Optimization of Formulation and Operating Parameters for Ginkgo biloba Extract Nanosuspension by Wet Ball Milling Using a Box–Behnken Design

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In the present work, the formulation of nanosuspension of Ginkgo biloba extract (GBE) and the operating parameters of wet ball milling (WBM) was optimized using the Box–Behnken design (BBD) method. The key formulation factors evaluated were GBE concentration, milling speed, milling time, and sodium dodecyl sulfate (SDS) concentration. A quadratic model with the correlation coefficient ($R^2$) value of 0.9276 was established based on the experimental data, implying that this model is significant; meanwhile, the analysis of variance (ANOVA) showed that the SDS concentration was the most significant factor on particle size, followed by GBE concentration, milling speed, and milling time was the slighter significant factor. The GBE nanosuspension with the particles size of 171.2 nm was prepared under the optimized conditions of GBE concentration (7.459 g/L) in combination with milling time (1.492 h), milling speed (9.253 m/s), and SDS concentration (0.846 g/L). The freeze-dried nanosuspension exhibited spherical particle morphology with uniform size by scanning electron microscopy. In addition, the melting behavior of the raw material and milled GBE was analyzed by differential scanning calorimetry and it was found that the melting point decreased due to the decrease of particle size. Furthermore, the dissolution rates of the optimized GBE showed better dissolution properties than the unprocessed GBE. The results show that it is feasible to prepare GBE nanosuspension on a commercial scale by WBM to increase the bioavailability of GBE.

1. Introduction

Ginkgo biloba is one of the oldest and most commonly used herbs. It has been used in traditional Chinese medicine for thousands of years [1]. Ginkgo biloba extract (GBE) is a kind of herbal medicine extracted from Ginkgo biloba leaves with antioxidant, antifungal, antibacterial, antiviral, antiasthmatic, and antitumor activities and has been widely used for the treatment of tinnitus [2], cerebral insufficiency [3], retinal [4], depression [5], diabetic nephropathy [6], and other diseases. It is currently one of the best-selling phytomedicine.

Unfortunately, the bioavailability of GBE is poor due to its low aqueous solubility, thus limiting its formulation development and clinical applications [7]. Literature has reported that nanotechnology-based drugs provide another approach to improve the therapeutic effects and bioavailability of drugs. Nanostructured systems has the potential to enhance the activities of herbal extracts, decrease the treatment dose, promote sustained release of active ingredients, reduce unwanted effects, and improve activity. Thus, incorporating herbal medicines with nanotechnology has become an alternative strategy. Meanwhile, nanosuspensions have been suggested as a new method for water-insoluble drug delivery. It has the ability to improve oral absorption and bioavailability of drugs for the merits of enhanced surface area, rapid onset of action, higher saturation solubility, and higher adhesiveness to gastrointestinal epithelium [8]. Many
researchers have successfully reduced the particle size by adopting some nanosizing methods [9], such as self-emulsifying drug delivery systems [10], freeze drying [11], film dispersion-homogenization [12], and supercritical anti-solvent [7, 13, 14].

However, the commercial applications of nanosizing methods mentioned above are limited due to the disadvantage of excessive amount of solubilizer, difficulty in particle size control, and high cost. Wet ball milling (WBM) technique is regarded as one of the most successful top-down nanosizing techniques. It is an aqueous grinding process, which reduces the drug particle size to nanoscale by high-speed friction, collision, and extrusion between grinding beads [15–18]. Various water-insoluble drugs like Rapamune®, Emend®, Tricor®, and Megace ES® [19, 20] have been studied so far for bioavailability enhancement by this method since it has the unique advantages of high efficiency, high drug concentration, and continuous operation ability. Nevertheless, there is no previous systematic study on the preparation of GBE nanoparticles by WBM technique. The Box–Behnken design (BBD) method is a powerful and accurate statistical technique, which can simultaneously observe the relationship between the effects of individual parameters on the responses and minimize the number of experimental runs [21–25].

In this work, some efforts have been made to optimize the conditions for preparing GBE by WBM based on BBD method. Furthermore, GBE concentration, milling speed, milling time, and sodium dodecyl sulfate (SDS) concentration on GBE particle size were investigated systematically by BBD method.

