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Formulation Design of a Highly Hygroscopic Drug (Pyridostigmine Bromide) for its Hygroscopic Character Improvement and Investigation of In Vitro/In Vivo Dissolution Properties

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ABSTRACT Pyridostigmine bromide (PB) sustained-release (SR) pellets were developed by extrusion-spheronization and fluid-bed methods using Taguchi experimental and 2^3 full factorial design. In vitro studies, the 2^3 full factorial design was utilized to search for the optimal SR pellets with specific release rate at different time intervals (release percent of 2, 6, 12, and 24 hr were 6.24, 33.48, 75.18, and 95.26%, respectively) which followed a zero-order mechanism (n = 0.93). The results of moisture absorption by Karl Fischer has shown the optimum SR pellets at 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH chambers from 1 hr–4 weeks, attributing that the moisture absorption was not significantly increased. In the in vivo study, the results of the bioavailability data showed the T_{max} (from 0.65 ± 0.082 hr–4.82 ± 2.12 hr) and $AUC_{0-30\ hr}$ (from 734.88 ± 230.68 ng/mL.hr–1454.86 ± 319.28 ng/mL.hr) were prolonged and increased, as well as C_{max} (from 251.87 ± 27.51 ng/mL–115.08 ± 14.87 ng/mL) was decreased for optimum SR-PB pellets when compared with commercial immediate-release (IR) tablets. Furthermore, a good linear regression relationship (r = 0.9943) was observed between the fraction dissolution and fraction absorption for the optimum SR pellets. In this study, the formulation design not only improved the hygroscopic character of PB but also achieved the SR effect.

KEYWORDS Sustained release pellets, Taguchi experimental design, 2^3 full factorial design, Zero-order mechanism, In vitro-in vivo relationship, Hygroscopic character

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INTRODUCTION

Nerve agents (NAs) pose a significant threat when used in wars or terrorist attacks. The two most widely known cases in which NAs were used was the 1991 Persian Gulf War and the 1995 sarin attack on the Tokyo subway system.

Thousands were killed or injured in the attack (Sidell et al., 1997; Kang et al., 2002).

Pyridostigmine bromide (PB), a carbamate derivative of reversible acetylcholinesterase (AChE) inhibitor, first approved by Food and Drug Administration (FDA) in 1955, was used to treat a neuromuscular disease called myasthenia gravis. Until 2003, FDA announced approval of PB as a pretreatment drug of neural toxicity for a combat to increase survival after exposure to "Soman" nerve gas poisoning (FDA, 2003). The "Soman" nerve gas takes only two minutes for 50% of the AChE-Soman complexes to "age", permanently inactivating AChE. Therefore, it is significant to take PB before wars or terrorist attacks that could provide competition with NA (Soman) to bind the AChE site. For this reason, it has decreased the peripheral cholinergic toxicity, as well increased survival after exposure to "Soman" nerve gas poisoning (Dirnhuber & Green, 1978). The approved dose (one 60 mg tablet every 8 hr) used for myasthenia gravis is higher than the dose (one 30 mg tablet every 8 hr) used for pretreatment to protect against Soman. There is a 180 mg extended release coated tablet (Mestinon®) in the market for the twice-daily administration against myasthenia gravis. However, there are no low dose sustained-release (SR) dosage forms of PB to prevent against NAs at present.

Because of its high aqueous solubility, high hygroscopic property (its rapid transformation from solid to liquid state under the ambient condition), short elimination half-life (1 ~ 2 hr) and exhibition of side effects, a modified released dosage form of PB such as microparticles has been developed (Hegazy et al., 2002). However, high initial release was observed in all the formulations of this paper and drug release might not fulfill the SR requirement. Moreover, this paper did not aim to discuss defect of high hygroscopic property of PB and to provide its solution.

Pellets are usually found in the pharmaceutical, agricultural, and polymer industries (Vervaet et al., 1995). The numerous advantages of pellets are discussed in previous papers (Bechgaard & Hegermann-Nielsen, 1978; Gandhi et al., 1999). The most popular method of producing pellets is by the extrusion-spheronization technique. This process was first reported by Reynold (1970), (Conine & Hadley, 1970; Reynold, 1970) and involves four steps. Among the specific pelletization processes, the processing equipment employed and formulation characteristics are critical for the development of pelletized pharmaceutical products, it is actually that which dictates both the successful production and the nature of the pellet dosage forms (e.g., size, size distribution, and resultant shape) (Ragnarsoson & Johansson, 1988, Lövgren & Lundberg, 1989). In order to achieve the SR purpose of pellet prepared by extrusion and spheronization, film coating is effectively used to modify the release of active ingredients from pellets. There are many materials that are found to be suitable for the production of controlled-release coating, e.g. Surelease®, Eudragit®, or Aquacoat ® (Porter, 1997).

In the last few years, statistical tools have been used for investigation of variables of the manufacturing process and formulations in pharmaceutical aspects (Chariot et al., 1987; Hileman et al., 1993; Bodea & Leucuta, 1997). In order to readily reach our goal, a computer optimization technique, based on a fraction factorial design (Taguchi orthogonal array) and 2^3 full factorial design utilizing polynomial equations were used to search for the optimum formulation, and efficiently quantify the influences of formulation variables on the drug release.

In our research, as PB is a highly soluble and hygroscopic drug, first, the core pellets were prepared by water-insoluble excipient (Avicel pH 102) using extrusion-spheronization method and the Taguchi experimental design was further used to estimate formulations and process parameters of extrusion-spheronization. Then, the surface of core pellets were coated with the seal layer coating (Opadry II layer), the sustained layer coating (Surelease® layer), and the waterproof layer coating (Opadry II HP) using fluid-bed method; and the 2^3 full factorial design was utilized to search for the optimal formulation with specific release percent at different time intervals and drug release mechanism (n-value). By using these manufacture methods and materials, we expected that the drug release system would exhibit SR (zero-order release mechanism) effect and improve the defect of drug hygroscopic character. Furthermore, the pharmacokinetics of the optimal PB-SR pellets was evaluated using rabbits to explore the relationship between in vitro release and in vivo absorption.

