The effect of polysorbate 20 and polysorbate 80 on the solubility of quercetin

Tristiana Erawati, Dewi Isadiartuti, Bintari Damartha Anggalih

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: Quercetin acts as an antioxidant, anti-inflammatory, wound healing, and anti-aging so quercetin can be used as a topical preparation. However, it has low solubility in water at 0.01 mg/ml at 25°C. Increasing the solubility of quercetin in water was done by the addition of surfactants.

Objective: This study compared the solubility of quercetin in Polysorbate 20 (P20) and Polysorbate 80 (P80) in a citrate buffer medium pH 4.5±0.2.

Methods: The surfactants Polysorbate 80 and Polysorbate 20 differ in their alkyl chain length. Polysorbate 80 has an alkyl chain length of 18, while Polysorbate 20 has an alkyl chain length of 12. The concentrations of surfactant are above, below, and at the critical micelle concentration (CMC) values. The concentrations of quercetin were determined at the maximum wavelength by spectrophotometric method.

Results: The results of the quercetin solubility test without surfactant were 3.89±0.59 mg/L. The results of the quercetin solubility test by adding Polysorbate 20 at a concentration of 42.0 ppm; 57.5 ppm; and 73.0 ppm were 3.62±0.72, 4.04±0.23 and 8.35±1.97 mg/L, respectively. While the solubility of quercetin by adding Polysorbate 80 at a concentration of 4.0 ppm, 11.5 ppm, and 19.0 ppm was 11.15±0.72, 11.37±1.23 and 14.17±1.96 mg/L, respectively. The solubility of quercetin is greater after the addition of surfactant Polysorbate 20 only at the concentration above the CMC value and the solubility of quercetin is greater with the addition of surfactant Polysorbate 80 at all concentrations. Surfactant Polysorbate 20 increases the solubility of quercetin in citrate buffer pH 4.5±0.2 only at concentrations above the CMC value of 2.14 times. Polysorbate 80 can increase the solubility of quercetin in citrate buffer pH 4.5±0.2 at concentrations below, at, and above CMC by 2.87, 2.92, and 3.63 times, respectively.

Conclusion: Polysorbate 80 can increase the solubility of quercetin in citrate buffer pH 4.5±0.2 higher than Polysorbate 20.

Correspondence: Dewi Isadiartuti, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115, Surabaya, Indonesia.
Tel.: +62.85100684948 - Fax: +62.0315935249.
E-mail: dewi-i@ff.unair.ac.id

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Introduction

Quercetin, which has the chemical name 2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxychromen-4-one, is a compound belonging to the flavonoid group that is widely found in onions, apples, and tea leaves.[1] According to the Biopharmaceutical Classification System (BCS) classification system, quercetin is included in the BCS II group, which means it has low solubility and high permeability.[2] The solubility of a drug in water affects the concentration of the drug that penetrates. The more drug in the dissolved state, the greater the availability of the drug to penetrate,[3] so a method is needed to increase the solubility of quercetin. An effort to increase the solubility of a drug that has a small solubility or is practically insoluble in water is the addition of surfactants.[4] The addition of surfactants can increase the solubility of drugs that have low solubility with the formation of micelles. Micelles have several advantages including having amphiphilic properties, self-assembly ability, namely the process of spontaneous self-assembly and can maintain stability in solution, and being easy to manufacture.[5-7] Surfactant comes from the word surface active agent. Surfactants are substances that adsorb on surfaces or interfaces to reduce surface tension or reduce the interfacial tension of a liquid.[4]

Surfactants can form micelles when a critical concentration is reached. The critical concentration, when these micelles are formed, is called critical micelle concentration (CMC).[8] When the surfactant has reached the CMC value, the water-insoluble drug is trapped in the micellar core so that the interaction between hydrophobic drugs and water decreases and solubility increases.[9,10] Non-ionic surfactants such as Polysorbate 80 and

