Research Article

Optimization of Semha-Pinas Extract Orodispersible Tablets Using Response Surface Methodology

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Abstract
The original dosage form of the Semha-Pinas herbal formula, an expectorant in Thai traditional medicine, is in a pill form. However, it is inconvenient to use because it must be powdered and dissolved in hot water or juice of *Citrus x aurantium* L. before use. The development of a new dosage form presents a challenging prospect. This work aimed to develop Semha-Pinas extract orodispersible tablets based on the response surface methodology using the Box-Behnken design. Firstly, Semha-Pinas extract was tested for its safety in HepG2 cells. The safe extract was further developed as orodispersible tablets. Four levels of three factors — compressional force (500–2,000 psi), the quantity of microcrystalline cellulose (0–15%), and the quantity of croscarmellose sodium and sodium starch glycolate (0:8–6:2%) — were screened using the one factor at a time technique. The Box-Behnken design has three levels for each factor: 1,000–2,000 psi, 5–15%, and 2:6–6:2%, respectively. Tablet thickness, hardness, friability, and disintegration time were the four responses that were monitored. The results indicated the safety of the Semha-Pinas extract, even at a concentration of 5 mg/mL. The optimal orodispersible tablet formulation had a compressional force of 1,500 psi, microcrystalline cellulose of 10%, and croscarmellose sodium to sodium starch glycolate of 4:4%. In summary, this study successfully fabricated Semha-Pinas extract orodispersible tablets using response surface methodology, achieving the desired property of fast disintegration. Moreover, these findings can serve as a valuable reference for pilot scale and industrial scale production.

Keywords: Box-Behnken design, Expectorant, One factor at a time, Piperine, Thai traditional medicines

1 Introduction

Response surface methodology (RSM) is a group of statistical and mathematical methods that can be used to develop, improve, and optimize processes. It also plays a significant role in the development, formulation, and design of new products as well as the enhancement of the designs of already existing products [1]. The central composite designs and Box-Behnken designs are the most popular for standard second-order response surface designs [1]. The Box-Behnken designs are less experimental compared with central composite designs. It lacks the corner points of the central composite designs; therefore, all the factors will never be high or low at once (no extreme combinations). In a Box-Behnken design, each factor has 3 levels, as opposed to the central composite design that has 5 levels, which are 2 levels outside the setting [2].

Mucus-filled coughs are a sign of ailments of respiratory tract infections. Sometimes, it can happen as a reaction to irritation caused by allergens or irritants. It can be treated by expectorants or mucolytic agents such as Semha-Pinas (SHPN) used in Thai traditional medicine. It is composed of six herbal ingredients: seeds of *Brassica* spp., leaves of *Blumea balsamifera* (L.) DC., fruit pulps of *Terminalia chebula* Retz., fruits of *Piper nigrum* L., fruit peels of *Citrus hystrix* DC., and fruits of *Coriandrum sativum* L., in an equal weight ratio [3]. The pill is the original dosage

form of the SHPN. It is inconvenient to use because it must be powdered and dissolved in some solutions, such as hot water or juice of *Citrus x aurantium* L. before use [3]. The development of a new dosage form presents a challenging prospect. Orodispersible tablet, also known as ODT, is an uncoated solid dosage form that dissolves quickly when placed on the tongue. It can be disintegrated in saliva [4]. Utilizing water can occasionally be avoided if the disintegration occurs quickly enough. ODT provides further improves patient acceptability and compliance by ease of use [5]. ODT can be prepared using a variety of methods, such as lyophilization, direct compression, tablet molding, flash heat processing, and 3D printing [5], [6]. The two manufacturing processes that are most frequently employed are direct compression and lyophilization. A major drawback of direct compressed ODT is that it can be difficult to find the proper balance between fast disintegration and enough mechanical strength [5]. Lyophilized ODT is frequently more fragile than compressed ODT and therefore needs special packaging to protect the fragile tablet from environmental effects and guarantee stability. In general, compressed ODT disintegrates more slowly than lyophilized ODT. However, lyophilized ODT has a longer processing time and a higher production cost [7].

Previously, the authors developed effervescent tablets of Semha-Pinas extract (SHPNE), but water is needed to disperse/dissolve the tablets [8]. In this paper, we expanded the previous work by developing a new dosage form of SHPNE. The objective of this work was to develop a SHPNE ODT to promote rapid disintegration resulting in ease of use and improve patient convenience and compliance. The use of water can be avoided for this dosage form. Furthermore, this work used RSM to evaluate the effect of compressional force, quantity of binder, and quantity of superdisintegrants on the physical properties of SHPNE ODT to strike a balance between fast disintegration and enough mechanical strength. Furthermore, dissolution tests and chemical analyses were also done.

2 Materials and Methods

2.1 *Materials*

Piperine with a purity of 98.78% was bought from Chengdu Biopurify Phytochemicals Ltd., Sichuan, China. Sucralose was purchased from Krungthepchemi Co., Ltd., Bangkok, Thailand. Fumed silica was bought from P.C. Drug Center, Bangkok, Thailand. Magnesium stearate was obtained from Changzhou Kaide Imp. & Exp. Co., Ltd., Changzhou, China. Microcrystalline cellulose (MCC) (Comprecel® M102) was purchased from Maxway Co., Ltd., Bangkok, Thailand. Mannitol (Mannogem® EZ Spray Dried, SPI Pharma, Delaware, USA) was given as a gift from DKSH (Thailand) Ltd, Bangkok, Thailand. Croscarmellose sodium (CCS) and sodium starch glycolate (SSG) were also gifts obtained from Onimax Co., Ltd., Bangkok, Thailand. Other reagents were analytical grade and solvents were analytical grade.

