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Statistical Optimization of Novel Traditional Chinese Medicine (Liu-Wei-Di-Huang-Wan) Pellets Prepared by Extrusion-Spheronization Method

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ABSTRACT

Traditional Chinese Medicine (TCM) has received broader attention recently, but the applications are limited due to the lack of more complete study of their pharmaceutical properties. TCM often consists of numerous ingredients and generally involves a complicated manufacturing process, but studies on the interaction of the active ingredients and excipients are rare. In this study, the development of a novel dosage form of a widely utilized TCM (Liu-Wei-Di-Huang-Wan, LWDHW) was investigated. Firstly, Differential Scanning Calorimetry (DSC) was used to study the incompatibility between the active ingredients of LWDHW and three excipients commonly used in the manufacturing of TCM products. Secondly, a novel pellet dosage form of LWDHW was prepared using the extrusion-spheronization method, and L16 (2^{15}) orthogonal experimental design method was applied to perform an overall investigation of different variables. The frequently used excipient, hydroxypropyl methylcellulose (HPMC), was compressed with resultant optimal pellets to investigate the feasibility of a new LWDHW tablet dosage form. Finally, a 2^3 full factorial design was used in drug release studies to explore the dissolution profile of pellets and tablets at various parameters. The pellets prepared in this study exhibited good compression properties and formed a harder and tighter structure during the compression process, the resultant tablets showed a slower initial release rate and higher stability than pellets. Optimal LWDHW pellet and tablet dosage forms were obtained by adjusting the variables of the extrusion-spheronization process. The results from this study may provide a novel way of preparing LWDHW as well as other TCM dosage forms.

Key words**:** traditional Chinese medicine, Liu-Wei-Di-Huang-Wan, extrusion-spheronization, orthogonal experimental design, full factorial design

INTRODUCTION

The applications of natural herbs are becoming increasingly extensive, for instance, Shosaikoto displayed mild anti-inflammatory action and significantly increased the anti-inflammatory effect of prednisolone[(1)]. The combined treatment of traditional Chinese medicine (TCM) and glibenclamide, an antidiabetic agent, has also been shown that TCM may facilitate the antidiabetic effect of glibenclamide[(2)]. Although TCM has been used over thousands of years in China, its application in drug therapy is not as high as western medicine. The main reason for this lies in the use of a mixture of natural crude drugs, which does not allow for the same level of consistency in quality as seen in western drugs and the complex components of TCM make scientific analysis more difficult. Furthermore, the interactions between the complex ingredients of TCM and its excipients have not been determined. However, with good planning, the pharmaceutical properties of ingredients used in TCM can be studied more completely using modern science. Experimental design methods can increase the efficiency in the complete evaluation of formulations and are applied in this study.

Recently, certain types of TCM have received greater attention and the efficacy and validity of some herbal mixtures have also been established. Liu-Wei-Di-Huang-Wan (LWDHW), a pellet dosage form prepared from six herbs, namely Rehmanniae radix, Corni fructus, Dioscoreae rhizoma, Alismatis rhizoma, Moutan cortex and Polyporus, has received considerable attention because of its therapeutic effect on Alzheimer's disease involving an anti-free radical mechanism[(3-5)]. However, LWDHW pellets possess some disadvantages, such as the tendency to mold, difficulty in

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swallowing due to its larger size, and different batches of LWDHW products are not identical in terms of pharmaceutical properties owing to their complex composition and preparation manner. Furthermore, during the preparation of LWDHW pellets, herbal extracts are diluted by certain kinds of excipients, and interactions between the active ingredients and excipients may exist. These interactions may accelerate the decomposition of the active ingredients and alter the bioavailability of the TCM. However, the possible interactions between Chinese herbs and excipients have not attracted much attention. Nevertheless, it is important to understand the problems with potential batch-to-batch variation of TCM products and the interactions between Chinese herbs and excipients. In the first part of this study, the possible effects of three excipients - Era-Tab®, lactose and Avicel PH102®, on the stability of two major active ingredients of LWDHW (paeonol and loganin) were investigated with the use of Differential Scanning Calorimetry (DSC). These excipients are commonly used in the manufacture of Chinese herbal products.

The extrusion-spheronization method was described by Smith Kline and French in 1950[6]. It is used to prepare different kinds of pellets[7-8] including slow-release pellets without subsequent coating[9]. However, its use in the preparation of TCM has not been studied. In this study, a novel pellet dosage form of LWDHW was prepared by the extrusion-spheronization method. The four main steps in the extrusion-spheronization process are dry blending, wet mixing, extruding and spheronizing using a spinning serrated plate. To investigate the effects of the manufacturing process and conditions on the pharmaceutical properties of the finished products, an L16 (2^{15}) orthogonal array experimental design method was applied and the data was compared statistically using analysis of variance (ANOVA)[10]. The effects of six important variations, namely the ratio of two kinds of hydroxypropyl methylcellulose, water content, extrusion speed, extrusion screen size, spheronization time and spheronizer speed, on the pellet properties were analyzed. In the second part of this study, extrusion–spheronization was shown to be a suitable technique for the production of TCM pellets and the optimal conditions for the preparation of pellets were obtained. The tablet dosage form is more widely used clinically. The resultant pellets were not only filled into empty capsules, but compressed with HPMC to evaluate the feasibility of TCM tablets. *In vitro* dissolution tests and stability tests were carried out to evaluate the differences between pellets and tablets.

MATERIALS AND METHODS

I. *Materials*

Rehmanniae radix (*Rehmannia glutinosa (Gaertn.) Libosch.)*, Corni fructus (*Cornus officinalis Sieb. et Zucc.*), Dioscoreae rhizoma (*Dioscorea opposita Thunb.*), Alismatis rhizoma (*Alisma orientalis (Sam.) Juzep.*), Moutan cortex (*Paeonia suffruticosa Andr.*) and Polyporus (*Poria cocos (Schw.) Wolf*) were purchased from Chinese medical shops. Paeonol (Nacalai Tesque Inc., Kyoto) and Loganin (Estrasythese B.P.62-69730 GENAY France) were used as standards for HPLC analysis. Fluorescein (Sigma-Aldrich Chemie Gmbh P.O. F-6377, Lot 79H0023, Germany) and Ferulic acid (Sigma-Aldrich Chemie Gmbh P.O. F-3500, Lot 79K1615, Germany) were used as internal standards for paeonol and loganin, respectively.

Avicel PH102® (Microcrystalline cellulose, Asahi Chemical Industry Co., Ltd., Japan), Era-Tab® (Rice starch, Erawan Pharmaceutical Research and Laboratory Co., Ltd., Thailand) and lactose monohydrate (New Zealand Lactose Co., Ltd., New Zealand) were used as supplied. HPMC 4000 cps (Methocel K4M) and HPMC 15000 cps (Methocel K15M) were purchased from Berwind Pharmaceutical Services Inc. (U.S.A.). All other chemicals were of analytical grade.

II. *Differential Scanning Calorimetry*

The interactions between two major active ingredients of LWDHW (paeonol and loganin) and three excipients (Avicel PH102®, Era-Tab® and lactose) were measured by DSC (DSC 7, Perkin-Elmer, USA). The individual powder samples of the active ingredients and excipients, as well as physical mixtures of active ingredient and excipients (1 : 1), were dried in a vacuum oven for 24 h to remove water. Each sample (8 mg) was placed in sealed flat-bottom aluminum sample pans and scanned from 25 to 300°C at a heating rate of 10°C/min.

III. *Preparation of LWDHW Pellets*

Rehmanniae radix, Corni fructus, Dioscoreae rhizoma, Alismatis rhizoma, Moutan cortex and Polyporus were separately grounded into powder and dried by a hot air oven at 40°C for 8 h. The six Chinese herbal powders were then sieved using a 40# mesh and mixed according to a certain ratio in clinical use (Rehmanniae radix : Corni fructus : Dioscoreae rhizoma : Alismatis rhizoma : Moutan cortex : Polyporus = 8 : 4 : 4 : 3 : 3 : 3). The LWDHW powder mixtures were used to prepare different pellets under different conditions. Three different excipients (Avicel PH102®, lactose and Era-Tab®) were used in this study. LWDHW powder mixtures (125 g) were mixed with different excipients (20 g) and HPMC (2 g) for 15 min in a planetary mixer (KSMC50, Taiwan). Appropriate amounts of water were then added to the dry blends and mixed for an additional 4 min to obtain a wet mass. The wet mass was passed through an extruder (SY-86070-4, Taiwan) and the extrudate was placed in a spheronizer (SY-86070-3, Taiwan) and spheronized at different speeds for different spheronization times. The pellets were collected and dried in a hot air oven at 40°C for 12 h.

