±0.8	4190±215	51.3±1.3
12	11.2±0.8	3880±207	77.3±0.9

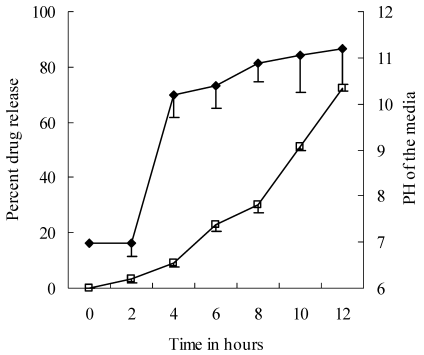

Fig. 9. Percent Drug Release and pH of Simulated Micro-Environmental Solutions of the Optimized Formulations during Dissolution (n=3, in Water at 100 rpm)

◆, pH of simulated microenvironmental solutions; □, percent drug release.

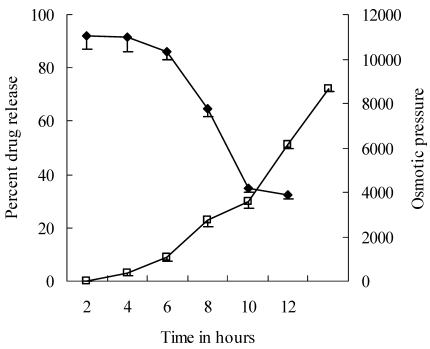

Fig. 10. Percent Drug Release and Micro-Environmental Osmotic Pressure of the Optimized Formulation during Dissolution (n=3 in Water at 100 rpm)

◆, osmotic pressure (mmol·l^{-1}); □, percent drug release.

where k is the pH and t is the time in hours.

Accumulatively theophylline release and micro-environmental osmotic pressure of the optimized formulation during dissolution were showed in Table 4 and Fig. 10. During the first 6 h of dissolution, the formulation maintained a relatively high micro-environmental osmotic pressure, and 6 h later, the micro-environmental osmotic pressure dropped obviously. Taking time as independent variable and micro-environmental osmotic pressure as dependent variable, micro-environmental osmotic pressure–time curve could be made and expressed by multiple linear regression as the following:

$$\pi=7.4t^4-182.07t^3+1385.5t^2-4147.2t+15115 \quad (2\le t\le 12,\ r=0.9999) \tag{6}$$

where π is the micro-environmental osmotic pressure in the

formulation and t is the time in hours.

At the time interval between 2 and 6 h, although micro-environmental pH jumped from 7.0 to 10.4 (Table 4) because of the dissolution of sodium phosphate, the solubility of theophylline change little (Table 3), so theophylline release was not accelerated patently. At the first 6 h of the dissolution, micro-environmental osmotic pressure was relatively high and theophylline was pumped out by the osmotic pressure. Six hours later, most sodium chloride had dissolved and sodium phosphate became the main osmotic agent. Micro-environmental osmotic pressure during this period declined greatly due to the much lower osmotic pressure of sodium phosphate solution,[23] and micro-environmental pH was elevated due to the alkalinity of sodium phosphate. Despite of the decrease of micro-environmental osmotic pressure, theophylline release from the optimized formulation increased due to the promoted theophylline solubility in the alkaline micro-environmental solution.

Drug release from osmotic pump systems can be theoretically described as the following[24]:

$$\frac{dM}{dt} = \frac{AC_s}{h}(L_p\sigma\Delta\pi + P) \quad (7)$$

where dM/dt is the drug release rate, A is the membrane surface area, h is the membrane thickness, C_S is the drug solubility, $\Delta\pi$ is the osmotic pressure difference across the membrane, L_p is the mechanical permeability, σ is the reflection coefficient, $L_p\sigma$ is permeability coefficient of water, and P is the permeability coefficient drug through the coating membrane.

Micro-environmental osmotic pressure and micro-environmental pH in the optimized formulation are changed dynamically with time during dissolution, and they can be expressed by Eqs. 5 and 6 respectively. The solubility of theophylline is changed with micro-environmental pH, and it can be expressed by Eq. 1. In this study, the osmotic pressure of dissolution medium is negligible compared with the osmotic pressure in the formulation, so $\Delta\pi$ can be substituted by π. Combining Eq. 1 with Eqs. 5—7, theophylline release from the developed system can be described as the following:

$$\frac{dM}{dt} = \frac{AC_s}{h}(L_p\sigma\pi + P) \quad (8)$$

$$C_S = 0.0209k^4 - 0.5864k^3 + 6.0873k^2 - 27.791k + 54.183 \quad (3 \le k \le 12) \quad (1)$$

$$k = -0.0039t^4 + 0.1246t^3 - 1.4305t^2 + 7.1065t - 2.4167 \quad (2 \le t \le 12) \quad (5)$$

$$\pi = 7.4t^4 - 182.07t^3 + 1385.5t^2 - 4147.2t + 15115 \quad (2 \le t \le 12) \quad (6)$$

Conclusion

A chronopharmaceutical theophylline delivery system for the nocturnal therapy of asthma was obtained. The developed system was composed of a tablet-in-tablet (TNT) core and a controlled porosity coating membrane. Theophylline release from the developed system follows a biphasic (slow-fast) pattern. Microenvironmental osmotic pressure and microenvironmental pH of the developed formulation were proved to be two dominant factors for the biphasic release. The first slow theophylline release was dominated by the osmotic pressure originated from the dissolution of sodium chloride. Micro-environmental pH of the developed formulation increased gradually, 8 h after dissolution, micro-environmental pH of the formulation exceeded near to 11, theophylline solubility increased at this alkaline micro-environment and thus the fast theophylline release was formed. By adjusting the components of the TNT core, this osmotic pump system may be applicable for the biphasic delivery of other drugs.

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