2.2 *Preparation of SHPNE*

The study utilized various dried plant samples including *Brassica* spp. seeds, *B. balsamifera* leaves, *T. chebula* fruit pulps, *P. nigrum* fruits, *C. hystrix* fruit peels, and *C. sativum* fruits. These plant samples were procured from Charoensuk Osod in Nakhon Pathom, Thailand. A plant taxonomist, Nirun Vipunngeun from the College of Pharmacy at Rangsit University, identified the plant species to confirm their authenticity, and the voucher specimens were coded accordingly and deposited at the Drug and Herbal Product Research and Development Center, College of Pharmacy at Rangsit University. Each plant sample was ground separately, and equal weights of all the plants were mixed together to make a total of 1.44 kg. This mixture was then infused in boiling water (14.4 L) for 10 min and centrifuged at 5,000 rpm for 10 min at 25 °C using a Universal 320R model by Hettich Holding GmbH & Co., Tuttlingen, Germany. The resulting solution was vacuum filtered, freeze-dried for 18 h, and kept in a desiccator until further use.

2.3 *Cytotoxicity test of SHPNE*

The potential toxicity of SHPNE was evaluated *in vitro* using a mitochondrial-based MTT cell viability assay. HepG2 cells were seeded at a density of 1×10^4 cells per well in a 96-well culture plate and incubated overnight at 37 °C with 5% CO_2. The cells were treated with varying concentrations of SHPNE (ranging from 0–10 mg/mL) or a positive control of 200 μM hydrogen peroxide for 24 h. After the incubation

period, 100 µL of 0.5 mg/mL MTT solution in culture medium was added to each well, and the cells were incubated for an additional 3 h. The resulting formazan was dissolved in 100 µL of dimethyl sulfoxide, and the optical density was measured at 570 nm using a microplate reader (Bio-Rad Laboratories, California, USA). The experiment was performed three times independently, and the percentage of cell viability was calculated by comparing the results to the non-treated group [9].

2.4 *Preparation of SHPNE ODT*

The SHPNE ODTs were formulated with various ingredients, including freeze-dried SHPNE as the active ingredient, fumed silica as an adsorbent and glidant, sucralose as a sweetener, magnesium stearate as a lubricant, CCS and SSG as superdisintegrants, MCC as a binder, and mannitol as a diluent. All the ingredients were sieved through a 40-mesh sieve, except for sucralose and magnesium stearate, which were passed through a 60-mesh sieve. The prototype formulation contained 13.33 g of freeze-dried SHPNE and 6.67 g of fumed silica per 100 g of the mixture. The two ingredients were blended by mortar and pestle and sieved through a 40-mesh sieve. The mixture was then mixed with half of the mannitol for 5 min (Mixture A). A premix was prepared by mixing 0–6 g of CCS, 2–8 g of SSG, 0.2 g of sucralose, 1 g of magnesium stearate, 0–15 g of MCC, and half of the mannitol (to adjust the total weight to 100 g) for 3 min (Mixture B). Mixture A and Mixture B were then combined and mixed for 5 min. The resulting powder mixture weighed 300 mg (equivalent to 40 mg of SHPNE per tablet) and was compressed into individual tablets using a hydraulic press with a pressure gauge.

2.5 *Factor level tests using the OFAT method*

The OFAT method was utilized to determine the appropriate levels of three factors: compressional force, MCC quantity, and CCS/SSG quantity. Each factor was tested at four different levels: compressional force at 500, 1000, 1500, and 2000 psi; MCC quantity at 0, 5, 10, and 15%; CCS/SSG quantity at 0:8, 2:6, 4:4, and 6:2%. The physical properties of the resulting SHPNE ODTs were assessed based on five criteria, including weight and weight variation, diameter and thickness, hardness, friability, and disintegration time (DT). However, only tablet thickness, hardness, friability, and DT were compared.

2.6 *Box-Behnken design for optimization of SHPNE ODTs*

Three levels of each factor obtained from the OFAT method were included in the Box-Behnken design. They varied as 1000, 1500, and 2000 psi for compressional force; 5, 10, and 15% for MCC; and 2:6, 4:4, and 6:2% for CCS and SSG.

The physical characteristics of the SHPNE ODTs derived from the experimental design were analyzed using the Design-Expert® program (version 11) (Stat-Ease, Inc., Minnesota, USA). ANOVA was performed to determine a quadratic model of each response and the coefficients based on actual values were also presented. The contour plots of the responses of tablet thickness, hardness, friability, and DT were generated. A design space was established that ensured tablet hardness was between 6–8 kP, friability was no more than 1%, and DT was no more than 3 min. A point within the design space was chosen for the verification step, and the actual values obtained from the optimal condition were compared to the predicted values. The percent error was calculated.

2.7 *Determination of SHPNE ODTs' physical properties*

The weight of twenty SHPNE ODTs was measured using an analytical balance (Entris224i-1S, Sartorius AG, Göttingen, Germany). The mean value and standard deviation (SD) were calculated and reported. The weight variation was determined using Equation (1).

$$\textit{Weight variation}\ (\%) = \left(\frac{\textit{Individual weight} - \textit{Average weight}}{\textit{Average weight}}\right) \times 100 \quad (1)$$

The diameter and thickness of twenty SHPNE ODTs were measured using a digital thickness tester. The mean value and SD were reported.

The hardness of ten SHPNE ODTs was evaluated by a hardness tester (TBH 220 TD, Erweka GmbH, Heusenstamm, Germany). The mean value and SD were reported.

