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Preparation of Quercetin Nanocrystals by Planetary Ball Mill to Increase the Solubility and the Dissolution Profile

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ABSTRACT

The objectives of this study are to prepare and characterize quercetin nanocrystals by planetary ball milling and to evaluate the influence on the solubility and the dissolution profile. Coarse quercetin was pretreated with ultrasonication prior to wet milling using bead size of 0.1 mm at 800 rpm for 15 and 30 minutes respectively. Particle size analysis showed that the milling process for 15 minutes yielded particles with d50% and d99% of 71.5 nm and 389.9 nm respectively, mean particle size 367.7 nm, the PDI value of 0.338 and the zeta potential 0.17 mV. The increase in milling time to 30 minutes yielded particles with d50% and d99% of 68.3 nm and 352.3 nm respectively, mean particle size 289.9 nm, the PDI of 0.308 and the zeta potential 0.31 mV. There was a significant increase in the solubility of quercetin nanocrystals (P<0.01). Quercetin nanocrystals showed an increase in dissolution profile in 0.1 N hydrochloric acid medium.

Keywords: Quercetin, nanocrystal, planetary ball mill, solubility, dissolution profile.

INTRODUCTION

Quercetin (3,3',4'5'-7 pentahydroxy flavon) is a bioflavonoid with a wide range of biological and pharmacological activities such as antitumor [1], antibacterial [2], antioxidant, anti-inflammation [3], obtains protection against osteoporosis, pulmonary and cardiovascular diseases [4]. Its potential to be developed as an active pharmaceutical ingredient (API), however, is limited by poor solubility and low rate of dissolution leading to low bioavailability in rats (< 17%) and in human (1%) [5,6].

Particle size reduction by nanosizing has been applied to improve the solubility of quercetin, this is achieved by preparation of nanocrystals [7,8] or quercetin loaded nanoparticles [9,10]. The former is considered as low cost and less toxic technique due to the absence of organic solvent, the latter is preferred to enhance the stability of quercetin by incorporation to a polymer, however being limited by low drug loading and the use of organic solvent. Kakran et al. (2012) reported optimization of producing quercetin nanocrystals using three fabrication methods: high pressure homogenization, bead milling and cavi-precipitation. All methods reduced the particle size to the nanometer range; the wet milling process using agitating bead mil produced the lowest particle size (276.7 nm) with highest saturation stability (25.59 $\pm$ 1.11 µg/mL) [7].

The aim of the present study is to produce and characterize quercetin nanocrystals by using planetary ball mill, a new generation of ball mill type equipment which apply combination of revolution and rotating speed resulting

higher energy for particle size reduction [11]. The increase in solubility and dissolution profile of quercetin nanocrystals is examined in comparison to coarse quercetin.

MATERIALS AND METHODS

Materials

Quercetin was purchased from Baoji Guakong Bio-Technology (China); tween 80 (polysorbate 80) was purchased from Bratachem Ltd. (Indonesia), distilled water, ethanol pro analysis (Merck), and hydrochloric acid (Merck).

Preparation of nanocrystals

Nanocrystals was produced by wet milling method using planetary ball mill (Fritsch Pulverisette 7 Premium Line). Suspensions of quercetin (10% w/w) and tween 80 (2.5%) in distilled water were pretreated in ultrasonic bath (Elmasonic S 80 (H)) for 30 minutes at 3^{o}C. The suspensions (15 grams) were then mixed with 20 grams of zirconium oxide milling beads of size 0.1 mm. The milling process was performed at a speed of 800 rpm for 15 and 30 minutes respectively, with a pause for 15 minutes at every 3-minute rotation. The equipment was set at a reverse mode to obtain counter direction rotating type which has higher energy impact. After milling for 15 and 30 minutes respectively, the nanosuspensions were freeze-dried (Freeze Dryer Modulyo, Edwards) and kept in the desiccator before used.

Particle size analysis

Particle size analysis was performed by using photon correlation spectroscopy (Delsa^{TM} Nano C Particle Analyzer, Beckman Coulter). Quercetin nanocrystals in the dried original dispersion medium were dispersed in 10 mL of distilled water and analyzed at 25^{o}C. Data such as particle size distribution, the mean particle size, and the polydispersity index (PDI) were obtained.

