



# Physicochemical Characterization and *In Vitro* Dissolution Test of Quercetin-Succinic Acid Co-crystals Prepared Using Solvent Evaporation

## Çözücü Buharlaştırma ile Hazırlanan Kersetin-Süksinik Asit Ko-kristalinin *In Vitro* Çözünme Testi ve Fizikokimyasal Karakterizasyonu

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### ABSTRACT

**Objectives:** Quercetin is one of the flavonoids with a polyhydroxyaromatic structure. Quercetin has been proposed to exhibit a bioWactivity against oxidative stress. However, quercetin has poor solubility in aqueous media. The purpose of this study was to investigate the physicochemical properties and dissolution rates of quercetin-succinic acid co-crystals.

**Materials and Methods:** The quercetin-succinic acid co-crystals were prepared in 1:1 molar ratio using solvent evaporation. X-ray diffraction, differential thermal analysis, infrared spectroscopy, and scanning electron microscopy were performed to determine the physicochemical properties of quercetin-succinic acid co-crystals. Dissolution was studied in medium citrate buffer with 2% SLS for 60 min using USP II (paddle) apparatus at 100 rpm and 37°C.

**Results:** Based on diffractogram, thermogram, infrared spectrum, and microscopic capture, the physicochemical properties of quercetin-succinic acid co-crystals showed difference to those of quercetin. In addition, the *in vitro* dissolution test showed that the dissolution profile of co-crystals was significantly higher than pure quercetin.

**Conclusion:** This study suggests that the formation of quercetin-succinic acid co-crystals using solvent evaporation enhanced the physicochemical properties and dissolution rate of quercetin.

**Key words:** Characterization, dissolution, co-crystal, quercetin, succinic acid, solvent evaporation

### ÖZ

**Amaç:** Kersetin, polihidroksiaromatik yapıli flavonoidlerden biridir. Kersetinin oksidatif strese karşı bir biyoaktivite sergilediđi ileri sürülmüştür. Ancak, kersetin sulu ortamda zayıf çözünürlüđe sahiptir. Bu çalışmanın amacı kersetin-süksinik asit ko-kristalin fiziko-kimyasal özelliklerini ve çözünme oranlarını araştırmaktır.

**Gereç ve Yöntemler:** Kersetin-süksinik asit ko-kristali 1:1 molar oranında çözücü buharlaştırma ile hazırlanmıştır. Kersetin-süksinik asit ko-kristalinin fiziko-kimyasal özelliklerini belirlemek için X-ışını kırınımı, diferansiyel termal analiz, kızıl ötesi spektroskopi ve taramalı elektron mikroskopu kullanılmıştır. Çözünme, %2 SLS içeren sitrat tamponu içinde 100 rpm ve 37°C'de USP II (palet) aparatında 60 dakika çalışılmıştır.

**Bulgular:** Difraktogram, termogram, IR vemikroskopik tespate dayanarak, kersetin-süksinik asit ko-kristalin fiziko-kimyasal özelliklerinin kersetinden farklı olduđu görülmüştür. Ek olarak, *in vitro* çözünme testi, ko-kristalin çözünme profilinin saf kersetinden çok daha yüksek olduğunu göstermiştir.

**Sonuç:** Bu nedenle bu çalışma, çözücü buharlaştırma kullanılarak kersetin-süksinik asit ko-kristalinin oluşumunun kersetinin fiziko-kimyasal özelliklerini ve çözünme oranını artırdığını düşündürmektedir.

**Anahtar kelimeler:** Karakterizasyon, çözünme, ko-kristal, kersetin, süksinik asit, çözücü buharlaştırma

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Received: 04.01.2017, Accepted: 16.02.2017

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## INTRODUCTION

Poor solubility of active pharmaceutical ingredients (APIs) is one of the problems in the development of oral dose form because it may affect the bioavailability profile. Solubility and dissolution are major factors that determine the release of APIs in dissolution media.<sup>1</sup> Several strategies have been developed in order to improve the dissolution rate of oral dose forms, such as enhancing the solubility of APIs.

