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RESEARCH ARTICLE

# Formulation, physicochemical characterisation, and in vitro evaluation of quercetin-alginate microsphere system

Dewi Melani Hariyadi<sup>1,2</sup>, Shafa Azaria<sup>1</sup>, Lintang Arum Cindravani<sup>1</sup>, Annisa Dayu Syifa Ramadhani<sup>3</sup>, Yotomi Desia Eka Rani<sup>1</sup>, I Nengah Budi Sumartha<sup>3</sup>, Ahmad Dzulfikri Nurhan<sup>3,4</sup>, Toetik Aryani<sup>3,4</sup>, Mahardian Rahmadi<sup>3,4</sup>, Yashwant V. Pathak<sup>5</sup>, Chrismawan Ardianto<sup>3,4</sup>

<sup>1</sup> Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>2</sup> Nanotechnology and Drug Delivery System Research Group, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>3</sup> Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>4</sup> Biomedical Pharmacy Research Group, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>5</sup> USF Health Taneja College of Pharmacy, University of South Florida, United States

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## Correspondence

Chrismawan Ardianto  
Department of Pharmacy Practice  
Faculty of Pharmacy  
Universitas Airlangga  
Surabaya  
Indonesia  
chrismawan-a@ff.unair.ac.id

## Abstract

**Background:** Quercetin exhibits various pharmacological properties. Unfortunately, quercetin has problems related to solubility, stability, and bioavailability, so it is necessary to develop an appropriate drug delivery system for quercetin. **Objective:** The study aims to develop a quercetin microsphere system and determine the effect of sodium alginate concentration on the physical characteristics and release of quercetin from the microspheres. **Method:** Quercetin-alginate microspheres were formed using sodium alginate with concentrations: Formula 1 (F1) 1%; Formula 2 (F2) 1.5%; Formula 3 (F3) 2% combined with 0.5 M CaCl<sub>2</sub> using the ionotropic gelation and aerosolisation technique. **Results:** The study found that the higher alginate concentration significantly increased microsphere particle size (6.53 – 8.34 μm) and decreased drug loading (11.58% - 6.08%). In addition, too low or high alginate concentrations accelerated the quercetin release. Variations of alginate concentration did not significantly affect the encapsulation efficiency, polydispersity index, and moisture content properties. The kinetic release of the microsphere followed the Higuchi kinetics model with the diffusion-controlled mechanism. **Conclusion:** This study successfully developed an alginate microsphere system controlling quercetin release. In addition, variations in sodium alginate concentration affect the particle size, drug loading, and cumulative release of quercetin from the alginate microsphere system.

## Introduction

Quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one] is a secondary metabolite from plants that are known to have anti-inflammatory, antioxidant, antiviral, and anticancer activities (Wang *et al.*, 2022). In addition, quercetin also has an effect on the central nervous system in various diseases such as depression, stroke, and Alzheimer's (Anggreini *et al.*, 2019; Zu *et al.*, 2021; Ardianto *et al.*, 2023). Furthermore, quercetin supplementation affects

metabolic syndrome diseases such as diabetes and non-alcoholic fatty liver disease (Al-Maamari *et al.*, 2021; Dhanya, 2022). All of the above evidence shows quercetin's potential to exhibit pharmacological activity. Unfortunately, quercetin has several problems related to solubility, stability, and bioavailability. Its chemical instability and short biological half-life reduce its effectiveness (Cai *et al.*, 2013). The stability of quercetin depends on pH and temperature. Exposure to the strong acidic pH of the stomach degrades quercetin into phenolic acid (Wang *et al.*, 2016). This

condition was a problem because quercetin absorption takes place in the large intestine (Zhang *et al.*, 2023).

In order to achieve the greatest therapeutic impact, it is necessary to enhance the physicochemical properties of quercetin by making it more water-soluble, bioavailable, and less susceptible to gastrointestinal side effects. Developing a drug delivery system using a microsphere system can potentially overcome quercetin's problem related to its physicochemical characteristics (Rohilla *et al.*, 2016). Microspheres are usually referred to as spheres particles with a diameter between 1 and 1000  $\mu\text{m}$ . Microspheres are a drug delivery system that potentiates the efficiency and stability of a drug (Hariyadi *et al.*, 2021). The development of the microsphere system reveals the drug into the system, which protects it from the outside environment. Utilising this system also increases drug absorption, which directly raises the drug's bioavailability. The microsphere system also improves the specificity of drug accumulation at specific sites (Liu *et al.*, 2006; Yawalkar *et al.*, 2022).