Zeta potential measurements

The zeta potential was determined by using electrophoretic light scattering (Delsa^{TM} Nano C Particle Analyzer, Beckman Coulter). Ten mg of quercetin nanocrystals in the dried original dispersion medium were dispersed in 10 mL distilled water and analyzed at 25^{o}C.

Particle morphology

The morphology of quercetin nanocrystals was characterized by using Transmission Electron Microscope (TEM Tecnai G2 20S-Twin, FEI) at 100kV accelerating voltage. Quercetin nanocrystals in the dried original dispersion medium were dispersed in distilled water, 5µL was dropped onto TEM grid carbon film and dried for 30 minutes at 25^{o}C [12].

The morphology of coarse quercetin was analyzed by using Scanning Electron Microscope (S-3400 N SEM, Hitachi). Coarse quercetin was placed onto gold coated SEM carbon tape and analyzed at 15 kV accelerating voltage at 25^{o}C.

X-ray diffraction (XRD)

XRD analysis was performed by using X-ray diffractometer (X'pert PRO, PAN analytical) equipped with copper Kα radiation (40 kV, 20 mA). The scanning was done from 5^{o} to 50^{o} 2θ for quercetin nanocrystals and coarse quercetin respectively.

Solubility test

Excess quercetin nanocrystals were added into 10 mL of distilled water. The nanosuspensions were pre-homogenized for 1 minute by sonication (Elmasonic S 80 (H)) and centrifuged at 21,000 x g force (Biofuge Primo R Centrifuge, Thermo Scientific) [12]. Four mL of the supernatant were taken and analyzed by spectrophotometer UV-Visible (Shimadzu 1700 Pharmaspec) for quercetin concentration. The method of analysis was validated by measuring the absorbance of quercetin at concentration range 4 – 12 µg/mL at the maximum wavelength of 255.8 nm. Experiments were carried out in triplicate, data were presented as the average value ± standard of deviation. The solubility of coarse quercetin in 2.5% tween 80 solution and that of coarse quercetin only were also determined.

Dissolution test

The dissolution rate profile was obtained by using the USP apparatus 2 (SR18 Dissolution Station, Hanson Research) at 100 rpm speed in 0.1 N hydrochloric acid medium at 37±0.1°C for 45 minutes. Suspension of quercetin nanocrystals (5 mg/mL) in water was added to the medium, drug concentration was determined by Spectrophotometer UV-Vis at time intervals. The dissolution profile of coarse quercetin suspension in water (5 mg/mL) was also determined.

Statistical analysis

The solubility data were analyzed statistically using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Particle size study

Suspension of coarse quercetin and 2.5% tween 80 in aqueous solution was pretreated with ultrasonic to improve the dispersion capability with expectation to obtain an effective particle size reduction [13]. Combination of ultrasonication followed by planetary ball milling at 800 rpm obtained particles in nanometer size with the morphology represented in Figure 1.A and that of the coarse particle in Figure 1.B. The milling process for 15 minutes yielded particle size with d50% and d99% of 71.5 nm and 389.9 nm respectively, mean particle size 367.7 nm, the PDI value of 0.338 and the zeta potential 0.17 mV. The increase in milling time to 30 minutes showed better results, with d50% and d99% of 68.3 nm and 352.3 nm respectively, mean particle size 289.9 nm, the PDI of 0.308 and the zeta potential 0.31 mV. It was reported that quercetin (10% w/w) in 2% tween 80 aqueous suspension subjected to agitating ball mill at 2000 rpm with the bead size of 0,4-0,6 mm obtained particle size with d50% and d95% of 280 nm and 728 nm respectively, the PDI of 0,121 and zeta potential –21.5±0.09 mV [7].

