Understanding the combination of fractional factorial design and chemometrics analysis for screening super-saturable quercetin-self nano emulsifying components

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Received 16 January 2022 • Accepted 15 February 2022 • Published 5 April 2022

Citation: Pratiwi G, Ramadhiani AR, Shiyan S (2022) Understanding the combination of fractional factorial design and chemometrics analysis for screening super-saturable quercetin-self nano emulsifying components. Pharmacia 69(2): 273–284. https://doi.org/10.3897/pharmacia.69.e80594

Abstract
Quercetin is formulated in a super saturable - self-nano emulsifying (SS-SNE) to increase its stability and bioavailability. This study focuses on the screening design for SS-SNE components with a fractional factorial design (FrFD) approach and chemometric analysis. The FrFD method was chosen because it provides comprehensive benefits. The oil components used are canola and grape seed oil. Croduret 50-SS was selected as a surfactant and PEG 400 as a co-surfactant. The interaction of SNE components was evaluated using FTIR-ATR instrumentation. SNE droplet morphology was observed using a transmission electron microscope (TEM). The selected formulas were grape seed oil as oil phase at 19.6%, croduret at 60%, and PEG 400 as co-surfactant with a concentration of 16.6%. The selected formula has a droplet size of 133.27 nm, PDI of 0.181, the zeta potential of 17.00 mV, electrophoretic mobility of 1.332 µm/cm/Vs, emulsification time of 10.05 seconds, a viscosity of 370.147 mPa.s, and a drug load of 31.70 mg/mL. The components of grape seed oil, croduret, and PEG 400 resulted in a quercetin carrier SNE formula that met the criteria. FrFD design and chemometric analysis in the screening process can help determine the selected formula very effectively and efficiently.

Keywords
chemometrics, cluster analysis, design of experiment, fractional factorial design, nanoemulsion, principal component analysis, quercetin, SNE

Introduction
Quercetin has the basic structure of flavonols, one of the six sub-class of flavonoid compounds. Quercetin or 3,3',4',5,7-pentahydroxyflavonone has pharmacological activities as an antidiabetic (Srinivasan et al. 2018), anti-inflammatory (Cheng et al. 2019), anticancer (Li et al. 2018; Tang et al. 2020) and antivirals (Ferreira et al. 2018). Quercetin, as an aglycone form of flavonoids, has low bioavailability and poor pharmaceutical stability. The low bioavailability of quercetin is due to its low water solubility (Dwi et al. 2018). Therefore, we need a breakthrough in the formulation of delivery system with a more effective and efficient approach.

Self-nano emulsifying drug delivery system (SNEDDS) has been developed to form emulsions with nanometer
size to increase oral bioavailability (Anwer et al. 2021). The modified self-nano emulsifying (SNE) formulation was done manually or semi-designed using pseudo ternary diagrams (Puppala and Lakshmi 2019). However, pseudo ternary use still requires a large number of trials and cannot predict the optimum conditions effectively (Ahmad et al. 2013). This approach is different from the application of the design of experiment (DoE). Evaluation using the DoE application with the fractional factorial design (FrFD) method will provide a more advantage at the screening stage. This method is carried out simultaneously and comprehensively compared to trial and error using a large number of samples. The resulting SNE characteristics provide qualitative information and quantitative effects, using the DoE mathematical modeling approach, with the FrFD method. The use of FrFD is also more effective and efficient because it uses a small number of samples.

The FrFD approach with mathematical modeling can provide qualitative information and quantitative influence on the characteristics of the formula. However, the analysis on the run of the FrFD design could not obtain information about grouping based on the formula’s characteristics and the correlation between responses. This information is critical in evaluating the response to further optimization procedures. Therefore, it is a novelty in the FrFD analysis combined with the chemometric approach. FrFD evaluation can be combined with chemometric analysis using principal component analysis (PCA) and cluster analysis (CA) techniques. The factors observed were grape seed oil and canola oil components linked to croduret 50-SS and PEG-400. The responses observed as parameters were droplet size, PDI, zeta potential, mobility, emulsification time, viscosity, and drug load. It is hoped that in the future, SSQ-SNE formulations can improve the quercetin delivery system.

**Materials and methods**

**Chemicals and materials**

Quercetin was purchased from Sigma-Aldrich. Grape seed oil under the Aceites Borges brand name and Palmtop canola oil is obtained from a local Palembang supermarket. Croduret 50-SS from Croda, PEG-400, and aquadest were purchased from Bratachem.

**Preparation of super saturable quercetin - self-nano emulsifying (SSQ-SNE)**

SNE was prepared by dissolving quercetin with carrier oil using vortex followed by ultrasonication for 5 minutes at room temperature. Surfactants and co-surfactants are added to the oil-quercetin solution. The homogeneous mixture was placed in a rotary shaker (25–30 °C for 12 hours) and allowed to stand again for 12 hours (Ogino et al. 2021; Shiyan et al. 2022).