Based on the conditions and problems related to the quercetin properties, research focusing on improving the properties of quercetin per oral route using a microsphere system is needed. To develop a microsphere system, sodium alginate was used in this study. Alginate is an anionic polysaccharide polymer with biocompatible properties as a carrier for oral delivery systems (Hariyadi *et al.*, 2017). In this study, an evaluation was carried out on the physical characteristics (particle size, particle morphology, entrapment efficiency, drug loading, swelling index) as well as the *in vitro* release of the quercetin from microspheres.

## Methods

### Materials

The quercetin-alginate microsphere system ingredients include quercetin hydrate (Tokyo Chemical Industry, Japan), sodium alginate (Sigma-Aldrich, USA),  $\text{CaCl}_2$  (Merck, Germany), ethanol 96% (Merck, Germany), and purified water.

### Formulation of quercetin-alginate microsphere system

The preparation of the quercetin-alginate microsphere system was divided into three formulas depending on the concentration of sodium alginate: Formula 1 (F1) 1%; Formula 2 (F2) 1.5%; Formula 3 (F3) 2%. Other ingredients, besides conditioned alginate, share the same composition: quercetin hydrate 200 mg,  $\text{CaCl}_2$  0.5 M, ethanol 96% 20 mL, and purified water added until

the preparation volume reaches 300 mL. The quercetin-alginate microsphere system was made using the principle of ionotropic gelation and aerosolisation techniques. Sodium alginate was dissolved in water, and quercetin was dissolved in ethanol 96%. The sodium alginate and quercetin solutions were combined until homogeneous and sprayed on the  $\text{CaCl}_2$  solution. Homogenisation of the quercetin-alginate mixture with  $\text{CaCl}_2$  was carried out for an hour at 1000 rpm using a magnetic stirrer. The microsphere particles were filtered and washed with water to remove residual  $\text{CaCl}_2$ . Freeze drying was applied at  $-60^\circ\text{C}$  for 48 hours.

### Microsphere size and distribution evaluation

An evaluation of the microspheres' size and particle size distribution was carried out by observing 300 particles at 400x magnification using a light microscope. The micrometric method was used to analyse microsphere size distribution. Additionally, the following formula was used to calculate the Polydispersion Index (PDI):

$$PDI = \frac{\text{standard deviation}}{\text{average particle size } (\mu\text{m})}$$

### Swelling index evaluation

Swelling index evaluation of quercetin-alginate microspheres was carried out by dispersing 50 mg of microspheres in 5 ml of phosphate buffer saline pH  $6.8 \pm 0.05$ . The microspheres were left in the medium for five hours. A Millipore filter was used to collect the microspheres, and their mass was measured. The swelling index calculation is carried out using the formula:

$$\% \text{swelling index} = \frac{[\text{wet} - \text{dry microsphere (g)}]}{\text{dry microsphere (g)}} \times 100\%$$

### Moisture content evaluation

Moisture content observations were carried out using a moisture analyser (Mettler-Toledo, Moisture Analyzer HC103, Switzerland). Three replications were performed. The following formula was used to calculate the percentage of moisture content:

$$\% \text{moisture content} = \frac{\text{wet microsphere (g)}}{\text{dry microsphere (g)}} \times 100\%$$

### Drug loading and entrapment efficiency evaluation

Drug loading and entrapment efficiency (EE) were evaluated by examining the quercetin concentration in the microsphere using a UV spectrophotometer. An amount of 100 mg quercetin-alginate microspheres was dispersed in a mixture of phosphate buffer saline pH  $6.8 \pm 0.05$  contained Tween 80 2% and examined at a wavelength of 370 nm. Replication was carried out three times for each formula.

$$\%drug\ loading = \frac{quercetin\ (mg)}{microsphere\ (mg)} \times 100\%$$

$$\%EE = \frac{drug\ loading}{theoretical\ drug\ loading} \times 100\%$$

**In vitro release evaluation of quercetin from microspheres**

The quercetin release test from microspheres was carried out using microspheres equivalent to 15 mg quercetin suspended in a 100 mL mixture of phosphate buffer saline pH 6.8 ± 0.05 contained Tween 80 2%. The mixture was stirred at a speed of 100 rpm and incubated at a temperature of 37 ± 0.5°C using a thermoshaker to obtain a model of the human body. At 0, 15, 30, 45, 60, 90, 120, 180, 240, and 300 minutes, a 5.0 ml sample was taken from the In vitro release medium. A 5.0 ml phosphate buffer saline pH 6.8 ± 0.05 containing Tween 80 2% was used to replace the sample in the in vitro model medium. Samples from the in vitro model medium were filtered using a 0.45 µm millipore filter. A UV spectrophotometer with a wavelength of 370 nm was used to calculate the quercetin release concentration. The Wurster correction factor equation was used to calculate quercetin concentrations. Three replications were used for every formula during testing. The slope value (b) from the regression equation for the quercetin release test was used to analyse the quercetin release rate. This

study tested the kinetics model using Zero-order, First Order, Higuchi, and Korsmeyer-Peppas.