The PDI is a measure of the broadness of particle size distribution, the value of 0.10-0.20 considered as a relatively narrow distribution, while > 0.5 a very broad distribution [7]. In this study, the PDI of 0.308 after 30 minutes milling indicates a midrange value of particle distribution. The zeta potential correlates with the stability of the nanocrystals after processing or during storage, the absolute value should be at least 30 mV if the stabilization is based on the electrostatic effect only. In the present study, tween 80 (a nonionic surfactant) is used, which stabilize the nanocrystals by steric effect, this may give an explanation to the zeta potential of 0.17 and 0.31 obtained for nanocrystals after milling 15 and 30 minutes respectively [14]. Particle size analysis of samples after 60 days of storage confirmed that the nanocrystals remained in the nanometer size range.

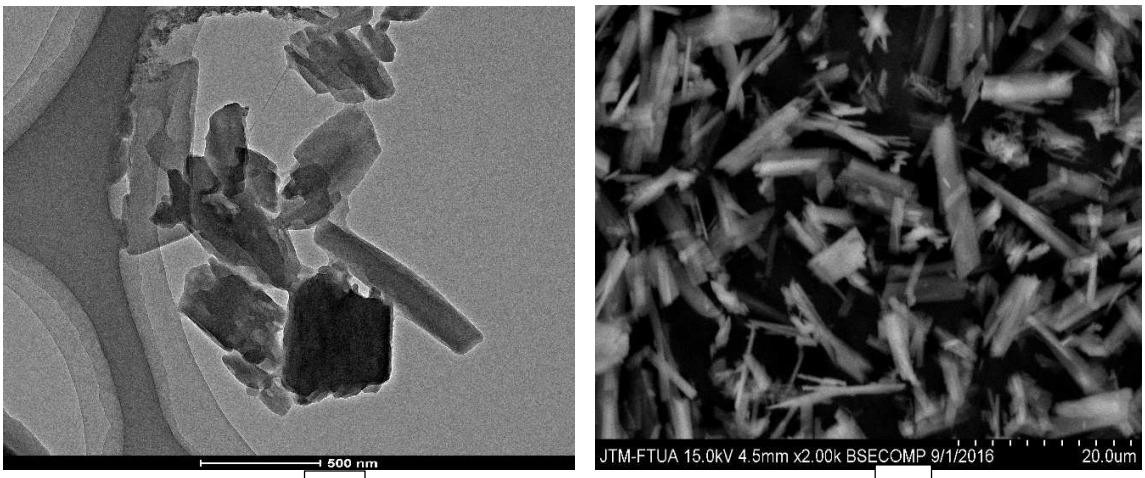

Figure 1. The microphotographs of quercetin nanocrystals in the dried original dispersion medium by TEM (A) and coarse quercetin by SEM (B)

X-ray diffraction

Figure 2 shows that coarse quercetin and the nanocrystals are in a crystalline state. There was no transformation to an amorphous state observed after the nanomilling process. However, there was a reduction in peak intensity at 27^{o}; 12^{o} and 10^{o} from 7476.54; 3993.51; 3993.51 (coarse quercetin) to 6988.67; 3362.95; 1046.55 (15 minutes milling) and 6449.86; 1905.43; 918.23 (30 minutes milling) respectively. This indicates a decrease in the crystallinity index and supports the reduction in particle size to nanometer range.