Quercetin (Q) (3',4',3,5,7-pentahydroxyflavon) is a polyphenolic flavonoid with potential antioxidant activity against oxidative stress. However, Q exhibits a poor solubility in aqueous media.<sup>2,3</sup> Previous studies showed that several strategies were used to improve the solubility of Q, such as solid dispersion<sup>3</sup> and complexation with  $\beta$ -cyclodextrin.<sup>4</sup>

Co-crystallization is one of methods used to improve the solubility profile of APIs.<sup>5</sup> Co-crystals are produced by combining two or more stoichiometrically neutral compounds through hydrogen bonding and  $\pi$ - $\pi$  interaction.<sup>6</sup> Co-crystal formers are commonly inert or exhibit no pharmacology activity.<sup>7</sup> Succinic acid (S) is a co-crystal former that is freely soluble in aqueous medium. It has been reported the co-crystals of S and sildenafil citrate with 1:1 molar ratio increase the solubility of sildenafil citrate by 5-fold.<sup>8,9</sup> It has been demonstrated that the formation of co-crystals can improve several properties of APIs, such as bioavailability, stability, hygroscopic properties, compressibility, and flowability.<sup>5</sup> Smith et al.<sup>10</sup> successfully produced the 1:1 molar ratio co-crystal of Q-caffeine. Moreover, the co-crystals enhanced solubility up to 14-fold and up to 10-fold compared with those of Q and Q dihydrate, respectively.

The two most common methods in the formation of co-crystals are solvent evaporation and grinding.<sup>11</sup> Solvent evaporation, commonly called slow evaporation, involves two or more molecules in certain stoichiometric amounts. The slow evaporation process facilitates the formation of hydrogen bonds between the components. This method produces larger amount of crystals and relatively more homogenous in particle size distribution compared with those produced by other methods. Moreover, slow evaporation can be conducted at room temperature.<sup>12,13</sup> It has been reported that solvent evaporation was successfully conducted to produce Q-isonicotinamide co-crystals and Q-theobromine dehydrate co-crystals.<sup>14</sup>

The present study investigated the physicochemical properties and the dissolution profile of QS co-crystals (Co-QS). Co-QS was prepared in 1:1 molar ratio using solvent evaporation. Powder X-ray diffraction (PXRD), differential thermal analysis (DTA), infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were performed to characterize Co-QS. Dissolution studies were also undertaken to evaluate the dissolution rate of Co-QS.

## MATERIALS AND METHODS

The materials used in this study were Quercetin (Tokyo Chemical Industry Co., Ltd., Japan), sodium lauryl sulphate (E Merck, Germany), citric acid (E Merck, Germany), S (E Merck, Germany), pro-analytical methanol (E Merck, Germany).

### *Preparation of quercetin-succinic acid co-crystal with solvent evaporation method*

Co-QS was prepared using solvent evaporation. For the formation of Co-QS, 365.5 mg (1 mmol) of Q and 134.5 mg (1 mmol) of S were dissolved in methanol. The dissolved Q was then poured into the S solution. The mixture was stirred and evaporated at room temperature. When evaporation was completed, the resulting Co-QS was then dried at 40°C for 12 hours and transferred into a desiccator.

### *Preparation of physical mixture quercetin-succinic acid*

The physical mixture (Pm) of Q and S was prepared by mixing the pure components with the same proportion used in the formation of Co-QS.

### *Thermal analysis*

Thermal analysis of the sample was performed on DTA (Mettler Toledo FP-90, USA). The temperature calibration was conducted using indium prior to the test. Samples (5 to 7 mg) were placed in sealed aluminum pans and scanned at heating rate of 10°C/min in the temperature range of 50-350°C.

### *Powder X-ray diffraction analysis*

Diffraction patterns were collected on PXRD (Philips X'Pert, Holland) with electricity conditions of 40 kV, 40 mA. Samples were packed into a sample holder and pressed on clean glass slides to ensure uniformity of powder thickness. Measurements were performed over 5° to 40° of 2 $\theta$  range. The diffraction patterns of the Pm of Q, S, and Co-QS were compared.

### *Fourier transform infrared spectroscopy analysis*

FTIR spectra were obtained using an FT-IR Jasco 5300 (USA). Ten milligram samples were mixed homogeneously with potassium bromide powder. The mixture was pressed to produce a pellet. Spectra were collected over the range of 4000 to 400 cm<sup>-1</sup>.