**Design of experiment for screening component SSQ-SNE**

The experimental design for screening the constituent components of SNE was carried out using the FrFD \(2^{4-1}\) approach. The formulation design was determined by factors including the type of oil (A; canola oil and grape seed oil), the concentration of surfactants (B; %), the concentration of co-surfactants (C; %), and oil concentration (D; %). The FrFD approach uses two levels (upper limit +1 and lower limit -1) in a certain portion. The category choice is used for A and numeric factors for B, C, and D in preparing the formula design. Canola and grape seed oil use a lower limit of 14% and an upper limit of 20%, respectively. The croduret concentration range uses a lower limit of 30% and an upper limit of 60%. Co-surfactant PEG 400 uses a lower limit range of 0% and an upper limit of 20%.
observed and measured consisted of droplet size (R₆; d.nm), polydispersity index (R₅; n), zeta potential (R₄; mV), electrophoretic mobility (R₃; µmcm/Vs), emulsification time (R₂; seconds), viscosity (R₁; mPa.s) and drug load (R₀; mg/mL). The complete design and data of the eight experiment runs are shown in Table 1.

### Chemometrics analysis for study at run formula

The data obtained were also analyzed using a chemometric approach with the PCA and CA methods. The PCA-CA method was processed using Minitab 17 series software (Minitab, State College, PA, USA). Evaluation at this stage is not part of modeling and prediction optimization, but evaluation of 8 runs and the correlation between responses (Kartini et al. 2020; Shiyan et al. 2021).

### Droplet size, polydispersity index, zeta potential, and mobility

The optimum droplet diameter, polydispersity index (PDI), and zeta potential of SSQ-SNE formula were measured using a particle size analyzer Zetasizer Nano ZSP (Malvern Panalytical, UK) by applying the dynamic light scattering (DLS-PSA) method. Data was collected in triplo (n=3) and presented in the form of mean ± standard deviation. The data processing used Zetasizer 7.12 (Malvern Panalytical) software which helped the analysis run, in order to obtain results in the form of particle size (d.nm), PDI, zeta potential (mV) and electrophoretic mobility (µmcm/Vs).

### Measurement of emulsification time, viscosity, and drug load

Emulsification is essentially the process of dispersing SSQ-SNE in aqueous media to form a nanoemulsion. A total of 1 mL of SSQ-SNE is dropped into 500 mL of media. The dispersing process is conditioned at 37 °C on the magnetic stirrer with a stirring rate of 120 rpm. Observations were made on time it took from the start of the drop until the nanoemulsion was formed. Visual observations were made by looking at the nanoemulsion efficiency, transparency, phase separation, and quercetin droplets. The nanoemulsion formed was characterized by the complete dissolution of SSQ-SNE in the medium (Shiyan et al. 2021). SSQ-SNE viscosity measurement uses an Oswald viscometer in mPa.s units (Yadav et al. 2014). The quantity of quercetin contained in SNE was measured by centrifugation at 3500 rpm for 30 minutes. The precipitate formed is weighed as quercetin, which does not enter the system.

### Percentage of clarity studies

A total of 100 µL of SSQ-SNE was emulsified into 10 mL of aqua pro injection. Clarity (transmittance; %) was determined using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, USA) at a wavelength of 650 nm and the blank solution is purified water.

### Thermodynamic stability studies

Stability tests for SSQ-SNE and nanoemulsions using heating-cooling and freezing methods in selected formulas. Centrifugation studies were carried out at 3500 rpm for 30 minutes, and visual observations were made to confirm phase separation, precipitation, instability, cracking, or cream formation (Jumaryatno et al. 2018).

### Morphology characterization and interaction studies

The morphology of nanoemulsion globules or droplets was identified using a transmission electron microscope (TEM). The TEM instrumentation used was JEM 2100 (Jeol, Tokyo, Japan). The interaction of SNE constituent components was identified using Fourier transform infrared spectrophotometry-attenuated total reflectance (FTIR-ATR) Nicolet iS5 (Thermo Scientific, USA). Spectra readings were carried out on SSQ-SNE, quercetin material, oil (canola and grape seed), surfactant (croduret 50-SS), and co-surfactant (PEG 400). IR spectra readings were carried out at a wavenumber between 4000 cm⁻¹ to 500 cm⁻¹.

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**Table 1. Design and complete experimental results of the FrFD 2³⁻.**

