ORIGINAL ARTICLE

Physical characteristics of quercetin pulmospheres using combination of alginate-carrageenan: Effect of polymer concentration

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ABSTRACT

Indonesia is the second country with the highest number of tuberculosis (TB) cases in the world and the first in Southeast Asia, according to WHO Global Report 2020. Quercetin has been tried as an alternative therapy and was found effective. This study aims to optimize quercetin pulmospheres using combination polymers and study its characteristics as an inhalation delivery system. Combination polymers provide the advantages of safe, mucoadhesive, and compact pulmospheres. Pulmospheres were made as formula F1, F2, and F3 (polymer ratios of 1:1, 1:2, and 1:3), respectively. Pulmospheres were made with quercetin 0.2%, alginate-carrageenan (total concentration of 1.8%), and $CaCl_2$ 0.5 M. Characterization of particle size, morphology, moisture content (MC), yield, drug loading, and entrapment efficiency (EE) were conducted. The yield range was from 83.89 to 86.30% ± 4.59%. MC range was from 4.23 to 5.12% ± 0.05%. Particle size was <3 μm (between 2.19 and 2.76 ± 0.149 μm), spherical shape and smooth surface. EE range was 60.69% ± 4.45% to 77.86% ± 1.74% and the drug loading range was 1.66–2.09% ± 0.15%. F2 formula with a polymer ratio of 1:2 was the best quercetin pulmospheres. Potential pulmospheres will then be recommended for *in vitro* release and *in vivo* study.

Key words: Alginate, carrageenan, physical characteristics, pulmosphere, quercetin, tuberculosis

INTRODUCTION

Tuberculosis (TB) is one of the complex diseases caused by *Mycobacterium tuberculosis*. Indonesia is the second country with the highest number of TB cases in the world and this has been making various efforts to target Indonesia to be TB-free by 2030.[1-3]

Quercetin shows antibacterial activity against *M. tuberculosis* and is effective as TB therapy.[4-6] Sasikumar *et al*.[6] and Nguyen *et al*.[7] shows that quercetin has antibacterial activity, reduces manifestations of TB, and reduces side effects.[8] Fortunato *et al*. found that quercetin inhalation can cause anti-asthmatic effects by inhibiting histamine release and inflammatory mediators such as tumor necrosis factor-alpha, interleukin (IL)-1β, IL-6, and IL-8 from mast cells stimulated by immunoglobulin E.[9] However,

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quercetin is chemically unstable[10] and solubility in water is only 0.01 mg/mL (25°C).[11] Therefore, quercetin needs to be formulated in an inhalation system.

The inhalation pulmonary delivery system offers advantages such as localized drugs to the lungs, dose reduction, and reduces side effects.[12,13] A dry powder inhaler is a simple and easy device and microsphere can be formulated with this into the lungs.[14,15]

Microspheres are delivery systems with a diameter of 1–1000 μm and for the pulmonary route are called pulmosphere.[16] Advantages of the pulmosphere reduce the frequency, reduce unwanted reactions, and improve patient compliance.[16]

Alginate is an anionic polysaccharide derived from brown algae that consists of α-L-guluronate (G) and β-D-mannuronate (M) that are biocompatible, low toxicity, mucoadhesive and can be used for lung delivery.[17-19] However, porous structures and poor mechanical properties cause burst release.[20] Ca^{2+} is needed to form an "egg-box" to entrap the drug.[21,22] Combination with other polymers can result in smaller sizes and higher entrapment efficiency (EE).[23]

Carrageenan is a sulfate polysaccharide extracted from red algae consisting of (1,3)-D-galactopyranose and (1,4)-3,6-anhydro-alpha-D-galactopyranose and sulfate group.[24] Kappa-carrageenan is selected because of its strong gelling properties, mucoadhesive, good entrapment, and controlled release.[18,19,25] Increased carrageenan concentration escalates the thickness of the matrix and slow release.[15] Gull *et al*. found a decreased rate of drug release is caused by strong polyelectrolyte complexes.[26]

