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Formulation Design of an HPMC-Based Sustained Release Tablet for Pyridostigmine Bromide as a Highly Hygroscopic Model Drug and its In Vivo/In Vitro Dissolution Properties

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Pyridostigmine bromide (PB), a highly hygroscopic drug was selected as the model drug. A sustained-release (SR) tablet prepared by direct compression of wet-extruded and spheronized core pellets with HPMC excipients and exhibited a zero-order sustained release (SR) profile. The 2^3 full factorial design was utilized to search an optimal SR tablet formulation. This optimal formulation was followed zero-order mechanism and had specific release rate at different time intervals (released % of 1, 6, and 12 hr were 15.84, 58.56, and 93.10%). The results of moisture absorption by Karl Fischer meter showed the optimum SR tablet could improve the hygroscopic defect of the pure drug (PB). In the in vivo study, the results of the bioavailability data showed the T_{max} was prolonged (from 0.65 ± 0.082 hr to 4.83 ± 1.60 hr) and AUC_{0-t} (from 734.88 ± 230.68 ng/ml.hr to 1153.34 ± 488.08 ng/ml.hr) and was increased respectively for optimum PB-SR tablets when compared with commercial immediate release (IR) tablets. Furthermore, the percentages of in vitro dissolution and in vivo absorption in the rabbits have good correlation. We believe that PB-SR tablets designed in our study would improve defects of PB, decrease the frequency of administration and enhance the retention period of drug efficacy in vivo for personnel exposed to contamination situations in war or terrorist attacks in the future.

Keywords highly hygroscopic drug; sustained-release tablet; direct-compression method; 2^3 full factorial design; zero-order mechanism; in vitro-in vivo relationship

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INTRODUCTION

Pyridostigmine bromide (PB) is a carbamate derivative of reversible acetylcholinesterase (AChE) inhibitor that has some properties, such as high water-solubility, high hygroscopic character (its rapid transformation from solid to liquid state under ambient condition), short elimination half time (1~ 2 hr) and exhibition of side effects (Hegazy et al., 2002; FDA, 2002; McEvoy, 1992).

By 1995, PB had been approved by the Food and Drug Administration (FDA) to treat a neuromuscular disease called myasthenia gravis. In 2003, FDA announced approval of PB as a pretreatment drug of neural toxicity for combat situations to increase survival after exposure to "Soman" nerve gas poisoning (FDA, 2003).

Presently, the approved dose for PB (one 60 mg of immediate-release (IR) tablet every 8 hr) used for myasthenia gravis is higher than the dose (one 30 mg of IR tablet every 8 hr) used for pretreatment to protect against Soman by United States military personnel (FDA, 2003). There is a 180 mg of extended release coated tablet (Mestinon®) on the pharmaceutical market for twice-daily administration against myasthenia gravis. However, there are no lower dose sustained-release (SR) forms of PB to protect against nerve agents at present. Clearly, the need to maintain an 8 hr schedule of PB under the conditions of actual or anticipated combat stress is a major practical deficiency in medical defense against nerve agents.

Pellets are one of common dosage forms that found in the pharmaceutical, agricultural, and polymer industries (Vervaet et al., 1995). The extrusion-spheronization technique is most popular method of producing pellets. Generally, the pellets prepared by extrusion-spheronization technique do not have an SR effect. In order to achieve the SR behavior of pellets

prepared by extrusion and spheronization, film coating or tableting are effectively used to modify the release of active ingredients from the pellets (Bodmeier, 1997; Chopra, et al., 2002; Chuo, et al., 1998; Gandhi et al., 1999; Lundqvist et al., 1997; Maejima et al., 2001). Tableting compare to film coating, it has some advantages, such as cost effectiveness, simple fabrication process, and decrease of spend on time and manpower, and dividability. Generally, tableting for controlled release preparations is the compaction of barrier-coated particles into disintegrating multiple unit tablets (Lundqvist et al., 1997). One challenge in the multiple unit tablets is maintaining the modified drug release after compaction, as the application of the compaction pressure can lead to structural changes in the film coating and, consequently, altered drug release, as reviewed by Bodmeier (1997). To protect the coating from such changes, excipients are usually incorporated in the tablet formulation. The compression-induced changes in the structure of a film coating may depend on formulation factors, such as the type and amount of coating, the properties and structure of the core pellets and the incorporation of excipient particles (Bashaiwoldu et al., 2004; Dashevsky et al., 2004). In the following literature compilation the focus lies on the compression of reservoir pellets, although some examples of other coated particles, such as powders and microparticles, are also included (Aulton et al., 1994).