| Run | A  | B  | C  | D  | R₁ | R₂ | R₃ | R₄ | R₅ | R₆ | R₀ |
|-----|----|----|----|----|----|----|----|----|----|----|----|
| 1   | Canola | 60 | 30 | 20 | 26.88 ± 1.33 | 0.406±0.005 | 25.27 ± 1.32 | 2.210 ± 0.10 | 10.07 ± 0.15 | 668.01 ± 19.13 | 17.41 ± 0.78 |
| 2   | Grape seed | 60 | 30 | 14 | 43.12 ± 2.07 | 0.345 ± 0.009 | 28.40 ± 0.89 | 2.186 ± 0.02 | 12.43 ± 0.15 | 676.49 ± 34.58 | 25.94 ± 1.04 |
| 3   | Grape seed | 60 | 10 | 20 | 130.07 ± 7.41 | 0.534 ± 0.016 | 23.10 ± 1.51 | 1.812 ± 0.12 | 10.46 ± 0.10 | 283.49 ± 9.04 | 29.01 ± 1.26 |
| 4   | Canola | 30 | 10 | 20 | 146.47 ± 18.66 | 0.510 ± 0.023 | 21.67 ± 0.57 | 1.728 ± 0.03 | 28.93 ± 1.42 | 942.27 ± 94.73 | 46.79 ± 0.96 |
| 5   | Canola | 60 | 10 | 14 | 266.53 ± 12.01 | 0.408 ± 0.034 | 23.47 ± 1.05 | 1.705 ± 0.02 | 19.37 ± 0.38 | 1216.73 ± 70.98 | 33.86 ± 2.16 |
| 6   | Grape seed | 30 | 30 | 20 | 164.00 ± 0.79 | 0.391 ± 0.027 | 23.70 ± 0.53 | 1.862 ± 0.04 | 9.67 ± 0.55 | 946.63 ± 101.29 | 35.28 ± 2.04 |
| 7   | Canola | 30 | 30 | 14 | 26.74 ± 0.51 | 0.330 ± 0.012 | 19.13 ± 0.85 | 1.376 ± 0.07 | 11.47 ± 1.21 | 851.85 ± 21.84 | 28.72 ± 0.89 |
| 8   | Grape seed | 30 | 10 | 14 | 121.10 ± 5.12 | 0.528 ± 0.032 | 16.64 ± 0.35 | 1.336 ± 0.06 | 19.47 ± 0.55 | 786.09 ± 25.35 | 42.69 ± 1.73 |

**Note:** (A) Oil type, (B) Surfactant concentration (%), (C) Co-surfactant concentration (%), (D) Oil concentration (%), (R₁) Zeta potential, (R₂) Electrophoretic mobility, (R₃) Emulsification time, (R₄) Viscosity, (R₅) Drug load.
Result and Discussion

Fractional factorial design for screening component SSQ-SNE

The FrFD approach to screening provides a more effective and efficient measure. Statistical data from the fitting model on all evaluated responses are presented in Table 3. The droplet size model has an $R^2$ value of more than 0.7, with an adjusted $R^2$ value of 0.9985. Predicted $R^2$ is 0.9929, and the difference between adjusted $R^2$ and predicted $R^2$ is less than 0.2. The value of adequate precision reinforces the model if the value is more than 4. The polydispersity index also shows an adequate response in modeling. The fitting model is included in the right criteria for predicting the selected or optimal formula by considering the value of $R^2$, adjusted $R^2$, predicted $R^2$, adequate precision, and press (Pratiwi et al. 2019; Shiyan et al. 2019).

Zeta potential is an essential parameter in determining the best formula for SSQ-SNE. The results of the fitting of the model for zeta potential, $R^2$ value 0.9837, adjusted $R^2$ 0.9428, predicted $R^2$ 0.7385, and adequate precision 15.72. Overall, the statistical evaluation of each parameter or response is very suitable for use in prediction. Based on the fitting model results, all responses have the same index also shows an adequate response in modeling. The equation and model for the response to droplet size

Table 2. Visual observation of SNEDDS and formed nanoemulsion.

| Run | Visual SNEDDS | SNEDDS color | Precipitation on SNEDDS | Clarity (% T) | Nanoeumulsion |
|-----|----------------|--------------|------------------------|---------------|--------------|
| 1   | No separation  | Clear        | No                     | 75.35 ± 1.33  | 98.67 ± 0.60 |
| 2   | No separation  | Yellow       | No                     | 71.97 ± 0.63  | 99.99 ± 0.01 |
| 3   | No separation  | Yellow       | No                     | 62.06 ± 1.24  | 98.63 ± 0.58 |
| 4   | No separation  | Yellow       | No                     | 49.37 ± 1.47  | 44.42 ± 0.59 |
| 5   | No separation  | Tawny        | No                     | 64.52 ± 1.17  | 99.54 ± 0.51 |
| 6   | No separation  | Tawny        | No                     | 54.04 ± 0.49  | 75.62 ± 0.61 |
| 7   | No separation  | Tawny        | No                     | 66.67 ± 0.68  | 99.67 ± 0.51 |
| 8   | No separation  | Tawny        | No                     | 52.44 ± 0.96  | 99.66 ± 0.57 |

Note: The transmittance nanoemulsion was measured from the SNEDDS emulsification results with a dilution of 500 times.