### *Scanning electron microscopy analysis*

Approximately 10 mg samples were dispersed on glass slides and coated with gold aluminum (10 nm). Co-crystals were observed under SEM (JEOL, Japan) with electricity conditions of 20 kV and 12 mA. Photomicrographs were taken at magnification of 600x for S, and 2500x for Q and Co-QS.

### *Dissolution profile studies*

The dissolution studies were conducted using USP II (paddle) apparatus containing 900 mL of citrate buffer (pH 5.0 $\pm$ 0.05) with SLS 2% at temperature 37 $\pm$ 0.5°C with paddle speed 100 rpm for 60 min. The samples were withdrawn at 5, 10, 15, 30, 45, and 60 min. The samples were filtered immediately through a 0.45  $\mu$ m membrane filter and the Q concentrations were measured using UV-Vis spectrophotometry at the maximum wavelength of Q of 336.95 nm. The data were calculated as percentage (%) dissolved and dissolution efficiency at 60 min (DE<sub>60</sub>). Statistical analysis was conducted using one-way ANOVA with  $\alpha$ = 0.05 (95% CI).

## RESULT AND DISCUSSION

### Diffractogram analysis

The PXRD pattern of Co-QS was compared with the patterns of pure components, Q and S, and Pm-QS (Figure 1). The results showed that the diffractogram of Q gave strong interference at  $2\theta = 10.78^\circ, 12.43^\circ, 14.15^\circ, 15.84^\circ, 24.43^\circ, 10.78^\circ, 24.43^\circ, 26.44^\circ, 27.41^\circ,$  and  $28.31^\circ$ . The diffractogram result of S showed a specific interference at  $2\theta = 16.02^\circ, 19.99^\circ, 26.10^\circ, 31.41^\circ, 32.40^\circ,$  and  $38.52^\circ$ . These diffractogram results are comparable with those of previous studies.<sup>4,8</sup>

The PXRD patterns of Pm-QS in a 1:1 molar ratio showed all intense unique peaks of the components. The diffractogram of the Pm reportedly appeared as a superposition between peaks of active ingredient and those of the co-crystal former.<sup>4,8</sup> However, the diffractogram of Co-QS showed several unique peaks at  $2\theta = 10.01^\circ, 13.23^\circ, 21.98^\circ,$  and  $44.52^\circ$ . The different PXRD pattern of Co-QS from those of the constituent Q and S suggests the formation of a co-crystal phase.

### DTA thermogram analysis

The thermogram for Q, S, Pm-QS, and Co-QS were determined using DTA. DTA is known as the fastest measurement method to detect co-crystal formation.<sup>8</sup> The result showed that the thermogram of Q had an endothermic peak at  $325.4^\circ\text{C}$  ( $\Delta H = 111 \text{ J/g}$ ), representing the melting point of Q. The thermogram of S showed an endothermic peak at  $189.8^\circ\text{C}$  ( $\Delta H = 22.8 \text{ J/g}$ ), indicating the melting point of S (Figure 2). Previous studies showed that Q demonstrated specific endothermic peaks at  $326^\circ\text{C}$  and  $147^\circ\text{C}$ . The endothermic peak at  $147^\circ\text{C}$  is produced due to the release of entrapped water molecules in the crystal. This may shift the melting temperature of Q to the lower level compared with those of anhydrous Q.<sup>15,16</sup>

Nevertheless, the thermogram of Pm-QS and Co-QS showed a new endothermic peak at  $251.3^\circ\text{C}$  ( $\Delta H = 1.06 \text{ J/g}$ ), which might represent the melting point of Co-QS (Figure 3). The result showed that the melting point of Co-QS lay between the melting points of its components. This result is in agreement with a previous study that showed the melting point of a co-crystal product, which presented between the melting points of its raw materials.<sup>4</sup> Moreover, the melting point of Co-QS shifted to the lower level, suggesting the fusion of S and Q in co-crystal form. Taken together, the shifting of the melting temperature of Co-

QS suggests the possible interaction between Q and S in the formation of a co-crystal.