IV. *L16 (2^{15}) Orthogonal Array Experimental Design*

Orthogonal array experimental designs were performed

Table 1. Two levels of six variables in experimental design

Variables	Avicel PH102®		Lactose		Era-Tab®	
	Low level(1)	High level(2)	Low level(1)	High level(2)	Low level(1)	High level(2)
A: Ratio of HPMC (K15M:K4M)	0 : 2	1 : 1	0 : 2	1 : 1	0 : 2	1 : 1
B: Amount of water content (mL)	60	65	45	65	55	60
C: Extrusion speed (rpm)	30	50	30	50	30	50
D: Extrusion screen size (mm)	1.0	1.5	1.0	1.5	1.0	1.5
E: Spheronization time (min)	5	10	5	10	5	10
F: Spheronizer speed (rpm)	700	900	700	900	700	900

Table 2. L16 (2^{15}) orthogonal array design matrix

Run	A 1	B 2	A×B 3	C 4	error 5	E×F 6	error 7	D 8	error 9	error 10	F 11	C×D 12	E 13	error 14	error 15
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
3	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
4	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
5	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2
6	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1
7	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1
8	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2
9	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
10	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1
11	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1
12	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2
13	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1
14	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2
15	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2
16	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1

A: Ratio of HPMC, B: Amount of water content, C: Extrusion speed, D: Extrusion screen size, E: Spheronization time, F: Spheronizer speed

and the responses were measured as roundness and yields. The variables are given in Table 1. Six variables A to F represent ratio of HPMC, amount of water content, extrusion speed, extrusion screen size, spheronization time and spheronizer speed, respectively. Each variable occurs at two suitable levels (low level (1), high level (2)). The experimental design matrix is shown in Table 2. It contains six variables in this L16 (2^{15}) orthogonal array design, so the remaining columns can be used to estimate three interactions (variable A×B, variable C×D and variable E×F) and errors (Table 2). For example, in the first experiment (Run 1), variables A, B, C and D are all of low level, that is, 2 g of HPMC (K4M) and 60 mL of water were used to obtain a wet mass, which was then passed through an extruder of 1.0-mm screen size with an extrusion speed of 30 rpm. The extrudate was processed in a spheronizer at 700 rpm for 5 min, and the resultant pellets were dried in a hot air oven and collected. All 16 formulations were performed in the same manner in random order. The experimental results were analyzed with the design-expert® software (Version 6).

V. *Morphological Characterization of Pellets*

The morphology of the intact pellets was investigated using optical microscopy and scanning electron microscopy (JSM 5300, Japan).

VI. *Size Distribution of Pellets*

The various batches were fractioned into eight particle size ranges using 1400, 1180, 1000, 850, 710, 600 and 425 μm sieves on a moving (vibrating) sieve shaker for 15 min. The pellets obtained from the various sieves were weighed, and the largest fraction pellets were used for the determination of roundness and yield percentage.

VII. *Roundness*

Pellet roundness was evaluated by optical microscopy. The roundness index was determined by elongation (E), calculated as the length (R_1) divided by the diameter (R_2) of pellets[11,12]. At least 70 pellets of each sample were determined.

$E = R_1 / R_2$

R_1 : length of pellets

R_2 : diameter of pellets

VIII. *Yield*

The yield of pellets (% w/w) was calculated as the weight of the largest fraction pellets (850-1000 μm pellets prepared with Avicel PH102® and Era-Tab® and 1180-1400 μm pellets prepared with lactose) obtained from size analysis distribution divided by the sum of the total weight of pellets.

IX. *Preparation of LWDHW Capsules and Tablets*

The pellets of selected particle size ranges for the optimized formulation were weighed and filled into empty capsules without adjuvants. In addition, the individual pellets were mixed with 0.1 g of HPMC, fed into a die (10 mm in diameter), and compressed using an automated Carver press under a pressure of 135 kg/cm^2. The capsules and tablets were placed in a desiccator for further studies.

X. *Drug Release*

Dissolution studies were carried out using a standard USP XXIV dissolution apparatus. The basket method and paddle method were used for capsules and tablets, respectively. The temperature of the dissolution medium was maintained at 37±0.5°C and the rotation speed of the basket or paddle was adjusted to 75 rpm. The capsules and tablets were introduced into 500 mL of 0.1 N hydrochloride solution (pH 1.2) or 0.1 M phosphate buffer medium (pH 6.8). 5-mL samples were withdrawn at specific time intervals (10, 20, 30 min, 1.0, 1.5, 2.0, 3.0, 6.0, 8.0 and 12.0 h) and analyzed by HPLC. Withdrawn samples were immediately replaced with fresh dissolution medium. All experiments were performed in triplicate. The conditions of HPLC analyses are described as follows : Paeonol was analysed using LiChrosorb RP-C 18 column with methanol – 0.01 M potassium dihydrogenphosphate solution (pH 2.3) (60:40, v/v) as mobile phase and UV detector at 275nm. Loganin was analysed using LiChrosorb RP-C 18 column with methanol – 0.01 M potassium dihydrogenphosphate solution (pH 2.3) (55:145, v/v) as mobile phase and UV detector at 235 nm. To explore the dissolution profile at various parameters, a 2^3 full factorial design was used and the variables and design matrix are listed in Table 3. The effects of dosage form (variable A), type of excipient (variable B) and pH value of dissolution medium (variable C) on the release profile of different conditions were evaluated. A low level of variable A represents the capsule dosage form, while a high level represents the tablet dosage form. The two levels of variable C represent the dissolution medium at pH 1.2 and pH 6.8 respectively. As the two levels of variable B represent two different excipients, the analyses of the three excipients were classified into two sections. The dissolution studies of capsules and tablets prepared by different excipients were performed to compare the release characteristics in pH 1.2 and 6.8 dissolution media. The time required for 60% release ($T_{60\%}$) of paeonol and loganin were used to evaluate the difference between different formulations. Furthermore, the dissolution characteristics ($T_{60\%}$) were compared by using the difference factor (f_1) and similarity factor (f_2). The two fit factors are defined by the equations below[13,14] :

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum R_t} \times 100\%$$

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

Table 3. A design matrix of 2^3 full factorial design

Formulation (Random No.)	Independent variables		
	A: Dosage form	B: Type of excipient*	C: pH value
5	Capsules	Excipient A	6.8
1	Tablets	Excipient A	6.8
6	Capsules	Excipient B	6.8
7	Capsules	Excipient A	1.2
2	Tablets	Excipient B	6.8
3	Tablets	Excipient A	1.2
8	Capsules	Excipient B	1.2
4	Tablets	Excipient B	1.2