HPMC is important for extended-release formulations; especially as a hydrophilic matrix tablet for oral SR preparation (Alderman, 1984; Hogan, 1989; Sung et al., 1996; Sánchez-Lafuente et al., 2002; Vazquez et al., 1992). When a matrix tablet comes into contact with an aqueous solution, the tablet surface becomes wet, and the polymer starts to partially hydrate to form a gel layer. There follows an expansion of the gel layer when water permeates into the tablet increasing the thickness of the gel layer and soluble drug diffuses through the gel barrier. Over a period of time, the tablet outer layers become eroded or broken down and finally the tablet core completely dissolves (Ford et al., 1985; Hogan, 1989). Drug release achieving a sustained effect is dependent on expansion and erosion of the tablet gel layer. The drug release mechanism from an HPMC matrix system has also been mathematically modeled by the Higuchi equation, first-order equation, zero-order equation and simple exponential equation (Kim & Fassihi, 1997; Lee, 1985; Peppas & Sahlin, 1989; Ritger & Peppas, 1987b). However, up to now, it has been less studied than that concerning HPMC (SR material) as an external excipient incorporated in wet-extruded and spheronized core pellets for tableting to achieve SR effect.

On the basis of the above-mentioned reasons, a case for the necessity for design of PB-SR dosage form in pretreatment nerve gas will be developed and investigated. The PB-SR dosage forms would be developed not only to solve difficulties of third or fourth daily administrations for immediate release (IR) dosage forms but to improve hygroscopic defects of PB as well. In our laboratory, a once-daily oral administration PB-SR pellet has been developed by extrusion-spheronization and fluid-bed methods to overcome the hygroscopic defect of PB and achieve the zero-order release mechanism (Huang et al., 2007).

In this research, another sustained release technique will further be developed to prepare the PB-SR dosage form. This technique will serve as the PB-SR tablet being prepared by direct compression of wet-extruded and spheronized core pellets (i.e. it has been fabricated in a previous study) (Huang et al., 2007) with HPMC excipients, and the 2^3 full factorial design was utilized to search for the optimal PB-SR formulation. By using these manufacturing methods and materials, we intended that the optimal PB-SR formulation would follow with specific release percentage at different sampling time points, improve drug hygroscopic property and exhibit SR effect (zero-order release mechanism). Furthermore, it is using rabbits to evaluate the pharmacokinetics, to explore the relationship between in vitro release and in vivo absorption for the optimal PB-SR tablets.

MATERIALS AND METHODS

Materials

PB (Paragon Technology, Inc., California) was used as a model drug for pretreatment drug of neural toxicity. Hydroxypropyl methyl cellulose (HPMC K4M and K100M, Dow Chemical Company, Le., S.A.) was used as SR material, and monohydrate lactose (New Zealand Lactose Co., Ltd., New Zealand) and microcrystalline cellulose (Avicel pH 102, Asahi kasei Corporation, Japan) were used as tablet diluent. Magnesium stearate (Degussa, Germany) was used as a lubricant and other chemicals used in this study were analytic grade.

Preparation of Granules (External Excipients)

The granulation formulations for 2^3 full factorial design are listed in Table 1. The required quantities of excipients (Avicel pH 102, lactose, HPMC K4M and K100M) were sieved through a 40-mesh screen respectively. Then, the excipient powders were mixed by planetary mixer, and the binder solutions (70 ml H_2O/80 ml ethanol) were added to the formulations to render a wet mass. The wet mass was manually sieved through an 18-mesh sieve and the granules were oven dried at 40°C for 4 hr. After drying, the granules were sieved at 425, 500, 600, 710, 850, 1000, 1180, and 1440 μm with the Octagon Digital series 2000 sieve shaker, and granule sizes between 1000 ~ 850 μm were selected for further tableting.

Preparation of PB-SR Tablets

The optimum core pellets that were described in former research (Huang et al., 2007) were chosen for further tableting in this study. According to Table 2 (2^3 full factorial design matrix), magnesium stearate was dissolved in acetone

TABLE 1
Granule Formulations for 2^3 Full Factorial Design

Excipients	Batch Size of Granule (F3)	Batch Size of Granule (F4)	Batch Size of Granule (F7)	Batch Size of Granule (F8)
Avicel pH 102 (g)	37.5	37.5	–	–
Lactose (g)	37.5	37.5	–	–
HPMC K4M (g)	75	–	150	–
HPMC K100M (g)	–	75	–	150
Total amount (g)	150	150	150	150