### FTIR spectrum analysis

An infrared spectrophotometer was used to evaluate the interaction between Q and S molecules in the formation of the co-crystal. The IR spectrum of Q demonstrated specific peaks corresponding to -OH at  $3411 \text{ cm}^{-1}$ , C=O at  $1667 \text{ cm}^{-1}$  and  $1612 \text{ cm}^{-1}$ , and C-O-C at  $1522 \text{ cm}^{-1}$ ,  $1319 \text{ cm}^{-1}$  and  $1168 \text{ cm}^{-1}$ . This result is in agreement with previous studies.<sup>4</sup> The IR spectrum of S showed the specific absorbance for -OH at  $2932 \text{ cm}^{-1}$  and  $2647 \text{ cm}^{-1}$ , and for C=O at  $1202 \text{ cm}^{-1}$ ,  $1693 \text{ cm}^{-1}$ , and  $1309 \text{ cm}^{-1}$ , confirming previous reports.<sup>5,8</sup> Accordingly, FTIR analysis of Pm-QS showed a combination of specific absorbance from Q and S. This result suggests that the Pm did not facilitate any interaction between Q and S molecules in the mixture. This finding is in agreement with previous reports showing that there is no interaction between the active compound with its cofomer in the Pm as demonstrated by the superimposition spectrum in the IR spectrum.<sup>13,17</sup>

The IR spectrum of Co-QS demonstrated several unique new IR peaks at  $3424 \text{ cm}^{-1}$  representing functional group -OH and at  $1830 \text{ cm}^{-1}$ , representing the C=O functional group. The absorbance representing the OH functional group was notably shifted and the peak intensities were markedly decreased compared with those of Q and S. These results suggest that there are a number of functional groups involved in chemical bonding between Q and S in co-crystal form. Furthermore, the absorbance peak representing the -OH functional group of Q and the absorbance peak representing the carboxylic functional

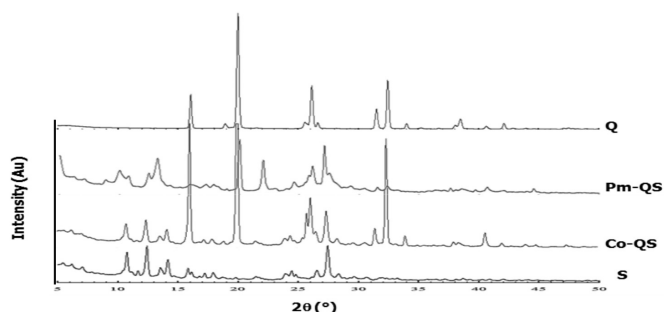


Figure 1. Diffractogram of quercetin, succinic acid, physical mixture, and Co-crystal

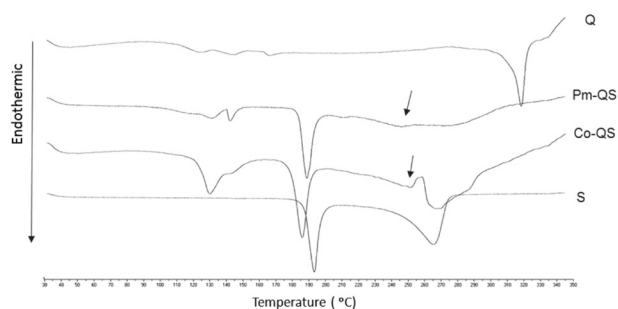


Figure 2. Thermogram of quercetin, succinic acid, physical mixture and Co-crystal QS

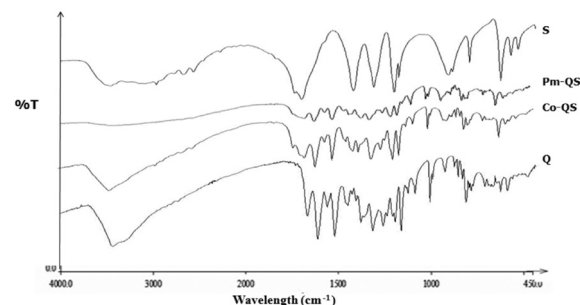


Figure 3. IR Spectrum of quercetin, succinic acid, physical mixture and Co-crystal

group of S were shifted, which suggests possible bonding between these functional groups in co-crystal form.

#### Photomicrograph SEM analysis

Photomicrographs of Q, S, and Co-QS are shown in Figure 4. The photomicrographs were captured at 600 times magnification for AS and 2500 times for Q and Co-QS. The results showed that the crystal size of S was approximately 300  $\mu\text{m}$  diameter. This is in agreement with research that stated that the particle size of S lay between 50 to several hundred  $\mu\text{m}$ .<sup>18</sup> Moreover, the photograph of S showed a different crystal habit compared with those of Q. On the other hand, the photomicrograph of Co-QS demonstrated smaller and more homogeneous particles as compared with those of its raw materials.