MATERIALS AND METHODS

Materials

PB (Paragon Technology, Inc., California) was used as a model drug for treatment of myasthenia gravis

and was developed as a pretreatment drug of neural toxicity. Microcrystalline cellulose (Avicel pH 102, Asahi Kasei Corporation, Japan) was used as a spheronizing aid, hydroxypropyl methyl cellulose (HPMC K4M, Dow Chemical Company, Le., South Africa) was used as a binder, and Surelease®/E-7–19010 (Colorcon, Indiana) was used as a SR material. Opadry II and Opadry II HP (Colorcon, Japan) were chosen as seal-coating and waterproofing material for pellet coating, respectively. Other chemicals used in this study were of analytic grade.

Preparation of PB Core Pellets

Preparation parameters of the extrusion-spheronization process for each batch, evaluated by Taguchi experimental design are listed in Table 1. The total batch size of each formulation was 240 g. First, the required quantities of excipients (184 g of Avicel pH 102 and 8 g of HPMC K4M) were sieved through a 40-mesh screen and mixed with the model drug (48 g of PB) for 10 ~ 15 minutes in a planetary mixer. Then, the binder solutions (with different ratios of ethanol/water) were added to the formulations to render a wet mass. Next, the wet mass was extruded through a ram extruder and spheronized on a plate spheronizer (Shang Yuh Machine Co., Ltd., Taiwan). After the extrusion-spheronization process, the core pellets were oven-dried at 40°C for 4 hr. The manufacturing run order was randomized.

Microscopic Image Analysis (Determination of Pellet Shape)

Variety of factors can determine the shape of pellets. In this study, an elongated character was selected as

TABLE 1 **Taguchi Experimental Design Matrix and Response Results for Measures**

	Actual values of independent variables						Responses	
No.	A_1	B_1	C_1	D_1	E_1	F_1	X_1 (elongation)	X_2 (yield %)
1	1.0	700	5	30	2	90/70	2.31	44.85
2	1.0	700	5	60	4	110/50	2.48	57.15
3	1.0	700	10	30	2	110/50	2.29	47.48
4	1.0	700	10	60	4	90/70	2.26	58.62
5	1.0	1100	5	30	4	90/70	1.39	59.71
6	1.0	1100	5	60	2	110/50	1.29	50.92
7	1.0	1100	10	30	4	110/50	1.17	56.67
8	1.0	1100	10	60	2	90/70	1.19	51.62
9	1.2	700	5	30	4	110/50	1.49	51.57
10	1.2	700	5	60	2	90/70	1.71	51.95
11	1.2	700	10	30	4	90/70	1.64	51.15
12	1.2	700	10	60	2	110/50	1.51	45.28
13	1.2	1100	5	30	2	110/50	1.17	34.75
14	1.2	1100	5	60	4	90/70	1.21	47.82
15	1.2	1100	10	30	2	90/70	1.09	36.66
16[c]	1.2	1100	10	60	4	110/50	1.08	32.81

	Levels	
Independent variables	Low level	High level
Extrusion screen opening (mm) (A_1)[a,b]	1.0	1.2
Spheronization speed (rpm) (B_1)[a]	700	1100
Spheronization time (min) (C_1)	5	10
Extrusion speed (rpm) (D_1)	30	60
Mixing time of active drug and ingredients (min) (E_1)	2	4
Ratio of water/ethanol (mL) (F_1)	90/70	110/50

[a]The independent variables have a significant effect on pellet elongation ($p < 0.05$).
[b]The independent variable has a significant effect on pellet yield % ($p < 0.05$).
[c]The optimal core pellets were chosen for further coating.

the pellet shape. Firstly, 70 PB core pellets were selected for analysis by optical microscopic imaging. The image analyzer consisted of a computer system linked to a video camera and a microscope (40×). Pellet elongation was calculated using the following equation:

$$Elongation = \frac{\max\ feret\ diameter}{\min\ feret\ diameter} \qquad (1)$$

It should be noted that Feret diameter is the distance between two tangents on opposite sides of the particle, parallel to some fixed direction (Martin et al., 1993; Paterakis et al., 2002). The theoretical value of elongation for a perfect sphere is 1.

It was observed that when the core pellet shape was more spherical, the elongation value approached 1.

Sieve Analysis (Determination of Yield of Core Pellet)

The amount of each formulation was accurately weighed, then shaken at an amplitude of 5 mm for 10 min through a series of sieves (425, 500, 600, 710, 850, 1000, 1180, and 1440 μm) with the Octagon Digital series 2000 sieve shaker (Endecotts Limited, England). Afterwards, each sieve was reweighed to determine the weight fraction of pellets retained on each sieve after shaking. These weights were converted to a mass percentage (Rambali et al., 2001). According to the mass percentage data, the largest weight fraction of pellet retained on each sieve was defined as "the highest pellet yield %". The highest pellet yield % was used as a dependent variable in the Taguchi experimental design for the extrusion-spheronization process. In this study, the range of the highest pellet yield % was found at 1000 ~ 850 μm sieves.

Preparation of PB-SR Pellets

250 g optimum core pellets (1000 ~ 850 μm) were chosen for further coating. Pellet coating was carried out with laboratory sized fluid-bed equipment (Yen Chen Machinery Co., Ltd., Taiwan) fitted with Wuster-insert. According to Table 2 (2^3 full factorial design matrix), the seal coating, the SR coating, and the waterproof coating have been finished, respectively. The structure drawing of SR pellet is shown in Fig. 1.

Seal Coating (Runs 2, 5, 7, and 8 of 2^3 Full Factorial Design Matrix)

A freshly prepared 25 g, 10% w/w aqueous solution of plasticized hydroxypropyl methylcellulose concentrate (Opadry II) was used as a seal-coating material. Before the application of the polymeric dispersion (Surelease®), a seal-coat was applied to the PB core pellets in order to decrease roughness on the pellet surface.

