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Combination Quercetin and Poloxamer in a Solid Dispersion Binary System and the Antioxidant Activity Using DPPH and ABTS

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Abstract

Free radicals are unstable and highly reactive chemicals. Free radicals are extremely harmful since they may change the DNA sequence and promote the production of cancer cells, heart disease, Alzheimer's, and inflammatory disorders. Substances such as antioxidants are required to neutralize unstable free radicals such as vitamin C, vitamin E, Q10, and flavonoids like quercetin. The characteristic of quercetin is that it is less soluble in water, which limits the effect of antioxidants. This research aims to investigate the incorporation of poloxamer 188 as a catalyst in solid dispersion and to assess its antioxidant properties through the application of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methodologies. There were 3 types of solid dispersion formulas (F1, F2, and F3) in this study that were observed in their organoleptic and characterized using particle size analyzers (PSA), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR). The results showed that F1 had the highest antioxidant content due to its high presence of poloxamer 188. The IC_{50} of F1 using DPPH and ABTS was 15.92 ppm and 48.57 ppm, respectively.

Keywords : ABTS; Antioxidants; DPPH; Solid Dispersions; Quercetin

1. Introduction

Reactive oxygen species (ROS) are free radicals, and excess ROS can disrupt the homeostasis in the human body, resulting in many kinds of disease [1,2]. Unbalanced ROS and antioxidant levels in the human body might trigger oxidative stress and damage to DNA cells. Oxidative stress also causes cancer, heart disease, Alzheimer's, diabetes, and kidney failure [3]. Supplementing the body with external antioxidants like quercetin, abundant in fruits and vegetables, can inhibit oxidative stress.

Studies have shown that flavonoid compounds like quercetin can reduce hypertension, heart disease, cancer, and inflammation [4]. Quercetin helps diabetics lower their blood glucose levels by inhibiting the aldose reductase enzyme, which is responsible for diabetes problems [5]. Ajami et al. (2024) reported that quercetin also strengthens the immune system of COVID-19 patients by decreasing blood levels of ferritin and C-reactive protein (CRP). Regrettably, the biopharmaceutical classification system (BCS) classifies quercetin as a hydrophobic chemical with high permeability and low solubility, placing it in class 2. This categorization will affect the dissolving rate of the formulation thus some methods are still being pursued to increase the solubility of quercetin [7].

Lucida and Zaini (2020) reported that adding PVP K-30 to quercetin can increase the solubility up to 436 times and as a result, the antioxidant effect increases with IC_{50} value (0.61 ppm) [8]. In 2022, Elisabeth and Mohammed found that solid dispersion using PEG 1000, 4000, and 6000 made the antioxidant quercetin work better than pure quercetin [9]. This study aims to develop a solid dispersion dosage of quercetin, utilizing the

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bottom-up method with freeze drying, to overcome its low bioavailability. Solid dispersion is a technique that involves dispersing one or more active ingredients with a carrier to increase their solubility and bioavailability. This study used poloxamer 188 as a carrier to entrap the quercetin. In this study, freeze-drying is one of the drying methods because it reduces thermal stress on the sample during the drying process, allowing for direct solid powder formation [10]. The antioxidant activity of a solid dispersion of quercetin-poloxamer 188 is then evaluated using DPPH and ABTS.

2. Experimental

2.1 Materials

Quercetin (Merck®), propylene glycol (Echemi®), Poloxamer 188 (Merck®), Ethanol 96% (Brataco®), Aquadest, ABTS (Sigma®), DPPH (Sigma®).

2.2 Instruments

Microplate reader UVM 340, particle size analyzer (Microtrac Nanotrac Wave II), Freeze dryer (Lyovapor L-200), Fourier transform Infrared Spectroscopy (Agilent Cary 630), Differential Scanning Calorimetry (Mettler Teledo STAR System).

2.3 Preparation of Quercetin Solid Dispersion

Table 1 shows the three formulas used in this study. One gram of quercetin was weighed for each of the formula while the poloxamer 188 was weighed in 56.5, 42.3, and 28.2 (%w/v) of the total components for F1, F2, and F3, respectively. The formulation process started with dissolving the powder of poloxamer 188 in ethanol, followed by quercetin. Next, the combination of quercetin and poloxamer 188 was homogeneously stirred using a magnetic stirrer for 1 hour at 250 rpm. The mixture was then stored at -80^{o}C to form a solid ice state and freeze-dried for 24 hours to get a solid dispersion powder.