#### Dissolution study (In vitro)

Dissolution profiles of Q, Pm-QS, and Co-QS are shown in Figure 5. The results show that Pm-QS increased the solubility of Q. Moreover, Co-QS achieved the highest solubility as compared with those of Pm-QS and Q. The  $DE_{60}$  value of Co-QS, Pm-QS, and Q were  $87.25 \pm 0.07\%$ ,  $73.90 \pm 3.27\%$ , and  $64.43 \pm 0.94\%$ ,

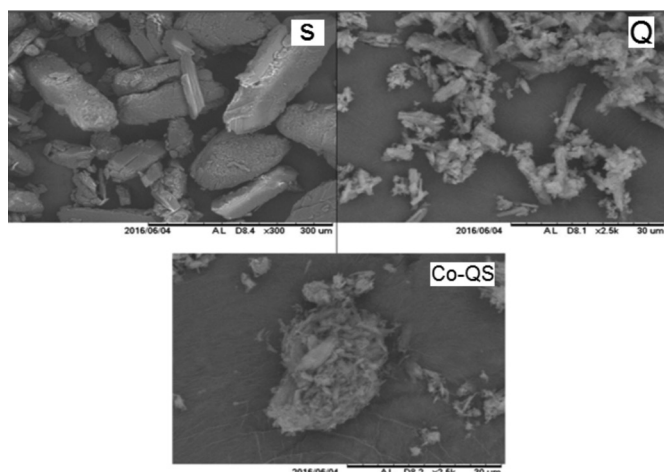


Figure 4. Photomicrograph of quercetin, succinic acid and Co-crystal

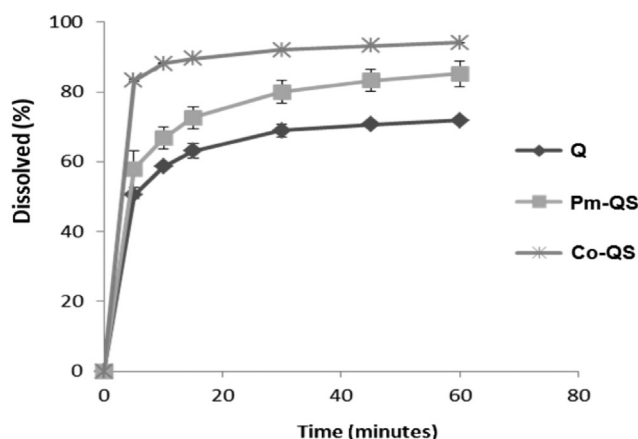


Figure 5. Dissolution rate of quercetin, physical mixture of quercetin-succinic acid, and quercetin-succinic acid co-crystal in buffer citrate medium (pH  $5.0 \pm 0.05$ ) with SLS 2% at temperature  $37 \pm 0.5^\circ\text{C}$

respectively. One-way ANOVA ( $p < 0.05$ ) showed that Co-QS significantly increased the dissolution rate of Q as compared with those of Q and Pm-QS. The enhancement of Q solubility by the Pm was likely due to the wetting effect of coformer S.<sup>4</sup> The higher dissolution rate of Co-QS compared with those of pure Q might be due to the formation of a new crystal lattice and the decrease in enthalpy energy as indicated previously by PXRD and DTA studies. In addition, it is suggested that the interaction between hydroxyl functional group from Q and the carboxylic functional group from S, conformer, facilitates the improvement of solubility profile of Q in aqueous medium.

## CONCLUSION

The formation of Co-QS was well-characterized by the shifting in melting temperature, the formation of a new crystal lattice as shown by the PXRD pattern, and the shifting in absorbance peaks, which represented the functional groups of Q and S. The dissolution study confirmed that the formation of Co-QS using solvent evaporation significantly improved the solubility profile of the compound. The possible potential of this research includes the development of pharmaceutical dose forms, especially solid dose forms.

## ACKNOWLEDGEMENT

The authors thank the Higher Degree Education Directorate for Funding the Research Grant, Faculty of Pharmacy Airlangga University for facilities support and our students Indah P, Rizka F for their support.

*Conflict of Interest:* No conflict of interest was declared by the authors.

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