Table 3. Statistical parameters for the overall response of the FrFD.

| Response | Parameter | Standar deviasi | Mean | CV (%) | Press | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Adequate precision |
|----------|-----------|-----------------|------|--------|-------|-------|----------------|-------------------|--------------------|
| $R_1$    |           | 3.23            | 115.61 | 2.80 | 334.39 | 0.9996 | 0.9985 | 0.9929 | 86.49 |
| $R_2$    |           | 0.02            | 0.432 | 3.95 | 0.01  | 0.9876 | 0.9565 | 0.8013 | 14.95 |
| $R_3$    |           | 0.86            | 22.67 | 3.81 | 23.89 | 0.9837 | 0.9428 | 0.7385 | 15.72 |
| $R_4$    |           | 0.04            | 1.78  | 2.49 | 0.06  | 0.9946 | 0.9812 | 0.9140 | 24.00 |
| $R_5$    |           | 1.54            | 15.00 | 10.29 | 76.20 | 0.9862 | 0.9518 | 0.7797 | 15.21 |
| $R_6$    |           | 60.38           | 796.45 | 116700 | 0.99 | 0.9859 | 0.9507 | 0.7746 | 17.85 |
| $R_7$    |           | 0.74            | 32.46 | 2.29 | 17.73 | 0.9982 | 0.9937 | 0.9712 | 45.10 |

Note: ($R_i$) Droplet size, ($R_j$) Polydispersity index, ($R_k$) Zeta potential, ($R_m$) Electrophoretic mobility, ($R_n$) Emulsification time, ($R_o$) Viscosity, ($R_p$) Drug load.
with a concentration of PEG-400 (AC) can reduce viscosity. Drug load is strongly influenced by the type of oil (A) and the concentration of PEG 400 (C).

The interaction of oil types with Croduret and PEG-400 for each response is shown in Fig. 3. The use of croduret with high grape seed oil concentrations will produce smaller droplet sizes than interactions with canola oil (Fig. 3A). The PDI was smaller when the PEG-400 concentration was higher, whether the interaction was in grape seed or canola oil. The lower PEG-400 concentration gave a greater PDI, especially its interaction with grape seed oil. The increase in the croduret concentration could increase the level of electrophoretic mobility. However, the interaction of canola oil and high concentrations of PEG-400 resulted in lower mobility (Fig. 3D). The emulsification time will be longer at the interaction of low concentration PEG-400 with canola oil, while the interaction with grapeseed oil still results in a faster emulsification time (Fig. 3E). The interaction between canola oil and high concentrations of croduret resulted in a high SNE viscosity (Fig. 3F). A high drug load was obtained at canola oil interaction with low concentrations of PEG-400 (Fig. 3G).

**Principal component analysis and cluster analysis on the FrFD**

The response data from the SSQ-SNE formulas that have been obtained were analyzed using a chemometric approach with the principal component analysis (PCA) and cluster analysis (CA) methods. The multivariate approach using...
PCA aims to simplify variables by reducing data from a large number of interrelated variables without changing existing information (Cui et al. 2021; Shiyan et al. 2021; Kim et al. 2022). CA technique is a method based only on information found in data that describes relationships and objects or is based on similar characteristics of these objects. CA analysis forms and separates groups with the closest relationship in more detail to provide more accurate information (Iaboni et al. 2020; García del Moral et al. 2021).

Fig. 4B is a score plot that shows the run formula grouping into 5 clusters. The score plot classifies the samples based on the run composition function and the resulting response (Talekar et al. 2019; Hong et al. 2021). Multivariate analysis was successful in grouping the runs at different distances from each other. The distance between runs or samples shows the similarity of characteristics. The further distance between the runs indicates little similarity in traits or characteristics (Shiyan et al. 2020; Setyawan 2022).

Figure 3. Graph of interactions between factors on the evaluated response, (A) droplet size (B) Polydispersity index, (C) zeta potential, (D) electrophoretic mobility, (E) time of emulsification, (F) viscosity, (G) drug load.
et al. 2021). The dendrogram in CA can group the same variables and have bonds in one group based on the value of closeness (similarly) (Szentmiklóssy et al. 2020). The characteristic similarity index is depicted in dendrogram form in Fig. 4C. Each run is classified based on its similarity. Run 1 and 2 have a closeness with a value of 97.82%; run 4 and 6 have a closeness of 96.94%; 7 and 8 have a value of 85.56%. The proximity of each run was also evidenced by a similar FTIR-ATR spectra pattern (Fig. 6A).

The loading plot aims to determine the variable of a sample or formula that most contributes to forming the principal component (PC) values. The contribution of the sample variables to the loading plot can be seen from a distance used. Data analysis using the PCA loading plot depicts the angle that shows a correlation between the responses of all formulas. The responses of R₁ and R₂, which form an adjacent angle (less than 45°), indicate a positive correlation. The electrophoretic mobility (R₃) of the droplets will increase with the high zeta potential (R₄) value. A negative correlation occurs between R₄ and R₅, which forms an angle close to 180°. A high polydispersity index (R₆) can reduce the zeta potential (R₇). The angle between the two vectors that are close to 90° indicates no correlation between responses.

