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Emulsion Formulation Optimization and Characterization of Spray-dried κ-Carrageenan Microparticles for the Encapsulation of CoQ10

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Abstract The present study is aimed to prepare κ-carrageenan microparticles for the encapsulation of model drug, coenzyme Q10 (CoQ10). A face-centered central composite design was employed to study the effects of three different formulation variables (κ-carrageenan, emulsifier, and oil). The powder yield was found inversely affected by the κ-carrageenan and oil concentration. The encapsulation efficiency was maximized in the region of the middle level κ-carrageenan concentration, the high level emulsifier concentration, and the low level oil concentration. The emulsifier concentration was the most influential variable on the particle size of powder. The optimal formulation was reported as 0.91% (w/v) κ-carrageenan concentration, 0.64% (w/v) emulsifier, and 1.0% (w/w) oil. Both differential scanning colorimeter and X-ray diffraction analyses proved that incorporation of CoQ10 into κ-carrageenan microcapsules resulted in amorphous powder with significantly (p<0.05) higher water solubility compared to pure CoQ10 and physical mixture in the crystalline form.

Keywords: κ-carrageenan, coenzyme Q10, spray drying, emulsion formulation, water solubility

Introduction

Coenzyme Q10 (CoQ10), also known as ubiquinone, is a lipophilic compound that synthesized endogenously in the human body (1,2). Found in most cell membranes, CoQ10 functions as a cofactor in the mitochondrial electron transport chain and is essential for the production of adenosine triphosphate (ATP) (3,4). Several studies have found the beneficial effects of CoQ10 in the treatment of cardiovascular disorders such as angina pectoris, hypertension, atherosclerosis, and congestive heart failure (1,5). Recently, CoQ10 has even been incorporated into dietary supplements, energy drinks, and skin care products (6,7). Owing to its high molecular weight (863.34 g/mol) and poor water solubility (<0.0001 mg/L), the bioavailability of CoQ10 in these commercial formulations is questionable (1,6).

Many attempts have been made to improve the bioavailability of CoQ10, such as using liposomes, lipid nanoparticles, and solid dispersion (8-10). The drawbacks of previous works have included the use of organic solvents and non-food-grade synthesis polymer. Hence, there is a great need to find an efficient, safe, and environmental friendly method to improve the solubility and bioavailability of CoQ10. An oil-in-water (O/W) emulsion can be a useful delivery vehicle for lipophilic, bioactive substances such as CoQ10 to improve the bioavailability and protect the compounds from the destructive effects of the environment (11). This simple method involves the use of edible oil (for CoQ10 dissolution) as a dispersed phase and a food-grade hydrophilic polymer as a continuous phase. The effect is attributed to the improved dispersion of the lipid phase, resulting in a more effective absorption (11). Unfortunately, O/W emulsions are kinetically and thermodynamically unstable systems that break down over time (11). One potential strategy to improve the stability of this system is spray drying the O/W emulsion to produce powders containing lipid droplets (11,12). κ-Carrageenan, a marine based polysaccharide, is extracted from red algae of the class *Rhodophyceae*, in which it is the major component for the maintenance of cell wall structure (13,14). Chemically, κ-carrageenan is a linear, sulphated polysaccharide composed of repeating units of 1,3-linked β-D-galactose sulphate and 1,4-linked 3,6-anhydro-α-D-galactose units

(15,16). Due to their advantageous properties such as nontoxicity, biocompatibility, and biodegradability, utilization of κ-carrageenan for the encapsulation of drug and food nutrients has received substantial attention in these recent years (17,18).

Depending on the technique of microencapsulation and its application, κ-carrageenan carrier can exists in various forms (16). Among the different microencapsulation technologies, spray drying is still the most favorable one used due to its easiness, low cost, and available equipment (19,20). Despite these advantages, in spray drying, the feed formulation and processing variables must be controlled adequately to avoid low yields sticking and high moisture content that often encounter in laboratory scale spray dryers (21).