This study used a combination of alginate and carrageenan to prevent porous pulmospheres and increase swelling index and encapsulation efficiency.[27] Kolesnyk *et al*. studied microspheres of alginate and carrageenan (1:1) resulted an increase in swelling index at neutral pH and sustained release.[28] Wijaksana *et al*. examined ciprofloxacin pulmosphere using a combination of alginate-carrageenan polymers resulting in small particle size, however, burst release occurred.[29] The optimum characteristics and also potential activity of quercetin as anti-TB have not studied comprehensively previously, therefore this study investigates quercetin pulmospheres with combination polymers of alginate and carrageenan (1:1, 1:2, and 1:3). Potential novelty of the quercetin pulmospheres produced by aerosolization technique could be beneficial for TB diseases.

MATERIALS AND METHODS

Materials

Quercetin and Sodium Alginate (Sigma-Aldrich Inc.,); Carrageenan (Danisco-Cultor); $CaCl_2.2H_2O$ (Solvay Chemicals International) were pharmaceutical grade. Na_2HPO_4, KH_2PO_4, NaCl, HCl, and NaOH were proanalysis (Merck); Maltodextrin and aqua demineralized were from PT. BrataChem.

Methods

Preparation of quercetin pulmospheres

The preparation of quercetin pulmospheres can be seen in Table 1.

A solution of 1.35 g of sodium alginate and carrageenan was made in 50 mL of aqua demineralized and mixed. Quercetin solution of 0.3 g was dissolved in 50 mL aqua demineralized and was mixed into polymer until homogeneous. $CaCl_2$ of 22.05 g was dissolved in 300 mL aqua demineralized. Dispersion of the quercetin solution in the polymer is sprayed on the $CaCl_2$ solution with a spray hole diameter of 35 μm, distance of 8 cm, and pressure of 40 psi. Droplets were stirred at 1000 rpm for 2 h. Pulmosphere was washed with aqua demineralized two times by centrifugation of 2500 rpm for 6 min. Pulmospheres were weighed and suspended in 5% maltodextrin solution and were freeze-dried at − 80°C for 96 h.

Fourier transform infrared of quercetin pulmosphere

Infrared (IR) spectra of sodium alginate, quercetin, carrageenan, and pulmospheres were recorded as a percentage of transmittance.

Yield

Yield is calculated as below formula:

$$\%Yield = \frac{Weight\ of\ pulmosphere}{Weight\ of\ quercetin + polymer + maltodextrin} \times 100\%$$

Particle size and morphology

Particle size was observed using an optical microscope and OpticaLab software. Morphology was observed using a Scanning Electron Microscope instrument.[28]

Table 1: Quercetin pulmosphere formula

Materials	Function	F1 (%)	F2 (%)	F3 (%)
Drug polymer				
Quercetin	Active agent	0.2	0.2	0.2
Alginate	Polymer	0.9	0.6	0.45
Carrageenan	Polymer	0.9	1.2	1.35
Aqua demineralized	Solvent	ad 150 mL		
Crosslinker				
Calcium chloride	Crosslinker	0.5 M	0.5 M	0.5 M
Aqua demineralize	Solvent	ad 300 mL		
Lyoprotectant				
Maltodextrin	Lyoprotectant	5	5	5

Moisture content

Moisture content (MC) was evaluated using MC Analyzer (METTLER Toledo HB43-S).

Drug loading and entrapment efficiency

Drug loading and efficiency were calculated using the following:

$$\%DL = \frac{Quercetin\,weight\,in\,pulmosphere\,(mg)}{Pulmosphere\,weight\,(mg)} \times 100\%$$

$$\%EE = \frac{Measured\,quercetin}{Theoretical\,quercetin} \times 100\%$$

Data analysis

ANOVA statistics with a degree of confidence of 0.95 (α = 0.05) were analyzed. If the significance value (sig) obtained is <0.05, it is a significant difference and further analysis was carried out using Honest Significant Difference.