## 2. Materials and Methods

### 2.1. Materials

GBE (compose of 96% flavonol glycosides and 24% terpene lactones), flavonol glycosides, and quercetin provided by Lunan Houpu Pharmaceutical Co., Ltd. (China) was used as a raw material for experiments. Microcrystalline cellulose, crosslinked carboxymethyl cellulose sodium, silicon dioxide, crosslinked povidone, magnesium stearate, and 80% ethanol supplied by New Era Pharmaceutical Co., Ltd. (China) were selected as the excipient to prepare Ginkgo biloba tablets. Methanol (purity 99.92%) and 25% hydrochloric acid were supplied by Sinopharm Chemical Reagent Co., Ltd. (China). The pure water made by the laboratory used in the experiment. In order to improve the solubility of the raw materials and promote the stability of the prepared suspension, it is necessary to add a certain amount of solubilizer. In this experiment, SDS purchased from SuZhou KuangShi Chemical Co. Ltd. (China) was selected as the solubilizer.

### 2.2. Preparation of GBE Nanosuspension

A nanoscale GBE (China) was selected as the solubilizer. SDS purchased from SuZhou KuangShi Chemical Co. Ltd. (China) was used as a raw material for experiments. Microcrystalline cellulose, crosslinked carboxymethyl cellulose sodium, silicon dioxide, crosslinked povidone, magnesium stearate, and 80% ethanol supplied by New Era Pharmaceutical Co., Ltd. (China) were selected as the excipient to prepare Ginkgo biloba tablets. Methanol (purity 99.92%) and 25% hydrochloric acid were supplied by Sinopharm Chemical Reagent Co., Ltd. (China). The pure water made by the laboratory used in the experiment. In order to improve the solubility of the raw materials and promote the stability of the prepared suspension, it is necessary to add a certain amount of solubilizer. In this experiment, SDS purchased from SuZhou KuangShi Chemical Co. Ltd. (China) was selected as the solubilizer.

### 2.3. Sample Characterization

The particle size and dispersion coefficient (PDI) of GBE were determined by a nanoparticle size analyzer N5 (Beckman Coulter, Inc., USA). A sample of GBE was diluted to an appropriate concentration and measured with a scattering angle of 90° at 25°C. Differential scanning calorimetry (DSC) was performed using a DSC TG209F3 (Netzsch, Inc., Germany) with standard aluminum sample pans and covers. Scanning electron microscopy (SEM) was performed using an EM-30 Plus (COXEM, Inc., Korea) to observe the surface morphology of GBE. Intelligent dissolution tester RCY-1400 T (Tianjin, China) and Shimadzu ultraviolet spectrophotometer UV-2401PC (USP, Hangzhou Coulomb Technology Co. LTD, China) was used to study the GBE dissolution rate changes before and after grinding. High-performance liquid chromatography (HPLC) was measured to study the influence of WBM processing on flavonol glycosides’ dissolution release using Waters HPLC (Waters Inc., America).

### 2.4. Design of Experiments

In order to describe the influence of the interaction between the factors on the GBE nanosuspension particle size, multiple regression analysis was performed on the basis of the experimental data. Experimental data was statistically analyzed by BBD using the software Design-Expert 10.0. The design consisted of 29 runs was carried out to optimize the levels of the selected independent factors: GBE concentration (A), milling speed (B), milling time (C), and SDS concentration (D). The codes and actual variables are presented in Table 1. The behavior of the process was explained by the second-order polynomial equation of quadratic order as follows:

\[
Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x_i^2, \tag{1}
\]

where \(Y\) denotes the value of the particle size, whereas \(\beta_0, \beta_i, \beta_{ij}, \) and \(\beta_{ii}\) represent the regression coefficient for the term intercept, linear, square, and interaction effects, respectively. Also, \(x_i\) and \(x_j\) are the independent variables.

### Table 1: Codes and actual variables and their levels in BBD.

| Codes | Variables                        | Level 1 (-1) | Level 2 (0) | Level 3 (+1) |
|-------|----------------------------------|--------------|-------------|-------------|
| A     | GBE concentration (g/L)          | 3.3          | 6.6         | 13.2        |
| B     | Milling speed (m/s)              | 6            | 8           | 10          |
| C     | Milling time (h)                 | 0.5          | 1           | 2           |
| D     | SDS concentration (g/L)          | 0.1          | 0.5         | 1           |
3. Results and Discussion