*Excipient A / Excipient B : Avicel PH 102® / Lactose or Era-Tab® / Lactose

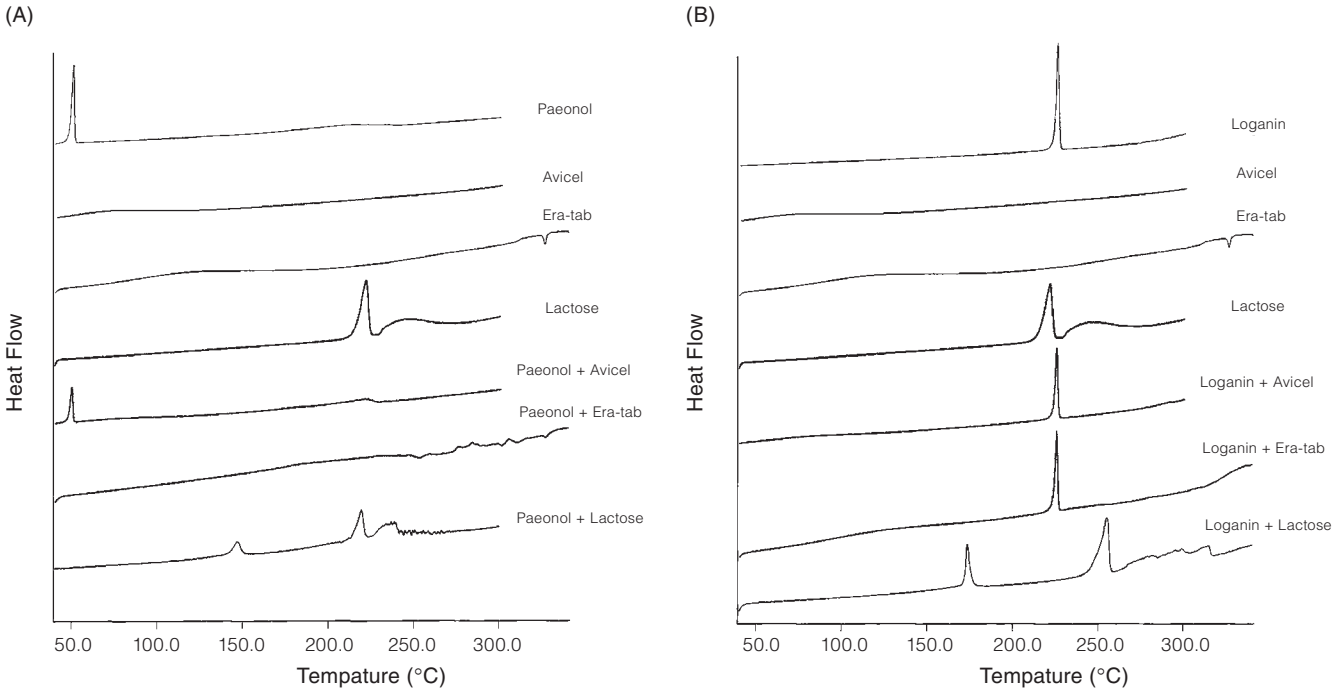


Figure 1. (A) DSC thermogram of paeonol and three different excipients (B) DSC thermogram of loganin and three different excipients.

where n is the number of dissolution sample times, T_t and R_t are the cumulative percentage released at each time point for the test and reference products, respectively.

XI. *Stability Studies*

LWDHW pellets and tablets were stored under three temperature conditions, 30, 37 and 45°C, and the relative humidity were 75%. A commercial LWDHW pellet dosage form was also studied to investigate the differences. Samples were taken at time intervals of 0, 30, 60 and 90 days. The active ingredients (paeonol and loganin) were extracted using 70% methanol and centrifuged at a speed of 110×g. The supernatants were analyzed by HPLC in triplicate for the content of the active ingredients.

RESULTS

I. *Differential Scanning Calorimetry (DSC)*

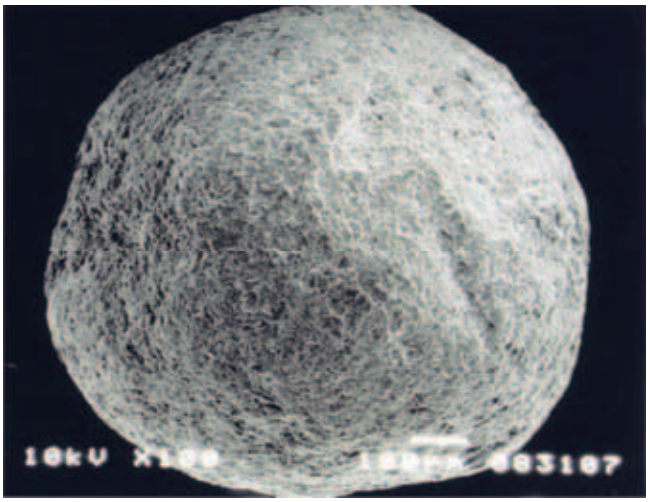
The thermograms of two active ingredients of LWDHW (paeonol and loganin) and three excipients were shown in Figure 1. The thermal curve of paeonol showed a sharp endothermic peak at 53.5°C (Figure 1A). This endothermic peak also appeared in the thermogram of the paeonol-Avicel PH102® mixture, but was absent in that of the Paeonol-Era-Tab® mixture. Besides, the thermogram of the paeonol-lactose mixture showed an absence of the peak at 53.5°C, but an appearance of new peak at 148°C. In the case of loganin (Figure 1B), the thermoanalysis of loganin-Avicel PH102® mixture and loganin-Era-Tab® mixture showed no transition in the examined temperature range. The sharp endothermic peak at 225°C did not disappear in both thermograms, indicating that no interaction occurred. However, from the thermogram of the loganin-lactose mixture, both the peak at 225°C (loganin) and the peak with a maximum at 220°C (lactose) disappeared, accompanied by the appearance of two new peaks at 175 and 250°C.

II. *Characterization of Pellets*

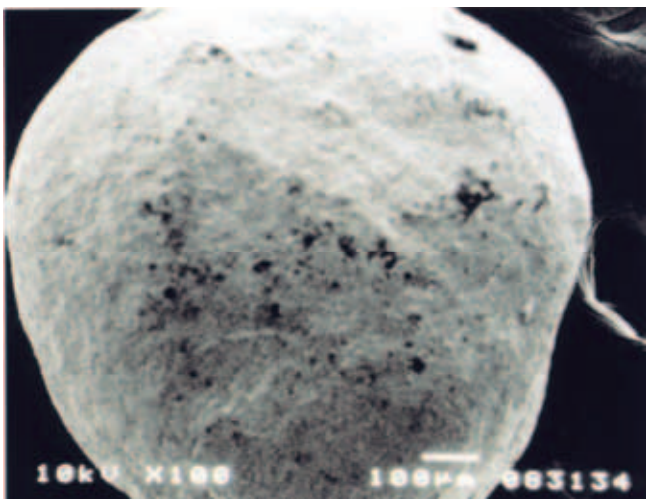
The LWDHW pellets were spherical in shape and exhibited a smooth surface as determined by optical and scanning electron microscopy on a representative sample. The SEM photographs of pellets prepared by three different excipients were shown in Figure 2. The pellets prepared by Avicel PH102® appeared to be rounder than the others. The size distribution of pellets of different formulations were studied using a sieve shaker. The pellets obtained from various sieves (1400, 1180, 1000, 850, 710, 600 and 425 μm) were weighed, and the largest fraction pellets were used to determine the roundness and yield percentage. In this study, 850-1000 μm pellets prepared using Avicel PH102® and Era-Tab® and 1180-1400 μm pellets prepared using lactose were selected.

The roundness and yield percentages of pellets prepared using different excipients are shown in Table 4. On comparison of the 16 formulations, pellets of runs 1, 3, 9, 10, 12, 13, 14 and 15 resulted in significantly less spherical pellets than the rest. The yield percentages of runs 1, 10, 12, 14 and 15 appeared to be higher than other runs.

(A)



(B)



(C)

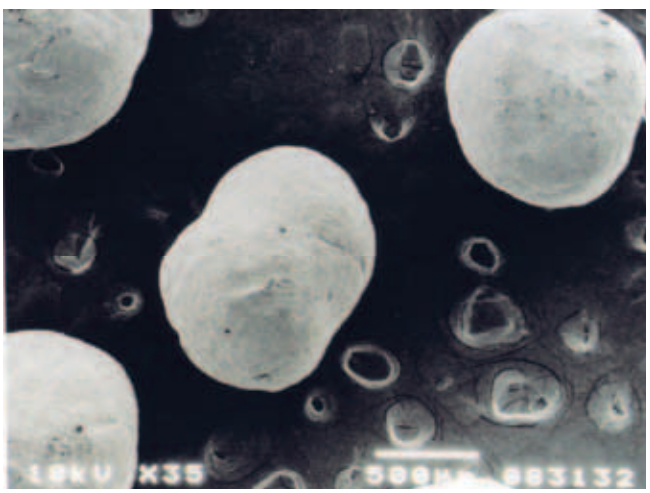


Figure 2. SEM photomicrographs of LWDHW pellets (A) Pellets prepared with Avicel PH102® (B) Pellets prepared with Era-Tab® (C) Pellets prepared with lactose.