Ultimate goal of this study is to prepare κ-carrageenan microparticles using spray drying technique for the microencapsulation of a poorly water-soluble model drug, CoQ10. A three factor, three level (3^2) face-centered central composite design (FCCCD) was employed to study the influences of formulation variables on the spray drying process and powder properties. The CoQ10-loaded κ-carrageenan microcapsules, obtained with optimized emulsion formulation were further characterized for their morphology, crystallinity, molecular changes, and water solubility.

Materials and Methods

Materials Wall material used in this study was κ-carrageenan extracted from *Eucheuma cottonii*, which obtained as gift from Tacara Sdn. Bhd. (Tawau, Malaysia). Core material was CoQ10 powder (purity: 99.7%; solubility: <0.0001 g/mL) procured from Kaneka Corporation (Osaka, Japan). Palm kernel oil and the emulsifier sodium stearoyl lactate (SSL) were generous gifts from Sime Darby Berhad (Kuala Lumpur, Malaysia) and Rikevita Sdn. Bhd. (Johor Bahru, Malaysia), respectively. Acetonitrile (99.9%), hexane (98.0%), and tetrahydrofuran (99.9%) were supplied by Merck KGaA (Darmstadt, Germany).

Preparation of CoQ10-loaded o/w emulsions The aqueous phase was first prepared by dispersing κ-carrageenan in deionized water heated to 70°C under constant magnetic stirring (700 rpm) for 1 h to ensure complete dispersion. Hydrophilic emulsifier (SSL) was then added to κ-carrageenan solution at different concentrations. The mixtures were stirred overnight to fully hydrolyze the emulsifier in κ-carrageenan solution. The core material (oil phase) was a mixture of CoQ10 dissolved in palm kernel oil. The content of CoQ10 in the oil mixture was 20% (w/w). The aqueous system was pre-homogenized with different amount of CoQ10-loaded palm kernel oil using a high speed rotor-stator mixer (L4RT; Silverson Machines Ltd, Chesham, England) at 5,000 rpm for 5 min in dark. The mixture was then further homogenized with a two stage valve homogenizer (APV Model 2000; APV Homogenisters, Albertslund, Denmark) at 300 bars with 2 cycles. The homogenized emulsions were directly subjected to spray drying.

Experimental design FCCCD was applied to study the interactions of variables on product characteristics for the optimization of emulsion formulation. Three formulation parameters were selected, namely κ-carrageenan concentration (X_1, % w/v), emulsifier concentration (X_2, % w/v), and oil concentration (X_3, % w/v). The measured dependent variables (responses) were emulsion droplet size (Y_1, μm), yield (Y_2, % w/w), encapsulation efficiency (Y_3, % w/w), powder particle size (Y_4, μm), water activity (Y_5), and moisture content (Y_6, % w/w). The complete design consisted of twenty experiments with eight factorial points, six axial points (on the cube's surface), and six center points is outlined in Table 1. Each analysis was performed in triplicate and the average values were taken as the response.

Spray-drying process The emulsions prepared were spray-dried on a bench-top Büchi Mini Spray Dryer (B-290; Büchi Labortechnik AG, Flawil, Switzerland) equipped with a 0.7 mm diameter nozzle and a drying chamber of 100 cm (height)x70 cm (diameter). The spray drying conditions were set at: inlet temperature (150°C), pump flow rate (4 rpm), aspiration rate (100%), and pressure (4 bars) for all the experiments. These conditions resulted in an outlet temperature of 87±5°C. The resultant spray-dried powders were kept in an air-tight, amber glass container at room temperature and stored in desiccator until further studies.