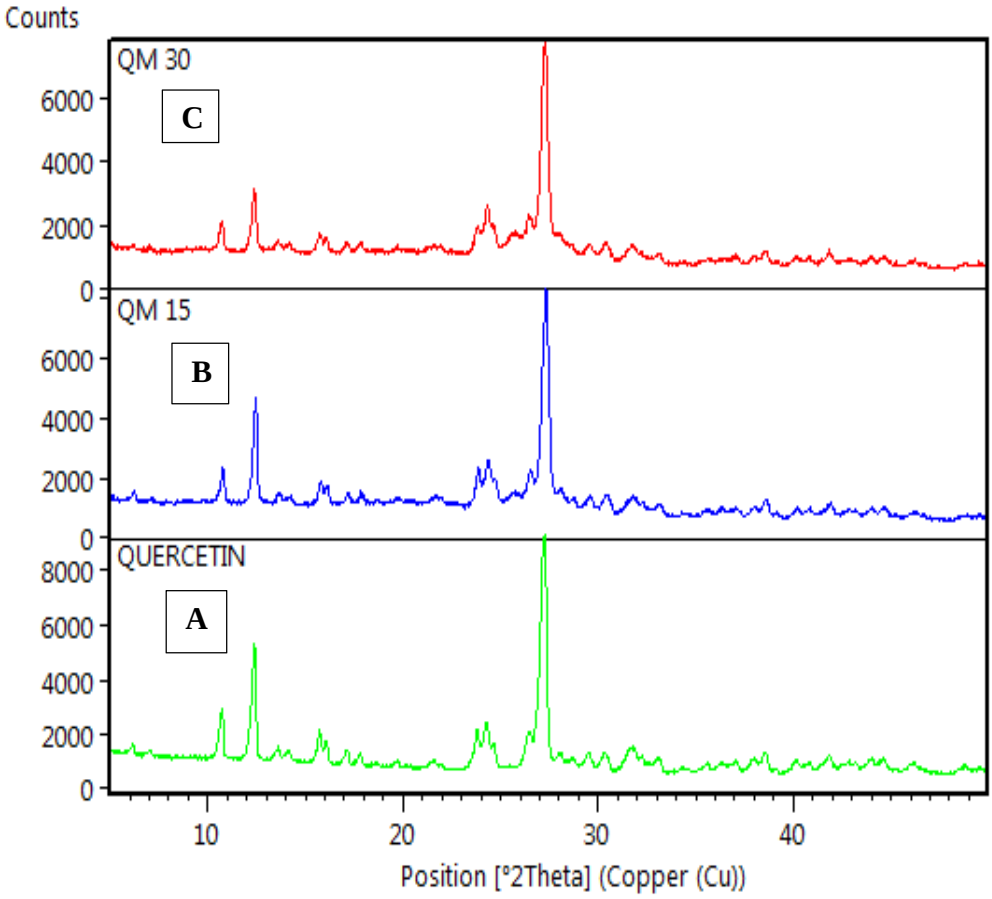

Figure 2. The XRD patterns of coarse quercetin (A), quercetin nanocrystals after 15 minutes milling (B) and after 30 minutes milling (C)

Solubility study

Figure 3 shows an increase in the solubility of quercetin nanocrystal over that of coarse quercetin or quercetin in 2.5% tween 80 solution. The solubility of coarse quercetin was 4.31±0.18 µg/mL and that of quercetin in 2.5% tween 80 solution was 5.54±1.29 µg/mL. There was an increase in the solubility of quercetin nanocrystals by four times to 18.03±1.53 µg/mL (milling 15 minutes); and approximately five times to 20.82±4.42 µg/mL (milling 30 minutes) in comparison to that of coarse quercetin. Statistical analysis by one-way ANOVA showed that the nanomilling of quercetin increase the solubility significantly ($P<0.01$).