TABLE 2
2^3 Full Factorial Design Matrix and Response Results for the Measure

	Actual Values of Independent Variables			Responses			
Formulation	A_2	B_2	C_2	Y_1 (R% 1 hr)	Y_2 (R% 6 hr)	Y_3 (R% 12 hr)	Y_4 (*n* value)
F1	K4M	Without granulation	200 mg:100 mg:100 mg	12.48	58.54	93.74	0.93
F2	K100M	Without granulation	200 mg:100 mg:100 mg	9.07	50.75	85.25	0.93
F3	K4M	With granulation	200 mg:100 mg:100 mg	54.94	100.15	99.75	0.48
F4	K100M	With granulation	200 mg:100 mg:100 mg	27.16	92.65	100.03	0.75
F5	K4M	Without granulation	400 mg:0 mg:0 mg	9.80	46.94	78.24	0.95
F6	K100M	Without granulation	400 mg:0 mg:0 mg	6.82	39.91	71.20	0.88
F7	K4M	With granulation	400 mg:0 mg:0 mg	34.11	98.12	101.74	0.69
F8	K100M	With granulation	400 mg:0 mg:0 mg	33.62	91.55	98.38	0.65

	Levels	
Independent Variables	Low Level	High Level
HPMC type (A_2)	K4M	K100M
With or without granulation (B_2)	Without granulation step	With granulation step
Ratio of HPMC/lactose/Avicel pH 102 (C_2)	200 mg:100 mg:100 mg	400 mg:0 mg:0 mg

(4% w/v) and smeared in a die at first. Then, 400 mg external excipients and 300 mg optimum core pellets were mixed manually and tablets were prepared with a hydraulic press (Carver Ltd.) under 130 ± 5 Kg/cm^2. The diameter of tablets was 12.5 mm.

In Vitro Dissolution Studies of PB-SR Tablets

USP XXIV dissolution test was carrying out in vitro release studies for PB-SR tablets. The dissolution apparatus (Hanson Research Corporation, USA) conditions were set with the temperature: 37 ± 0.5°C, the dissolution medium: 900 ml of water and the paddle speed: 50 rpm. Samples of the drug solution passed through a 0.45 μm filter at 10 and 30 min, 1, 2, 3, 4, 6, 8, 10, and 12 hr were collected to an automatic fractional collector (VanKel, a Member of Varian, Inc.). The amount of the dissolved drug was determined by HPLC (Hitachi, Ltd., Japan) at 269 nm. No interference due to either the dissolved HPMC, Avicel pH 102, or magnesium stearate occurred. Each kind of tablet was tested for six replicates.

Scanning Electron Microscopy

The PB-SR tablets were mounted onto stubs using double-sided adhesive carbon tape and dried overnight. Then, using a FINE COAT JFC-1100E ion sputter (JEOL Ltd., Japan) to vacuum coat with gold in an argon atmosphere for SR tablets about 2 min. The coating thickness of SR tablets was obtained of 200 Å. The surface and cross section of SR tablets were examined under a scanning electron microscope (JSM-5300, JEOL Ltd., Japan).

2^3 Full Factorial Design

In this study, the 2^3 full factorial design was also followed former research (Huang et al., 2007) that used to estimate three independent variables of formulations for PB-SR tablets including HPMC type, with or without granulation and ratio of Avicel pH 102/lactose/HPMC. The dependent variables (responses) were selected as the percentages of drug released after three different sampling time points (1, 6, and 12 hr) and drug release mechanism (*n* value). The combination of

independent variables and the results of the responses for each experiment are listed in Table 2. A commercially available statistical software package (DESIGN EXPERT V 6.0.3, Minneapolis) was used to evaluate of the results by analysis of variance (ANOVA).

Release Mechanism of PB-SR Tablets

This study used the simple exponential equation $\frac{Mt}{M\infty} = Kt^n$ to analyze the release mechanism of PB from SR tablets (Peppas & Sahlin, 1989; Ritger & Peppas, 1987a, 1987b).

An *n* value equal to 0.50 is defined as Fickian diffusion drug release, *n* value between 0.5 ~ 1 is defined as non-Fickian (anomalous) drug release and *n* value equal to 1.0 is defined as Case II transport referring to zero-order.

Exposure to Conditions of Accelerated Stability

The optimum SR tablets were put into 100 ml unsealed glass-brown bottles and placed inside to 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH of pre-equilibrated humidity chambers (TAICHY HRM-80B, Terchy Industrial Co., Ltd., Taiwan). At sampling time points (1 hr to 4 weeks), a bottle was pulled from the humidity chambers and the moisture content of the SR tablets was tested by Karl Fischer moisture meter (Model MKS-1s, Ken Kyoto Electronics Manufacturing Co., Ltd., Japan). The moisture content was determined at the particular time point (see Table 4) and compared with the data of freshly-prepared core pellets that had been described in previous studies (Engineer et al., 2004; Huang et al., 2007; Lin et al., 2003).

