INTRODUCTION

Many nanoparticle drug delivery systems have been investigated because of their advantages over conventional dosage forms [1–3]. Solid lipid nanoparticles (SLNs) are one method of drug delivery and the first generation of a lipid nanoparticle system. There is interest in using them to deliver various drugs and substances. SLNs have several advantages: controlled release of drugs, avoidance of organic solvents, low toxicity, biocompatibility, enhanced permeability, and bioavailability [4]. Moreover, SLNs can incorporate both hydrophilic drugs, and they have a high potential for containing lipophilic drugs such as FZ. SLNs are solid lipids derived from nano-emulsions formed by changing oil with a solid lipid [4]. Many solid lipids have been used as SLNs in products such as waxes, cholesterol, triglycerides, and fatty acids [5]. The saturated 14-carbon (Ma), 16-carbon (Pa), and 18-carbon (Sa) chain fatty acids have generally been used as a lipid matrix to formulate SLNs in the pharmaceutical field. They are solid at the body or room temperature, have low toxicity and are biocompatible in the body [6]. The physicochemical properties and melting points of those fatty acids are different because of their structure's number and the length of their carbon chain. Their other properties affect the performance of SLNs [6]. FZ is a broad-spectrum drug that is used both orally and locally to treat fungal infections or relieve their symptoms. FZ is only slightly soluble in water. Several methods have been used to prepare FZ in various dosage forms, and trial-and-error has been the traditional approach. This method is laborious, unpredictable, expensive, and time-consuming [7, 8]. Response surface methodology (RSM) is an option for correcting these points and optimize several pharmaceutical formulations, and one popular type of RSM is used to optimize the simplex centroid design [9]. This research aimed to prepare FZ-loaded SLNs using the simplex centroid design and the effects of fatty acids to optimize the SLNs as small-sized nanoparticles with narrow size distribution. Hot emulsification was used to prepare the FZ-loaded SLNs and to evaluate their PDI, particle size, zeta potential, and pH.

MATERIALS AND METHODS

Materials

FZ was bought from TCI (Tokyo, Japan). Ma, Pa, and Sa were purchased from Acros Organics (Belarus, Belgium). Polysorbate 80 was bought from PC Drug Co. Ltd. (Bangkok, Thailand).

Preparation of FZ-loaded SLNs

This approach used emulsification in means of hot homogenization [4]. An aqueous phase and an oil phase were prepared independently. The water phase was accurately weighted with polysorbate 80 dissolved and mixed in distilled water. The oil phase comprised different amounts and types of solid lipids: Sa, Pa, Ma (table 1), and FZ. Each phase was heated to 80 °C. As the two phases were heating to the setpoint, the oil phase was poured continually into the aqueous phase. Concurrently, homogenization (7000 rpm for 2 min) was used to mix the two phases to form an emulsion. The emulsion size was minimized using a probe sonicator at 100% amplitude for 15 min. The nano-sized emulsions were cooled to room temperature to form SLNs subjected to a physicochemical characterization test.

Design of experiment and analysis of statistics

The resulting types and concentrations of the FZ-loaded SLNs were evaluated with respect to their PDI, particle size, zeta potential, and pH, using the simplex centroid design. The composition of the FZ-loaded SLNs is shown in table 1. The three key ingredients, namely Sa (X1), Pa (X2), and Ma (X3), were identified as independent factors. The following equations were used to determine the limits of each ingredient.

\[0 \leq X_1 \text{ or } X_2 \text{ or } X_3 \leq 100 \% \quad (1)\]

\[X_1 + X_2 + X_3 = 100 \% \quad (2)\]

\[X_1 + X_2 + X_3 = 0.24 \text{ (g)} \quad (3)\]
The three key components of the FZ-loaded SLNs formulations were changed concurrently, and the total amount was adjusted to 100% (0.24 g).

The particle size, PDI, zeta potential, and pH (Y1-Y4, respectively) were dependent variables, as shown in Table 1. Design-Expert® software (version 9, USA) was used to estimate the responses of all model formulations (11 runs in random order, 3-factor, and 3-level). To optimize the SLNs to get small-sized nanoparticles with a narrow size distribution, numeric optimization was conducted, and the highest value of the desirability index of Ma, Pa, and Sa estimated by the software was selected. The consequent experimental and predicted values of the dependent responses were compared, and the accuracy and reliability were evaluated [9, 10]. Analysis of statistics used analysis of variance (ANOVA).

Characterization of PDI, Particle size, and pH of FZ-loaded SLNs
Malvern Zetasizer Nano ZS (Malvern, UK) was used to characterize the FZ-loaded SLNs regarding their PDI particle size, and pH. An appropriate amount of distilled water was used to dilute a sample, which was used to fill a zeta cuvette. The cuvette was set in the machine’s sample holder to examine its physicochemical properties. A pH meter (Mettler Toledo seven easy, Switzerland) was used to measure the pH of the SLNs.

RESULTS AND DISCUSSION
Table 1 shows the physicochemical properties of the FZ-loaded SLNs. The particle sizes ranged between 16.49 nm and 56.65 nm, and they were tiny. Fig. 1A shows the relationship between independent factors of the impact of Sa, Pa, and Ma on particle size. The large particle size could be seen only in the SLNs with a large ratio of Sa and Ma (red area of the figure). However, the particle size response surface shows a small blue area around the Sa conner, suggesting a small particle size when the FZ-loaded SLNs contained only Sa. This result was the opposite of another finding that preparing nanoparticles with a fatty acid with a longer carbon chain yields a larger size than other fatty acid prepared SLNs [6]. The higher viscosity and melting point of the fatty acid with the longer carbon chain may cause this phenomenon. The higher viscosity and melting point of fatty acids could disturb the efficacy of the homogenization process, resulting in larger particle size [6]. However, the amount of fatty acid to formulate the SLNs in this study was significantly lower than in the other report [6]. Hence, those factors might not explain the phenomenon that occurred in this study.