TABLE 2 **Formulations of PB-SR Pellets for Film Coating in 2^3 Full Factorial Design**

	Actual values of independent variables			Responses				
Run	A_2	B_2 (g)	C_2	Y_1 (Released % 2 hr)	Y_2 (Released % 6 hr)	Y_3 (Released % 12 hr)	Y_4 (Released % 24 hr)	Y_5 (*n*-value)
1	Uncoating	37.5	Coating	74.233	97.3839	97.2081	95.7432	0.5884
2	Coating	37.5	Uncoating	2.7108	25.4891	75.2222	89.5425	0.9525
3	Uncoating	52.5	Coating	39.8369	86.5402	93.8622	94.0056	0.7163
4	Uncoating	52.5	Uncoating	44.2538	97.452	98.7047	99.9584	0.6895
5	Coating	52.5	Uncoating	1.7095	14.1331	48.1715	78.1408	0.9898
6	Uncoating	37.5	Uncoating	52.8258	95.879	100.077	98.6013	0.6726
7	Coating	52.5	Coating	12.7402	70.6747	88.9283	94.5168	0.8392
8	Coating	37.5	Coating	44.4697	94.6268	100.661	99.121	0.7012

Independent variables

Uncoating or coating seal layer (25 g Opadry II) (A_2)
Quantity of coating sustained release layer (Surelease) (B_2)
Uncoating or coating waterproof layer (25 g Opadry HP II) (C_2)

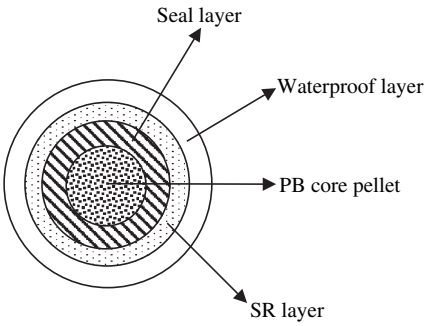

FIGURE 1 **Illustration of Structure of the PB-SR Pellet.**

Sustained-release Coating

Polymeric coating formulations for SR coating were prepared prior to pellet coating by diluting each Surelease® formulation with purified water to 12.5% w/w total solids content. The diluted dispersions were mechanically stirred for at least 30 min before being used for coating and the stirring was continued during the coating process.

Waterproof Coating (Runs 1, 3, 7, and 8 of 2^3 Full Factorial Design Matrix)

Because PB is a highly hygroscopic drug, a freshly prepared 10% w/w aqueous solution of plasticized polyvinyl alcohol concentrate (Opadry II HP) was used as a waterproof coating as protection from environmental moisture.

In vitro Dissolution Studies of PB-SR Pellets

In vitro release studies of PB-SR pellets were carried out with USP XXIV dissolution apparatus (Hanson Research Corporation, USA) in 900 mL water maintained at 37 ± 0.5°C as dissolution medium with a paddle speed of 50 rpm. Samples of the drug solution passed through a 0.45 μm filter at 10 and 30 min with an interval of 1, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hr and were collected using an automatic fractional collector (VanKel, a Member of Varian, Inc., USA). The amount of the dissolved drug was determined by HPLC (Hitachi, Ltd., Japan) at 269 nm. No interference from any of the dissolved HPMC K4M, Avicel pH 102, Opadry II, Opadry II HP or Surelease® occurred. Six replicates for each kind of pellet were tested.

Scanning Electron Microscopy

The uncoated (core pellets) and coated pellets were dried overnight and then mounted onto stubs using double-sided adhesive carbon tape. The uncoated (core pellets) and coated pellets were vacuum-coated with gold in an argon atmosphere using an FINE COAT JFC-1100E ion sputter (JEOL Ltd., Japan) for about 2 min to obtain a coating thickness of 200 Å. The surface of uncoated (core pellets) and coated pellets were examined under a scanning electron microscope (JSM-5300, JEOL Ltd., Japan).

Taguchi Experimental Design

The formulations and processes of PB core pellets were evaluated using Taguchi experimental design [L_{16} (2^{15})] that allowed investigation of six independent variables at two levels after performing 16 experiments shown in Table 1. Taguchi has envisaged an orthogonal design, a new method of designing experiments using a special set of arrays. Taguchi's orthogonal arrays are essentially fractional factorial experiments that allow simultaneous evaluation of all non-numerical or numerical parameters with a relatively small number of experiments. These standard arrays stipulate a method of conducting the minimal number of experiments to derive complete information on all factors affecting performance parameters. It assumes the main effect of variables without interaction with the variables (Li et al., 2002, 2003; Yang & Zhu, 2002).

In this study, each independent variable in the pellet preparation process, such as extrusion screen opening (A_1), spheronization speed (B_1), spheronization time (C_1), extrusion speed (D_1), mixing time of active drug and ingredients (E_1), and ratio of water/ethanol (F_1) were estimated by experimental analysis based on a Taguchi orthogonal table. Dependent variables were pellet elongation (X_1) and pellet yield % (X_2). Consequently, the PB core pellets were optimized.

2^3 Full Factorial Design

Factorial design is a useful tool to characterize multivariables. It allows for the possibility of separating the important factors from those which are not, and identifying any possible interaction between them. Single response optimization, even though widely used, could lead to misleading results, since different

release curves could show the same percentage of drug released at a single reference time (Montgomery, 1996).