The DT of six SHPNE ODTs was evaluated using a disintegration tester (Model: BJ-2, Tianjin Guoming Medicinal Equipment Co., Ltd., Tianjin, China). Water at 37 ± 0.5 °C was used as a disintegration medium. The average values and SD were reported.

Eleven tablets were cleaned with a soft brush, then weighed as W_1 using an analytical balance. Friability was evaluated using a friability tester (Model: CS-2, Tianjin Guoming Medicinal Equipment Co., Ltd., Tianjin, China). The friability tester's drum was turned 100 times at a speed of 25 rpm. The tablets were then taken out of the drum, cleaned, and weighed again as W_2. The friability was calculated using Equation (2).

$$Friability\ (\%) = \left(\frac{W_1 - W_2}{W_1}\right) \times 100 \quad (2)$$

2.8 *Morphology test by field emission scanning electron microscope*

Surface and cross-sectional morphologies of the optimal SHPNE ODT were evaluated by field emission scanning electron microscope (FESEM) (Model: Sigma 500VP, Carl Zeiss, Deutschland, Germany). The optimal SHPNE ODT was gold-coated using a sputter coater (Q150R ES Plus, Quorum, East Sussex, United Kingdom). Coating parameters were coating material of 99.97% gold, sputter current of 20 mA, sputtering time of 2 min, and sputtering vacuum pressure of $1.0e^{-1}$ mbar. FESEM parameters were a system vacuum of approximately $5.03e^{-7}$ bar VP Mode) a gun vacuum of approximately $7.54e^{-10}$ bar, a temperature of approximately 21.0 °C, a humidity of approximately 55% RH, electron high tension of 15.0 kV (FESEM), the working distance of approximately 8.5 mm (SEM), a scan speed of 6 times per second, signal/detector of SE2, and current of 2.270 A.

2.9 *Piperine content analysis*

The marker chosen for SHPNE ODTs was piperine, which was measured using high-performance liquid chromatography (HPLC) coupled with a photodiode array detector. To carry out the analysis, ten optimal SHPNE ODTs were pulverized and weighed for 300 mg (n = 3). Methanol was added, and the mixture was ultrasonicated for 30 min, cooled, and then adjusted to volume by adding methanol. The resulting solution was mixed and filtered through a nylon syringe filter (0.45 μm) before being analyzed by the validated HPLC method. The isocratic elution system was performed on an InfinityLab Poroshell 120 EC-C18 column (150 × 4.6 mm, i.d., 4 μm) with a constant temperature of 25 °C. The mobile phase was a mixture of methanol and ultrapure water at a ratio of 70:30 v/v, with a flow rate of 1 mL/min. The injection volume was 10 μL, and the photodiode array detector was set at 340 nm [8].

2.10 *Dissolution test*

Three optimal SHPNE ODTs were tested for piperine dissolution from the tablets using dissolution apparatus II (Model: 72-600-400, Hanson Research Corp., California, USA). The paddle speed was controlled at 100 ± 1 rpm. The 0.5% sodium lauryl sulfate aqueous solution (900 mL) at 37 ± 0.5 °C was used as a dissolution medium. At 1, 3, 5, 10, 15, 30, 45, 60, 90, and 120 min, the medium was sampled for 5 mL. To keep a constant volume of the dissolution, fresh medium was added. The removed medium was filtered and subjected to HPLC analysis. The dissolution profile of piperine from the optimal SHPNE ODTs was created.

2.11 *Statistical analysis*

The statistical software SPSS Statistics 22.0 (IBM, New York, USA) was used to perform a One-way analysis of variance (One-way ANOVA) and Tukey HSD post hoc analysis to compare the differences between multiple groups. A *p*-value of less than 0.05 at a 95% confidence interval was considered statistically significant.

3 Results

3.1 *Cell viability*

The cytotoxic effects of SHPNE in HepG2 cells were measured by MTT assay. Treatment of HepG2 cells with SHPNE for 24 h indicated that cell viability was not significantly reduced at SHPNE levels up to 0.01 mg/mL (cell viability of 89.98 ± 0.79%). The

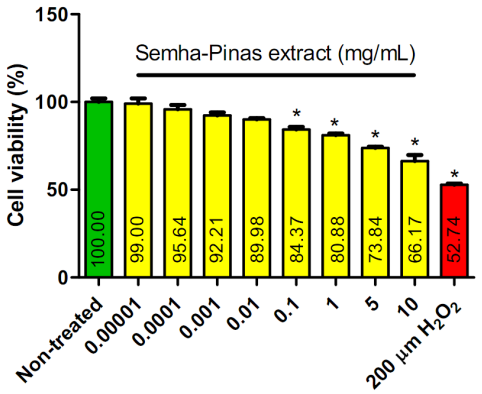

Figure 1: *in vitro* cytotoxicity of SHPNE in HepG2 cells compared with the non-treated control group and the positive control group (200 µM hydrogen peroxide). The significance was presented as * when the *p*-value < 0.05.

positive control (200 µM hydrogen peroxide) gave a cell viability of 52.74 ± 0.71%. The SHPNE equal or less than 1 mg/mL provided cell viability of greater than 80%. However, a significant decrease in cell viability less than 70% was found at concentrations of 10 mg/mL (cell viability of 66.17 ± 3.58%), therefore this concentration was considered to be toxic of this recipe (Figure 1).