Physicochemical properties of spray-dried CoQ10 in κ-carrageenan microparticles

***Emulsion droplet size analysis*:** The oil droplet size distributions of emulsions were determined using a laser diffraction particle size analyzer (Mastersizer 2000; Malvern Instruments, Malvern, UK). Refractive index of emulsion was set as 1.4569. Emulsions were added drop by drop to the circulating deionized water until laser obscuration fell between 4.5-5.0%. The samples were stirred continuously at constant speed (1,500 rpm) throughout the measurement to ensure the homogeneity of the samples. In this study, the powder particle size was reported as volume weighted mean D [4,3] ($=\Sigma n_i d_i^4 / \Sigma n_i d_i^3$, where n_i is number of particles with diameter d_i) in which the mean diameter is based on the volume frequency.

***Yield*:** The yield was calculated as the ratio of the powder weight collected at the end of the spray drying process to the initial amount of solids added in the emulsion before spray drying including κ-carrageenan, SSL, and CoQ10.

$$\text{Yield } (\%) = W_f / W_i \times 100 \quad (1)$$

Where W_f is the weight of spray-dried powder collected and W_i is the weight of total solids in the dispersion before spray drying.

***Encapsulation efficiency (EE)*:** The methods described in Bule *et al.* (4) and Yoo *et al.* (22) were adapted to determine the EE of CoQ10 with some modifications. Two hundred milligrams of spray-dried CoQ10 powder was weighed into a boiling tube, and 20 mL hexane was added to the accurately weighed CoQ10 and ultrasonic-treated for 30 min in the dark using an ultrasonic system (Power Sonic 420;

Hwashin Technology, Seoul, Korea) at room temperature. After agitation, the yellow-orange-colored hexane fraction was filtered through a 0.45 μm syringe filter and diluted ten times with hexane. The CoQ10 was quantified by HPLC (Series 200; Perkin Elmer, Waltham, MA, USA) according to the AOAC Official Method 2008.07 (7). The separation was performed using an Ascentis™ C18 column (4.6x150 mm, 5 μm; Supelco, Bellefonte, PA, USA). The mobile phase consisted of acetonitrile, tetrahydrofuran, and water (55:40:5, v/v/v), which was filtered through a 0.45 μm nylon membrane filter and degassed for 15 min before use. The elution was performed at a flow rate of 1.0 mL/min. The column temperature was maintained at 25°C. Detection was performed with a UV detector at 275 nm for a run time of 20 min. The concentration of CoQ10 present in the microcapsules was calculated based on the calibration curve obtained (y=16,834x+11,851).

$$EE\ (\%)=A_{actual}/A_{theoretical} \times 100 \quad (2)$$

Where A_{actual} is the actual amount of CoQ10 encapsulated and $A_{theoretical}$ is the amount of CoQ10 added to the formulation.

***Powder particle size*:** The spray-dried CoQ10 powder was determined using a laser diffraction particle size analyzer (Mastersizer 2000; Malvern Instruments) with a dry powder dispersion accessory. Refractive index of CoQ10 powder was set as 1.456. The particle size of spray dried CoQ10 powder was expressed as surface weighted mean D [3,2] ($=\Sigma n_i d_i^3/\Sigma n_i d_i^2$, where n_i is number of particles with diameter d_i).

***Moisture content*:** Samples (0.5 g) were weighed in triplicate and dried in an oven (UNB 100; Memmert GmbH & Co. KG, Schwabach, Germany) at 105°C for 24 h until constant weight.

$$\text{Moisture content (\% w/w)} = \text{Water loss during drying/Sample weight} \times 100\% \quad (3)$$

***Water activity (A_w)*:** A_w of spray-dried CoQ10 powder was determined using an AquaLab (Series 3TE; Decagon Devices Inc., Pullman, WA, USA), with a ±0.001 sensitivity. The temperature was maintained at 25.0±0.1°C.