**Selected formulas and verification of results**

The best formula for screening can be predicted by the model obtained from the FrFD. The most critical stage in prediction is to determine the level of importance and goals of each response. The target droplet size is 50 nm, with an important level value of 5. The polydispersity index has a lower limit of 0.33 and an upper limit of 0.54 with an in-range target and an important level value of 3. Zeta potential in the FrFD2⁺ experiment produces a range of 16.64–28.40 mV. Considering this response is related to stability, the prediction stage uses a target of 25 mV and the value of importance 4. Electrophoretic mobility in the in-range target with a level of importance of 3 is positively correlated with zeta potential. The emulsification time and viscosity were determined with minimum targets with importance values of 5 and 4. Considering the super saturable-SNE formulated, the target set for drug load must be a maximum with a level of importance of 4.

The SNE components selected were grape seed as the oil phase, croduret as a surfactant, and PEG-400 as a co-surfactant with concentrations of 19.6%, 60%, and 16.6%, respectively. The desirability value is an essential indicator in determining the selected formula mixture in the SSQ-SNE formulation. The desirability at the prediction stage obtained a value of 0.751. High desirability values (close to 1) indicate the ability of the FrFD design to produce perfect predictions and proper screening procedures (Shiyan et al. 2019; Indrati et al. 2020). The desirability value provides an overview of the similarity between the predicted value and the actual observation. The composition of SSQ-SNE selected in FrFD 2⁻¹ obtained optimum results with a droplet size of 112.84 d.nm, a polydispersity index of 0.487, a zeta potential of 25 mV, mobility of 1.932 µm/cm/Vs, an emulsification time of 8.60 seconds, a viscosity of 364.72 mPa.s, and drug load of 27.64 mg/mL.

**Characterization and evaluation of selected SSQ-SNE**

**Appearance, drug load, and viscosity**

The visuals observed include color, odor, separation, and precipitation. SSQ-SNE is yellowish, clear, slightly thick due to the addition of surfactant and a slightly pungent odor of oil. The yellow color of SNE is affected by quercetin (Fig. 5C). In general, drug load is used to determine drug solubility in SNE components (Indrati et al. 2020). The drug load parameter in this study indicated the level of quercetin saturation in the SNE system. The selected formula in the saturated state had a drug load of 31.70 ± 1.15 mg/mL.

Viscosity on SNE will affect the ease of use and the formation of nanoemulsion droplets. The low viscosity is due to the smaller globule size of oil (Anwer et al. 2021). The SNE form, which resembles a gel character, has a high viscosity so that after contact with water, it produces a relatively longer dispersion (emulsification time runs slowly). In contrast, low viscosity (which does not resemble a gel) will emulsify more easily. The SSQ-SNE viscosity in the selected formula is 370.15 ± 7.69 mPa.s, still has suitable viscosity with emulsification time of fewer than 5 minutes.

**Emulsification time**

Emulsification time describes the length of time to produce nanoemulsion from SNE when it encounters gastrointestinal fluids. The selected formula showed an emulsification time of fewer than 5 minutes in a medium of 10.05 ± 0.33 seconds. The faster the SNE turns into nanometer-sized droplets, the faster the drug will dissolve and be absorbed into the blood vessels.
Emulsification rate is positively correlated with viscosity, referring to the loading plot (Fig. 4D) of vectors $R_5$ and $R_6$ forming an angle of less than 45°. SNE with high viscosity will spread slowly or emulsify slowly, while SNE with low viscosity will emulsify more easily.

**Morphology, droplet size, and polydispersity index**

The instrument used to determine droplet morphology was transmission electron microscopy (TEM). The observations show that the form of nanoemulsion particles produced is spherical (Fig. 5A, B). Droplet size is a crucial characteristic in assessing a good nanoemulsion. The selected formula has a droplet diameter of 133.27 ± 0.64 nm. The droplet size is calculated from the volume, intensity, and bimodal distribution, assuming spherical particles. Droplet size is an essential factor in the SNE formulation, as it determines the rate of drug release, absorption, and increases bioavailability (Anwer et al. 2021; Cardona et al. 2021). The droplet diameter also depends on the type of oil phase formulated because it affects the formation of oil globules (Indrati et al. 2020). Discussing nanoemulsion not only focuses on droplet size but also the polydispersity index (PDI), which provides information on size homogeneity (Shiyan et al. 2022). Theoretically, the higher the PDI value, the lower the uniformity of globule size from nanoemulsion. PDI is the standard deviation value from the mean particle size used as the uniformity parameter for the nanoemulsion evaluation. The polydispersity index value is getting below 1, indicating the uniformity of the nanoemulsion size formed. The measurement results in the selected formula, the PDI value is 0.181 ± 0.01.