Polysorbate 20 are non-toxic and non-irritating, widely used in cosmetics, food products, and oral and topical drug formulations.[11] Polysorbate 80 and Polysorbate 20 have benefits as solubilizers because they have HLB ≥ 15.[4] Non-ionic surfactants can also be a better solvent than ionic surfactants.[5] Non-ionic surfactants have lower CMC values than ionic surfactants and have more micellar-forming molecules than ionic surfactants.[12]

The length of the hydrophobic alkyl chain can affect the surfactant's ability to increase solubility. The longer the alkyl chain of hydrophobic groups, the greater the volume of the inner micelle so that more drug compounds are accommodated in the micelles, resulting in increased drug solubility.[12] Based on research conducted by Sheeder and Kanojia (2008) gliclazide which has a water solubility of 37.32 g/ml at 25^{o}C by the addition of surfactant Polysorbate 80 can increase to 2,465.5 g/ml at the same temperature. Another study of candesartan cilexetil shows that it has a water solubility of 1.4 mg/ml at 36^{o}C and can increase to 10.3 mg/ml with the addition of the surfactant Polysorbate 20 at the same temperature.[13] Polysorbate 80 has a hydrophobic alkyl chain of 18, while Polysorbate 20 has a hydrophobic alkyl chain length of 12.[12] Based on this background, a study was conducted on the effect of Polysorbate 80 and Polysorbate 20 on the solubility of quercetin in a citrate buffer medium pH 4.5±0.2. The use of citrate buffer at pH 4.5±0.2 as a medium was used to maintain the stability of quercetin. Quercetin is stable at acidic pH.[14] Quercetin will decompose more quickly under alkaline conditions, at pH >5 the quercetin solution in water is degraded.[15]

This study compared the solubility of quercetin in Polysorbate 20 and Polysorbate 80 in citrate buffer medium pH 4.5±0.2 with concentrations above, below, and at the surfactant CMC values. The addition of Polysorbate 20 and Polysorbate 80 with a concentration above the CMC value is expected to increase the solubility of quercetin because micelles have been formed. To prove that micelles have been formed, the value of CMC was determined by a spectrophotometric method using iodine as a hydrophobic probe.

Materials and Methods

The material used in this study, the quercetin hydrate was brought from Sigma Aldrich Co.Ltd. (China), Polysorbate 20 Cosmetic Grade brought from Chemical Indonesia Multi Sentosa (Surabaya, Indonesia), Polysorbate 80 Cosmetic Grade brought from PT Brataco (Bekasi, Indonesia), Ethanol p.a. brought from Sigma Aldrich Co.Ltd. (Germany), Aqua Demineralization brought from PT Brataco (Surabaya, Indonesia), Citric Acid brought from Sigma Aldrich Co.Ltd. (Austria) and Sodium Citrate brought from Sigma Aldrich Co. Ltd. (Germany).

Determination of cmc value of polysorbate 20 and polysorbate 80 in citrate buffer ph 4.5±0.2

Values of CMC were observed using spectrophotometry and the addition of iodine as a hydrophobic probe. An amount of 1 gram of iodine is dissolved in 2 g of potassium iodide (KI). Furthermore, the potassium iodide/iodine (KI/I_2) solution was diluted in 100 mL of citrate buffer pH 4.5±0.2 as a standard solution of KI/I_2. Polysorbate 20 solutions are prepared at concentrations of 30 to 75 ppm in citrate buffer pH 4.5±0.2 as well as Polysorbate 80 solutions are prepared at concentrations from 1 to 32 ppm in citrate buffer pH 4.5±0.2. The standard solution of KI/I_2 was added as much as 25 μL into each solution of Polysorbate 20 and Polysorbate 80, then shaken until homogeneous. The mixture formed was stored at room temperature and dark place for 12 hours before the measurements. Then the absorbance was measured using a UV-Vis spectrophotometer with a maximum wavelength of 353.5 nm.