In this study, 2^3 full factorial design was used to estimate three independent variables in the formulations of PB-SR pellets including coated or uncoated seal layer, quantity of sustained release layer (Surelease), and coated or uncoated waterproof layer; and dependent variables were selected as the percentages of drug released after four different times (2, 6, 12,and 24 hr) to detect the burst effect and ensure complete drug release and drug release mechanism (*n*-value). The different formulations of the factorial design consisted of all possible combinations of all factors at all levels and were conducted in a fully randomized order. The matrix of the experiments and results of the responses for every experiment are listed in Table 2. The statistical evaluation of the results was carried out by analysis of variance (ANOVA) using a commercially available statistical software package (DESIGN EXPERT V 6.0.3, Minneapolis, Minnesota, USA).

Release Mechanism of SR Pellets

The release mechanism of PB from SR pellets can be analyzed by the simple exponential equation

$$\frac{Mt}{M\infty} = Kt^n \qquad (2)$$

(Korsmeyer et al., 1983; Ritger & Peppas, 1987a,b Peppas & Sahlin, 1989).

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.

High Performance Liquid Chromatography (HPLC) Conditions

The drug-released percent in vitro study and drug-absorbed percent in vivo study of the PB-SR pellets were determined according to a previous study (Michaelis, 1990). The liquid chromatographic system consisted of a Hitachi L-7100 multisolvent delivery system pumps, a Hitachi L-7200 autosampler, and a Hitachi L-7455 diode array detector. The sample was detected at 269 nm and the column was a reverse-phase C18 (Purospher® STAR RP-18 endcapped, Merck, Germany).

Exposure to Stability Conditions for Estimating the Hygroscopic Character of PB

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

In vivo Absorption Study

Six healthy male rabbits weighing 3 ~ 4 kg were used in this study in accordance with a protocol approved by the Institutional Review Board-Use and Care of Animals at Kaohsiung Medical University. All rabbits were placed on a fast for 12 hr, but were allowed free access to water. After oral administration of optimum PB-SR pellets, the ear was shaved and an ear artery was cannulated using 24 g IV catheter and 2 ~ 3 mL of blood samples were taken at appropriate intervals. The samples were centrifuged 3000 rpm for 10 min, and plasma was kept frozen pending analysis. The analysis method and extraction method of PB plasma were determined according to a previous study (Michaelis, 1990). About 2 weeks (wash out period) after oral administration of optimum PB-SR pellets, the commercial IR-tablets followed above-mentioned methods proceeded to study further.

The measured plasma concentrations were used to calculate the area under the plasma concentration-time profile from zero time to the last concentration time point ($AUC_{(0-t)}$). The $AUC_{(0-t)}$ was determined by the trapezoidal method. The $AUC_{(0-\infty)}$ was determined by the following equation:

$$AUC_{(0-\infty)} = AUC_{(0-t)} + \frac{C_t}{K_e} \qquad (3)$$

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. The Wanger-Nelson model (Wanger & Nelson, 1964) was used to calculate the percentage of the PB dose absorbed (Roshdy et al., 2002; Takka et al., 2003; Huang et al., 2004).

$$FA_t = \frac{(C_t + K_e \times AUC_{(0-t)})}{K_e \times AUC_{(0-\infty)}} \quad (4)$$

Where FA_t is the fraction of drug absorbed at time t, C_t is the concentration of drug 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 vivo absorption values were directly related to in vitro dissolution data to complete the in vitro–in vivo correlations (IVIVC). Linear regression analysis was applied to the IVIVC plots and coefficient of relation (r-value), slope, and intercept values were calculated. All other comparisons were made using ANOVA analysis.

RESULTS AND DISCUSSION

PB is a chemical, characterized with a high moisture absorption rate. Placed under the ambient condition, it will liquefy in less than 10 minute. In this study, the water-insoluble excipient (Avicel pH 102) was used as the material to manufacture the core pellets and the core pellets were further coated with waterproof materials in order to reduce the moisture absorption rate and achieve the desired release effect.

Evaluation of Preparation Process Variables of PB Core Pellets Using Taguchi Experimental Design

The quality of the produced pellets (e.g. size, the size distribution, and the shape of the pellets) is important for the uniformity of filling into hard gelatin capsules or success of the coating procedure. The purpose of the pelletization process is to produce spherical particles of acceptable size and size distribution.

The prerequisite for successful processing is the proper shape of the pellets. Calculation of the shape factor (elongation) is an important parameter for evaluating pellet shape.

The extrusion screen opening and the spheronization speed are very important parameters in the pellet shape of this study. Higher spheronization speeds and larger extrusion screen openings resulted in increasingly spherical pellets. In previous papers it was observed that the diameter of extrusion screen openings determines the size of the pellet, however, the influence of the opening diameter of extrusion screen on the pellet roughness has not been mentioned. According to papers in the past (Baert & Remon, 1993), it is found that extruded plastic cylinders are rounded in the form of pellets because of the frictional forces as well as the rotational forces. Cylinders transform into cylinders with rounded edges, then to dumb-bells and elliptical particles, and eventually to perfect spheres. When the rate of spheronization plate is higher, the frictional forces and rotational forces are also relatively higher, and the pellet becomes rounder. Table 1 shows the results of pellet elongation calculated by Eq. (1). The results of statistical evaluation by ANOVA analysis showed that extrusion screen opening and spheronization speed have p-values less than 0.05, which indicates that they have a significant effect on elongation. As extrusion screen opening and spheronization speed increased, pellet elongation significantly decreased and this tendency resulted in increasingly spherical pellets. If the experiments are ranked according to the quality obtained, we note that experiments 15 and 16 yielded the best results, because their elongation value approached 1.

The yield percent of PB core pellets determined by sieve analysis and a pellet distribution range of each batch between 1000 ~ 850 μm sieves that were chosen as a response (dependent variable) for the Taguchi experimental design. The yield percent for the range at 1000 ~ 850 μm is listed in Table 1. According to the results of ANOVA analysis of the orthogonal experimental design presented that extrusion screen opening had a p-value less than 0.05. In other words, extrusion screen opening significantly affected the yield %. As diameter of the extrusion screen opening increased, pellet yield % significantly decreased, because the diameter of extrusion screen opening determines the size of the pellet. In this study, the range of the highest pellet yield % (dependent variable) was set at 1.0 mm ~ 850 μm sieves. While the core pellets were prepared by the extruded plastic cylinders that passed through the 1.0 mm

extrusion screen opening, the highest pellet yield was higher as compared to those that were prepared by way of passing through the 1.2 mm extrusion screen opening.