RESULTS AND DISCUSSION

Fourier transform infrared (FTIR) of pulmosphere

Fourier transform infrared (FTIR) of the pulmosphere can be seen in Table 2.

FTIR spectra from all pulmospheres predict intergroup interactions by the shift in the peak of the guluronic block because the COO^- cluster of the guluronic block reacts crosslinking with Ca^{2+} ions.[30] This reaction forms *an* "egg-box" structure that entraps quercetin. Peak shift of manuronate block occurs because the COO^- bond of the manuronate binds to the Ca^{2+} ion of the sulfate group from the carrageenan.[31] This interaction results in stronger pulmosphere and reduces pores.[32] The loss of IR peaks from quercetin is due to the interaction between quercetin and alginate that changes the crystal structure of quercetin to amorphous.[30]

Yield and moisture content

Yield and MC calculations are shown in Table 3. One way ANOVA showed that the increase in ratio of polymers did not make a significant difference to yield indicated by Sig = 0.642 (>0.05). Formula F3 had a higher MC than formula F1 and F2 due to the hydrophilic nature of carrageenan so that pulmosphere entraps more water molecules.[15] Ni *et al*. (2017) states that ideal microparticle to deliver drugs to the lungs has MC of <10%.[33]

Particle size

Particle size is shown in Table 3. Significant differences were shown (Sig = 0.005 [<0.05]). Particle size sequentially is F1 < F3 < F2. Li *et al*. found that an increased carrageenan ratio contributes to the size.[34] However, continuous increase in carrageenan ratio results in decreasing size. Particles are in accordance with inhalation requirements (1–5 μm).[35]

Entrapment efficiency

EE and loadings are shown in Table 3. Formulas had significant differences and F2 has the highest efficiency. Rosita *et al*. showed the increased viscosity of the polymer-quercetin solution causes quercetin entrapped to be reduced.[36] Hariyadi *et al*. explained that the larger the particle size, the higher the number of drugs entrapped.[37] Drug loading was <3% [Table 3]. F1 and F2 as well as F1 and F3 demonstrated significant differences, while F2 and F3 had no significant differences. Suggestions for increasing drug loading are by increasing the drug concentration.[38]

Table 2: Fourier transform infrared spectra of pulmosphere

Materials	Bonds	Wave number (cm^{-1})			
		Raw materials	F1	F2	F3
Quercetin	Phenolic OH	3405.24–3284.66	3261.77	3262.23	3229.11
	C=O ketone	1664.14	-	-	-
	C=C aromatic	1608.65–1564.11	-	-	-
	C-H internal field aromatic	1318.81	-	-	-
	C-H external field aromatic	940.54; 820.56; 639.70	621.38	643.60	695.41
	C-O-C	1262.33; 1197.70; 1166.74	1148.05	1148.50	1147.97
Sodium alginate	OH	3615.71–3181.37	3600.82–3261.77	3567.67–3262.23	3629.92–3229.11
	C-H	2895.76	2929.70	2923.84	2916.86
	C=O	1592.77	1609.43	1625.63	1643.64
	Carboxyl (C-O)	1296.36	1355.25	1351.22	1359.36
	Guluronate	884.91	927.15	928.67	926.40
	Manuronate	810.48	842.67	844.65	844.65
Kappa-carrageenan	Sulfate ester (S=O)	1223.51	1240.89	1249.00	1247.81
	Glycosidic bond	1061.19	1075.77	1077.02	1077.21
	3.6-anhydrogalactose	922.24	927.15	928.67	926.40
	Galactose-4-sulfate	841.94	842.67	844.65	844.65