Table 4. Roundness and yield percentages of pellets prepared by three different excipients

Run	Roundness			Yield percentage (%)		
	Avicel PH102®	Lactose	Era-Tab®	Avicel PH102®	Lactose	Era-Tab®
1	1.24	1.35	1.24	63.75	63.66	64.78
2	1.10	1.11	1.09	36.43	36.59	35.88
3	1.27	1.32	1.22	49.97	48.91	45.05
4	1.17	1.19	1.14	47.25	47.57	51.59
5	1.13	1.15	1.12	35.89	33.33	30.05
6	1.18	1.24	1.17	42.78	53.46	46.03
7	1.19	1.23	1.21	49.69	41.54	43.40
8	1.11	1.18	1.12	40.55	41.36	38.70
9	1.31	1.28	1.30	48.79	45.50	46.81
10	1.32	1.33	1.31	56.22	65.61	64.42
11	1.13	1.17	1.14	40.79	46.23	44.20
12	1.29	1.30	1.23	60.29	70.07	62.54
13	1.28	1.33	1.27	40.19	48.09	38.49
14	1.30	1.32	1.39	57.02	63.90	60.85
15	1.35	1.33	1.35	60.87	63.41	69.62
16	1.10	1.16	1.11	34.31	38.24	27.16

III. *Experimental Design of the Preparation of LWDHW Pellets*

The orthogonal experiments were performed in random order and the responses were measured as roundness and yields of the pellets respectively. The results obtained from a duplicate of the L16 (2^{15}) orthogonal experiments are shown in Table 5 and 6. Variables D and F (extrusion screen size and spheronizer speed) were observed to have an important influence on the roundness of the pellets and the yield of pellets prepared with Avicel PH102®.

IV. *In vitro Dissolution Study*

The release profiles of capsules and tablets prepared by different excipients are shown in Figures 3A-D. Two dosage forms (capsule and tablet) and dissolution media of two different pH values (pH 1.2 and 6.8) were studied. On comparing the release characteristics of capsules and tablets under different dissolution conditions, the release rates of capsules were faster than tablets, indicating that the effect of dosage form on release characteristics was significant. The time required for 60% release of paeonol and loganin ($T_{60\%}$) were used to evaluate the difference between different formulations. Two fit factors (f_1 and f_2) of tablets prepared with different excipients were calculated and listed in Table 7. The f_1 values of pH 1.2 and 6.8 dissolution media were close to zero, while the f_2 values were between 50 and 100 for different tablets.

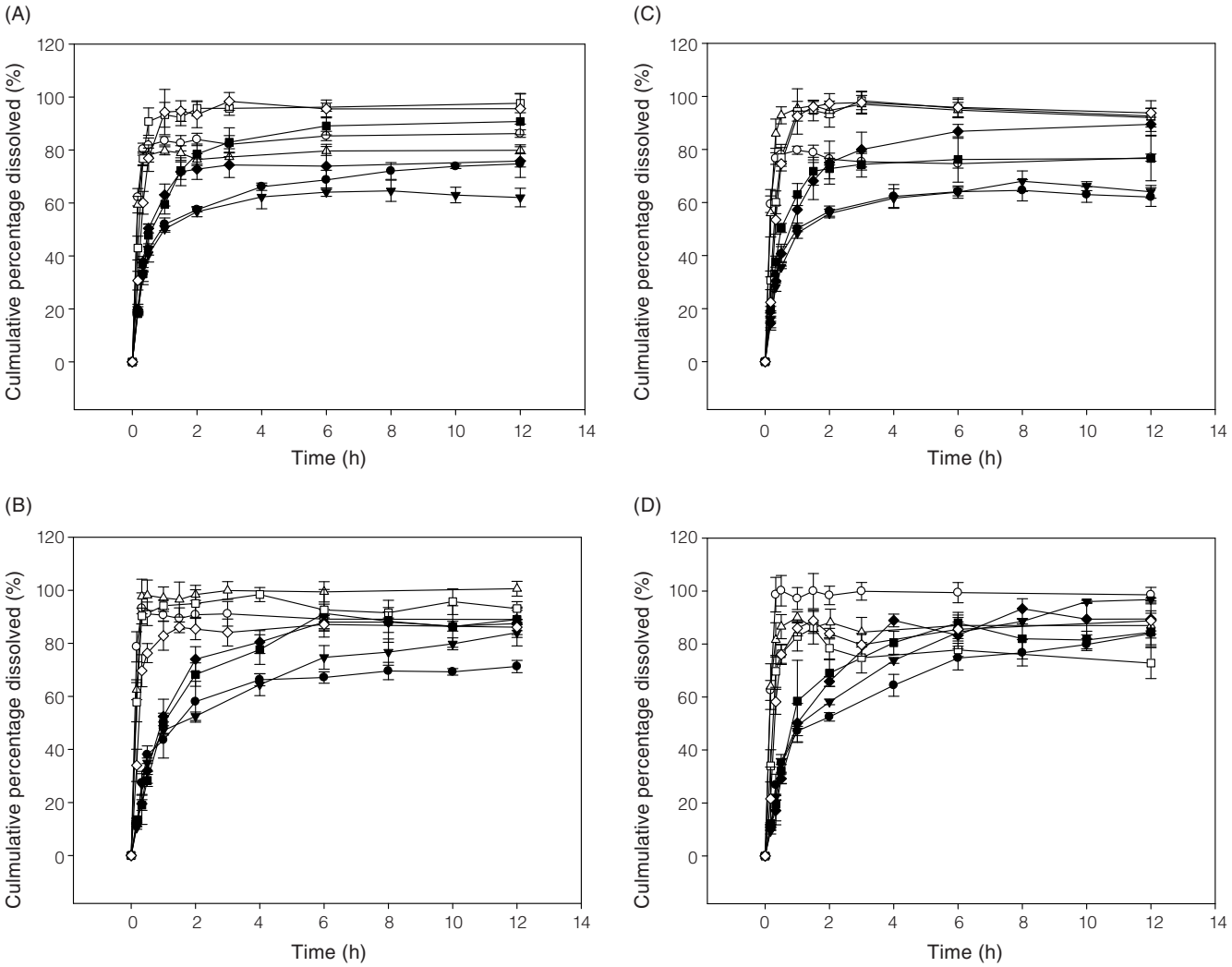


Figure 3. Release profile of different formulations of 2^3 full factorial design (A) Release profile of paeonol from different formulations (excipient A: Avicel PH102®; excipient B: Era-Tab®) (B) Release profile of loganin from different formulations (excipient A: Avicel PH102®; excipient B: Era-Tab®) (C) Release profile of paeonol from different formulations (excipient A: Era-Tab®; excipient B: lactose) (D) Release profile of loganin from different formulations (excipient A: Era-Tab®; excipient B: lactose).
◆ Tablets / excipient A / pH 6.8 ■ Tablets / excipient B / pH 6.8 ▼ Tablets / excipient A / pH 1.2 ● Tablets / excipient B / pH 1.2 ◇ Capsules / excipient A / pH 6.8 □ Capsules / excipient B / pH 6.8 △ Capsules / excipient A / pH 1.2 ○ Capsules / excipient B / pH 1.2

V. *Stability Studies*

The effects of temperature on the contents of paeonol and loganin in different formulations were shown in Figures 4 and 5. No significant difference was observed between commercial LWDHW pellets and other dosage forms for paeonol at different temperatures (Figures 4A-C). For the content of loganin under different conditions, the pellets and tablets prepared in this study exhibited similar degradation behavior, which was superior to that of commercial LWDHW pellets (Figure 5A-C). Besides, the degradation of paeonol under 45°C/75% R.H. was faster than that under 30°C/75% R.H. and 37°C/75% R.H., but the difference was less distinct for loganin, indicating that the influence of temperature on paeonol in LWDHW pellets was higher than that on loganin.

DISCUSSION

It has been suggested that DSC can be a useful method for predicting compatibility during preformulation studies[15,16]. Although thermal analysis cannot replace classical long-term stability tests completely, DSC allows for fast evaluation of possible incompatibility between formulation components, deriving from the appearance, shift or disappearance of peaks and/or variation in the corresponding ΔH. It has been considered a useful tool and applied as the first step for the screening of candidate excipients, because of its speed and convenience.