As a result of the low *p*-value (*p*-value: 0.0783) for mixing time of active drug and ingredients, the possible importance of this factor cannot be overlooked. When the mixing time of active drug ingredients was longer, the yield of pellets at 1000 ~ 850 μm sieves was higher. This was presumably due to water/ethanol acting as binders during wet massing; they could thoroughly mix with active drug and ingredients to form wetter masses than the shorter mixing time. Shorter mixing time would produce finer powders.

If the experiments are ranked according to quality (high yield % as well as sufficient roundness of PB core pellets) obtained, we note that the best result is from experiment 16, followed by experiment 15. The core pellets with the best results (experiment 16) were chosen for further coating.

In-vitro Dissolution Study and Release Mechanism of Drug Release From PB-SR Pellets

The dissolution profiles of all formulations required by 2^3 full factorial design are shown in Fig. 2. Table 2 showed the overall formulations (R1 ~ R8 formulations) coating two different quantities of Surelease (37.5 and 52.5 g). Formulations R2, R5, R7, and R8, on the other hand, were coated with seal layer (Opadry II), and the amount of coating was fixed at 25 g. Moreover, formulations R1, R3, R7, and R8 were coated with 25 g waterproof layer (Opadry II HP), respectively.

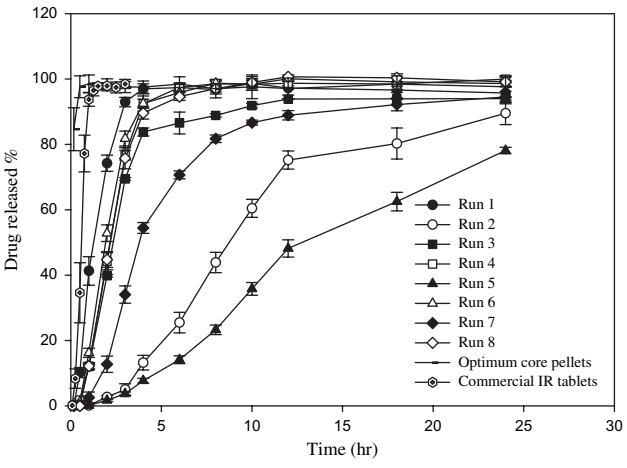

FIGURE 2 **Dissolution Profiles of Optimum Core Pellets, Commercial IR Tablets and Optimum PB-SR Pellets Using 2^3 Full Factorial Design.**

According to Fig. 2, the release rate of PB core pellet was quite rapid, 97% had been released within 30 minutes thus there was no evidence of the SR effect. In this study, using the Surelease as a SR material could produce an extended drug release effect. The release rate of formulations R1 ~ R8 were all-slower than the uncoated core pellets. The quantity of Surelease coating not only influenced the release rate but also determined the drug release mechanism. When the pellet was coated with a high level of Surelease, the drug release was slower than the pellets coated with a low level of Surelease. The observed differences in release rates are attributed to the thickness of the Surelease coating; the thicker the Surelease coating, the relatively longer the diffusion path length between the pellet core and the dissolution medium. Conversely,it was observed that the thinner the Surelease coating, the faster the release rate of the drug.

There is a clear difference in PB release pattern between the formulations of preparation process coating and uncoating seal layer, while the presence of seal layer formulation (R2, R5, R7, and R8) appears to decrease the overall PB release rate from the PB-SR pellets. This tendency was due to the surface structure of the extrusion-spheronization core pellet; if the surface of the extrusion-spheronization core pellet was coated with a seal layer, the core pellet surface becomes smoother than the core pellet coating without a seal layer, as seen in Fig. 3a,b.

Due to this reason, the core pellets were coated with a SR layer in addition to the seal layer coating, and a complete film was formed over the surface of SR pellets. In contrast to the core pellets that were not coated with a seal coating, the film of SR pellet produced some cracks, so that the drug release rate was faster than the extrusion-spheronization core pellet coated with a seal coating. For example, the release rate of formulation R4 was faster than formulation R5, because the film of formulation R4 was cracked, as shown in Fig. 3c,d.

As a result of PB being a hygroscopic drug; the coating of a waterproof layer prevented the influence of moisture. However, the use of waterproof material (Opadry II HP) in this study caused, instead, an increase in drug release rate. According to the manual of Colorcon Corporation, the adhesive force of

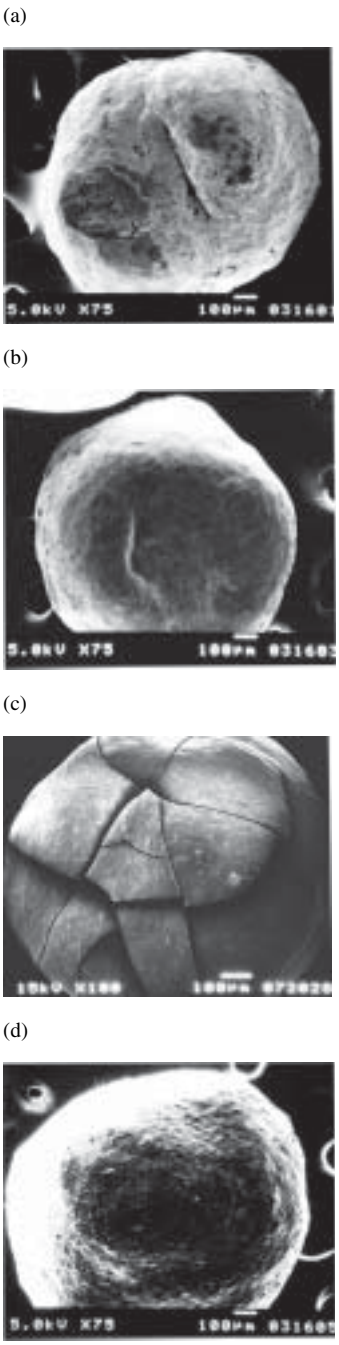

FIGURE 3 **Photograph of SEM (a) Optimum Core Pellet (Uncoating), (b) Optimum Core Pellet Coating With Seal Layer, (c) Run 4 and (d) Run 5 Observed From SR Pellets Using 2^3 Full Factorial Design.**