In Vivo Absorption Study

According to a protocol approved by the Institutional Review Board-Use and Care of Animals at Kaohsiung Medical University, six New Zealand white rabbits weighing 3 ~ 4 kg were used in this study. All rabbits had been placed on a fast for 12 hr and allowed free access to water. After oral administration of optimum PB-SR tablets, the ears were shaved and an ear artery was cannulated using 24 g I.V. catheter for 2 ml of blood samples taken at sampling time points. The blood samples were put in heparinized tube, and centrifuged at 3000 rpm for 10 min. After the centrifugation, the plasma was kept frozen until analysis. The analysis method and extraction method of PB plasma were determined according to a previous study (Michaelis, 1990). The drug concentrations of PB-SR tablets were comparing with those of PB-IR tablets that described in former research (Huang et al., 2007).

The plasma concentrations of measuring for PB were used to compute the area under the concentration versus time profile ($AUC_{(0-t)}$ and $AUC_{(0-\infty)}$). The $AUC_{(0-t)}$ was estimated by the trapezoidal rule. The $AUC_{(0-\infty)}$ was calculated by the following equation:

$$AUC_{(0-\infty)} = AUC_{(0-t)} + \frac{C_t}{K_e}$$

K_e was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline. The C_{max} and T_{max} were obtained directly from the data. It is using the Wanger-Nelson model (Wanger & Nelson, 1964) to calculate the absorbed percentage from PB-SR tablets (Huang et al., 2004; Takka et al., 2003).

$$FA_t = \frac{(C_t + K_e \times AUC_{(0-t)})}{K_e \times AUC_{(0-\infty)}}$$

where FA_t is the absorption fraction of drug at time *t*, C_t is the drug concentration in the plasma at time *t* and K_e is the elimination rate constant. The elimination rate constant K_e was calculated from the mean plasma concentration-time profile after administration of IR tablets. The in vitro dissolution data were directly related to in vivo absorption data to complete the in vitro–in vivo correlations. Linear regression analysis was applied to the in vitro–in vivo correlation plots and coefficient of relationship (r value), slope and intercept values were calculated. All other comparisons were performed by using ANOVA.

RESULTS AND DISCUSSION

Evaluation of In-Vitro Dissolution Study and Release Mechanism from PB-SR Tablets Using 2^3 Full Factorial Design

The dissolution profiles of all formulations required by 2^3 full factorial design are shown in Figure 1. Table 2 shows the overall formulations (F1~F8 formulations) contained two types of HPMC (K4M and K100M), formulation F1, F2, F3, and F4, on the other hand, contained other excipients (Avicel pH 102 and lactose) as well, and the ratio of HPMC/lactose/Avicel pH 102 was fixed 2:1:1. Formulations F1, F2, F5 and F6 had not proceeded to the granulation step, and formulations F3, F4, F7 and F8 acted as granulation formulations.

According to Figure 1, the release rate of PB core pellet was quite rapid; 97% had been released within 30 min, thus there was no evidence of an SR effect. In this study, using the HPMC as an SR material could produce an extended drug release effect. The release rate of formulations F1~F8 were all slower than the wet-extruded and spheronized core pellets. The release of PB in different formulations was governed by the viscosity grade of the HPMC. The release rate of PB increased as HPMC viscosity decreased, for example (F5 and F6), because of the higher porosity, lower gel-strength, faster erosion, and lower tortuosity polymeric nature of HPMC K4M

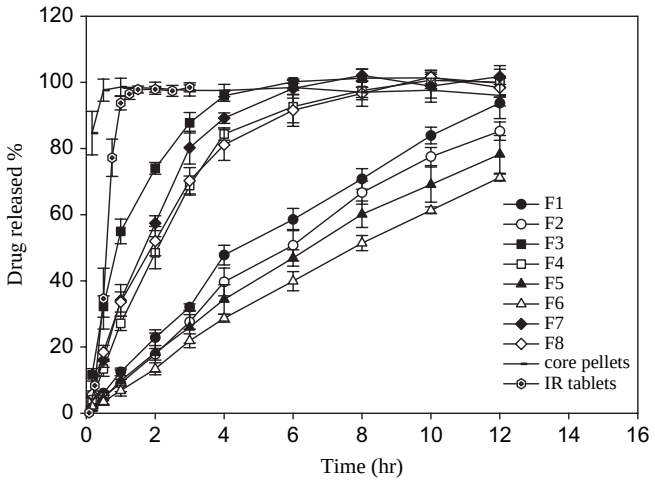

FIGURE 1. Dissolution profiles of optimum core pellets, IR tablets, and PB-SR tablets using 2^3 full factorial design.

(F5; viscosity = 4000 cps), it had a higher release rate in comparison to HPMC K100M (F6; viscosity = 10,000 cps).