Avicel PH102® was found to be compatible with both paeonol and loganin (Figure 1). On the contrary, lactose was found to be incompatible with both active ingredients. Although lactose is generally considered inert and unreactive

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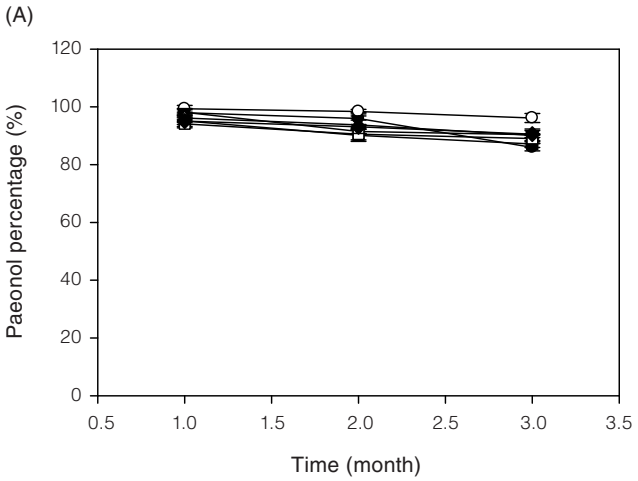
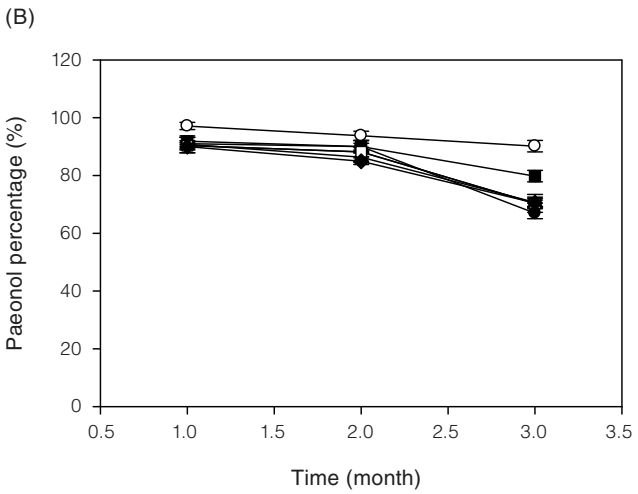




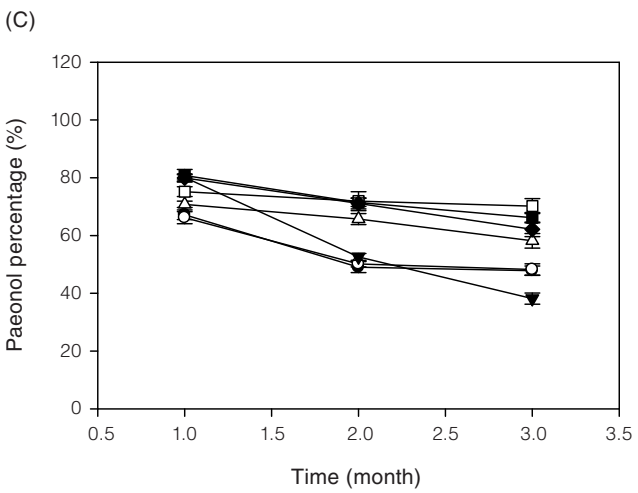
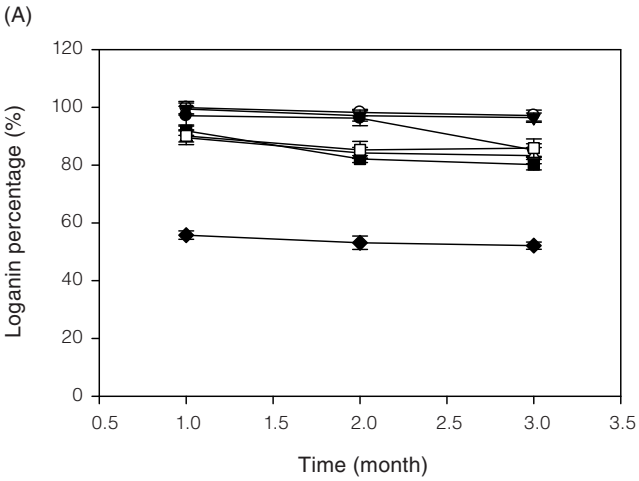
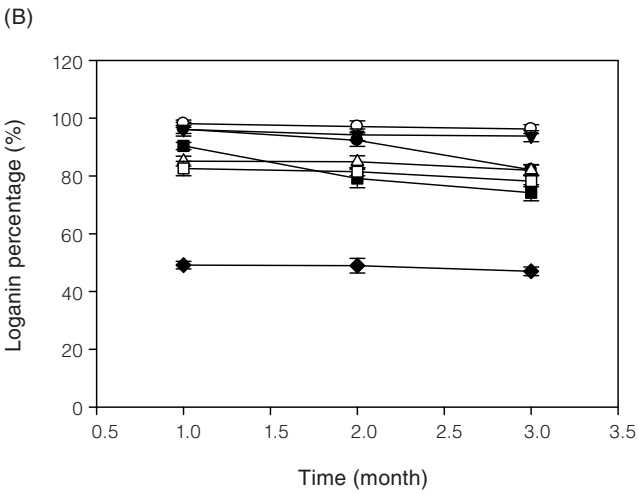


Figure 4. Paeonol contents of pellets and tablets stored under three conditions (A) 30°C/75% R.H. (B) 37°C/75% R.H. (C)45°C/75% R.H. ●Avicel PH102 / pellet ○Era-Tab / pellet ▼lactose / pellet △Avicel PH102 / tablet ■Era-Tab / tablet □lactose / tablet ◆commercial LWDHW pellet.





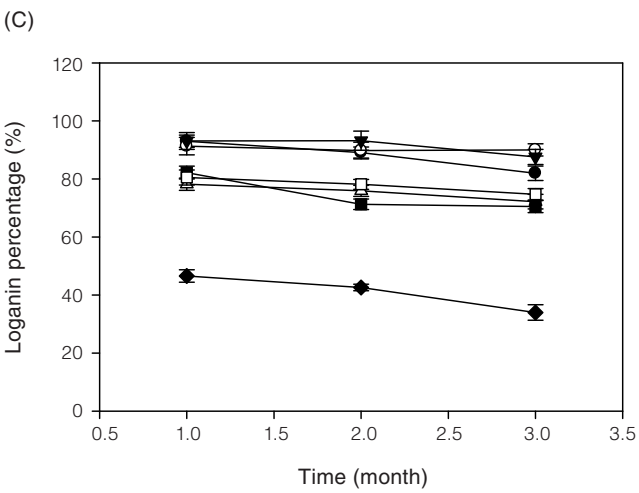


Figure 5. Loganin contents of pellets and tablets stored under three conditions (A) 30°C/75% R.H. (B) 37°C/75% R.H. (C) 45°C/75% R.H. ●Avicel PH102 / pellet ○Era-Tab / pellet ▼lactose / pellet △Avicel PH102 / tablet ■Era-Tab / tablet □lactose / tablet ◆commercial LWDHW pellet.

and was most commonly used in pharmaceutical dosage forms, its incompatibility with different drugs had been reported[17,18]. The effects of different excipients (microcrystalline cellulose and lactose) on the stability of Indorenate hydrochloride were described by Villalobos-Hernández and Villafuerte-Roble[16]. Lactose was observed to influence the stability of Indorenate hydrochloride to a larger extent, due to a specific interaction of the primary amino group of the

Table 5. Analysis of variance for roundness of pellets prepared with different excipients

Source of Variation[a]	Degrees of Freedom	Avicel PH102®				Era-Tab®				Lactose			
		Sum of Squares	Mean square	F	prob>F[b]	Sum of Squares	Mean square	F	prob>F[b]	Sum of Squares	Mean square	F	prob>F[b]
A	1	0.004225	0.004225	5.32	0.0606	0.000441	0.000441	0.26	0.6342	0.000605	0.000605	0.45	0.5251
B	1	0.005625	0.005625	7.08	0.0654	0.006561	0.006561	3.81	0.1083	0.002016	0.002016	1.52	0.2644
A×B	1	0.00198	0.00198	2.49	0.1654	0.000196	0.000196	0.11	0.7494	0.001232	0.001232	0.93	0.3730
C	1	0.00483	0.00483	6.08	0.0871	0.000841	0.000841	0.49	0.5156	0.000252	0.000252	0.19	0.6781
E×F	1	0.000144	0.000144	0.18	0.6851	0.000	0.000	0.000	1.0000	0.001116	0.001116	0.84	0.3951
D	1	0.015	0.015	19.20*	0.0047	0.017	0.017	9.60*	0.0269	0.014	0.014	10.19*	0.0188
F	1	0.068	0.068	85.44*	<0.0001	0.083	0.083	48.39*	0.0009	0.061	0.061	46.20*	0.0005
C×D	1	0.000650	0.000650	0.82	0.4004	0.000272	0.000272	0.16	0.7072	<0.0001	<0.0001	6.322E003	0.9392
E	1	0.007921	0.007921	9.97	0.0514	0.00801	0.00801	4.66	0.0834	0.008118	0.008118	6.10	0.0501
Residual	6	0.000794	0.000132			0.009601	0.00172			0.007981	0.00133		
Total	15	0.1092				0.1259				0.09632			

*[a] A: Ratio of HPMC, B: Amount of water content, C: Extrusion speed, D: Extrusion screen size, E: Spheronization time, F: Spheronizer speed
*Significant : [b]<0.0500

drug with the aldehyde group of lactose. Besides, lactose was found to interact with ketoprofen[18] and incompatibility can also be assumed in the case of a clenbuterol-lactose mixture[17]. The interactions between drugs and excipients could be predicted with a DSC compatibility analysis and a suitable excipient should be chosen to ensure the optimum stability of the product. In this study, Avicel PH102® was more suitable for LWDHW pellets than lactose and Era-Tab®.