Table 1 also shows the PDI value, an indicator of the distribution of the particle sizes, which ranged between 0.258 and 0.676. All PDI values were lower than 0.7, indicating that the sizes of all the FZ-loaded SLNs were in a relatively narrow size distribution [4]. Fig. 1B shows PDI’s response surface for the effects of Sa, Pa, and Ma. The red area indicates the broad size distribution of the SLNs containing a proportion of Pa and Ma. In contrast, a narrow size distribution appears in the SLNs with large Sa and Pa ratios (blue color in the response surface) but is insignificant.

Table 1 displays the zeta potential of all the formulations of the FZ-loaded SLNs. They ranged between -7.47 mV and -12.2 mV. The carboxylic acid groups displayed on the molecular structures of the solid lipids affect the negative charge of the SLNs [11]. Fig. 1C shows the response surface for the amounts of the three components on the zeta potential. The zeta potential response surface shows a large red area around the Ma conner, referring to a high zeta potential (lower negative charge). In contrast, a higher negative zeta potential charge appeared in the SLNs with large Sa and Pa ratios. This result correlates with another study that a fatty acid with longer carbon chains led to increased zeta potential [6].

The pH of the FZ-loaded SLNs was around 4.63–4.77 (table 1). This indicates that the SLN system was a weak acid because of the carboxylic acid group’s ionization of the solid lipid’s molecular structure. Fig. 1D shows the response surface for the influence of the three components on pH, and there was a no significant difference in pH values.

The reliability of the dependent responses (Y1-Y4) was tested using the corresponding residual plot shown in Fig. 2A-D. There was random scattering in line from bottom to top in the randomized run. This means that data lay within a 95% confidence interval [11]. Fig. 2E-H shows the linear correlation between actual and predicted values. The correlation coefficient (R²) of all response variables was between 0.8944 and 0.9978. The high values of R² indicate a solid linear correlation [9]. The corresponding residual and the linear correlation plots imply that the response surface’s accuracy and reliability were relatively high.
Table 1: The simplex centroid design and response values

| Formulation | Actual value of independent variables | Response values |
|-------------|--------------------------------------|-----------------|
|             | Sa (g), X<sub>1</sub> | Pa (g), X<sub>2</sub> | Ma, X<sub>3</sub> | Particle size (nm), Y<sub>1</sub> | PDI, Y<sub>2</sub> | Zeta potential (mV), Y<sub>3</sub> | pH, Y<sub>4</sub> |
| S1          | 0 | 0 | 0.24 | 24.27±0.76 | 0.44±0.009 | -7.77±1.42 | 4.75±0 |
| S2          | 0 | 0.12 | 0.12 | 29.65±0.67 | 0.67±0.021 | -7.73±0.42 | 4.66±0 |
| S3          | 0 | 0.24 | 0 | 46.9±17.25 | 0.64±0.047 | -8.26±1.48 | 4.77±0 |
| S4          | 0.04 | 0.16 | 0.04 | 42.97±1.17 | 0.31±0.045 | -10.23±1.52 | 4.69±0 |
| S5          | 0.04 | 0.04 | 0.16 | 44.67±1.09 | 0.63±0.260 | -7.47±0.67 | 4.63±0 |
| S6          | 0.08 | 0.08 | 0.08 | 49.58±4.20 | 0.42±0.050 | -9.5±0.85 | 4.63±0 |
| S7          | 0.12 | 0 | 0.12 | 56.65±1.36 | 0.63±0.260 | -8.4±1.61 | 4.63±0 |
| S8          | 0.12 | 0.12 | 0 | 16.49±1.12 | 0.35±0.047 | -12.23±1.33 | 4.63±0 |
| S9          | 0.16 | 0.04 | 0.04 | 29.37±3.70 | 0.25±0.021 | -10.4±2.28 | 4.77±0 |
| S10         | 0.24 | 0 | 0 | 19.83±0.39 | 0.28±0.015 | -8.62±1.56 | 4.68±0 |
| S11         | 0.24 | 0 | 0 | 17.97±1.72 | 0.31±0.036 | -8.74±2.42 | 4.67±0 |

Table 2 shows the independent factors and response variables of the optimal FZ-loaded SLN formulation. The optimized formulation, which contained 0.190 g Sa, 0.048 g Pa, and 0.002 g Ma, was selected for preparation. This optimum formulation was selected from the small-sized nanoparticles' criteria with narrow size distribution by numeric optimization. The highest desirability index of Sa, Pa, and Ma estimated by the software was 0.976. This implied that the models could optimize all factors [9]. The experimental values of the particle size and the PDI of the optimal formulation did not differ significantly from predicted values, and they lay within the 95% CI. The percentage prediction error of the particle size and PDI were in the range of 8.12 to 15.35. These results suggest that the good prediction from the model confirmed the validity and reliability of existing response variable models.

CONCLUSION
The FZ-loaded SLNs were prepared by using hot emulsification. The effect of various types and amounts of fatty acids on the physicochemical properties of the SLNs was studied using the simplex centroid design. The particle size of the SLNs was in the nanometer range, and the PDI displayed a narrow size distribution. There was a negative charge on the zeta potential. The pH of the FZ-loaded SLNs was around 4.63–4.77, indicating that the SLN system was a weak acid. The results showed that the experimental values matched the expected response variables very well. Therefore, the experimental design using computer software could efficiently identify the optimal FZ-loaded SLN formulation.

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AUTHORS CONTRIBUTIONS
All authors have contributed equally.

CONFLICT OF INTERESTS
The author declares no conflict of interest.

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