Determination of quercetin maximum wavelength without surfactant

The observation of the maximum wavelength using the Hitachi UH 5300 UV-Vis Spectrophotometry was determined by observing the absorbance of the working standard solution of quercetin with concentrations of 8.0 and 16.0 ppm in citrate buffer pH 4.5±0.2, between wavelengths of 240-400 nm. The maximum wavelength of quercetin determined is the wavelength that provides the greatest absorbance.

Determination of quercetin maximum wavelength with surfactants

The solution of Polysorbate 20 and Polysorbate 80 was made in citrate buffer pH 4.5±0.2 with each concentration of 1000 ppm. Each solution of Polysorbate 20 and Polysorbate 80 at 1000 ppm concentration was pipetted 80 μL and then put into a 10.0 mL volumetric flask. Then 80 μL of 1000 ppm quercetin was added to each volumetric flask, then mixed with the solution and diluted with citrate buffer pH 4.5±0.2 to 10.0 mL. Then the absorbance of the solution was observed using a UV-Vis spectrophotometer Hitachi UH 5300 at a wavelength of 240-400 nm. The resulting spectrum was compared with the spectrum of the standard working solution of 8.0 ppm quercetin.

Standard curve determination of quercetin without and with surfactants

Preparation of the standard curve of quercetin without surfactant was made with a concentration of 2.0; 4.0; 8.0; 12.0; 16.0; 20.0 ppm and the standard curve of quercetin in the presence of surfactant made with a concentration of 4.0; 8.0; 12.0; 16.0; 20.0 ppm. The working standard solution that had been prepared was observed for absorbance at the maximum wavelength of quercetin using a Hitachi UH 5300 UV-Vis spectrophotometer. Then an absorbance curve for the concentration of the working standard solution was made. From these data, the standard curve equation and linear regression of the curve can be obtained.

Quercetin solubility test

The solubility test of quercetin was carried out in citrate buffer and citrate buffer solvent with the addition of a surfactant. This solubility test uses surfactant concentrations below, right, and above the CMC value of each surfactant. The solubility test was carried out at a temperature of 30±0.5^{o}C by dissolving 50 mg of quercetin in 25 ml of citrate buffer and stirring using a Memmert shaking water bath at a speed of 140 rpm until a saturated solubility was formed. A 5.0 ml sample of quercetin was taken at 240 minutes and then filtered through 0.45 μm Millipore filter paper. The filtered sample was taken with a pipette of 4.0 ml volume, then 1.0 ml was added and the absorption was observed with Hitachi UH 5300 UV-Vis Spectrophotometer at the maximum wavelength of quercetin. The quercetin solubility test was replicated three times.

Results

CMC value of polysorbate 20 and polysorbate 80 in citrate buffer ph 4.5±0.2

The CMC values of Polysorbate 20 and Polysorbate 80 were 57.5 ppm and 11.5 ppm, respectively. It can be seen in Figure 1.

Maximum wavelength of quercetin without and with surfactants

Observation of absorbance of a standard solution of quercetin 8.0 ppm and 16 ppm showed the maximum wavelength of quercetin without additional surfactant in citrate buffer pH 4.5±0.2 was at 368 nm. The results of determining the maximum wavelength of quercetin can be seen in Figure 2a. Observations of the maximum wavelength of quercetin without the addition of Polysorbate 20 and Polysorbate 80 and the addition of surfactants Polysorbate 20 and Polysorbate 80 can be seen in Figure 2b and 2c, respectively.

Quercetin standard curve without and with surfactant

The results of observations of quercetin absorption without and with surfactants can be seen in Table 1. In the table, there is an equation of a linear regression line with absorbance as the y-axis and the concentration of the standard solution of quercetin as the x-axis. On each standard curve of quercetin, the coefficient of the calculated relation is greater than the coefficient of the relation of the table so that the equation of the line obtained shows a linear relationship between the increase in the concentration of quercetin and the absorbance obtained so that it can be used to determine the concentration of quercetin.