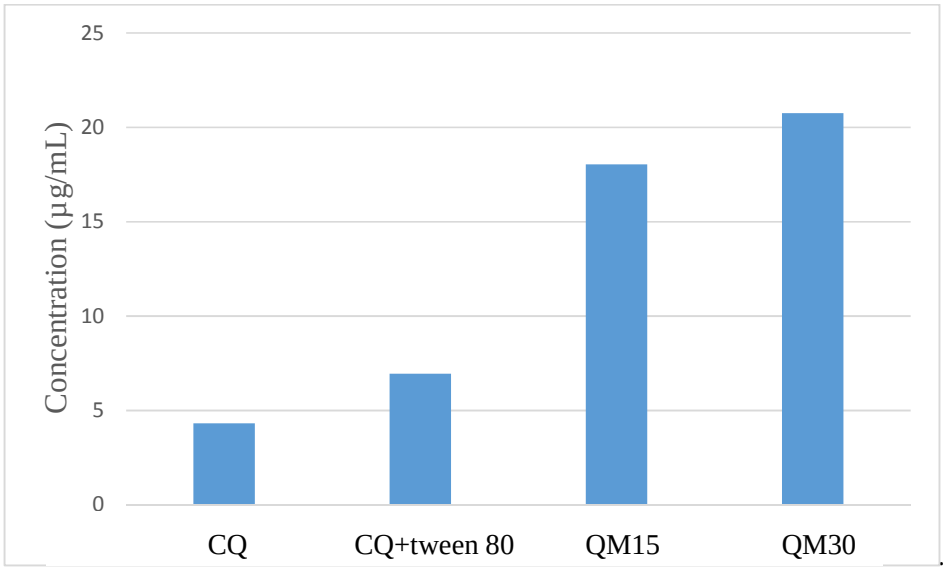

Figure 3. The mean solubility of coarse quercetin (CQ), coarse quercetin and 2.5% tween 80 (CQ + tween 80), nanocrystals after 15 minutes milling (QM15) and after 30 minutes milling (QM30)

Dissolution study

Quercetin is expected to dissolve in the acidic gastric medium due to the narrow absorption window character and the instability in the alkaline pH [15]. In this study 0.1 N hydrochloric acid solution was employed as medium. The dissolution study was conducted for coarse quercetin and the nanocrystals after 30 minutes milling. At 5th minutes of dissolution, coarse quercetin dissolved for 14.83±1.58% and the nanocrystal 32.50±0.53% respectively. After 45 minutes the amount of drug dissolved was 13.42±0.27% and 36.03±1.75% for coarse quercetin and quercetin nanocrystal respectively. There was an increase in the amount of quercetin nanocrystals dissolved in comparison to coarse quercetin, however it was lower than the increase in solubility. The amount of drug dissolved remained the same after 15 minutes although drug concentration in the dissolution medium had been kept below the saturation solubility. There are many factors influencing the rate of quercetin dissolution such as the chemical stability of quercetin after the milling process which yet to be studied.

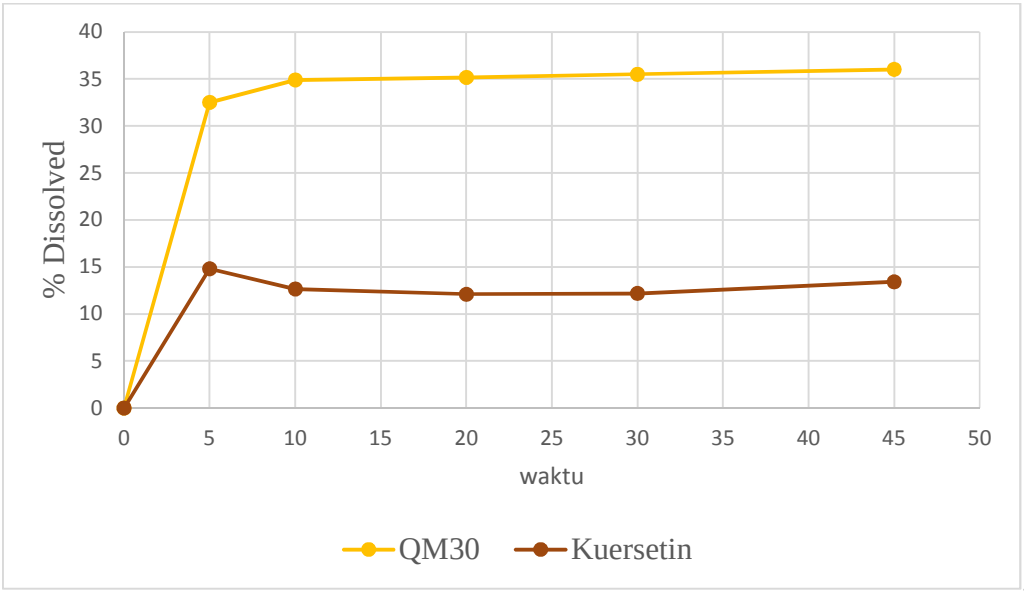

Figure 4. The dissolution profile of quercetin nanocrystals (QM30) and coarse quercetin (Kuersetin)

CONCLUSION

Quercetin nanocrystal has been obtained by using pretreatment with ultrasonication followed by planetary ball milling at 800 rpm with a significant increase in the solubility and an increase in the dissolution profile.

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