During the course of extrusion and spheronization, pellet properties are affected by different preparation conditions. The variance between different formulations and the effective variables were discussed in detail by experimental analyses. Six important variables, including the ratio of two kinds of HPMC (variable A), water content (variable B), extrusion speed (variable C), extrusion screen size (variable D), spheronization time (variable E) and spheronizer speed (variable F), were chosen to investigate their effects on the pharmaceutical properties of pellets. An L16 (2^{15}) orthogonal array design was employed to obtain optimal formulation and the data was compared statistically using analysis of variance (ANOVA).

The effects of the individual variables (variables A, B, C, D, E and F) and their interactions (A×B, C×D and E×F) in the orthogonal design of experiments could be calculated and the statistical differences were assessed using ANOVA[19]. A summary of the results from the 16 experiments in terms of the roundness of the resultant pellets prepared with three excipients and an analysis of variance is shown in Table 5. Variables D and F (extrusion screen size and spheronizer speed) were observed to have an important influence on the roundness of the pellets prepared with Avicel PH102®. The response values of the effects[20] showed that the effects of variables D and F were positive (Figure 6). Increasing variables D and F led to an increase in the roundness of pellets from 1.251 to 1.189 and 1.285 to 1.115, respectively, indicating that that the best result could be obtained using a larger screen size and higher spheronizer speed. The correlation between the response (roundness) and the effective experimental variables (D and F) could be calculated and the polynominal equation obtained is as follows:

$$Y = 1.22 - 0.031x_4 - 0.065x_5$$

where the code variables x_4 and x_5 represent variables D and F, respectively.

In order to identify the equation, the results of the experimentally obtained and equation-predicted response were calculated and shown in Figure 7A. The predicted value demonstrated a good agreement with the experimental data ($r = 0.95$), thus supporting our results that variable D and F were the only significant effects in this part. This result could probably be attributed to a more regular shape of pellets obtained from an extrusion screen size of 1.5-mm and the frictional force was larger under higher spheronization speed (900 rpm) than a lower spheronization speed (700 rpm).

The results from the yield studies are shown in Table 6. According to the results, the important factors influencing the

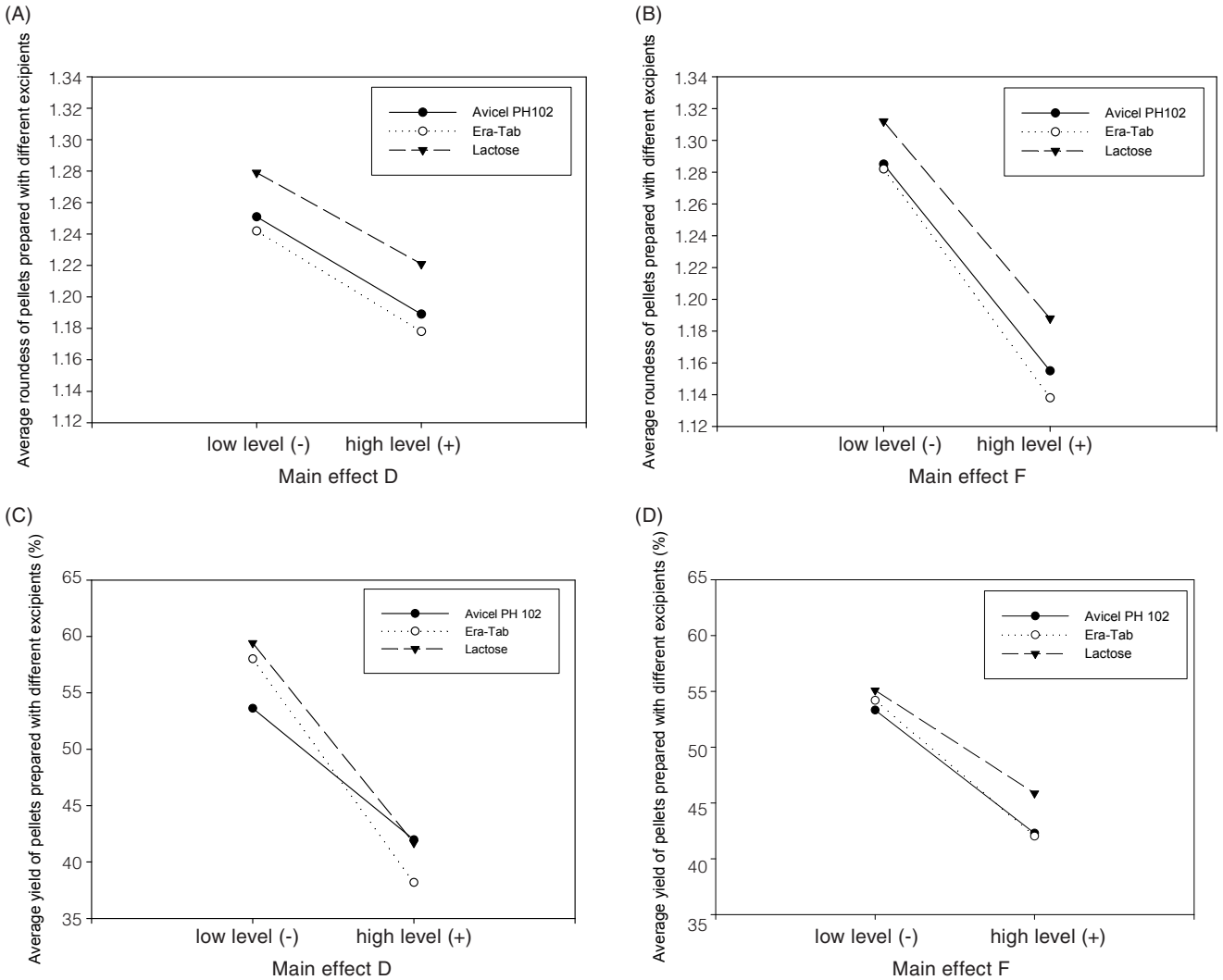


Figure 6. Plot of roundness and yield percentage for variables D and F (A) Roundness plot for variable D (B) Roundness plot for variable F (C) Yield percentage plot for variable D (D) Yield percentage plot for variable F.
(variable D : extrusion screen size, variable F : spheronizer speed)

yield of pellets prepared with Avicel PH102® were variables D and F (extrusion screen size and spheronization speed). This was similar to the results obtained from the roundness analysis. However, the two effects were negative for yield percentage. For instance, the yield percentage at a low level of variable D was 53.62%, compared to 41.98% at a high level (Figure 6). For variable F, the yield percentage at a low level was 53.31%, compared to 42.29% at a high level. Increasing the extrusion screen size (variable D) and spheronization speed (variable F) led to a decrease in the yield of pellets.

The polynominal equation obtained is as follows:

$$Y = 47.8 - 5.82\ x_4 - 5.51\ x_5$$

The equation-predicted value demonstrated good agreement with the experimental data (r = 0.90) (Figure 7B).