3.1. Optimization of WBM by BBD. After measurement, it can be seen from Table 2 and Figure 1 that only five experimental particle sizes are larger than 360 nm and most of the experimental particle sizes could be controlled between 170 nm and 250 nm. The experimental results were all right and within the expected results. PDI values for most formulation were less than or equal to 0.5, which is an indicator of good particle size uniformity. After applying multiple linear regressions, the following polynomial model describing the quantitative effect of studied formulation and operating variables and their first-order interaction on the response was obtained:

\[ Y = 201.66 + 51.19A + 21.04B - 18.58C - 162.22D \\
+ 48.35AB - 4.025AC - 124.65AD + 5.85BC - 32.63BD \\
+ 5.76CD - 6.35A^2 + 33.6B^2 + 0.02C^2 + 150.17D^2. \]

(2)

A positive sign of the terms in Equation (2) indicates a synergistic effect, while a negative sign indicates an antagonistic

| Run | GBE concentration (g/L) | Milling speed (m/s) | Milling time (h) | SDS concentration (g/L) | Particle size (nm) | PDI |
|-----|-------------------------|--------------------|-----------------|--------------------------|-------------------|-----|
| 1   | 3.3                     | 6                  | 1               | 0.5                      | 203.4             | 0.505|
| 2   | 13.2                    | 6                  | 1               | 0.5                      | 197               | 1.215|
| 3   | 3.3                     | 10                 | 1               | 0.5                      | 178.6             | 0.565|
| 4   | 13.2                    | 10                 | 1               | 0.5                      | 365.6             | 0.386|
| 5   | 6.6                     | 8                  | 0.5             | 0.1                      | 568.1             | 1.055|
| 6   | 6.6                     | 8                  | 2               | 0.1                      | 512.8             | 0.302|
| 7   | 6.6                     | 8                  | 0.5             | 1                        | 213.9             | 0.561|
| 8   | 6.6                     | 8                  | 2               | 1                        | 181.5             | 0.441|
| 9   | 3.3                     | 8                  | 1               | 0.1                      | 198.5             | 0.286|
| 10  | 13.2                    | 8                  | 1               | 0.1                      | 664.3             | 0.425|
| 11  | 3.3                     | 8                  | 1               | 1                        | 205.9             | 0.669|
| 12  | 13.2                    | 8                  | 2               | 1                        | 173.1             | 0.366|
| 13  | 6.6                     | 6                  | 0.5             | 0.5                      | 240.4             | 0.635|
| 14  | 6.6                     | 10                 | 0.5             | 0.5                      | 223.8             | 0.518|
| 15  | 6.6                     | 6                  | 2               | 0.5                      | 185               | 0.527|
| 16  | 6.6                     | 10                 | 2               | 0.5                      | 191.8             | 0.748|
| 17  | 3.3                     | 8                  | 0.5             | 0.5                      | 200.9             | 0.481|
| 18  | 13.2                    | 8                  | 0.5             | 0.5                      | 209.3             | 0.378|
| 19  | 3.3                     | 8                  | 2               | 0.5                      | 185               | 0.553|
| 20  | 13.2                    | 8                  | 2               | 0.5                      | 177.3             | 0.302|
| 21  | 6.6                     | 6                  | 1               | 0.1                      | 525.3             | 1.125|
| 22  | 6.6                     | 10                 | 1               | 0.1                      | 649.8             | 0.216|
| 23  | 6.6                     | 6                  | 1               | 1                        | 201.9             | 0.594|
| 24  | 6.6                     | 10                 | 1               | 1                        | 195.9             | 0.527|
| 25  | 6.6                     | 8                  | 1               | 0.5                      | 197               | 0.473|
| 26  | 6.6                     | 8                  | 1               | 0.5                      | 203.8             | 0.598|
| 27  | 6.6                     | 8                  | 1               | 0.5                      | 212               | 0.585|
| 28  | 6.6                     | 8                  | 0.5             | 1                        | 203.8             | 0.408|
| 29  | 6.6                     | 8                  | 1               | 0.5                      | 191.7             | 0.449|