3.2 *Effect of factors on SHPNE ODT properties obtained from screening step by OFAT method*

Screening for the factor levels of compressional force, MCC, and CCS:SSG was conducted using the OFAT method. The physical properties (thickness, hardness, friability, and DT) of the SHPNE ODTs were analyzed when the factor levels were altered, as depicted in Figure 2. Figure 2(a) illustrates the impact of compressional force on the properties of the SHPNE ODTs. Formulations without MCC and with a CCS:SSG ratio of 4:4% were prepared. Increasing the compressional force from 500–2,000 psi resulted in thinner tablets, with tablet thickness significantly decreasing from 4.12–3.49 mm (for a tablet diameter of 9.7 mm). Tablet hardness increased significantly from 2.34–8.39 kP. The friability of the tablet decreased with an increase in compressional force, with the compressional force of 500 psi yielding the highest friability (1.68%), exceeding the acceptable range of not more than 1.0%. Consequently, the compressional force of 500 psi will not be considered for further study. The DT ranged from 0.44–0.67 min as the compressional force increased from 500–2,000 psi. Therefore, the compressional force of 1,500 psi was selected for the subsequent screening steps due to its ability to provide suitable hardness, low friability, and a short DT.

Since formulations without MCC exhibited low tablet hardness, the next step involved varying the quantity of MCC while fixing the compressional force at 1,500 psi and CCS:SSG at 4:4%. Figure 2(b) demonstrates the effect of MCC on the properties of the SHPNE ODTs. MCC quantity ranged from 0–15%. Tablet thickness slightly decreased from 3.62–3.49 mm, while tablet hardness increased from 5.69–7.10 kP. However, increasing MCC quantity from 0–5% or from 10–15% did not result in a significant increase in hardness. The friability values remained unchanged as MCC quantity increased, ranging from 0.42–0.64%. There were no significant differences in DT when MCC quantity increased from 0–10%. However, an MCC quantity of 15% exhibited the longest DT of 0.75 min. Therefore, an MCC quantity of 10% was selected for the subsequent screening steps.

Figure 2(c) displays the effect of CCS:SSG on the properties of the SHPNE ODTs. When the CCS:SSG ratio was altered from 0:8%–6:2%, tablet thickness slightly decreased from 3.58–3.53 mm. Changing the CCS:SSG ratio did not significantly impact tablet hardness or friability. The use of a CCS:SSG ratio of 0:8% resulted in the longest DT compared to ratios of 2:6%, 4:4%, and 6:2%. Changing the CCS:SSG ratio from 0:8%–2:6% and 4:4% significantly reduced DT from 5.40–0.89 and 0.52 min, respectively. However, changing the CCS:SSG ratio from 4:4%–6:2% did not lead to any significant difference in DT. It appears that the CCS:SSG ratio significantly influenced DT.

To summarize, the compressional force of 500 psi was not selected due to its ability to provide the lowest hardness, resulting in excessive friability. The 0% MCC was not chosen because it provided the lowest hardness. Similarly, the 0:8% CCS:SSG ratio was not selected as it resulted in the longest DT.

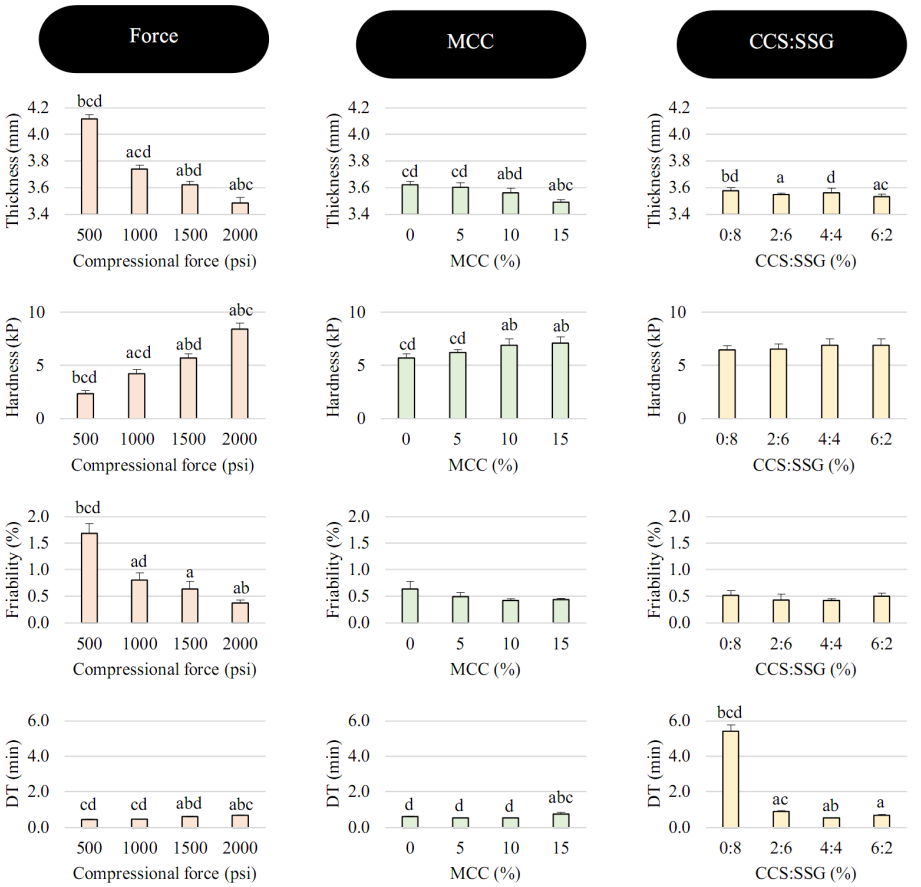

Figure 2: Physical properties, i.e., thickness, hardness, friability, and DT of SHPNE ODTs when (a) compressional force, (b) MCC, and (c) CCS:SSG were varied based on the OFAT method. The symbols a, b, c, and d represented significant values (p-value < 0.05) compared with other levels.