There is a clear difference in PB release pattern between the formulations of the preparation process with/or without granulation, while the presence of granulation formulations (F3, F4, F7, and F8) appeared to increase the overall PB release rate from the SR tablets. This tendency was due to the pore structure and pore size distribution within the tablet and tablet surface, which can be seen in Figure 2 (SEM photograph). Figure 2 (a) shows that formulation (e.g., F8) of tablets prepared with a granulation step have a rougher and more uneven surface than those prepared without a granulation step (e.g., F6). The surface of tablet prepared without a granulation step appears smooth (Figure 2(b)). Based on this reason, when an SR tablet (prepared with a granulation step) comes into contact with a dissolution medium, more water could permeate through the surface cracks or pores than that prepared without a granulation step (Lee et al., 1999). The cross section of tablets prepared with a granulation step (e.g., F8) showed that PB core pellets were distributed on one side of the tablet, and were not well mixed with external excipient granules (see Figure 2(c)). In the dissolution test, one side of PB core pellets within the tablet had an easier contact dissolution medium than PB core pellets with uniform distribution in external excipient powder (see Figure 2(d)); for this reason, the drug release rate of tablets with a granulation step was faster than those without a granulation step.

In Figure 1, these dissolution profiles indicate that drug release for tablets (without granulation step) of the optimized core pellets with pure HPMC powder (formulations F5 and F6) is slower than those with powder of the ratio of HPMC/lactose/Avicel pH 102 = 2:1:1 (formulations F1 and F2) after 4 hr. In accordance with these results, we may know that an increase in HPMC concentration would also increase the viscosity of the

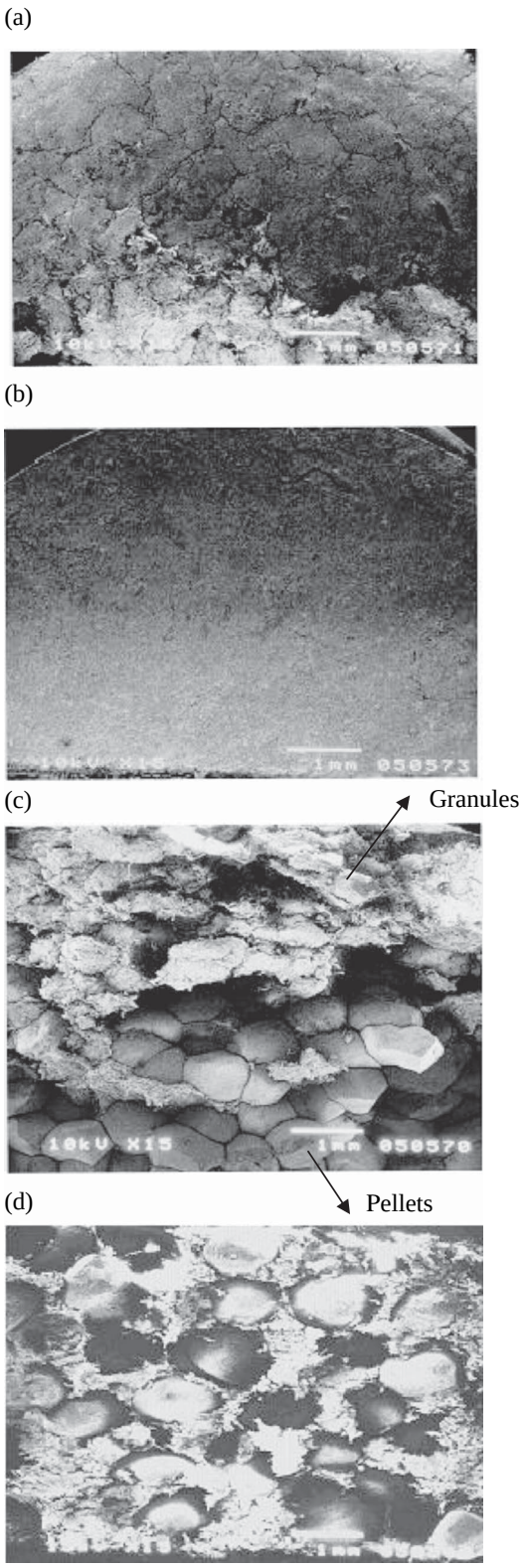

FIGURE 2. Photographs of SEM (a) Surface of tablet F8, (b) Surface of tablet F6, (c) Cross section of tablet F8 and (d) Cross section of tablet F6. Observed from tablets were prepared by the optimum core pellets with respective different HPMC external excipients formulations (magnification ×15).

surrounding fluid, which would increase gel-strength to cause a slower drug release rate. Lactose, a water-soluble diluent contained in tablets, could decrease the tortuosity of the tablets and diffuse outward forming channels. Therefore, the formulations of tablets containing a higher ratio of lactose will have a higher drug release rate.