Table 2: Particle size and dispersion coefficient (PDI) of GBE nanosuspension.
effect of the response. The quality of the developed model was evaluated based on the correlation coefficient ($R^2$) value. The $R^2$ value of 0.9276 assures the significant adjustment to the quadratic model to experimental data, and it provides an appropriate estimate of response in the studied range as shown in Figure 2. The analysis of variance (ANOVA) is the statistical tool which defines the significance and accuracy of developed quadratic response surface model. According to ANOVA results (Table 3), the ‘$F$ value’ of 12.81 and the ‘$P$ value’ less than 0.05 imply that this model is significant. The smaller the ‘$P$ value,’ the more significant is the corresponding coefficient. Obviously, SDS concentration is the most significant variable for the response with the smallest $P$ value of <0.0001 but milling time does not have an influence on the particle size with the highest $P$ value among the four variables. The same conclusion can be drawn from Figure 3 that four parameters have significant effects in the order (SDS concentration > GBE concentration > milling speed > milling time) on the GBE particle size. The ratio of signal-to-noise ratio defines the value of adequate precision which should be greater than 4, and the ratio of 13.825 for the preparation of GBE nanosuspension particles represents the adequate signal for the model was used to navigate the design space. Obviously, the points on the residual normal graph (Figure 4) are close to the straight line, which indicates the accuracy of the model, as well as the independence of the residuals.

The optimal conditions of GBE nanosuspension prepared were predicted by Derringer’s desirability function. Based on all the above results, GBE nanosuspension particle size with 171.2 nm was obtained under the optimum conditions of GBE concentration (7.459 g/L), milling speed (9.253 m/s), milling time (1.492 h), and SDS concentration (0.846 g/L). Table 4 summarized the optimum size of GBE particles prepared by other methods. It is seen from the table that the optimum size of GBE particles prepared by other methods were less than that of WBM; however, high-pressure homogenization needs high energy input and are highly inefficient compared with WBM. In addition, the downside of emulsion solvent evaporation involves high amounts of surfactants due to the high fraction of aggregates retrieved after the process. Meanwhile, it is difficult for liquid antisolvent precipitation and supercritical antisolvent to remove the residual solvent completely and meet the industrial level.

3.2. Effect of Influencing Factors on GBE Nanosuspension Particle Size. In order to gain a better understanding of their interactions with GBE nanosuspension particle size, three-dimensional (3D) response surface plots for the measured responses were formed based on the regression equation (Equation (2)). The 3D response surface analysis plots for particle size of microparticles are illustrated in Figures 5–8.

3.2.1. Effect of GBE Concentration. It is present that GBE concentration had a significant influence on GBE nanosuspension particle size with the ‘$P$ value’ of 0.0083 in Table 3. Figures 5 and 6 clearly illustrate that the GBE nanosuspension particle size increases with GBE concentration increases. This may be due to fact that GBE suspension has a strong viscosity at higher GBE concentration, and it alters or hinders the processing of nanosuspension on WBM. On the one hand, nanoparticles have the characteristics of large specific surface area, high surface energy, and extremely unstable energy state. On the other hand, there are strong van der Waals forces and electrostatic forces between the molecules and atoms on the surface of the nanoparticle and a large number of positive and negative charges accumulate on the surface, which can facilitate particle agglomeration. When a critical GBE concentration is reached, the addition of SDS is necessary for successful milling.
3.2.2. Effect of Milling Speed. Effects of milling speed varying at 6, 8, and 10 m/s on GBE nanosuspension particle size were exhibited in Figures 6 and 7. No clear trend was observed between the milling speed and particle size. Under low milling speed, the shear force provided by the stirring head rotation is not enough to open the cohesion between aggregates. In addition, under high milling speed, the collision between grinding ball and drug produces high energy and strong shear force, which increases the energy loss and reduces the dispersion efficiency. Thus, varying the milling speed, no significant changing in the particle size occurred.

3.2.3. Effect of Milling Time. The influence of the milling time was investigated at 0.5, 1, and 2 h, fixing all the other operating parameters at a certain level. It was observed that milling time does not have a significant effect on the particle size with the highest P value of 0.2834 compared with other variables, as it is possible to observe in Figures 5 and 8. Due to the fast ball milling process of GBE, the particles collide with the ball, which produces high heat and high power in short time. Consequently, the heat could not be discharged from the milling chamber in time and it will be conducive to the agglomeration of particles. However, when the critical particle size is reached, the kinetic energy provided by the milling balls is insufficient to break the particle and the heat was removed from the milling chamber with time elapsing.