Characterization of optimized spray-dried formulation

***Morphology*:** The surface morphology of raw materials and spray-dried CoQ10 microcapsules in κ-carrageenan was investigated by scanning electron microscope (SEM) (Leo 1455 VP; Carl Zeiss, Oberkochen, Germany) with Oxford Instruments INCA energy dispersive X-ray (EDX) spectrometer. A thin layer of samples were placed on the double-sided adhesive carbon tape, mounted on SEM aluminium stubs and coated with gold in a sputter coater (SCD 055; BAL-TECH, Lübeck, Germany) for 180 s, with a current of 20 mA. The coated samples were then analyzed using the SEM operating at an accelerating voltage of 20.00 kV with a magnification of 1,500x.

***Differential scanning calorimeter (DSC)*:** The thermodynamic behavior of coating raw materials and spray-dried powder were measured using DSC (DSC823e; Mettler Toledo, Greifensee, Switzerland). 5-12 mg of sample was weighed in 40 μL aluminum pan with lid and cool sealed. The reference pan was left empty. DSC measurements for coating materials, pure CoQ10, and the spray-dried CoQ10 were performed from 25 to 100°C at a heating rate of 5°C/min to investigate a possible melting of crystalline material in the samples.

***X-ray diffraction (XRD)*:** XRD studies were performed using a X-ray diffractometer (XRD-6000; Shimadzu, Kyoto, Japan) with Cu Kα radiation (λ=1.5406 Å), operating at 40 kV, 30 mA to detect any crystallinity present in raw κ-carrageenan, emulsifier, pure CoQ10, and CoQ10 in κ-carrageenan microcapsules. The samples were scanned from 5 to 50° with a scanning rate of 2°/min.

***Fourier transform infrared spectroscopy (FT-IR)*:** The molecular structure change of pure CoQ10 powder and spray-dried CoQ10 in κ-carrageenan microcapsules were characterized by FT-IR spectrometer (Spectrum 100; Perkin Elmer, Massachusetts, USA). The samples were scanned over the wave number range from 4,000 to 280 cm^{-1}.

***Solubility*:** Solubility of CoQ10 was determined at 25 and 37°C. Pure CoQ10, physical mixtures, or spray-dried CoQ10 in κ-carrageenan microcapsules equivalent to 25 mg of CoQ10 was added to 10 ml of distilled water. The mixture was vortexed for 2 min and subjected to shaking in a temperature controlled water bath shaker (ProTech; Saintifik Maju, Petalling Jaya, Malaysia) at 25 and 37°C for 24 h. After shaking, the solutions were centrifuged at 1,258*xg* for 15 min using a centrifuge (Kubota 5800; Kubota Corporation, Tokyo, Japan) and supernatant was filtered through a 0.45 μm syringe filter. The concentration of CoQ10 was determined by HPLC method as describe in EE analysis.

Statistical analysis The Design Expert (Version 6.0.10; Stat-Ease Inc., Minneapolis, MN, USA) statistical software was employed to design the FCCCD and to analyze the experimental data in response surface methodology (RSM). Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model proposed for the response surface analysis was given as follows:

$$Y=\beta_0+\sum^{k}\beta_i X_i+\sum^{k}\beta_{ii}X_i^2+\sum^{k-1}\sum^{k}\beta_{ij}X_iX_j \quad (4)$$

where β_0, β_i, β_{ii}, and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are coded value of the independent variables while k equals to the number of the tested factors (k=3). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic, and interaction terms were determined.

Results and Discussion

Preliminary investigation A preliminary study was conducted to determine the ranges of formulation variables to be used in designing FCCCD. κ-Carrageenan concentration of 1.5% (w/v) was selected as the highest concentration as the solution start to gel once it goes beyond this concentration. Gelation of κ-carrageenan solution may affect the emulsion mixing and cause clogging during the spray drying process. Emulsifier, SSL was added at concentration lower than that of coating material. Too high the emulsifier concentration increases stickiness of the spray-dried powder, which leading to clumping. The high level of oil concentration was set as 3.0% (w/w) to avoid low product yield (<30%), low EE, and large powder particle size. Since the aim of this work is to investigate the effects of emulsion formulation, the spray drying conditions were held constant throughout the experiment.