3.3 *Effect of factors on SHPNE ODT properties obtained Box-Behnken design*

After a screening step with the OFAT method, the effects of factors on the SHPNE ODT properties were investigated using a Box-Behnken design. Appropriate ranges for each factor were selected from the screening step, The effects of each factor (in terms of linear effects) can be explained similarly to the OFAT method in the previous section. The Box-Behnken design has been shown to account for all linear, interaction, and quadratic effects. It also reveals the effects of more complex factors than the OFAT method.

Response surfaces of tablet thickness, hardness, friability, and DT at different compressional forces are shown in Figure 3. The coefficient of responses of SHPNE ODTs, and their p-values of linear, interaction, and quadratic effects, and lack of fit are shown in Table 1. Figure 3(a) demonstrates that increasing compressional force (X_1), MCC (X_2), and CCS:SSG (X_3) decreased tablet thickness; except for medium and high compressional force, increasing CCS at high MCC gave slightly increased thickness. Table 1 shows that the tablet became significantly thinner as the compressional force increased. The term X_1^2 significantly increased tablet thickness. The terms X_2, X_3, X_1X_2, and X_2^2 insignificantly decreased tablet thickness while other terms insignificantly increased tablet thickness. Figure 3(b) demonstrates that increasing compressional force (X_1) increases tablet

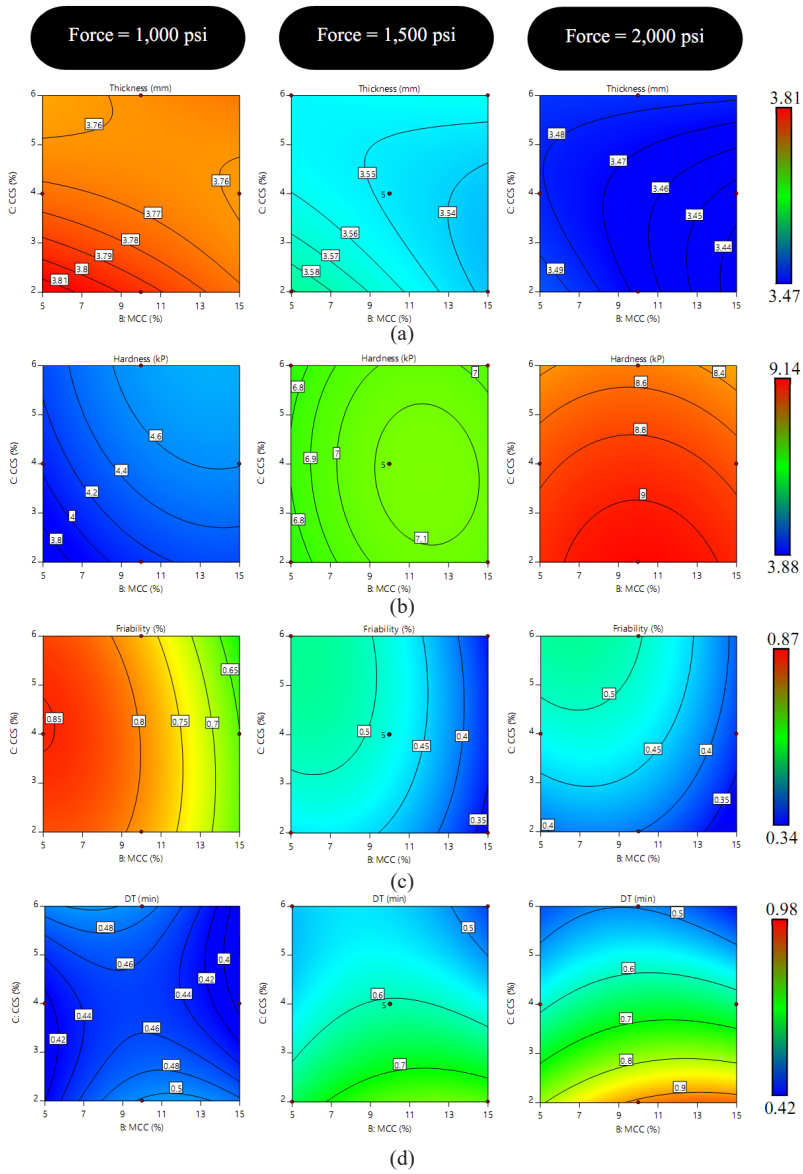

Figure 3: Contour plots of (a) thickness, (b) hardness, (c) friability, and (d) DT of SHPNE ODTs obtained from the Box-Behnken design when different compressional forces were applied.

hardness. Increasing MCC quantity (X_2) increased tablet hardness at low compressional force; at medium compressional force, maximum hardness could be found at medium to high MCC quantity; and at high compressional force, increasing MCC quantity did not affect hardness. In the case of CCS:SSG (X_3), increasing CCS:SSG increased hardness at low compressional force; hardness was similar when CCS:SSG increased at medium compressional force; and hardness was deceased when CCS:SSG increased at high compressional force. Table 1 shows that the terms X_1 and X_2 had a positive effect while other terms had negative effect on tablet hardness. However, only X_1 and X_1^2 affected hardness significantly. Figure 3(c) demonstrates that increasing compressional force (X_1) and MCC quantity (X_2) decreased tablet friability

while increasing CCS:SSG showed a small effect on tablet friability. Table 1 shows the terms X_1, X_2, X_2X^3, X_2^2, and X_3^2 decreased tablet friability while the other terms increased tablet friability. However, only X_1, X_2, and X_1^2 affected friability significantly. Figure 3(d) demonstrates that increasing compressional force (X1) prolonged DT, increasing CCS:SSG shortened DT while increasing MCC did not affect DT. Table 1 shows the terms X_3, X_1X_3, X_2X_3, X_1^2, and X_2^2 shortened DT while the other terms prolonged DT. Among them, only X_1, X_3, and X_1X_3 affected DT significantly.