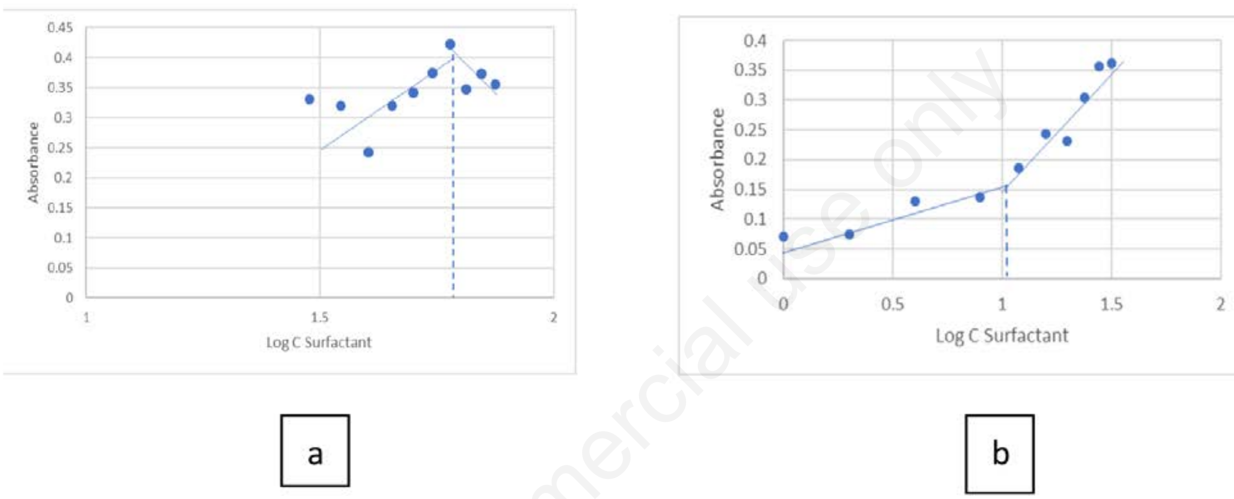

Figure 1. The Curve of log concentration of Polysorbate 20 *versus* absorbance of iodine (a); the curve of log concentration of Polysorbate 80 versus the absorbance of iodine (b).