In the manner described previously, the experiment results for pellets prepared with other excipients (Era-Tab® and lactose) are also discussed (Tables 5 and 6). Compared with the results from Avicel PH102®, the consequence was similar and regular. The effective variable analysis of lactose and Era-Tab® was in accordance with Avicel PH102®. The influence of extrusion screen size (variable D) was smaller than spheronizer speed (variable F) (Figures 6A and B). For instance, the y values obtained from the equation at a low level and a high level of extrusion screen size (variable D) were close, but big difference was observed when increasing spheronizer speed (variable F) from a low level to a high level. For yield studies (Figures 6C and D), extrusion screen size and spheronizer speed were effective variables, but it was obvious that extrusion screen size was more effective than spheronizer speed, indicating that spheronizer speed had a greater influence on the roundness of pellets, while extrusion screen size had a greater influence on the yield of pellets. Furthermore, the type of excipient did not significantly affect the experimental results.

Table 6. Analysis of variance for yield percentage of pellets prepared with different excipients

Source of Variation[a]	Degrees of Freedom	Avicel PH102®				Era-Tab®				Lactose			
		Sum of Squares	Mean square	F	prob>F [b]	Sum of Squares	Mean square	F	prob>F [b]	Sum of Squares	Mean square	F	prob>F [b]
A	1	30.61	30.61	0.84	0.3938	56.06	56.06	2.74	0.1489	5.03	5.03	0.16	0.7021
B	1	1.24	1.24	0.034	0.8596	8.34	8.34	0.41	0.5468	68.93	68.93	2.21	0.1879
A×B	1	1.75	1.75	0.048	0.8335	11.14	11.14	0.54	0.4884	1.40	1.40	0.045	0.8394
C	1	33.15	33.15	0.91	0.3761	125.83	125.83	6.15	0.0512	48.62	48.62	1.56	0.2587
E×F	1	60.57	60.57	1.67	0.2439	35.67	35.67	1.74	0.2348	29.13	29.13	0.93	0.3715
D	1	542.31	542.31	14.95*	0.0083	1569.94	1569.94	76.73*	0.0001	1231.13	1231.13	39.41*	0.0008
F	1	486.31	486.31	13.40*	0.0106	591.34	591.34	28.89*	0.0017	340.13	340.13	10.89*	0.0164
C×D	1	4.30	4.30	0.12	0.7425	6.01	6.01	0.29	0.6072	1.94	1.94	0.062	0.8116
E	1	6.67	6.67	0.18	0.6831	10.71	10.71	0.52	0.4966	88.31	88.31	2.83	0.1437
Residual	6	36.29	6.05			122.76	20.46			187.42	31.24		
Total	15	1384.62				2537.81				2002.03			

**[a] A: Ratio of HPMC, B: Amount of water content, C: Extrusion speed, D: Extrusion screen size, E: Spheronization time, F: Spheronizer speed
*Significant : [b]<0.0500

In vitro dissolution tests were studied for pellets prepared under optimal conditions obtained from experimental design. In addition, the release profile of tablets compressed by different pellets was investigated to evaluate the differences between two dosage forms. Although it was clear that the release rates of capsules were faster than tablets, an indication that the effect of dosage form on release characteristics was significant, it was difficult to observe the influence from excipient and pH value. Therefore, the release characteristics were evaluated by 2^3 full factorial design, with the analyses of the three excipients classified into two sections.

The effects of the individual variables A, B and C

Figure 7. Plot of predicted value versus actual value for pellets prepared with Avicel PH102® (A) Roundness analysis (B) Yield percentage analysis.

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Table 7. The differences of two fit factors (f_1 and f_2) of tablets prepared with different excipients in pH 1.2 and pH 6.8 dissolution media

	Avicel PH102® and Lactose		Avicel PH102® and Era-Tab ®		Era-Tab ® and Lactose	
	pH 1.2	pH 6.8	pH 1.2	pH 6.8	pH 1.2	pH 6.8
Paeonol	$f_1 = 9.82$	$f_1 = 4.38$	$f_1 = 7.94$	$f_1 = 9.91$	$f_1 = 5.01$	$f_1 = 13.27$
	$f_2 = 61.22$	$f_2 = 71.6$	$f_2 = 63.14$	$f_2 = 52.97$	$f_2 = 76.30$	$f_2 = 50.61$
Loganin	$f_1 = 9.08$	$f_1 = 9.13$	$f_1 = 11.10$	$f_1 = 6.33$	$f_1 = 5.60$	$f_1 = 9.68$
	$f_2 = 57.07$	$f_2 = 54.55$	$f_2 = 55.86$	$f_2 = 66.57$	$f_2 = 71.93$	$f_2 = 56.57$

f_1 (difference factors) : The f_1 value is zero when the two dissolution profiles are identical.
f_2 (similarity factors) : The f_2 value between 50 and 100 suggests the two dissolution profiles are similar.

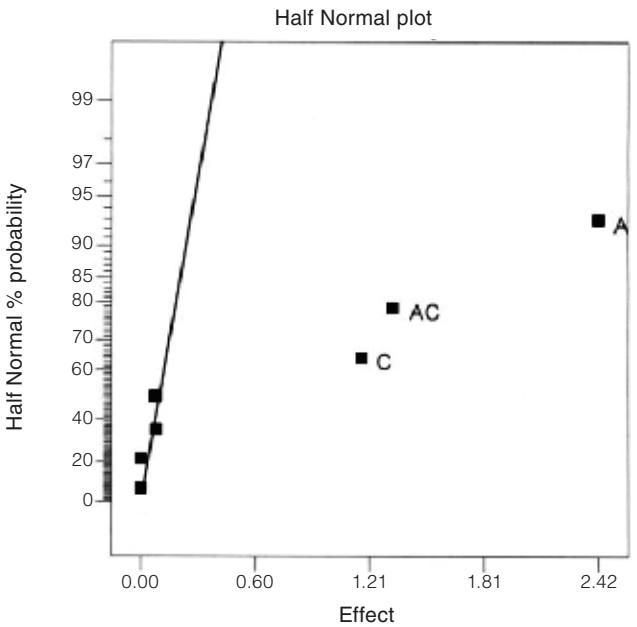

Figure 8. Factorial analysis of the effect of individual variables (variable A, B and C) and their interactions on paeonol release (excipient A: Avicel PH102®; excipient B: lactose).

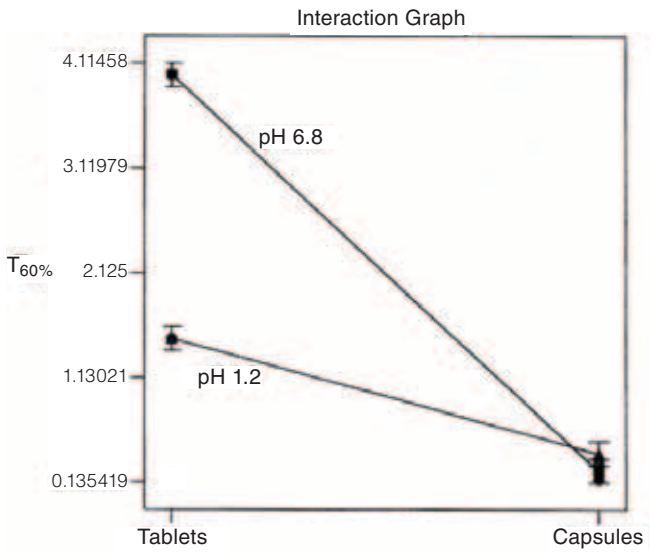

Figure 9. The dosage form - pH value of dissolution medium interaction (A×C interaction) of tablets prepared with different excipients. (excipient A: Avicel PH102®; excipient B: lactose).

(dosage form, type of excipient and pH value of dissolution medium) and the interactions between the three variables in the factorial design of excipients could be calculated using a table of contrast coefficients[20]. From these contrasts, we could estimate the 7 factorial effects as well as the normal probability plot of these effects, as shown in Figure 8. The important effects that influenced the $T_{60\%}$ of different formulations seemed to be the main effects of dosage form (variable A), pH value (variable C) and dosage form - pH value interaction (A×C interaction), which were far from the line.