3.4 *Design spaces and optimal formulation*

The design space where the tablet hardness was 6–8 kP, the friability was not more than 1%, and the DT was not more than 3 min were produced at different compression forces. Thus, the three design spaces were obtained as shown in Figure 4. Design spaces revealed that the desired properties were achieved when the compressional force of 1,500 psi was applied for all MCC and CCS:SSG levels. While design space was not achieved when compressional forces of 1,000 and 2,000 psi were applied.

The optimal formulation was selected at the verification step. The compressional force of 1,500 psi, the MCC of 10%, and the CCS to SSG of 4:4% were chosen as the optimal formulation. This formulation had a tablet weight of 298.31 ± 0.96 mg, a diameter of 9.72 ± 0.01 mm, a thickness of 3.54 ± 0.03 mm, a hardness of 7.26 ± 0.63 kP, a friability of 0.45 ± 0.03%, and a DT of 0.56 ± 0.03 min. None of the tablets had a weight variation exceeding 7.5% which was within the acceptable range for a tablet's weight of 130–324 mg. For all predicted responses, the percent errors between predicted and actual values were less than 10% (Table 2).

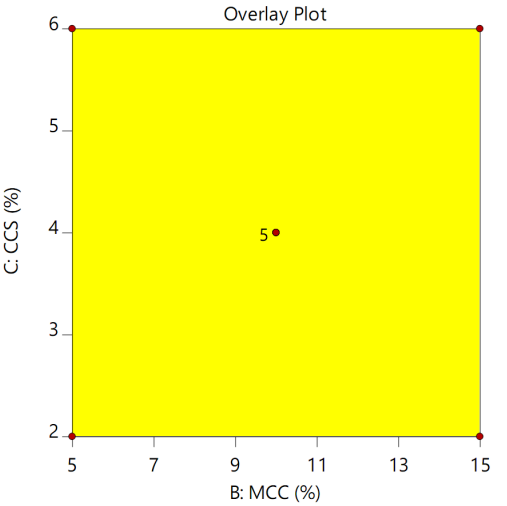

Figure 4: Design spaces where the SHPNE ODTs had a hardness of 6–8 kP, friability of not more than 1%, and disintegration time of not more than 3 min when compressional forces of 1,500 psi were applied.

The morphology of the optimal SHPNE ODTs was evaluated using a mobile phone camera and FESEM. Photographs obtained from the mobile phone camera exhibited a smooth surface with brown spots distributed over the tablet [Figure 5(a)]. FESEM photomicrographs showed that the tablet had a

Table 1: Coefficient table of responses, i.e., thickness, hardness, friability, and DT of SHPNE ODTs, and their *p*-values of linear, interaction, and quadratic effects, and lack of fit

Response	Intercept	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2	Lack of fit
Thickness	3.55	–0.15	–0.01	–0.004	–0.005	0.01	0.02	0.07	–0.0002	0.01	-
p-values	-	< 0.0001*	0.2297	0.7048	0.7208	0.3832	0.3011	0.0013*	0.9853	0.3807	0.1825
Hardness	7.12	2.20	0.14	–0.01	-0.15	-0.30	–0.03	–0.42	–0.18	–0.10	-
p-values	-	< 0.0001*	0.2988	0.9281	0.4072	0.1239	0.8873	0.0380*	0.3118	0.5722	0.1342
Friability	0.48	–0.16	–0.08	0.02	0.02	0.03	–0.01	0.15	–0.05	–0.02	-
p-values	-	0.0013*	0.0451*	0.5258	0.6325	0.5265	0.7893	0.0110*	0.3196	0.6702	0.0497*
DT	0.61	0.10	0.01	–0.11	0.02	–0.10	–0.04	–0.05	–0.05	0.04	-
p-values	-	0.0021*	0.7425	0.0019*	0.5903	0.0146*	0.2130	0.1564	0.1388	0.2074	0.2947

An asterisk (*) denotes the significant terms (*p*-value < 0.05). Terms X_1, X_2, and X_3 were compressional force, MCC, and CCS:SSG, respectively.

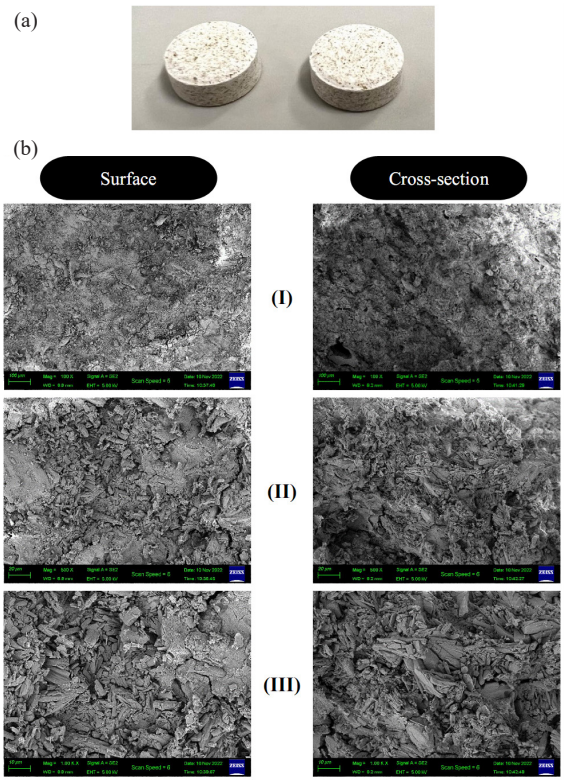

Figure 5: Morphology of the optimal SHPNE ODT obtained from (a) mobile phone camera and (b) FESEM in surface view and cross-sectional view with magnification (I) ×100, (II) ×500, and (III) ×1,000.

smooth surface corresponding to the photograph from a mobile phone. FESEM photomicrographs found slight roughness at the surface. Moreover, the inside of the tablet was dense, compact, and homogenous [Figure 5(b)].