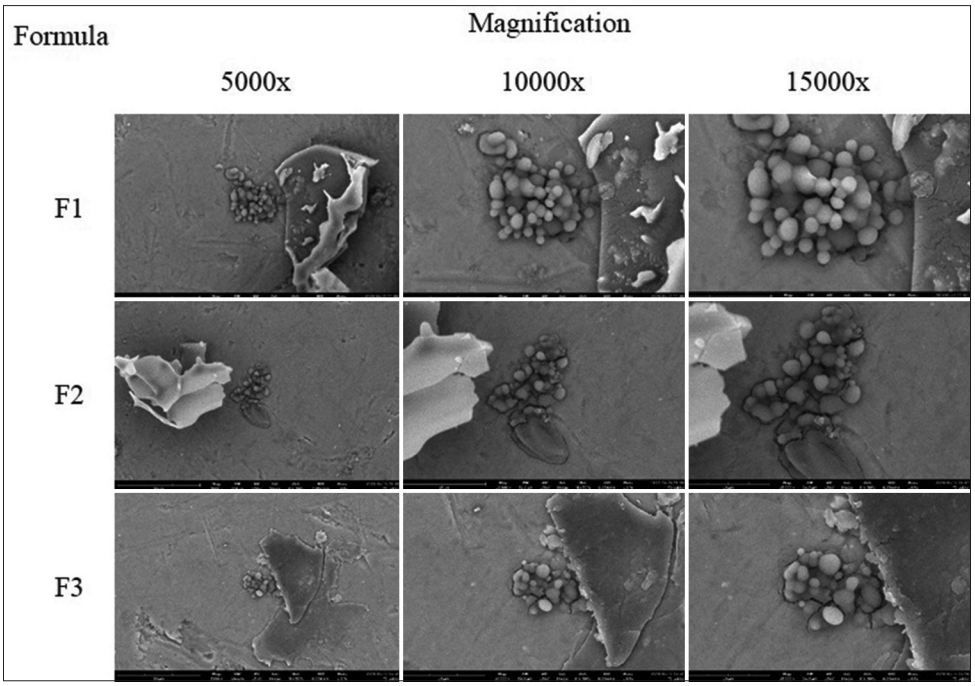

Figure 1: Morphological of quercetin pulmosphere using scanning electron microscope at ×5000, ×10,000, and ×15,000 magnification

Table 3: Quercetin pulmosphere characteristics

Parameter	Mean±SD		
	F1	F2	F3
Yield (%)	84.69±1.27	86.30±4.59	83.89±2.42
MC (%)	4.23±0.08	4.48±0.22	5.12±0.05
Particle size (μm)	2.19±0.029	2.76±0.149	2.39±0.17
EE (%)	60.69±4.45	77.86±1.74	72.90±2.90
Drug loading (%)	1.66±0.12	2.09±0.15	2.01±0.14

MC: Moisture content, SD: Standard deviation, EE: Entrapment efficiency

Morphological of the pulmosphere

Quercetin pulmospherse morphology is shown in Figure 1.

Morphology showed spherical shape and a smooth surface as good and efficient properties to enter the lungs.[39,40]

Formula F2 with a polymer ratio of 1:2, exhibited the most optimal results compared to those of the F1 and F3 formulations. Although sizes were small in all formulas, at a polymer ratio of 1:2, it has the biggest size but still in inhalation requirement.[25] Li *et al*. stated that the more the double gel is formed, the more alginate and carrageenan molecules that are connected.[34] However, continuous addition of the carrageenan, decrease double gels formation and resulting smaller particle size. Large particle size causes more amount of drugs entrapped and increases EE and drug loading.[23] Overall, the F2 pulmosphere showed the most optimal results of characteristic compared to the F1 and F3. Some recommendations for future study are needed.

CONCLUSION

The quercetin pulmospheres were successfully formed from the combination of alginate-carrageenan polymers with a 1:2 polymer ratio and showed the best characteristics (particle size, morphology, drug loading, and high EE). Potential development of the quercetin pulmospheres is needed including *in vitro–in vivo* release and activity study.

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Conflicts of interest

There are no conflicts of interest.

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