**Zeta potential and electrophoretic mobility**

The zeta potential describes the repulsion between the droplets. The strength of the attraction or repulsion is determined by hydrogen bonds and van der Waals bonds. The zeta potential value away from zero will be more stable because it minimizes aggregation. Zeta potential as the main parameter can describe the stability of nanoemulsion. The droplet in the selected formula has a zeta potential value of 25.03 ± 2.53 mV with a negative charge (Fig. 5G). The negative charge is caused by the presence of free fatty acids in the formula (Balakumar et al. 2013). The zeta potential value that is ahead of zero theoretically shows a more stable nanoemulsion. In addition to the zeta potential, the electrophoretic mobility clarifies the study of nanoemulsion stability. This parameter describes the velocity of the droplet. The higher the zeta potential value, both positive and negative charges, the higher the electrophoretic mobility value (Pratiwi et al. 2019). The electrophoretic mobility on the selected SSQ-SNE was 1.332 ± 0.19 μm cm/V s.
Analysis of SSQ-SNE components using FTIR-ATR

The interaction analysis of constituent materials used FTIR instrumentation based on vibrations in each SNE component (Pratiwi et al. 2020; Shiyan et al. 2022). The spectral patterns on the SNE constituent components of quercetin, canola oil, grapeseed oil, croduret 50-5S, and PEG-400 are presented in Fig. 6. The spectral patterns of the eight runs on FrFD at first glance look similar, but in a more detailed evaluation, the intensity at the peak is different. SNE has a typical peak at wavenumbers 3300–3600 cm\(^{-1}\), 2800–3500 cm\(^{-1}\), 2200–2400 cm\(^{-1}\), and a fingerprint area of 500–1800 cm\(^{-1}\). The spectral pattern of the selected SSQ-SNE can be observed in Fig. 6B with the ratio of the components used. Quercetin spectra (Fig. 6B) have typical peaks that widen in 3000–3600 cm\(^{-1}\). The peak was lost in the SSQ-SNE spectra (Fig. 6B). Based on the FTIR-ATR spectra pattern and droplet morphology of TEM, quercetin was successfully incorporated into the oil globule system (SNE). Theoretically, verification is carried out by evaluating changes of spectral patterns in each component and the SNE.

Thermodynamic stability of SSQ-SNE and nanoemulsions

Physical stability is carried out to determine the maximum storage time leading to separation of the emulsion phase (creaming or cracking). Heating cooling was chosen as an accelerated thermodynamic stability test method because, with a short time, the kinetic stability of SNE could be known through the phase separation that occurred. Observations on the stability of SNE and...
nanoemulsions were carried out visually to see their clarity, physical changes such as creaming, cracking, and the formation of deposits. The stability testing results using the heating-cooling and free-thaw method showed that the selected SNE and nanoemulsion formulas remained stable (Table 5). SNE and nanoemulsions show no phase separation (Fig. 5C–E).

Conclusion

The FrFD design and chemometric analysis in the screening process of the SSQ-SNE formulation have proven to be effective and efficient. SSQ-SNE comprises grape seed oil, croduret, and PEG 400 to produce a formula that meets the criteria. Screening results can be continued at the optimization stage with more comprehensive factors and responses. The formula developed is following the target in increasing the solubility and bioavailability of quercetin.

Table 5. The SSQ-SNE and nanoemulsion stability test.

| Parameters     | SSQ-SNE Stability | Color       | Clarity (%T)* | Nanoemulsion Stability | Color       | Clarity (%T)* |
|----------------|--------------------|-------------|---------------|-------------------------|-------------|---------------|
| Before test    | –                  | Clear yellow | 98.45 ± 0.84  | –                       | Clear       | 99.87 ± 0.16  |
| Centrifugation | Stable             | Clear yellow| 98.69 ± 1.65  | Stable                  | Clear       | 98.90 ± 0.45  |
| Heating-Cooling| No separation      | Clear yellow| 95.53 ± 1.06  | No separation           | Clear       | 98.46 ± 1.14  |
| Freeze-Thaw    | No separation      | Clear yellow| 95.72 ± 1.10  | No separation           | Clear       | 98.85 ± 0.97  |

Funding

This study was supported by Direktorat Riset dan Pengabdian Masyarakat Direktorat Jendral Riset dan Pengembangan Kementrian Riset, Teknologi dan Pendidikan Tinggi with contract number 849/SP2H/LT/MONO/LL2/2020, in accordance with the Assignment Agreement Letter Implementation of the 2020 Research Program Number: B/87/E3/RA.00/2020.

Acknowledgment

The author is grateful, and this research is facilitated by the Biomaterials and Drug Delivery System (BiDDS) Research Group and Department of Pharmacy STIKES ‘Aisyiyah Palembang. Thanks to the Phytopharmaceutic Research Center (PRC), Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya. Thanks to the PT DKSH Indonesia.

References

Ahmad J, Amin S, Kohli K, Mir SR (2013) Construction of pseudoternary phase diagram and its evaluation: Development of self-dispersible oral formulation. International Journal of Drug Development & Research 5(2): 84–90.
Altamimi MA, Kazi M, Hadi Albgomi M, Ahad A, Raish M (2019) Development and optimization of self-nanoemulsifying drug delivery systems (SNE DDS) for curcumin transdermal delivery: an anti-inflammatory exposure. Drug Development and Industrial Pharmacy 45(7): 1073–1078. https://doi.org/10.1080/03639045.2019.1593440
Anwer MK, Iqbal M, Aldawarsi MF, Alalawi A, Ahmed MM, Maharram MM, Erzeldin E, Mahmoud MA, Imam F, Ali R (2021) Improved antimicrobial activity and oral bioavailability of delafloxacin by
self-nanoemulsifying drug delivery system (SNEDDS). Journal of Drug Delivery Science and Technology 64: e102572. https://doi.org/10.1016/j.jddst.2021.102572