Table 2: Predicted values, actual values, error and lower to upper of 95% confidence interval of the prediction for each response of SHPNE ODTs

Parameters	Predicted Values	Actual Values	Error (%)*	95% CI
Thickness (mm)	3.55	3.54 ± 0.03	–0.28	3.52–3.58
Hardness (kP)	7.12	7.26 ± 0.63	1.93	6.76–7.48
Friability (%)	0.48	0.45 ± 0.03	–6.67	0.39–0.58
DT (min)	0.61	0.56 ± 0.02	–8.93	0.54–0.67

* Error = (Actual value – Predicted value) × 100/Actual value

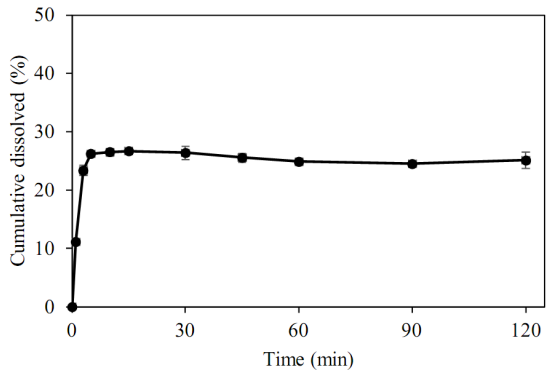

Figure 6: Dissolution profile of piperine from the optimal SHPNE ODTs (n = 3) when 900 mL of 0.5% sodium lauryl sulfate aqueous solution was used as dissolution medium.

The dissolution profile of piperine from the optimal SHPNE ODTs is shown in Figure 6. The maximum piperine dissolution was reached within 5 min in 0.5% sodium lauryl sulfate aqueous solution. After that, there was no piperine dissolved for 120 min.

4 Discussion

This work developed the SHPNE ODTs containing 40 mg of SHPNE. A preliminary study found that 150 mg of the Semha-Pinas herbal recipe was equivalent to approximately 40 mg of its extract. The dosage regimen of this recipe is 300 to 600 mg of Semha-Pinas herbal powder three times a day before meals. The recommended dosage of the SHPNE ODTs should be 2–4 tablets per time. This is the major drawback of the traditional herbal recipe, which must be used in high doses to reach therapeutic effects.

ISO 10993-5 defines cytotoxicity as the condition in which the viability of cells is reduced by 30% or more [10]. So, the extract at a maximum concentration of 5 mg/mL was considered safe due to the cell viability being higher than 70%. This calculation is based on the average amount of blood circulating in an adult, which is estimated to be around 5 L [11] with an assumption that the whole extract was absorbed [12], [13]. The intake dose of SHPNE should not exceed 25.0 g per time, which is equivalent to 93.75 g of Semha-Pinas herbal powder. Therefore, the usual dose (0.08–0.16 g/dose or 0.24–0.48 g/day of SHPNE, or 0.3–0.6 g/dose or 0.9–1.8 g/day of Semha-Pinas herbal

powder) will be safe for hepatic cells. Nevertheless, the whole extract could not be absorbed completely. First-pass metabolism is the most important factor in lowering the concentration of a drug upon reaching the site of action or systemic circulation [14]. In addition, several factors affect drug absorption upon oral administration: physicochemical properties of active substances and drug formulations, gastrointestinal content, gastrointestinal surface area and blood flow, intestinal motility, breakdown by gastrointestinal secretions, metabolism, pH, irritation of mucous membranes by drugs, vomiting, and coadministration of other drugs [15].

Based on factor level screening by the OFAT method, the compressional force was the main factor influencing various tablet properties [8], [16]–[18]. Increasing the compressional force not only decreases the tablet thickness but also increases the tensile strength of the table [19]. Increasing the compressional force can prolong the DT of rapidly disintegrating aspirin tablets [20]. A previous study showed comparable results to our current study, in which increasing the compressional force from 500–1,500 psi decreased tablet thickness and friability, and increased tablet hardness and DT of *Thunbergia laurifolia* Lindl. leaf tablets [21]. Similar results were also found in the development of herbal effervescent tablets of Semha-Pinas extract [8] and plain tablets of Chatuphalathika extract [18] and Prasakanphlu powder [22], when 1,000 to 2,000 psi were applied. The compressional force highly affected the DT of Prasakanphlu tablets prepared by the wet granulation method, when low quantity of superdisintegrant: 2% SSG was used. However, the compressional force slightly affected DT when a higher quantity of superdisintegrant: 3% and 4% SSG was used [22].