Table 1: Quercetin Solid Dispersion Formulation

Formula	Components				
	Quercetin	Poloxamer 188 (%)	Polypropylene glycol (PPG)	Ethanol (ml)	Aquadest ad 150
Formula 1 (F1)	1 g	56.5	10%	50	Aquadest ad 150
Formula 2 (F2)	1 g	42.3	10%	50	Aquadest ad 150
Formula 3 (F3)	1 g	28.2	10%	50	Aquadest ad 150

2.4 Antioxidant Activity Assay

a. Preparation of ABTS Reagent

This study followed Setiawan et al. (2018) to prepare ABTS. Seven mg of ABTS powder was weighed and mixed with 4.0 mg potassium persulfate. Each powder was dissolved in 5 ml of ethanol and incubated for 24 hours in a dark room. Next, each solution was mixed into a 25 ml measuring flask and added with aquadest. Scanning wavelength was performed from 600-800 nm to determine the maximum wavelength [11].

b. Antioxidant Test Using the ABTS Method

Ten milligrams of vitamin C as a positive control were weighed and dissolved in 10.0 ml of ethanol. The 100 ppm stock solution was then diluted to 25 and 50 ppm using three replications. Each well contains 90 µl of samples and 30 µl of ABTS reagent. After a 10-minute incubation, the absorbance at ʎ was measured.

c. Preparation of DPPH Reagent

Martysiak-Zurowska and Wenta (2012) provided the DPPH preparation method for this study. DPPH powder was weighed at 2.0 mg and dissolved in 10.0 ml of ethanol, followed by incubation for 15 minutes in the room. Twenty milligrams of samples were weighed and dissolved in 10.0 ml of ethanol. The wavelengths were scanned from 400 to 700 nm [12].

d. Antioxidant Test with the DPPH Method

Each well contained 30 µl of DPPH solution and a 90 µl positive control sample. We read the mixture at 517 nm after 15 minutes of incubation. We used the following equation to determine inhibitory activity:

$$\% \text{ inhibition} = \frac{(\text{Negative control absorbance} - \text{sample absorbance})}{\text{Negative absorbance}} \times 100\%$$

2.5 Solid Dispersion Result Evaluation

a. Organoleptic Evaluation

The organoleptic observation encompassed the visual appearance, shape, color, and odor of the solid dispersion of quercetin-poloxamer 188.

b. PSA Evaluation

Particle size analyzer was used to observe the particle size distribution from each formula. Fifty milligrams of each sample were weighed and dissolved in 5,0 ml of distilled water. Next, the particle size and polydispersity index (PDI) were observed with Nanotrac Microtrac II software.

c. DSC Evaluation

The study used differential scanning calorimetry to assess the temperature characteristics and melting point of each sample. Four milligram samples of F1, F2, and F3, pure quercetin, pure poloxamer 188, and PM (physical mixture) of quercetin-poloxamer in a crucible were weighed. The temperature of DSC was set between 20-350°C and increased by 10°C every minute.

d. FTIR Evaluation

Fourier transform infrared spectroscopy analysis was performed on samples of F1, F2, and F3, pure quercetin, pure poloxamer 188, and PM. Spectrum wavenumber was carried out in the range 650 – 4000 cm^{-1}.

3. Results and Discussion

a. Organoleptic Evaluation Results

The results of the organoleptic examination of three formulas displayed different colors and textures but retained the same shape and odor. Figure 1 illustrates the tendency for the color to shift towards brilliant yellow as the amount of poloxamer 188 used as a carrier decreases. Variations in the amount of poloxamer 188 added to each formula contribute to the changes in color by affecting the resulting physicochemical features, including color.