**Data analysis**

Statistical data analysis was carried out using the one-way ANOVA and post hoc Tukey test.

**Results**

**Quercetin-alginate microsphere system physicochemical characterisation**

Varying alginate concentrations produce different microsphere sizes. The size of the F1 microspheres was not significantly different compared to F2. Meanwhile, the size of the F1 microspheres is much smaller than F3. The range of microsphere sizes produced in this study was 6.53 ± 0.91 to 8.34 ± 0.46 µm. Meanwhile, there is no difference in the PDI value for each formula. The PDI range was 0.0010 to 0.0193 (Table I).

F2 has a smaller tendency for the swelling index than F1 and F3, but it is insignificant. The swelling index results ranged from 664.217 ± 284.249% to 858.210 ± 407.612%. Furthermore, there was no significant difference in the % moisture content results for F1, F2, and F3. The % moisture content results ranged from 8.93 ± 0.30% to 9.46 ± 1.49% (Table I).

**Table I: Physical characterisation of quercetin-alginate microsphere system**

Formula	Particle size (µm) ± SD	Polydispersity Index	Swelling Index (%) ± SD	Moisture content (%) ± SD
F1	6.53 ± 0.91 <sup>†</sup>	0.0193	830.190 ± 385.052	9.46 ± 1.49
F2	7.847 ± 0.20	0.0010	664.217 ± 284.249	9.29 ± 0.23
F3	8.34 ± 0.46	0.0030	858.210 ± 407.612	8.93 ± 0.30

F1 (Alginate 1%); F2 (Alginate 1.5%); F3 (Alginate 2%), <sup>†</sup>Significant vs F3 (p < 0.05)

**Quercetin-alginate microspheres drug loading and entrapment efficiency**

The study demonstrates that drug loading decreased with increasing alginate concentration. F1 and F2 had significantly greater drug loading compared to F3. However, there was no significant difference between

drug loading F1 and F2. The range of the drug loading was 6.08 ± 0.87% to 11.58 ± 0.79 %. Meanwhile, variations in alginate concentration did not significantly affect entrapment efficiency. The range of the entrapment efficiency was 68.97 ± 7.67% to 76.77 ± 5.18 % (Table II).

**Table II: Drug loading and entrapment efficiency of quercetin-alginate microspheres system**

Formula	Drug loading (%) ± SD	Entrapment efficiency (%) ± SD
F1	11.58 ± 0.79 <sup>†</sup>	70.91 ± 4.15
F2	9.21 ± 0.79 <sup>†</sup>	76.77 ± 5.18
F3	6.08 ± 0.87	68.97 ± 7.67

F1 (Alginate 1%); F2 (Alginate 1.5%); F3 (Alginate 2%), <sup>†</sup> significant vs F3 (p < 0.05)

**Quercetin release profile from alginate microspheres**

Based on the release profile at 300 minutes, quercetin release from F2 was significantly lower compared to F3. In contrast, there are no noticeable statistical differences between F1 and F3. Difference statistical results occur in the release rate of quercetin. Compared to F1 and F3, the release rate of quercetin tended to be

lower in F2, but this variation was not statistically significant. The range of quercetin release in the microsphere system was  $0.0182 \pm 0.0094\%$  to  $0.0269 \pm 0.0092\%$  /minute (Table III). In addition, the relationship coefficient value in the Higuchi model is closest to one compared to other kinetic models, which indicates that the quercetin-alginate microspheres follow Higuchi release kinetics (Table IV).

**Table III: Release profile of quercetin-alginate microsphere system**

Time	Drug release rate (%/minutes $\pm$ SD)		
	Formula 1	Formula 2	Formula 3
0	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000
15	10.453 $\pm$ 6.006	9.108 $\pm$ 5.642	12.605 $\pm$ 2.645
30	14.173 $\pm$ 4.360	11.509 $\pm$ 7.551	17.256 $\pm$ 4.607
45	18.496 $\pm$ 2.569	14.088 $\pm$ 6.538	21.758 $\pm$ 3.557
60	24.615 $\pm$ 1.434	20.048 $\pm$ 0.911	25.053 $\pm$ 4.929
90	27.926 $\pm$ 1.672	23.784 $\pm$ 1.523	30.614 $\pm$ 4.575
120	31.488 $\pm$ 1.375	25.967 $\pm$ 3.721	34.989 $\pm$ 1.530
180	35.060 $\pm$ 4.525	29.141 $\pm$ 2.519	38.091 $\pm$ 0.405
240	38.028 $\pm$ 4.548	31.499 $\pm$ 3.501	41.445 $\pm$ 2.164
300	41.087 $\pm$ 4.132	31.732 $\pm$ 6.142 <sup>†</sup>	48.623 $\pm$ 3.862
<b>Drug release rate</b>	<b>0.0243 <math>\pm</math> 0.0067</b>	<b>0.0182 <math>\pm</math> 0.0094</b>	<b>0.0269 <math>\pm</math> 0.0092</b>