Opadry II HP response to tablet or pellet was stronger than other materials (e.g., HPC, HPMC, and Opadry), thereby causing a faster drug release. The adhesive force of Opadry II HP response to the Surelease pellet was stronger, subsequently destroying the structure of the Surelease film, and as a consequence, the drug release from the core pellet through the Surelease layer became faster. Adversely, if the outer layer of the Surelease pellet coating was not waterproof, the Surelease structure would have maintained a complete film, and the drug release rate would be slower than that of the pellet coated with waterproof material. For example, the release rate of formulation R7 was faster than formulation R5.

The results of examination of the dissolution parameters derived from $\frac{Mt}{M\infty} = Kt^n$ are shown in Table 2. The results indicate that the release of the PB-SR pellet is almost identical to the non-Fickian mechanism, as the *n*-values were between 0.5 ~ 1. However, the *n*-value of the formulation R5 approached 1.0 ($n = 0.99$); or in other words, the drug release rate maintained a constant release rate. In addition, the formulation R1 followed the Fickian diffusion mechanism (*n*-value approaching 0.5). In a previous paper, Zhang et al. pointed out that as the quantity of coating increase, not only does the drug release rate decrease, but the mechanism for the drug release rate changes from that defined by the square root of time to one that approaches zero order (Zhang et al., 1991). 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 had been utilized to screen the optimum PB-SR pellets and was then used them to carry out an IVIVC study.

Evaluation of Drug Release and Release Mechanism From SR-PB Pellets Using 2^3 Full Factorial Design

Main Effect of the Factors

From Table 3 it can be concluded that the primary effects of the three factors have statistical significance ($p < 0.05$) on the drug release percent at 2 and 6 hr, and the *n*-value. However, no statistical significance ($p > 0.05$) was found on the drug release percent at 12 and 24 hr. The results indicated that the three factors (seal layer, SR layer, and waterproof layer) could influence the drug release percent in an early stage and drug release mechanism. These findings correspond with the prior research (Zhang et al., 1991), which defined the transition point as the critical coating level and the physicochemical characteristics of the core would have significance for the drug release rate and

TABLE 3 ANOVA Test for Different Release Parameters From 2^3 Full Factorial Design

Source	Sum of squares	df[a]	Mean square	F Value	Probability > F
Released % of 2 hr (%)					
A_2	2783.30	1	2783.30	822.16	0.0222[b]
B_2	721.98	1	721.98	213.27	0.0435[b]
C_2	613.90	1	613.90	181.34	0.0472[b]
AB	12.34	1	12.34	3.65	0.3071
AC	162.89	1	162.89	48.12	0.0911
BC	404.02	1	404.02	119.35	0.0581
Released % of 6 hr (%)					
A_2	3712.26	1	3712.26	9.227E+0.05	0.0007[b]
B_2	248.41	1	248.41	61746.43	0.0026[b]
C_2	1689.91	1	1689.91	4.201E+0.05	0.0010[b]
AB	84.74	1	84.74	21064.46	0.0044[b]
AC	2281.04	1	2281.04	5.670E+0.05	0.0008[b]
BC	78.21	1	78.21	19439.26	0.0046[b]
Released % of 12 hr (%)					
A_2	738.61	1	738.61	19.76	0.1409
B_2	236.54	1	236.54	6.33	0.2409
C_2	427.55	1	427.55	11.44	0.1830
AB	145.05	1	145.05	3.88	0.2990
AC	682.77	1	682.77	18.27	0.1463
BC	22.26	1	22.26	0.60	0.5816
Released % of 24 hr (%)					
A_2	91.04	1	91.04	7.44	0.2237
B_2	33.56	1	33.56	2.74	0.3458
C_2	36.74	1	36.74	3.00	0.3332
AB	30.52	1	30.52	2.50	0.3593
AC	151.08	1	151.08	12.35	0.1765
BC	1.71	1	1.71	0.14	0.7720
Release mechanism (*n*-value)					
A_2	0.083	1	0.083	6274.79	0.0080[b]
B_2	0.013	1	0.013	965.82	0.0205[b]
C_2	0.026	1	0.026	1988.47	0.0143[b]
AB	1.163E-0.04	1	1.163E–0.04	8.77	0.2073
AC	0.015	1	0.015	1118.68	0.0190[b]
BC	5.602E-0.03	1	5.602E-0.03	422.44	0.0309[b]

A_2: Uncoating or coating seal layer (25 g Opadry II).
B_2: Quantity of coating sustained release layer (Surelease).
C_2: Uncoating or coating waterproof layer (25 g Opadry HP II).
[a]df: degrees of freedom.
[b]Significant at 95 % confidence level.

mechanism. According to results of the 2^3 full factorial design, we concluded that the slowest release rate of formulation was obtained when high levels of the seal coating and SR coating was followed by low levels of waterproof coating.

Interactions Between the Factors

An interaction is the failure of a factor to produce the same effect on the response at different levels from the other factor (Montgomery, 1996). The ANOVA results showed that the interaction of AB, AC, and BC had a significant influence on the release percent at 6 hr. The AB interaction especially, was synergistic, as it led to a decrease in the release percent at 6 hr; moreover, the interactions of AC and BC exhibited an opposite tendency that led to a decrease in the release percent at 6 hr. The interactions of AC and BC were also found to have a significant influence on the drug release mechanism.