Balakumar K, Raghavan CV, Selvan NT, Prasad RH, Abdu S (2013) Self nanoemulsifying drug delivery system (SNEDDS) of rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. Colloids and Surface R: Biointerfaces 112: 337–43. https://doi.org/10.1016/j.colsurfb.2013.08.025

Cai Y, Liu L, Xia M, Tian C, Wu W, Dong B, Chu, X (2022) SEDDS facilitate cinnamaldehyde crossing the mucus barrier: the perspective of mucus and Caco-2-HT29 co-culture models. International Journal of Pharmaceutics 614: e121461. https://doi.org/10.1016/j.ijpharm.2022.121461

Cardona MI, Domínguez GP, Echeverry SM, Valderrama IH, Bernkop-Schnürch A, Aragón M (2021) Enhanced oral bioavailability of rutin by a self-emulsifying drug delivery system of an extract of calyces from Physalis peruviana. Journal of Drug Delivery Science and Technology 66: e102797. https://doi.org/10.1016/j.jddst.2021.102797

Cheng SC, Wu YH, Huang WC, Pang JS, Huang TH, Cheng CY (2019) Anti-inflammatory property of quercetin through downregulation of ICAM-1 and MMP-9 in TNF-alpha-activated retinal pigment epithelial cells. Cytokine 116: 48–60. https://doi.org/10.1016/j.cyto.2019.01.001

Cui F, Kim M, Park C, Kim D, Mo K, Kim M (2021) Application of principal component analysis (PCA) to the assessment of parameter correlations in the partial-nitration process using aerobic granular sludge. Journal of Environmental Management 288: e112408. https://doi.org/10.1016/j.jenvman.2021.112408

Dhritlahre RK, Ruchika, Padwad Y, Saneja A (2021) Self-emulsifying formulations to augment therapeutic efficacy of nutraceuticals: From concepts to clinic. Trends in Food Science & Technology 115: 347–365. https://doi.org/10.1016/j.tifs.2021.06.046

Dwi S, Febrianti S, Zainul A, Retno S (2018) PEG 8000 increases effect and enhanced hepatoprotective function. Journal of Functional Foods 78: e104391. https://doi.org/10.1016/j.jff.2021.104391

Ferreira CGT, Campos MG, Felix JS, Santos MR, Carvalho OV, Diaz MAN, Fietto JLR, Bressan GC, Silva-Junior A, Almeida MR (2018) Evaluation of the antiviral activities of Bucharis dracunculifolius and quercetin on Equid herpesvirus 1 in a murine model. Research in Veterinary Science 120: 70–77. https://doi.org/10.1016/j.rvsc.2018.09.001

García del Moral LF, Morgado A, Esquivel JA (2021) Reflectance spectroscopy in combination with cluster analysis as tools for identifying the provenance of Neodictichum flint artefacts. Journal of Archaeological Science Reports 37: e103041. https://doi.org/10.1016/j.jasrep.2021.103041

Halder S, Islam A, Muht MA, Shill MC, Haider SS (2021) Self-emulsifying drug delivery system of black seed oil with improved hypotriglyceridemic effect and enhanced hepatoprotective function. Journal of Functional Foods 78: e104391. https://doi.org/10.1016/j.jff.2021.104391

Hong Y, Liao X, Chen Z (2020) Determination of bioactive components in the fruits of Cercis chinensis Bunge by HPLC-MS/MS and quality evaluation by principal components and hierarchical cluster analysis. Journal of Pharmaceutical Analysis 11(4): 465–471. https://doi.org/10.1016/j.jpha.2020.07.010

Iaboni DSM, Farrell SR, Chauhan BC (2020) Morphological multivariate cluster analysis of murine retinal ganglion cells selectively expressing yellow fluorescent protein. Experimental Eye Research 196: e108044. https://doi.org/10.1016/j.exer.2020.108044

Indrati O, Martien R, Rohman A, Nugroho AK (2020) Application of simplex lattice design on the optimization of andrographolide self-nanoemulsifying drug delivery system (SNEDDS). Indonesian Journal of Pharmacy 13(2): 124–130. https://doi.org/10.14499/indonesianjpharm31is2pp124

Jumaryatno P, Chabib L, Hayati F, Awaluddin R (2018) Stability study of Ipomoea reptans extract self-nanoemulsifying drug delivery system (SNEDDS) as anti-diabetic therapy. Journal of Applied Pharmaceutical Science 8(9): 11–14. https://doi.org/10.7324/JAPS.2021.110313

Kartini K, Putri LAD, Hadiyat MA (2020) FTIR-based fingerprinting and discriminant analysis of Aiptornia graveolens from different locations. Journal of Applied Pharmaceutical Science 10(12): 62–67. https://doi.org/10.7324/JAPS.2020.101208