<sup>†</sup> significant vs F3 ( $p < 0.05$ )

**Table IV: In vitro kinetics model of quercetin-alginate microsphere system**

Formula	R-value – Drug release kinetics model			
	Zero-order	First order	Higuchi	Korsmeyer-Peppas
F1	0.7917	0.8462	0.9484	0.9341
F2	0.7533	0.7922	0.9145	0.9079
F3	0.8341	0.8935	0.9682	0.9483

F1 (Alginate 1%); F2 (Alginate 1,5%); F3 (Alginate (2%))

**Discussion**

This study shows that a quercetin-alginate microsphere system was successfully developed. The development of the quercetin-alginate microsphere system was proven by evaluating the physicochemical characteristics of the microsphere and in vitro drug release. The microsphere formula used in this study results in spherical particles. Furthermore, this research confirms that the microspheres produced are monodisperse based on PDI results, which have a value  $<0.1$ . This finding confirms that the quercetin-alginate microsphere in this study has a narrow particle size distribution range (Raval et al., 2018). The variation of alginate concentration affects the particle size of the

microsphere. This study shows that increasing alginate concentration causes an increase in microsphere particle size. Increasing alginate concentration causes a rise in alginate viscosity, which slows down the movement of polymer molecules, making it more difficult for them to spread out. As a result, larger microspheres were formed. The range of microsphere sizes produced in this study was  $6.53 \pm 0.91$  to  $8.34 \pm 0.46 \mu\text{m}$ . It is well known that an ideal particle size for drug localisation in the colon is between 5 and 15  $\mu\text{m}$  (Nidhi et al., 2016). Therefore, this study verified that the microsphere sizes in all three formulas were suitable for delivery with a focus on colonic localisation.

In contrast to the results of microsphere size, increasing alginate concentration caused a significant decrease in drug loading. This result is likely caused by the lack of calcium ions in the microsphere system, resulting in a poorer polymer bond and decreased drug trapped in the microsphere (Mandal *et al.*, 2010). In contrast to drug loading, the entrapment efficiency of quercetin in microspheres is not influenced by variations in alginate concentration. The swelling index measures the capacity of alginate polymer bonds from microspheres to relax (Cadena-Velandia *et al.*, 2020). The drug release from the microsphere system is possibly impacted by this relaxation phenomenon. The tendency to lower the swelling index in F2 was confirmed in this study. All of the formulas used in this study had moisture contents below 10%, demonstrating the ability of the microsphere system to prevent drug aggregation. In addition, since moisture affects the stability of quercetin, the low moisture content value in this study naturally maintains the stability of quercetin in the microsphere form (Shan *et al.*, 2016; Haryadi *et al.*, 2019).

In the quercetin release evaluation, this study used a five-hour test duration to illustrate the gastrointestinal tract condition (Jain *et al.*, 2007). In this study, F2 had the smallest average release compared to the other. This result was consistent with the swelling index evaluation, in which F2 had the lowest swelling index value compared to the other formulas, leading to a more prolonged release of quercetin. In addition, the quercetin-alginate microsphere system developed in this study follows Higuchi release kinetics. This result indicates that quercetin is released from the microspheres through a controlled diffusion mechanism of the microsphere system (Paul, 2011).

The results of the quercetin-alginate microsphere system evaluation in this study show that the microsphere has the potential to enhance the pharmacological effects of quercetin. The microsphere system is likely to increase the effectiveness of quercetin, mainly because the presence of quercetin is controlled in the body. This feature is expected to reduce factors that trigger any diseases (Yang *et al.*, 2019; Cao *et al.*, 2023). Moreover, research from Bai *et al.* (2023) proves that there is an increase in the antioxidant activity of selenium in the microspheres form. Furthermore, developing this system also provides a greater anti-inflammatory effect (Cosco *et al.*, 2016). Considering that quercetin also has antioxidant and anti-inflammatory activity, it is certainly an advantage to make a quercetin-alginate microsphere system.

Although this research has succeeded in developing a quercetin-alginate microsphere system for the oral

route on colon localisation, further research is required, especially in elucidating the application of the drug delivery developed in this research by using in vivo pharmacokinetic and pharmacodynamic research.

## Conclusion

This study successfully developed an alginate microsphere system that controlled quercetin release. In addition, variations of sodium alginate concentration affect the particle size and cumulative release of quercetin from the alginate microspheres system.

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