The results of the dissolution parameters derived from $\frac{Mt}{M\infty} = Kt^n$ as shown in Table 2 indicate the release of the PB-SR tablets is almost identical to the non-Fickian mechanism, as the *n* values were between 0.5 ~ 1. However, the *n* value of the formulation F5 equal to 0.95 is close enough to one and hence assumes zero order kinetics. In addition, the formulation F3 followed the Fickian diffusion mechanism (*n* value approaching 0.5). However, the final goal measurement of the SR dosage form was expected to achieve a zero-order release mechanism (n value approaching 1). In this study, the experimental design will be utilized to screen the optimum PB-SR tablet (n value approaching 1) for further IVIVC study.

Lastly, the results of ANOVA from 2^3 full factorial design showed that the primary effects of the three factors (HPMC type, with or without granulation step and ratio of HPMC/lactose/Avicel pH 102) have statistical significance ($p < 0.05$) on the drug release percentage at 6 hr. The results indicated that these factors can influence the drug release percentage in a middle stage. However, the external excipients with or without granulation steps have statistical significance ($p < 0.05$) on the drug release percentage at 1, 6, 12 hr and *n* value. The results indicated that the formulations of external excipients with or without granulation steps not only influenced the release rate but also determined the drug release mechanism.

Optimization of Formulations

According to the standard of the USP monographs, the drug extended release dosage forms would have to specify the percentage of drug released after more than one time point. Therefore, the percentages of drug release after 1, 6, and 12 hrs were selected as response variables to determine an overall optimum drug released region in this study. The conditions of optimum formulation were selected to set the n value equal to 1, and the range of drug release percentage was restricted to 5% < R% of 1 h < 20%; 30% < R% of 6 h < 60%; 80% < R% of 12 h < 100%.

A commercially available statistical software package (DESIGN EXPERT V 6.0.3, Minneapolis) was used to infer the optimum formulation. Optimum response (predicted value) was inferred to find that release percentage of 1, 6, 12 hr and *n* value equal to 15.84, 58.56, 93.10, and 0.89 at A_2, B_2, and C_2 variables were set for HPMC K4M, without granulation and HPMC/lactose/Avicel pH 102=2:1:1, respectively. In order to verify these values, the optimum formulation was prepared according to the foregoing values of the factors and subjected to the dissolution test. The dissolution profile (observed value) of the optimum formulation and the predicted profile are shown in Figure 3. Using the FDA recommended similarity factor f_2 and difference factor f_1 to compare both profiles (FDA, 1997) and the results of f_2 value and f_1 value were 75.92 and 5.09. The results indicated that the dissolution profile (observed value) of the optimum formulation was similar to the predicted profile of the optimum formulation. The n value of optimum formulation was computed by simple exponential equation equal to 0.89, and showed not statistically significant ($p > 0.05$) value as that observed value compared with the predicted value ($n=0.89$). Further, this optimum formulation was selected to carry out in vivo-in vitro correlation study.

Open Disc Exposure to Accelerated Stability Conditions

In a preliminary study, the pure drug (PB) was put under ambient conditions; its rapid transformation from solid to liquid state occurred within 10 min. As well as this, a matrix tablet prepared by the mixture of pure drug (PB) with HPMC excipients also produced hygroscopic characteristics; it softened in 2 ~ 3 days under ambient conditions after fabrication.

In order to improve hygroscopic properties of the drug (PB), the core pellets were prepared by a water-insoluble excipient (Avicel pH 102) using the extrusion-spheronization method that was mentioned in a previous study (Huang et al., 2007) and PB-SR tablets were further prepared by direct compression of wet-extruded and spheronized core pellets with HPMC excipients. According to the results in Table 3, compared with wet-extruded and spheronized core pellets, the optimum SR tablets at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH chambers from 1 hr to 2 weeks did not show significant increase in moisture absorption. In other words, the tablet formulation had a more protective effect than the wet-extruded and spheronized core pellets in moisture absorption. In this study, the tablets were designed by an extrusion-spheronization

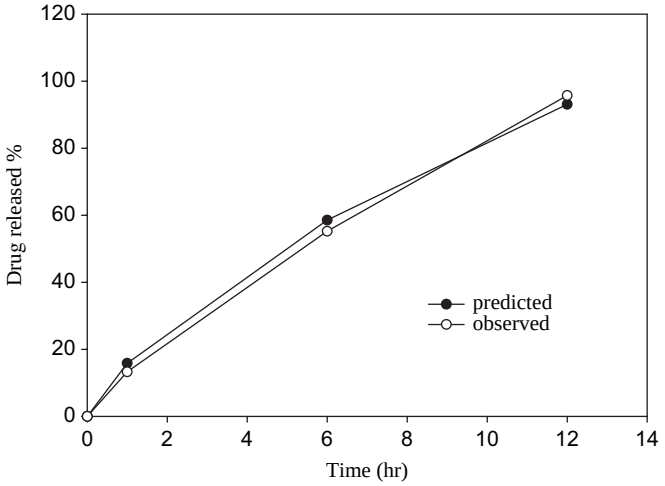

FIGURE 3. Comparison of the observed dissolution profile with the predicted profile of optimum SR tablets that sifted from 2^3 full factorial design.