Optimization of the emulsion formulation of spray-dried CoQ10 in κ-carrageenan microcapsules Table 1 summarizes the experimental results for the emulsion droplet size, yield, EE, powder particle size, moisture content, and a_w corresponding to 20 sets of formulation combinations. The ANOVA results (Table 2) showed that the reduced regression models for all of the response variables were highly significant at $p<0.05$, with satisfactory R^2 ranging from 0.8256 to 0.8801. According to Sin *et al.* (23), an R^2 value of more than 0.80 is adequate for prediction purposes. In addition, the absence of any lack of fit ($p>0.05$) further ascertains the reliability of the final reduced models.

Generally, the finer the emulsion produced, the more stable the emulsion and the better the retention of the core material embedded in the wall matrix (24). The high-pressure emulsification employed in this study resulted in the formation of emulsions with droplet sizes of 0.60-34.36 μm (Table 1). As seen in Table 2, κ-carrageenan concentration had the most influence on emulsion droplet size, as indicated by its highest quadratic term coefficient (β_1^2=12.60; $p<0.001$). The oil concentration showed a significant linear effect on emulsion droplet size at $p<0.01$.

From an industrial point of view, yield is the key parameter for a spray drying process, especially when working with high value products. Table 1 shows that most of the experimental runs produced a yield of less than 45%. This is understandable because the laboratory-scale spray dryer used in this work typically has a lower product yield in comparison to a pilot-scale spray dryer (25). Its smaller drying chamber does not guarantee that the particles are fully dried before they collide with the walls of chamber. The lumps of wet particles tend to stick to the walls of the drying chamber and cyclone, causing the loss of powder. As shown in Fig. 1A, the yield of the spray-dried powder increased initially at lower emulsifier concentrations (0.2-0.6%) up to a maximum value of 44.94%, after which it began to decline. However, the magnitude of the changes varied with the level of the oil concentration, as a result of significant synergism ($p<0.001$) between the emulsifier and the oil concentration.

Table 1. Face-centered central composite design (FCCCD) for optimizing the emulsion formulation of CoQ10-loaded κ-carrageenan microcapsules together with the experimental responses

Run order	Independent variables[1)]			Dependent variables (Responses)					
	X_1[2)]	X_2[3)]	X_3[4)]	Y_1[5)]	Y_2[6)]	Y_3[7)]	Y_4[8)]	Y_5[9)]	Y_6[10)]
1	1.00(0)	1.00(+1)	2.00(0)	2.74	40.97	98.37	11.59	2.52	0.373
2	1.00(0)	0.60(0)	3.00(+1)	1.32	39.10	95.78	15.30	1.61	0.309
3	0.50(-1)	0.60(0)	2.00(0)	22.05	45.20	90.74	20.30	1.39	0.369
4	1.50(+1)	1.00(+1)	1.00(-1)	16.85	30.25	95.69	5.89	1.09	0.274
5	1.00(0)	0.60(0)	2.00(0)	2.92	43.42	97.20	16.61	1.90	0.310
6	1.50(+1)	0.20(-1)	3.00(+1)	19.29	26.77	84.78	55.12	2.88	0.399
7	0.50(-1)	0.20(-1)	1.00(-1)	10.14	46.73	91.82	18.58	2.67	0.381
8	1.00(0)	0.20(-1)	2.00(0)	14.81	32.44	88.50	33.04	2.32	0.377
9	1.50(+1)	1.00(+1)	3.00(+1)	5.07	35.64	96.90	16.03	1.48	0.272
10	1.00(0)	0.60(0)	1.00(-1)	5.17	43.95	99.38	10.08	1.79	0.300
11	1.00(0)	0.60(0)	2.00(0)	3.20	44.34	95.88	17.37	1.85	0.307
12	1.00(0)	0.60(0)	2.00(0)	10.84	41.73	98.26	11.66	1.61	0.308
13	0.50(-1)	1.00(+1)	3.00(+1)	26.96	36.11	86.64	27.81	1.01	0.338
14	0.50(-1)	1.00(+1)	1.00(-1)	32.71	34.34	98.12	18.21	2.16	0.377
15	1.50(+1)	0.60(0)	2.00(0)	9.94	34.94	92.16	15.91	1.54	0.255
16	1.00(0)	0.60(0)	2.00(0)	2.99	43.29	97.49	12.19	1.85	0.280
17	1.50(+1)	0.20(-1)	1.00(-1)	34.36	38.27	91.13	19.16	2.57	0.247
18	1.00(0)	0.60(0)	2.00(0)	4.67	39.99	95.88	19.25	2.06	0.308
19	0.50(-1)	0.20(-1)	3.00(+1)	20.60	32.74	84.27	34.53	1.28	0.420
20	1.00(0)	0.60(0)	2.00(0)	3.35	44.03	95.40	12.30	1.71	0.288