Optimization of Formulations

In general, an optimal SR dosage form must have a minimal burst effect within most of the drugs are being released in a specific time period. The USP monographs for drug extended release dosage forms specify the percent of drug released after more than one time point; therefore the percentages of drug release after 2, 6, 12, and 24 hr were selected as the response variables. These time points were used to detect the burst effect at an earlier stage, and to ensure that most of the drug was released in a time period comparable to that the gastrointestinal residence time. Under these conditions, these four responses were then combined to determine an overall optimum region.

According to the results of overall formulations (formulations R1 ~ R8), using a commercially available statistical software package (DESIGN EXPERT V 6.0.3, Minneapolis) to infer the optimum formulation, the conditions of optimum formulation were selected to set the *n*-value equal to 1 and the range of drug release percent were restricted to 5% < R% of 2 hr < 20%; 30% < R% of 6 hr < 50%; 60% < R% of 12 hr < 80%; 90% < R% of 24 hr < 110%.

An optimum response (predicated value) was inferred to found with release percent of 2, 6, 12, 24 hr, and *n*-value of 4.89, 30.00, 80.00, 91.03%, and $n = 0.94$ at A_2, B_2, and C_2 values of 24.54, 33.22, and 0.00 g, respectively. To verify these values, the optimum formulation was prepared according to the above values of the factors and subjected to the dissolution test. The dissolution profile (observed values) of the optimum formulation and the predicted profile are shown in Fig. 4. The dissolution profile (observed values) of the optimum formulation with release percent of 2, 6, 12, and 24 hr were 6.24, 33.48, 75.18, and 95.26%. Both profiles were compared using the FDA recommended similarity factor f_2 and difference factor f_1 (FDA, 1997). The values of f_2 and f_1 were 70.78 and 6.74, respectively and do not exceed the critical value indicating equivalence to the release profile of the optimum formulation and the predicted profile. Besides, the *n*-value of optimum formulation as computed by simple exponential equation was 0.93, which is compared favorably with the predicted value ($n = 0.94$) and was not statistically significant ($p > 0.05$) as this value approached 1. This optimum formulation was chosen for a further IVIVC study.

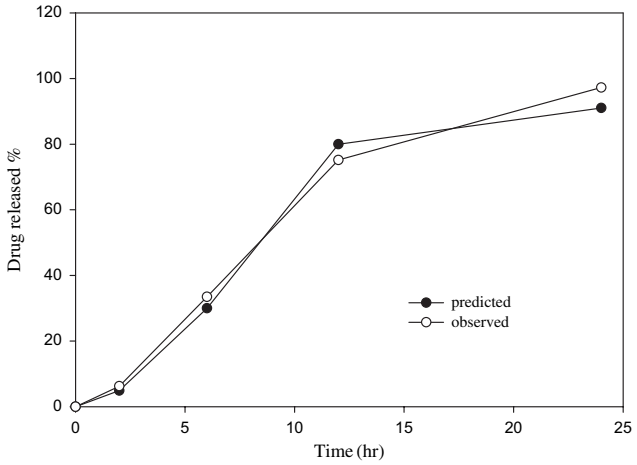

FIGURE 4 **Comparison of the Observed Dissolution Profile With the Predicted Profile of Optimum PB-SR Pellets That Inferred by 2^3 Full Factorial Design.**

Exposure to Accelerated and Long-term Stability Conditions

The pure drug (PB) and a matrix tablet with non-pellitized drug were studied in a preliminary study. According to the results of the preliminary study, the pure drug (PB) was put under the ambient condition, its rapid transformation from solid to liquid state within 10 minutes. Owing to a matrix tablet that was prepared by the mixture of a pure drug (PB) with HPMC excipients possibly to produce hygroscopicity, it was softened in 2 ~ 3 days under the ambient condition after fabricating. In order to improve hygroscopic character of pure drug (PB), first, the PB core pellets were prepared by water-insoluble excipient (Avicel pH 102) using extrusion-spheronization method, and then PB-SR pellets were prepared by fluid-bed coating of wet-extruded and spheronized core pellets with polymer solution. According to the results of Table 4, the rapid moisture absorption potential of uncoated pellets was increased after 4 hr at 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH chambers, respectively. Extending exposure time does not appear to further increase moisture absorption. The results showed that the core pellets were prepared by pure drug mixtures and water-insoluble excipient (Avicel pH 102) using extrusion-spheronization method could improve hygroscopic defect of pure drug (PB). In regard to the optimum SR pellets at 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH chambers from 1 hr–4 weeks, the moisture absorption was not significantly increased.

TABLE 4 Moisture Content of (A) Optimum PB-SR Pellets and (B) Uncoating Pellets (Optimum Core Pellets) on Storage

	Moisture content (%)		
Time	25°C/60 % RH	30°C/65 % RH	40°C/75 % RH
(A)			
Initial	2.36 ± 0.31	2.36 ± 0.31	2.36 ± 0.31
1 hr	2.40 ± 0.76	2.33 ± 0.62	2.54 ± 0.48
4 hr	2.28 ± 0.31	2.46 ± 0.13	2.63 ± 0.53
8 hr	2.34 ± 0.46	2.45 ± 0.26	2.52 ± 0.21
12 hr	2.31 ± 0.08	2.32 ± 0.02	2.43 ± 0.52
1 day	2.38 ± 0.53	2.91 ± 0.08	2.14 ± 0.23
4 days	2.49 ± 0.15	2.41 ± 0.15	2.90 ± 0.28
7 days	2.50 ± 0.15	2.25 ± 0.05	2.50 ± 0.20
2 weeks	2.76 ± 0.79	2.33 ± 0.29	2.48 ± 0.50
3 weeks	2.82 ± 0.56	2.54 ± 0.33	2.27 ± 0.17
4 weeks	2.86 ± 0.84	2.78 ± 0.68	2.87 ± 0.10
(B)			
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