TABLE 3
Moisture Content of (a) Optimum PB-SR Tablets and (b) Optimum Core Pellets on Storage

	Moisture Content (%)		
Time	25°C/60% RH	30°C/65% RH	40°C/75% RH
(a) Optimum PB-SR Tablets			
Initial	3.01 ± 0.45	3.01 ± 0.25	3.01 ± 0.45
1 hr	3.12 ± 0.36	3.13 ± 0.32	2.94 ± 0.48
4 hr	3.08 ± 0.35	3.42 ± 0.13	3.23 ± 0.53
8 hr	2.94 ± 0.56	3.25 ± 0.36	3.12 ± 0.21
12 hr	3.01 ± 0.38	3.02 ± 0.49	3.43 ± 0.54
1 day	3.38 ± 0.53	2.91 ± 0.07	3.17 ± 0.23
4 days	3.29 ± 0.25	3.41 ± 0.21	3.20 ± 0.38
7 days	3.54 ± 0.09	3.55 ± 0.71	3.50 ± 0.20
2 weeks	3.96 ± 0.32	3.73 ± 0.26	3.78 ± 0.57
3 weeks	4.52 ± 0.57	4.24 ± 0.12	4.37 ± 0.70
4 weeks	4.86 ± 0.54	4.78 ± 0.47	4.83 ± 0.20
(b) Optimum Core Pellets on Storage			
Initial	3.47 ± 0.15	3.47 ± 0.15	3.47 ± 0.15
1 hr	3.45 ± 0.36	2.93 ± 0.44	3.07 ± 0.34
4 hr	4.37 ± 0.18	3.92 ± 0.53	3.89 ± 0.23
8 hr	4.78 ± 0.75	4.59 ± 1.40	4.41 ± 0.21
12 hr	4.07 ± 0.87	4.82 ± 0.42	4.57 ± 0.30
1 day	4.89 ± 0.89	4.32 ± 0.30	4.09 ± 0.91
4 days	4.88 ± 1.88	4.58 ± 0.17	4.85 ± 0.77
7 days	4.19 ± 0.19	4.53 ± 0.16	4.30 ± 0.19
2 weeks	4.27 ± 0.86	4.47 ± 0.21	4.47 ± 0.28
3 weeks	4.16 ± 0.16	4.18 ± 0.35	4.99 ± 0.30
4 weeks	4.28 ± 0.37	4.45 ± 0.78	4.56 ± 0.78

Reference: Huang et al., 2007.

TABLE 4
Pharmacokinetic Parameters of PB After Oral Administration of Optimum PB-SR Tablets and Commercial IR Tablets

	PB-SR Tablets	IR-Tablets
T_{max} (h)	4.83 ± 1.60	0.65 ± 0.082
C_{max} (ng/ml)	137.56 ± 60.00	251.87 ± 27.51
$AUC_{0-24\,h}$ (ng/ml×h)	1153.34 ± 488.08	734.88 ± 230.68
$AUC_{0-\infty}$ (ng/ml×h)	1695.95 ± 617.65	906.82 ± 235.05
Ke (h^{-1})	0.14 ± 0.05	0.27 ± 0.03

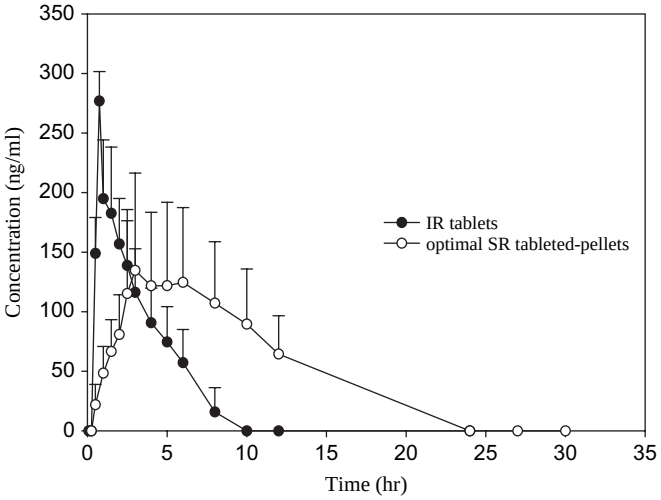

FIGURE 4. Mean plasma concentration of PB after administration of the optimum SR tablets and IR tablets in rabbits (n = 6).

method, and a direct-compression method not only obviously improved the hygroscopic character of PB but achieved SR effect as well.