There are several excipients that can be used as fillers for direct compression, such as anhydrous dibasic calcium phosphate, MCC, maltose, fructose, lactose, etc. In the case of ODT, mannitol is usually used due to its highly water-soluble property, free-flowing, sweetness, and cooling sensation [23]. However, using mannitol alone could not provide sufficient tablet hardness. Therefore, other diluents were added to promote adequate tablet hardness. MCC is the most commonly used tablet diluent. It serves multiple functions as a directly compressible diluent, disintegrant, binder, lubricant, glidant, etc. [24]. This work used MCC as a binder. It increased tablet hardness but decreased tablet thickness, resulting in decreased friability. The plasticity of MCC, coupled with its high surface area, high hygroscopicity, and relatively low bulk density, explain its unique binding properties. [25]. Despite high compactness at low pressure, MCC has poor flowability [26]. Although MCC increased tablet hardness, MCC did not prolong DT, as MCC also exhibited disintegration properties [23]. According to the mechanism of MCC, the high porosity of MCC is believed to contribute to tablet swelling and disintegration. This is caused either by the penetration of water into the hydrophilic tablet matrix by capillary action or by the breaking of hydrogen bonds. Moreover, MCC, by its nature, exhibited less elastic deformation and a faster water-wicking rate. These features allow the tablet to break down [24]. However, the present work found that increasing the MCC quantity had a small effect on DT. Furthermore, the previous work also reported that MCC was superior to spray-dried rice starch and spray-dried lactose [27].

CCS is a well-known tablet disintegrant. It is insoluble in water but swells to 4–8 times its original volume in contact with water [23]. Its fluid absorption and swelling properties facilitate the function of CCS as a superdisintegrant [28]. However, increasing CCS levels beyond 5% prolongs DT as a viscous gel layer forms and acts as a barrier to tablet disintegration [28]. It has occasionally been reported that the use of CCS above about 7.5% can prolong the DT of aspirin, ibuprofen, and ascorbic acid from rapidly disintegrating tablets [20]. SSG is the most commonly used superdisintegrant due to its superior swelling value and water absorption compared to CCS [29]. However, CCS was superior to SSG in terms of shortened DT [30] as found in the present work; increasing CCS content in superdisintegrants mixture better shortened DT compared with SSG.

The interaction between factors affecting tablet properties can be found in several studies [31]–[34]. This issue can be identified by the DOE rather than OFAT method [35], [36]. However, it has been difficult to demonstrate the mechanism due to the complex interactions of various factors. Interactions between factors obtained from the DOE approach may be comparable to synergistic or antagonistic effects of some factors or tablet excipients. An example is combining excipients with different physical properties,

such as brittle anhydrous dibasic calcium phosphate and ductile MCC, directly compressible sitagliptin tablets with excellent mechanical strength (greater than 2 N/mm^2 tensile strength), fast disintegration (DT less than 2 min), and rapid drug release [37]. Increasing the quantity of CCS and MCC had a synergistic effect on the wetting ratio and an antagonistic effect on wetting time. A significant interaction effect between CCS and MCC was also seen in the wetting ratio. Increased CCS and MCC concentrations led to increased water uptake [38]. In this case, the interaction effect of CCS and MCC should affect DT. In the present work, only the interaction effect of compressional force and CCS:SSG gave a significantly shortened DT.

Flowability of the powder mixture as well as die filling is a critical step in the pharmaceutical tableting step as it determines the tablet weight variation, which affects the consistency of drug content and tablet appearance [39]. This work found that none of the tablets had weight variation exceeding the acceptable range because weighed the powder mixture and compressed individually resulting in small weight variation. However, the pilot scale and production scale required weight variation are important parameters to be evaluated.

Piperine, a water-insoluble compound, exhibiting poor dissolution properties, is the rate-controlling step in the absorption process [40]. This study found that the ODT remaining after the dissolution test finished, contained high piperine content indicating that piperine was absorbed into water-insoluble excipients or remained in SHPNE. SHPNE was obtained from infusion in which the extraction solvent was boiling water. The solubility of piperine was temperature-dependent. Piperine solubility was increased when temperature increased [41]. However, a dissolution test was performed at 37 °C resulting in low SHPNE as well as piperine being dissolved. So, the dissolution of piperine from SHPNE ODT seems relatively low even though surfactant was added to the dissolution medium.

5 Conclusions

In the present study, SHPNE ODTs were successfully fabricated. The fabrication process relied on the implementation of RSM using the Box-Behnken design. Through a cytotoxicity test conducted on HepG2 cells, it was determined that SHPNE remained safe, even at a concentrated extract of 5 mg/mL. Capitalizing on this safety finding, the researchers developed the safe extract into ODTs. The study focused on evaluating three key factors: compressional force, quantity of MCC, and quantity of CCS and SSG. Initially, the factor levels were screened using the OFAT technique. Subsequently, three levels were selected for each factor and incorporated into the Box-Behnken design. By employing these design methodologies, the authors successfully established a design space encompassing desirable properties for the SHPNE ODTs. These properties included tablet hardness within the range of 6–8 kP, friability of 1% or less, and a disintegration time of 3 min or less. The optimal formulation for the SHPNE ODTs involved a compressional force of 1,500 psi, an MCC quantity of 10%, and a CCS to SSG ratio of 4:4%. Additionally, it was found that the low piperine marker could be effectively dissolved in a 0.5% aqueous solution of sodium lauryl sulfate. In summary, this study accomplished the fabrication of SHPNE ODTs through the utilization of RSM, resulting in ODTs with desired properties, notably rapid disintegration. The data derived from this study hold practical implications for the pharmaceutical industry, particularly in terms of enhancing the value of Thai herbs.

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Author Contributions

J.S.: methodology, investigation, formal analysis, writing - original draft; C.M.: conceptualization, methodology, data curation, formal analysis, investigation, project administration, resources, writing - original draft, writing - review & editing ; A.N.: methodology, investigation, formal analysis, writing - original draft; L.C.: conceptualization, methodology, supervision, resources, writing - original draft; N.C.: methodology, formal analysis, resources, writing - original draft;

O.N.: methodology, formal analysis, resources, writing - original draft. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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