TABLE 5 Pharmacokinetic Parameters of PB After Oral Administration of Optimum SR Pellets and Commercial IR Tablets

	PB-SR pellets	IR tablets
T_{max} (hr)	4.82 ± 2.12	0.65 ± 0.082
C_{max} (ng/mL)	115.08 ± 14.87	251.87 ± 27.51
$AUC_{0-30\ hr}$ (ng/mL.hr)	1454.86 ± 319.28	734.88 ± 230.68
$AUC_{0-\infty}$ (ng/mL.hr)	1676.22 ± 262.02	906.82 ± 235.05
Ke (hr^{-1})	0.09 ± 0.02	0.27 ± 0.03

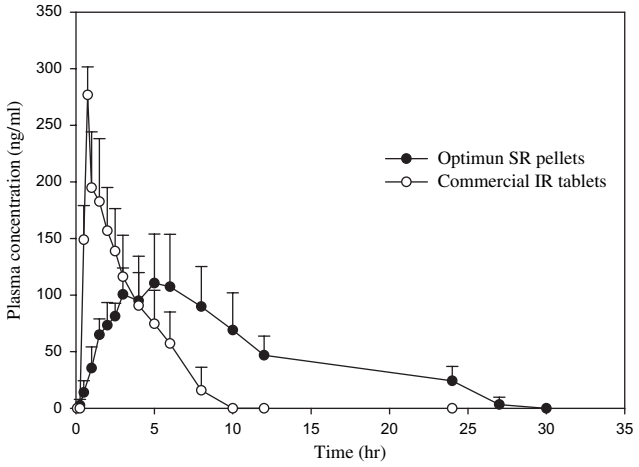

FIGURE 5 Mean Plasma Concentration of PB After Oral Administration of the Optimum SR Pellets and Commercial IR Tablets in Rabbits (n = 6).

This phenomenon showed that the surface of core pellets coated only with seal layer and sustained release layer could achieve the waterproof effect. From these results, it is observed that the formulation design not only has obviously improved the hygroscopic character of pure drug but also achieved SR effect in this study.

In vivo Absorption Study

The results of the mean pharmacokinetic parameters are summarized in Table 5, and the mean plasma PB concentration versus time profiles after the oral administration of the optimum PB-SR pellets and commercial IR tablets are shown in Fig. 5. The results showed that the optimum SR pellets of PB achieved a significantly ($p < 0.05$) prolongation of T_{max} value compared with the commercial IR tablets (T_{max} from 0.65 ± 0.082–4.82 ± 2.12 h). This suggests that the therapeutic period of PB was extended, after it was presented in an optimum SR formulation.

There are significant differences ($p < 0.05$) in the plasma level concentrations between the two dosage forms (optimum SR pellets and IR tablets). In addition, it was found that the results of release observed in the dissolution testing was also apparent in the plasma PB concentration profiles with a mean C_{max} of 115.08 ± 14.87 and 251.87 ± 27.51 ng/mL for the optimum SR pellets and IR tablets. The same results were also observed in the $AUC_{0-30\ hr}$ and $AUC_{0-\infty}$. There is a significantly noticeable difference ($p < 0.05$) in the AUC from the optimum SR pellets as compared to the IR tablets; the AUC of the optimum SR pellets is actually higher than the IR tablets, thereby demonstrating that the extent of absorption of PB is different.

Exploring a relationship between the in vivo absorption and in vitro drug release from a controlled-release dosage form is an important part of the dosage form development process (Qiu et al., 2003). A level

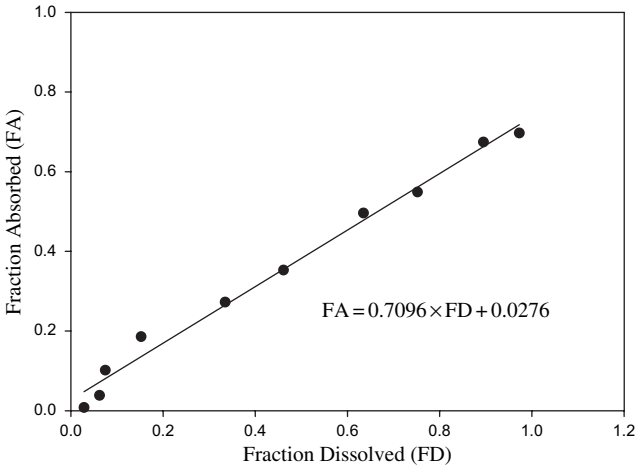

FIGURE 6 **Relationships Between the Percent Release and the Percent Absorbed for Optimum PB-SR Pellets in Rabbit (r = 0.9943).**

A in vitro-in vivo correlation was investigated using the percent dissolved versus the percent absorbed data for optimum SR pellets, using water as dissolution medium at 50 rpm. The apparent in vivo absorption profiles from the single dose study were calculated using the Wagner-Nelson method. The absorption profile versus percent release in vitro at the same point is shown in Fig. 6. A good linear regression relationship ($r = 0.9943$) was observed between the fraction dissolution and fraction absorption for the optimum SR pellets. This phenomenon indicated that the in vivo fraction absorbed could be predicted from in vitro dissolution data.

CONCLUSION

In this study, the optimum PB-SR pellets were developed by extrusion-spheronization and fluid-bed methods using Taguchi experimental and 2^3 full factorial designs. The optimum PB-SR pellets have good pellet properties, SR efficiency, and can overcome the defect of highly hygroscopic character of PB. The drug dissolution profiles of the drug delivery system exhibited zero-order release mechanism. Compared with IR tablet, the optimum PB-SR pellets exhibited a prolonged T_{max} and a relatively high AUC. This indicated that the optimum PB-SR pellets could decrease the dissolution rate of a high water-soluble drug and prolong its therapeutic period in vivo when compare with IR tablets. Finally, the once-daily PB-SR pellets with the desired in vivo performance were successfully designed.

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