Pharmacokinetics of In Vivo Absorption Study

According to the results shown in Table 4 and Figure 4, the optimum SR tablets of PB achieved a significant ($p < 0.05$) prolongation of T_{max} value compared with the commercial IR tablets that described in former research (Huang et al., 2007) after the oral administration of both tablet types. When the PB was presented in the optimum SR tablets, the therapeutic period of PB was extended.

There were also significant differences ($p < 0.05$) in the $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$ between the two dosage forms (PB-SR tablets and IR tablets). The mean C_{max} of 137.56 ± 60.00 ng/ml for the SR tablets was lower than the mean C_{max} of 251.87 ± 27.51 ng/ml for IR tablets; this phenomenon was also found in vitro dissolution testing for both tablet types. There was a significant noticeable difference ($p < 0.05$) in the AUC from the SR as compared to the IR tablets; the AUC of the SR tablet was actually higher than the IR tablet, thereby demonstrating that the extent of absorption of PB is different. When the PB is presented in SR tablets, the extent of absorption of PB is slower and more prolonged than IR tablets.

In Vitro-In Vivo Correlation Study

A level "A" in-vitro-in-vivo correlation was selected to investigate percent-dissolved versus percent-absorbed data for optimum PB-SR tablets in this study. In vitro dissolution study, the conditions were selected water as a dissolution medium at 50 rpm. The Wagner-Nelson model was used to calculate the percent absorbed of the PB-SR tablets in vivo absorption profiles from a single dose. The results of Figure 5 showed the fraction absorption (FA) in vivo versus fraction dissolution (FD) in vitro at the same point have a good linear regression relationship (r = 0.9871). This condition indicated that the in vivo fraction absorbed could be predicted in vitro fraction dissolved from dissolution data (Rao et al., 2001).

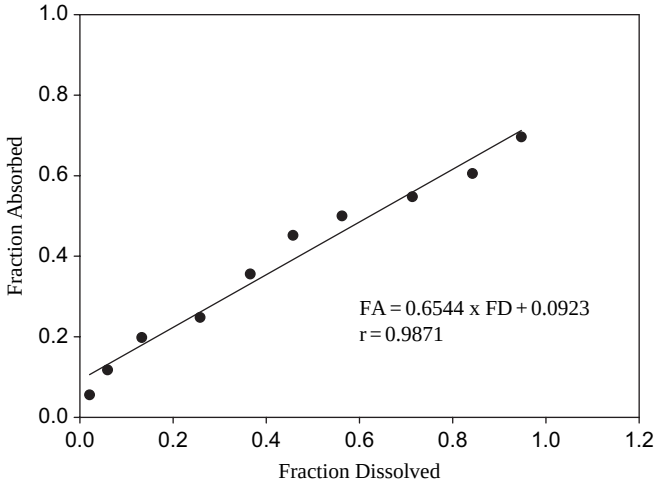

FIGURE 5. Relationships between the percent release and the percent absorbed for optimum PB-SR tablets in rabbits (r = 0.9871).

CONCLUSION

According to the results of ANOVA from 2^3 full factorial design, the parameters of HPMC type, with or without granulation step and ratio of HPMC/lactose/Avicel pH 102 for optimized PB-SR tablet fabrication were significant ($p < 0.05$) on the drug release percentage at 6 hr. 2^3 full factorial design was used to obtain a optimum SR tablet that showed specific release rates at different time intervals (release percentages of 1, 6, and 12 hr were 15.84, 58.56, and 93.10%) and followed the zero-order mechanism (n = 0.89). The results of moisture absorption by Karl Fischer meter showed that for the optimized SR tablet at 25°/60% RH, 30°/65% RH, and 40°/75% RH chambers from 1 hr to 2 weeks, the moisture absorption was not significantly increased. In this study, the PB-SR tablets did not only have SR efficiency but improved the hygroscopic defect of PB also. In the in vivo study, the PB-SR tablet showed a prolonged T_{max} and a relatively high AUC as compared with the IR tablet. In other words, the PB was prepared to SR tablet could decrease the dissolution rate of a high water-soluble drug and prolong its therapeutic period in vivo when compared with the IR tablet. Finally, we believe that the PB-SR tablet would improve defects of PB, decrease the frequency of administration and enhance the retention period of drug efficacy in vivo while personnel are exposed to "Soman" nerve gas attacks in the future.

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