Table 3: Summary of the analysis of variance (ANOVA) result for the quadratic model.

| Run | Sum of square | df | Mean square | F value | P value |
|-----|---------------|----|-------------|---------|---------|
| Model | 597267.40 | 14 | 42661.96 | 12.81 | <0.0001 |
| A | 31447.04 | 1 | 31447.04 | 9.44 | 0.0083 |
| B | 5313.02 | 1 | 5313.02 | 1.60 | 0.2271 |
| C | 4144.08 | 1 | 4144.08 | 1.24 | 0.2834 |
| D | 315770.96 | 1 | 315770.96 | 94.84 | <0.0001 |
| AB | 9350.89 | 1 | 9350.89 | 2.81 | 0.1160 |
| AC | 64.80 | 1 | 64.80 | 0.019 | 0.8910 |
| AD | 62150.49 | 1 | 62150.49 | 18.67 | 0.0007 |
| BC | 136.89 | 1 | 136.89 | 0.041 | 0.8422 |
| BD | 4257.56 | 1 | 4257.56 | 1.28 | 0.2771 |
| CD | 131.10 | 1 | 131.10 | 0.039 | 0.8456 |
| A² | 1733.28 | 1 | 1733.28 | 0.52 | 0.4825 |
| B² | 7324.44 | 1 | 7324.44 | 2.20 | 0.1602 |
| C² | 0.002 | 1 | 0.002 | 4.88×10⁻⁷ | 0.9995 |
| D² | 146268.83 | 1 | 146268.83 | 43.93 | <0.0001 |
| Residual | 466144.41 | 14 | 3329.60 | |
| Lack of fit | 463774.42 | 10 | 4637.74 | 78.28 | 0.0004 |
| Pure error | 236.99 | 4 | 59.25 | |
| Col total | 643881.81 | 28 | |
| Std. Dev. | 57.70 | | | |
| Mean | 270.94 | | | |
| C. V. (%) | 21.30 | | | |
| Press | 267504.25 | | Adequate precision | 13.825 |

Figure 3: Perturbation plots for the GBE particle size. (a) GBE concentration. (b) Milling speed. (c) Milling time. (d) SDS concentration.
Therefore, the heat exchange between the cooling circulating system and the milling chamber achieves dynamic balance, having no significant effect on the particle size.

3.2.4. Effect of SDS Concentration. The effect of SDS concentration on GBE nanosuspension particle size has been studied. 'P value' of <0.0001 given in Table 3 indicates that SDS concentration greatly influences the GBE nanosuspension particle size. As can be seen in Figures 7 and 8, SDS concentration had a positive effect on GBE nanosuspension particle size as it increased from 0.1 to 1 g/L. The addition of SDS is necessary for successful milling due to its highly

**Table 4:** Examples in the literature of GBE prepared by different methods.

| Methods                                           | Particle size (nm) |
|---------------------------------------------------|--------------------|
| Film dispersion-homogenization method [12]         | 141                |
| Emulsion solvent evaporation combined with freeze-drying [26] | 56.0               |
| Liquid antisolvent precipitation [27]              | 76.9               |
| Supercritical antisolvent [7]                      | 81.2               |
| This paper                                         | 171.2              |

**Figure 4:** The normal % probability distribution versus values of the model externally studentized residuals.

**Figure 5:** 3D response surface of interactive effect of (a) GBE concentration and (b) milling time.

**Figure 6:** 3D response surface of interactive effect of (a) GBE concentration and (b) milling speed.
hydrophobic nature of GBE and poor wettability. The high SDS concentration facilitates adsorption of the solubilizer molecules, which provides a barrier to agglomeration via steric or electrostatic stabilization effect. However, the surface area of nanosuspension particles is limited, which leads to limited adsorption capacity of the solubilizer. Excess SDS tends to form micelles and increases solubility of GBE due to the electrostatic attraction and the hydrogen bonds, resulting in Ostwald ripening and PDI reduction.

3.3. Characterization of GBE Nanosuspensions

3.3.1. SEM Analysis. In addition to particle size analysis, the SEM micrographs further confirmed that the milling process is effective in converting the original GBE particles into the nanometer range. Sample powders were placed on a silicon wafer, which was mounted on an aluminium pin stub. Gold was coated in vacuum by sputtering coater and observed by SEM at 15 kV voltage. SEM micrographs of GBE particles at different conditions are displayed in Figure 9. It was found that the raw material of GBE (Figure 9(a)) showed irregularly flake-shaped particle agglomerates of nonuniform size; conversely, the freeze-dried nanosuspension (Figures 9(b) and 9(c)) had particles of uniform size where most of the particles exhibit spherical morphology characteristic.