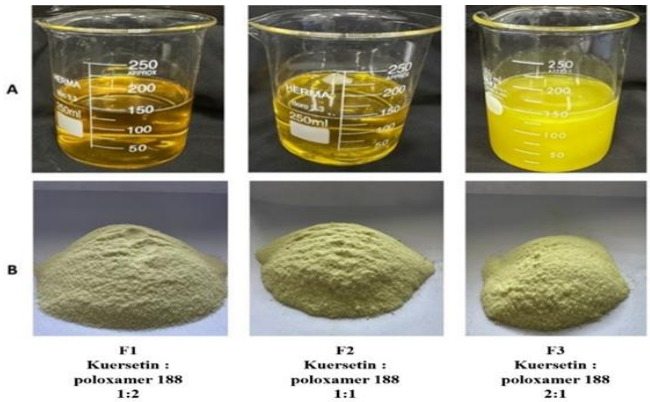

Figure 1. Color of formula solutions F1, F2, and F3 before freeze-drying (A); color of powder after freeze-drying (B).

b. PSA Evaluation Result

The PSA observation findings revealed that formula 1 generated the highest particle size, 2862 nm, followed by formulas 2 and 3 in 2018 and 786 nm, respectively (Table 2). It showed that the larger the particle size, the greater the poloxamer 188 in the formula. Therefore, study assumes that poloxamer 188 promotes agglomeration during the freeze-drying or lyophilization process.

Table 2. Particle size observation results

	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Particle size (nm)	2862 nm PDI : 0,674	2018 nm PDI : 0,128	786 nm PDI : 0,017

Based on the PDI value, all formulas were less than 0.7, suggesting that the particle size distribution is within acceptable ranges and does not lead to sedimentation. Large particle size often leads to agglomeration, and factors such as the amount of polymer supplied, the use of electrolytes, and the physical properties of the particle structure can contribute to sedimentation [13]. For example, in this study, the addition of poloxamer 188 can induce the solubility of quercetin. In addition, Szafraniec et al. (2019) found that a solid dispersion formulation of a bicalutamide drug that contained poloxamer 188 made it easier for the drug to dissolve and spread [14].

b. DSC Evaluation Results

Table 3 displays the results of observations made using DSC. The melting point of pure quercetin peaks at 316.05°C, while that of poloxamer 188 is 53.33°C. This is consistent with the theoretical melting point of quercetin, which is 315°C, and the melting point of poloxamer 188, which is 52–57°C [15, 16]. The melting point of the three formulas was in the range of 51.44°C to 53.26°C, indicating the melting point of poloxamer.

Table 3. Melting point results

Sample	Melting Point (^{O}C)
Pure Quercetin	316,05^{o}C
Poloxamer 188	53,33^{o}C
PM Quercetin Poloxamer 188	50,77^{o}C
F1 Quercetin Poloxamer 188 (1:2)	51,44^{o}C
F2 Quercetin Poloxamer 188 (1:1)	52,55^{o}C
F3 Quercetin Poloxamer 188 (2:1)	53,26^{o}C

Three formulations, as well as the physical mixture (PM), have a melting point close to 53.33 °C, which is that of poloxamer. Figure 2 illustrates that the melting point of quercetin in three formulas and PM was not visible. The undetectable melting point of quercetin in the PM is not due to crystallinity. Instead, the decomposition peak of poloxamer 188, with its higher intensity around 260°C, obscures it. This pattern is also observed in the three formulas, where the melting point of quercetin is similarly covered by the decomposition peak of poloxamer 188.