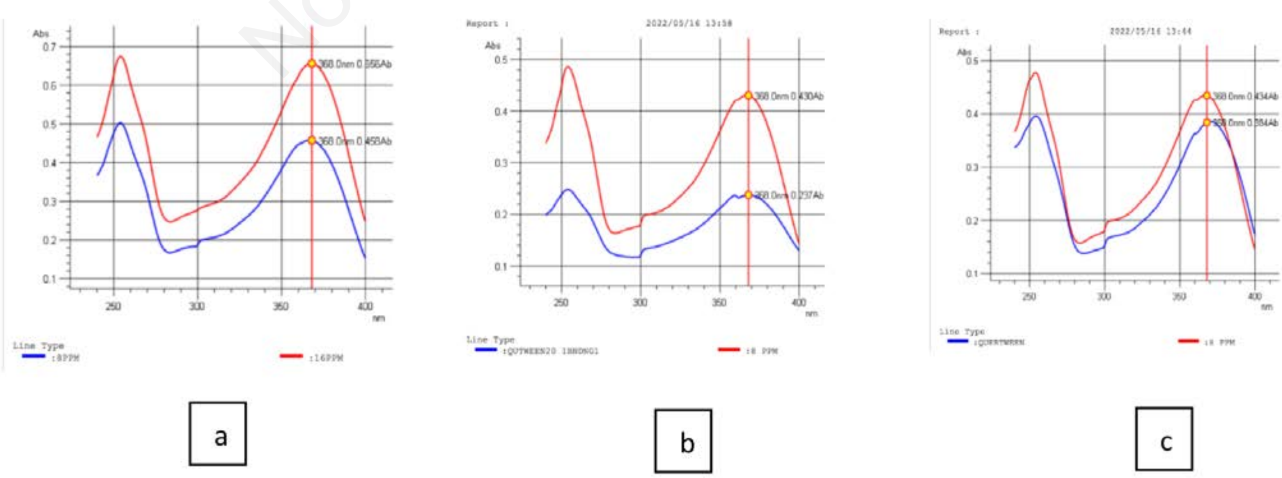

Figure 2. UV-Vis spectra of 8.0 ppm and 16 ppm quercetin in citrate buffer solution pH 4.5±0.2 (a); UV-Vis spectra of the effect of Polysorbate 20 (P20) on the absorbance of quercetin at a wavelength of 240-400 nm (b); UV-Vis spectra of the effect of Polysorbate 80 (P80) on the absorbance of quercetin at a wavelength of 240-400 nm (c).

Solubility test

The solubility test of quercetin in citrate buffer pH 4.5±0.2 was carried out with the addition of Polysorbate 20 and Polysorbate 80. Polysorbate 20 was added with a concentration of 42 ppm; 57.5 ppm; and 73 ppm, while Polysorbate 80 was added with a concentration of 4 ppm; 11.5 ppm; and 19 ppm. This solubility test was conducted to determine the effect of adding Polysorbate 20 and Polysorbate 80 in increasing the solubility of quercetin in citrate buffer pH 4.5±0.2. The results of the quercetin solubility test can be seen in Figure 3.

Based on the results of the HSD statistical test, there was no significant difference between the solubility of quercetin in the citrate buffer pH 4.5±0.2 and the solubility of quercetin in the citrate buffer with the addition of Polysorbate 20 at concentrations below and exactly the CMC value. Polysorbate 20 is able to increase the solubility of quercetin if added above the CMC value. Meanwhile, the solubility of quercetin in citrate buffer pH 4.5±0.2 with the addition of Polysorbate 80 at concentrations above, below, and just the CMC values showed a significant increase in solubility.

The addition of Polysorbate 80 below the CMC value increased the solubility 2.87 times and the addition of Polysorbate 80 at the CMC value increased solubility 2.92 times.

Discussion

The determination of the CMC value in this research using the UV-Vis spectrophotometry method, when the surfactant concentration is still below the CMC value, only a small amount of hydrophobic probe (iodine) is dissolved so that the resulting absorbance is still low, but when the surfactant concentration has reached the CMC value then a micellar system will be formed and the hydrophobic core of the micelles will dissolve the iodine as a hydrophobic probe so that there will be an increase in absorbance.[10] Iodine is used as a probe to determine the minimum concentration of micellar formation. The hydrophobic moiety of micelles is favored by iodine compared to interacting with KI in solution, so the increase in the absorbance of iodine in micelles can be an indicator of micellar formation.[16]

In Figure 2b and 2c it was showed the effect of adding Polysorbate 20 and Polysorbate 80 on the absorbance of quercetin, so to determine the level of quercetin with the addition of Polysorbate 20 and Polysorbate 80 in citrate buffer pH 4.5±0.2. It is necessary to make a standard curve according to the concentration of surfactant used. The shift in quercetin absorbance after the addition of surfactants can be caused by the polarity of the solvent.