The response values of the effects could be calculated and the values showed that the two main effects (dosage form and pH value) were negative, and dosage form - pH value interaction was negative, too. However, the main effects alone did not have much meaning when they were involved in significant interactions. Those interactions were the key to getting the optimal conditions. The results obtained from the dosage form - pH value interactions are shown in Figure 9 and can be described as follows: Dosage form had little effect on $T_{60\%}$ at pH 1.2 dissolution medium, compared with that at pH 6.8. Capsules at pH 1.2 dissolution medium tended to decrease the $T_{60\%}$ value, but the influence was minor compared with that at pH 6.8. Furthermore, pH value had little effect on $T_{60\%}$ at capsule dosage forms, compared with that at tablet dosage forms. This indicated that the effect of the pH value of the dissolution medium on the $T_{60\%}$ value was minor for pellets, but obvious for tablets. The results from the dissolution profiles and analyses of 2^3 experimental design were identical, indicating that the dosage form was the most effective variance on the $T_{60\%}$ value. For variable B (excipients), pellets or tablets prepared by different excipients showed similar dissolution profiles, and the analyses of variance showed that the type of excipient was not a significant variance. Therefore, the type of excipient made no difference in the preparation procedure or dissolution profiles. Two fit factors (f_1 and f_2) of tablets prepared with different excipients were calculated and listed in Table 7. The f_1 value was zero when the test and reference profiles were identical and increased proportionally with dissimilarity between two profiles. Moreover, the f_2 value was 100 when the profiles were identical. An f_2 value between 50 and 100 suggested that the two dissolution profiles were similar. All

the f_1 factors of the pH 1.2 and pH 6.8 dissolution media were close to zero and f_2 factors of between 50 and 100 were obtained for different tablets, demonstrating good agreement with the experimental data.

Using the same manner to analyze the difference between pellets and tablets prepared with Era-Tab and lactose, the results were identical, indicating that variable A, C and A×C interaction were the significant effects for the experimental design, and the type of dosage form (pellets and tablets) was the most significant variable for release profiles. For the same amount of active ingredients, the total surface area of pellets was larger than that of tablets, resulting in a faster release from pellets than tablets. Another reason for the slower release rate of tablets may be due to the addition of HPMC as an adjuvant, which may mitigate the destruction of pellets under compression pressure. Pellets were not destroyed or destroyed only to a small extent, forming a harder and tighter structure and resulting in a slower initial release rate for tablets[21]. SEM photomicrographs of the cross-sectional area of tableted pellets compressed by different pellets prepared with three excipients are shown in Figure 10. The integrity of pellets shown in these figures may confirm the opinion described previously.

The stability of commercial LWDHW pellets and six formulations prepared in this study was studied. No significant difference was observed between commercial LWDHW pellets and other dosage forms for paeonol at different temperatures (Figure 4A-C). However, on comparison of the LWDHW pellet and tablet dosage forms, LWDHW tablet dosage forms exhibited more stability for paeonol at higher temperature (Figure 4C). The content of paeonol at a time interval of 90 days was decreased to about 38-48% for pellets at 45°C/75%R.H., while that for tablets was 58-70%, indicating that the effect of temperature on paeonol degradation was less significant for tablets. Comparing the stability test results with DSC thermogram, the low melting point of paeonol (Figure 1A) might cause a higher degradation rate at higher temperature. On the other hand, the content of loganin in the pellets and tablets exhibited similar degradation behaviors and were superior to commercial LWDHW pellets. The content of loganin at a time interval of 90 days for commercial LWDHW pellets was decreased to 34-52%, lower than other formulations prepared in this study (Figure 5A-C). Another active ingredient of LWDHW, paeoniflorin, an active ingredient of Moutan cortex (besides paeonol), also exhibited similar results. The stability of paeoniflorin in the LWDHW pellets or tablets prepared in this study was better than that in commercial LWDHW dosage form (data not shown). Therefore, the LWDHW pellets and tablets prepared in this study may be properly prepared under optimal preparation conditions and demonstrated good dissolution and stability properties.

CONCLUSIONS

In this study, a novel TCM dosage form was prepared by the extrusion-spheronization method. In the manufacturing process, the type of HPMC and concentration, water content, extrusion speed and spheronization time did not

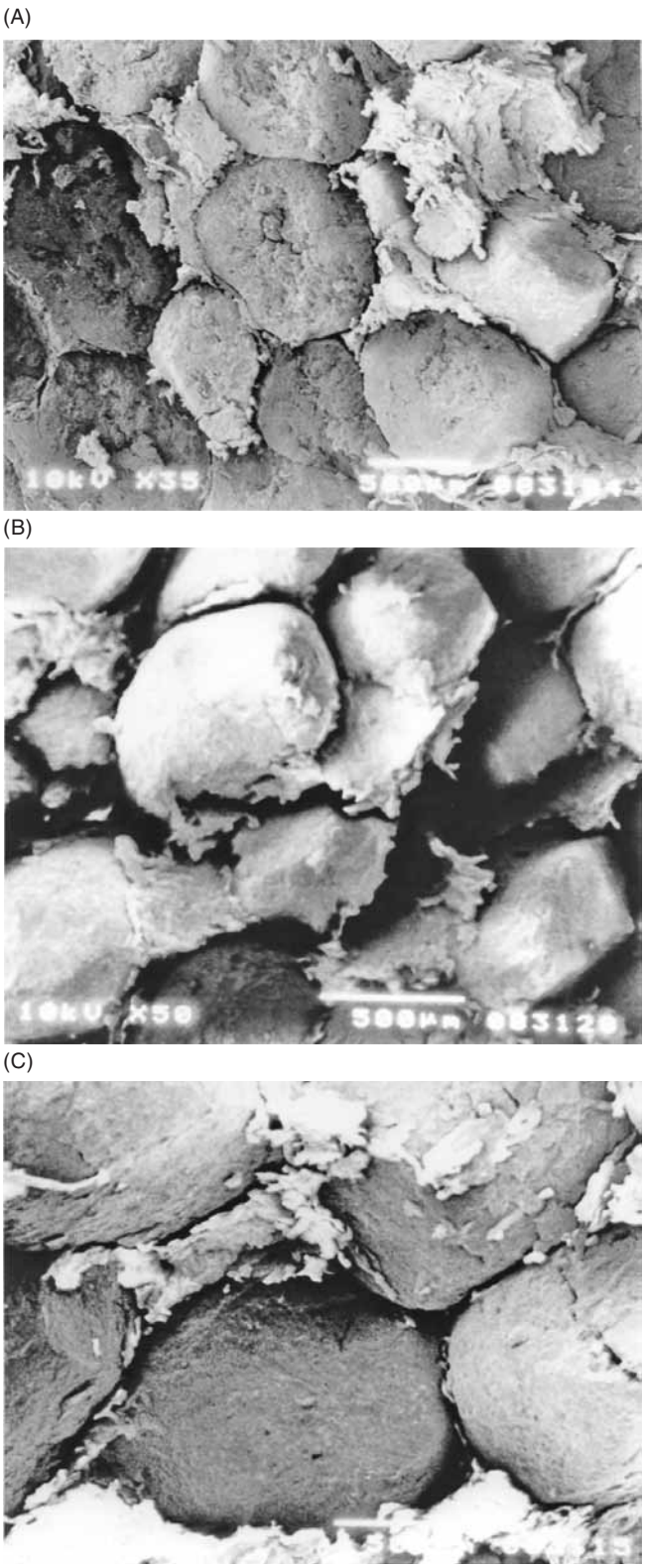

Figure 10. SEM photomicrographs of tableted LWDHW pellets (A) Pellets prepared with Avicel PH102® (B) Pellets prepared with Era-Tab® (C) Pellets prepared with lactose.

have significant effects on the studied responses. These variables can be fixed at convenient levels. On the other hand, the extrusion screen size and spheronizer speed are critical, with a more regular pellet shape obtained from a higher spheronizer speed and larger extrusion screen size. Conversely, increasing the extrusion screen size and spheronizer speed led to a decrease in the yield of pellets. By varying the size of the extrusion screen and spheronizer speed, it is feasible to obtain optimal pellets. The dissolution behavior showed that the effect of pH value on $T_{60\%}$ values was minor for tablets but significant for pellets, while the type of excipient was not a significant variance in the *in vitro* dissolution study, that is, the release profile of pellets prepared with different excipients exhibited similar characteristics. Although the effects of different excipients on stability was not obvious, the calorimetric studies showed a probable interaction between the active ingredient of LWDHW and lactose, suggesting that lactose may not be a suitable excipient for the LWDHW dosage form. In this study, the prepared pellets exhibited good compression properties and maintained their integrity during the compaction process. Tablets displayed prolonged release behavior and finer stability characteristics than pellets. The results from this study may provide a novel way of preparing LWDHW as well as other TCM dosage forms.

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