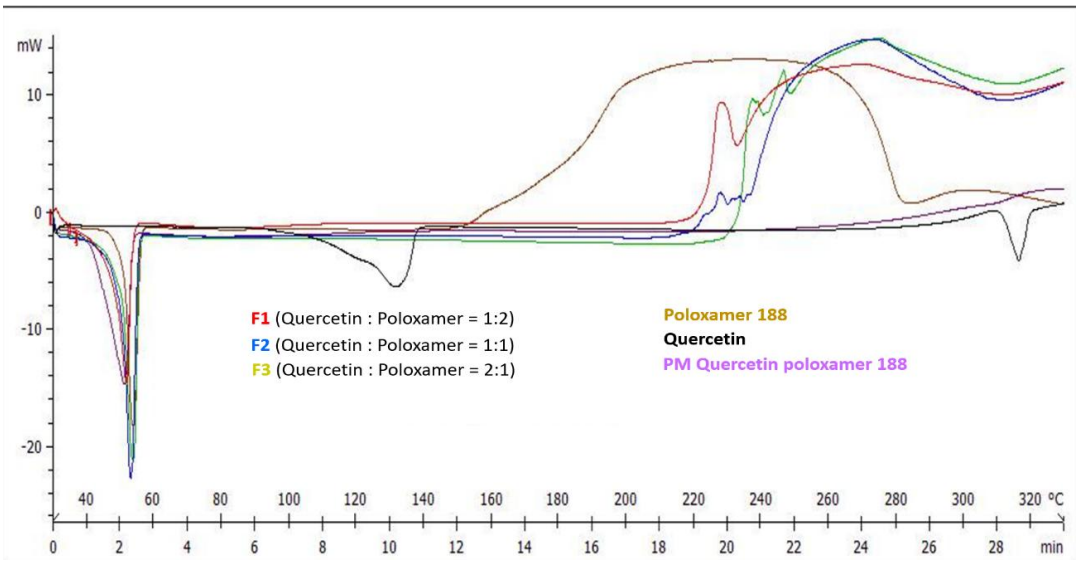

Figure 2. DSC overlay of dense disperses

C- Evaluation FTIR Results

The infrared spectrum of pure quercetin (the dark blue line) exhibits an absorption at 3272 cm^{1}, indicating the presence of OH functional groups. The C=O stretching peak appears at 1604 cm^{1}, the C-H stretching peak at 1239 cm^{1}, and the C-O-C stretching peak at 1161 cm^{1}. The green line represents the FTIR peaks for pure poloxamer 188. It showed an aliphatic C-H stretching peak at 2879 cm^{1}, an OH group at 1341 cm^{1}, and a C-O stretching peak at 1097 cm^{1}. This in line with reports from Wang et al. (2020) and Sharma et al. (2013).