3.3.2. DSC Analysis. The melting behavior for the raw material and milled GBE was analyzed by DSC. Measurements were carried out with a heating rate of 10 K/min, and the nitrogen purge gas flow rate was set to 25 mL/min. As can be seen in Figure 10(a), the DSC curve for the raw material had endothermal peaks at 88.7°C, while the DSC curve for the milled GBE in Figure 10(b) showed apparent endothermal peaks at 63.4°C. SDS did not exhibit any melting event during heating due to its amorphous nature (data not shown). However, the melting peak of the milled GBE was not as sharp as the raw material and slightly shifted. A GBE melting peak was clearly visible in the thermograms of all analyzed samples. The melting point depression decreases with the decrease of crystal size according to the Gibbs-Thomson equation; meanwhile, WBM also induces disorder of the drug lattice as a high energy process, which also lowers their melting point.

3.3.3. Dissolution Analysis. Generally, USP was carried out to investigate the dissolution rate of samples. GBE nanosuspension was first frozen in laboratory freezer (−20°C) and then freeze-dried. The obtained GBE solid powder and raw materials were, respectively, pressed into standard tablets weighing 14.7 mg with excipients (microcrystalline cellulose, crosslinked carboxymethyl cellulose sodium, silicon dioxide, crosslinked povidone, magnesium stearate, and 80% ethanol) in a certain proportion for dissolution test. Dissolution medium, volume, and temperature of dissolution medium were adopted: pure water, 900 mL and 37 ± 0.5°C, respectively. In addition, the paddle device with a speed of 50 r/min was considered. After the start of the test in certain periods of time (5, 10, 15, 30, 40, 60, and 120 min), 5 mL of the dissolved medium sample was drawn. Each sample was repeated thrice. In order to maintain a constant volume of dissolution medium, fresh medium was added to vessels. Samples were filtered through 0.45 μm Whatman filter paper and analyzed by spectrophotometry at 266 nm (raw material samples) relative to their respective medium to determine drug release. The formula is as follows:

\[
\% \text{Drug release rate} = \left( \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \right) \times 100\%,
\]

where the standard was 100% of drug release.
Dissolution release and error bar of raw materials and optimized GBE were shown on Figure 11. It should be pointed out that the optimized GBE showed better dissolution properties than the raw materials, especially at the beginning of 40 minutes. The dissolution rate of optimized GBE can reach 65% in the first 5 minutes and is fully released at 40 minutes, whereas the raw material of GBE can be completely dissolved in 60 minutes. It could be explained by the fact that particle size reduction and SDS concentration are major factors to the improvement of solubility.

The influence of WBM processing on the dissolution release of flavonol glycosides was studied by HPLC. Firstly, 3 g quercetin was added to 100 mL methanol solution to prepare the reference solution. Secondly, 10 g optimized GBE was added to 25 mL methanol-25% hydrochloric acid solution (4:1), and the solution was placed into water bath to heat and reflow for 30 minutes. Methanol was adopted to
dilute the solution to 50 mL after cooling to room temperature to prepare the test solution. The elution was employed using an Novapak C18 column (3.9 mm × 150 mm, 5 μm). The column was equilibrated at a flow rate of 1.0 mL/min with an injection volume equal to 10 μL; the mobile phase consisted of methanol (50%) and phosphoric acid solution (50%). Sample solutions were filtered with a 0.45 μm membrane syringe, and detected at 360 nm. The dissolution release of raw flavonol glycosides and optimized GBE were exhibited in Figure 12. The similarity factors calculated was 39, indicating that the optimized GBE shows significant differences and exhibits better dissolution properties compared with raw flavonol glycosides.

4. Conclusion
In this study, GBE nanosuspension with most nanoparticles below 200 nm and uniform size distribution is produced by WBM process with careful selection of influencing factors. Analysis of experimental results showed that the SDS concentration was the most significant factor on particle size, followed by GBE concentration and milling speed, and milling time was the slighter significant factor. Additionally, the optimal GBE nanosuspension particle with 171.2 nm was obtained using GBE concentration (7.459 g/L) in combination with milling time (1.492 h), mill rotation speed (9.253 m/s), and SDS concentration (0.846 g/L). Finally, the morphology, melting behavior, and dissolution rate were analyzed for the GBE nanosuspension, respectively. In conclusion, the WBM process proposed in the study proved to be a feasible way to improve the bioavailability of GBE and it can be used as a reference for the production of GBE particles.

Data Availability
The (data type) data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare no conflict of interest exits in the submission of this manuscript.

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