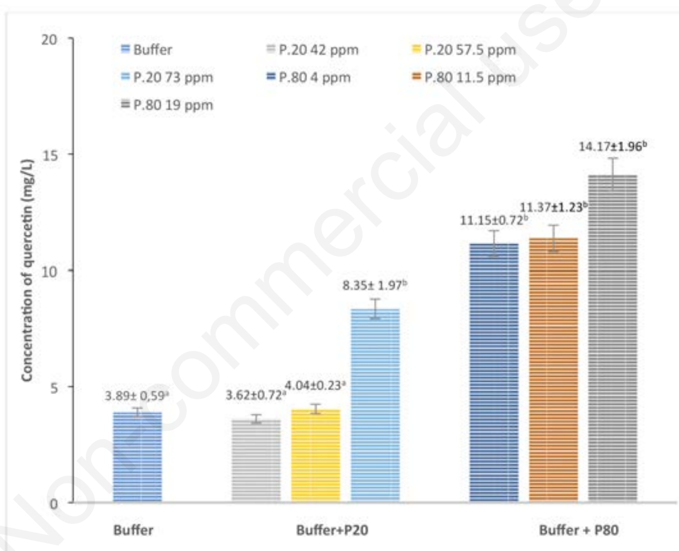

Figure 3. Solubility profile polysorbate 20 (P20) and polysorbate 80 (P80) in citrate buffer medium pH 4.5±0.2 (temperature 30±0.5°C) at 240 Minutes. The letters a, b indicate no significant difference between groups α 0.05 after the one-way ANOVA statistical test.

Table 1. Results of the Quercetin Standard Curve Without and With Surfactants.

Medium	λ max(nm)	Regression Equation	r_{count}	r_{table}
Citrate Buffer	368	0.0515x + 0.0275	0.9995	0.8114
P20 42.0 ppm + Citrate Buffer	368	0.0274x + 0.1167	0.9997	0.8783
P20 57.5 ppm + Citrate Buffer	368	0.0243x + 0.1430	0.9995	0.8783
P20 73.0 ppm + Citrate Buffer	368	0.0225x + 0.1200	0.9999	0.8783
P80 4.0 ppm + Citrate Buffer	368	0.0250x + 0.1134	0.9996	0.8783
P80 11.5 ppm + Citrate Buffer	368	0.0248x + 0.1153	0.9997	0.8783
P80 19.0 ppm + Citrate Buffer	368	0.0256x + 0.1055	0.9999	0.8783

P20: polysorbate 20 and P80: polysorbate 80.

The higher the HLB value, the more hydrophilicity will increase. The more surfactant is used, the hydrophilicity will increase so that the polarity increases.

The results showed that the solubility of quercetin by the addition of Polysorbate 80 greater than by the addition of Polysorbate 20. This can occur because the addition of surfactants can reduce the aggregation of drug particles so that it expands the drug surface and causes a wetting effect. This wetting effect appears when surfactants are added at low concentrations.[17] The statistical test of quercetin in citrate buffer pH 4.5±0.2 with the addition of Polysorbate 20 below and at the CMC value showed a non-significant increase. This can happen because Polysorbate 20 has a shorter hydrophobic chain length than Polysorbate 80. Nonionic surfactants with longer hydrophobic chain lengths show higher wetting abilities than surfactants with shorter hydrophobic chain lengths when used at very low concentrations.[18] The solubility of quercetin in citrate buffer pH 4.5±0.2 with the addition of Polysorbate 20 and Polysorbate 80 above CMC value increased significantly, increasing the solubility 2.14 and 3.63 times, respectively, compared to the solubility of quercetin in citrate buffer pH 4.5±0.2. The addition of Polysorbate 80 showed an increase in the solubility of quercetin in citrate buffer pH 4.5±0.2 greater than that of Polysorbate 20. This was because Polysorbate 80 had a longer hydrophobic carbon chain than Polysorbate 20. The longer the hydrophobic alkyl chain, the larger the internal volume of micelles, so that more drug compounds are accommodated in micelles resulting in increased drug solubility.[4]

Conclusions

Based on the results obtained, it can be concluded that surfactant Polysorbate 20 increases the solubility of quercetin in citrate buffer pH 4.5±0.2 only at concentrations above the CMC value of 2.14 times. Polysorbate 80 can increase the solubility of quercetin in citrate buffer pH 4.5±0.2 at concentrations below, right, and above CMC value by 2.87 times, respectively; 2.92 times; 3.63 times. Polysorbate 80 can increase the solubility of quercetin in citrate buffer pH 4.5±0.2 higher than Polysorbate 20.

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