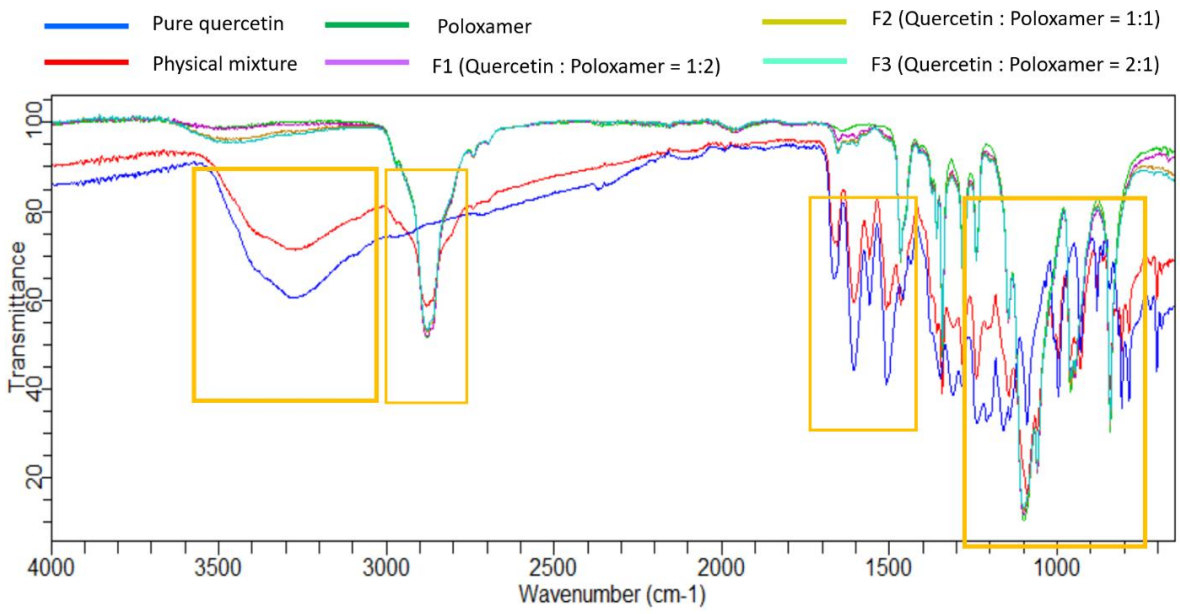

Figure 3. Observation results of quercetin formulae F1, F2, and F3 using FTIR

Figure 3 presents the FTIR spectra of various quercetin powder solid dispersions. It appears that the FTIR spectra of various formulas, F1, F2, and F3 of quercetin have been encapsulated into poloxamer 188, characterized by a decrease in the intensity of OH absorption at wave number 3513 cm^{-1}, C=O stretching at wavenumbers 1507 to 1644 cm^{-1}, and an increase in the intensity of aliphatic C-H stretching at peak 2877 cm^{-1}, OH group at 1341 cm^{-1}, and C-O at fingerprint region 840 to 1099 cm^{-1}. The similarity of the peak profiles of the three formulas with poloxamer 188 indicates the success of their encapsulation, including when compared to the physical mixture profile. The absence of a new peak in the encapsulation suggests that quercetin and poloxamer 188 did not form any new chemical bonds [19].

d. Antioxidant Activity Test Results

This study tested antioxidant activity using two methods, DPPH and ABTS (Table 4). The results revealed that formula 1 has the lowest IC_{50} value and the strongest antioxidant activity compared to the other formulations. Formula 1 has a higher antioxidant capacity, perhaps due to its higher poloxamer 188 than other formulas. Moreover, in this study, the antioxidant activity of formula 1 is also superior to that of pure quercetin and vitamin C.

There are some antioxidant methods to measure antioxidant capacity. It is not enough to use only one method to determine antioxidant due to each method has its own limitations (Salama et al., 2024). Therefore, in this study two antioxidant methods were used, DPPH and ABTS. According to Rumpf et al. (2023) and Wołosiak et al. (2021), antioxidant testing using the ABTS method is preferable to DPPH since it can be utilized in a wider pH range of 3–9. The ABTS approach offers the advantage of applying to both hydrophilic and lipophilic samples, while the DPPH method is exclusive to hydrophobic materials. The ABTS method is also good because it does not depend on the type of solvent. The ferric ion-reducing antioxidant potential (FRAP) method, on the other hand, can only be used with water-loving samples that have a pH of about 10 [21, 22].

Table 4. Presents the observation of IC_{50} values for quercetin solid dispersion using DPPH and ABTS

Sample	Score of IC_{50} (ppm)	
	DPPH	ABTS
F1 Quercetin : Poloxamer 188 (1:2)	15.92	48,57
F2 Quercetin : Poloxamer 188 (1:1)	24.50	54,76
F3 Quercetin : Poloxamer 188 (2:1)	45.21	59,52
Vitamin C	82.35	335.71
Quercetin	108.07	422.38

The antioxidant results between DPPH and ABTS were similar. The antioxidant activity tested with DPPH and ABTS produced comparable data, with higher concentrations of poloxamer showing greater antioxidant power. According to the classification of antioxidant activity, all the formulas tested using DPPH fell into the "very strong" category, while the ABTS methods indicated "strong" (Table 5).

Table 5. Classification of antioxidant activity based on IC_{50} value [23]

Score of IC_{50} (ppm)	Category
< 50	Very strong
50 – 100	Strong
101 – 150	Medium
151 – 200	Weak
> 200	Very Weak

Different antioxidant methods yield varying results due to their distinct mechanisms. In this study, antioxidant testing with ABTS generally showed higher results compared to the DPPH method. This pattern perhaps due to the DPPH reagent solely detects antioxidants in the hydrophobic region, whereas ABTS assesses both the hydrophobic and hydrophilic regions [24]. Therefore, ABTS method induced higher result of IC_{50} in this study. Elnaggar et al. (2024) reported similar findings, testing antioxidant activity from *Millingtonia hortensis* leaves using both DPPH and ABTS, and demonstrating that the IC_{50} measured using ABTS yielded higher value than DPPH. As a result, this study added poloxamer 188 to examine the increasing solubility of quercetin, followed by an antioxidant test. Moreover, in this study, all formulas gave higher IC_{50} compared to pure quercetin and vitamin C.

4. Conclusions

Formula 1 is superior to the other two formulas in terms of antioxidant activity. The IC_{50} value for formula 1 using the DPPH is 15.92 ppm, whereas the ABTS method yields 48.57 ppm.

5. Conflict of interest

The authors declare there are no conflicts of interest.

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