Kim M, Chang JW, Park K, Yang DR (2022) Comprehensive assessment of the effects of operating conditions on membrane intrinsic parameters of forward osmosis (FO) based on principal component analysis (PCA). Journal of Membrane Science 641: e119909. https://doi.org/10.1016/j.memsci.2021.119909

Li X, Zhou N, Wang J, Liu Z, Wang X, Zhang Q, Liu Q, Gao L, Wang R (2018) Quercetin suppresses breast cancer stem cells (CD44+/CD24-) by inhibiting the PI3K/Akt/mTOR-signaling pathway. Life Sciences 196: 56–62. https://doi.org/10.1016/j.lfs.2018.01.014

Ogino M, Nakazawa A, Shiokawa K, Kikuchi H, Sato H, Onoue S (2021) Krill oil-based self-emulsifying drug delivery system to improve oral absorption and renoprotective function of ginger extract. Pharma-Nutrition 19: e100285. https://doi.org/10.1016/j.jpha.nu.2021.100285

Pratiwi G, Murwanti R, Martien R (2019) Chitosan nanoparticle as a delivery system for polyphenols from meniran extract (Phyllanthus niruri L.). Formulation, optimization, and immunomodulatory activity. International Journal of Applied Pharmaceutics 11(2): 50–58. https://doi.org/10.22159/ijap.2019v11i2.29999

Pratiwi G, Susanti S, Shiyani S (2020) Application of factorial design for optimization of PVC-HPMC polymers in matrix film ibuprofen patch-transdermal drug delivery system. Indonesian Journal of Chemometrics and Pharmaceutical Analysis 1(1): 11–22. https://doi.org/10.22146/ijcpa.486

Puppala RK, Lakshmi VA (2019) Optimization and solubilization study of nanoemulsion budesonide and constructing pseudoternary phase diagram. Asian Journal of Pharmaceutical and Clinical Research 12(1): 551–553. https://doi.org/10.22159/ajpcr.2019.v12i1.28686

Setyawati EL, Rohman A, Setyowati EP, Nugroho AK (2021) The combination of simplex lattice design and chemometrics in the formulation of green tea leaves as transdermal matrix patch. Pharmacia 68(1): 275–282. https://doi.org/10.14497/pharmacia.68.e61734

Shiyani S, Arifin A, Amriani A, Pratiwi G (2020) Immunostimulatory activity of ethanol extract from Calotropis gigantea L. flower in rats against Salmonella typhimurium infection. Research Journal of Pharmacy and Technology 13(11): 5244–5250.

Shiyani S, Suryani RP, Mulyani LN, Pratiwi G (2022) Stability study of super saturable catechin-self nano emulsifying drug delivery system...
as antidiabetic therapy. Biointerface Research in Applied Chemistry 12(5): 5811–5820. https://doi.org/10.33263/BRIAC125.58115820

Shiyan S, Zubaidah, Pratiwi G (2021) Chemometric approach to assess response correlation and its classification in simplex centroid design for pre-optimization stage of catechin-SNEDDS. Research Journal of Pharmacy and Technology 14(11): 5863–5870. https://doi.org/10.52711/0974-360X.2021.01020

Srinivasan P, Vijayakumar S, Kothandaraman S, Palani M (2018) Anti-diabetic activity of quercetin extracted from Phyllanthus emblica L. fruit: In silico and in vivo approaches. Journal of Pharmaceutical Analysis 8(2): 109–118. https://doi.org/10.1016/j.jpapha.2017.10.005

Szentmiklóssy M, Török K, Pusztai É, Kemény S, Tremmel-Bede K, Rakszegi M, Tömösközi S (2020) Variability and cluster analysis of arabinoxylan content and its molecular profile in crossed wheat lines. Journal of Cereal Science 95: e103074. https://doi.org/10.1016/j.jcs.2020.103074

Talekar SD, Haware RV, Dave RH (2019) Evaluation of self-nanoemulsifying drug delivery systems using multivariate methods to optimize permeability of captopril oral films. European Journal of Pharmaceutical Sciences 130: 215–224. https://doi.org/10.1016/j.ejps.2019.01.039

Tang SM, Deng XT, Zhou J, Li QP, Ge XX, Miao L (2020) Pharmacological basis and new insights of quercetin action in respect to its anti-cancer effects. Biomedicine and Pharmacotherapy 121: e109604. https://doi.org/10.1016/j.biopha.2019.109604

Yadav P, Yadav E, Verma A, Amin S (2014) In vitro characterization and pharmacodynamic evaluation of furosemide loaded self nano emulsifying drug delivery systems (SNEDDS). Journal of Pharmaceutical Investigation 44(6): 443–453. https://doi.org/10.1007/s40005-014-0138-z

Zhang N, Zhang F, Xu S, Yun K, Wu W, Pan W (2020) Formulation and evaluation of luteolin supersaturatable self-nanoemulsifying drug. Journal of Drug Delivery Science and Technology 58: e101783. https://doi.org/10.1016/j.jddst.2020.101783