[1)]Independent variables are presented in actual levels (coded levels). [2)]X_1: κ-carrageenan concentration (%, w/v). [3)]X_2: Emulsifier concentration (%, w/v). [4)]X_3: Oil concentration (%, w/v). [5)]Y_1: Emulsion droplet size (μm). [6)]Y_2: Yield (%, w/w). [7)]Y_3: Encapsulation efficiency (%, w/w). [8)]Y_4: Powder particle size (μm). [9)]Y_5: Moisture content (%, w/w). [10)]Y_6: Water activity (a_w).

Table 2. Estimated regression coefficients and analysis of variance (ANOVA) for the reduced model of response variables

Model parameters	Regression coefficient					
	Dependent variables[1)]					
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Intercept						
X_0	5.20	42.00	96.21	15.10	1.82	0.30
Linear						
X_1, κ-Carrageenan concentration	-0.69	-2.93**[2)]	0.91	-0.73	0.11	-0.044***
X_2, Emulsifier concentration	0.51	0.04	3.52***	-8.09***	-0.35***	-0.019*
X_3, Oil concentration	-4.60**	-2.32*	-2.78***	7.69***	-0.20*	0.016*
Quadratic						
X_1^2	12.60***	-	-4.99***	-	-0.43**	-
X_2^2	-	-6.57***	-	8.90***	0.52**	0.042***
X_3^2	-	-	-	-	-	-
Interaction						
X_{12}	-10.08***	-	-	-5.66**	-0.26*	-
X_{13}	-	-	1.74*	-	0.41***	0.019*
X_{23}	-	4.08***	-	-4.02*	-	-0.029**
p value	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	<0.0001
R^2	0.8545	0.8256	0.8552	0.8801	0.8621	0.8904
Adjusted R^2	0.8026	0.7633	0.8035	0.8248	0.7817	0.8399
Lack of fit	0.1159	0.0830	0.0504	0.1474	0.0912	0.0927

[1)]Y_1=Emulsion droplet size (μm); Y_2=Yield (%, w/w); Y_3=Encapsulation efficiency (%, w/w); Y_4=Powder particle size (μm); Y_5=Moisture content (%, w/w); Y_6 =Water activity (a_w).

[2)]*Significance at 0.05 level; **Significance at 0.01 level; ***Significance at 0.001 level.

A high EE (>80%) reported in Table 1 indicated that the entrapment of CoQ10 in the κ-carrageenan microcapsules via spray drying was effective. A similar observation was achieved by Rascón-Díaz *et al.* (26), who reported that the retention of acetaldehyde in spray-dried yogurt incorporating κ-carrageenan exceeded 90%. At all oil levels, the EE increased as the concentration of κ-carrageenan increased to 1.0%, possibly due to the rapid crust formation around the atomized oil droplets that prevented CoQ10 leakage from the ruptured emulsion (Fig. 1B). This finding is in agreement with a previous study on the encapsulation of indomethacin, where a higher EE was achieved with a greater amount of polymer (27). In contrast, a poorer EE was noticed when a greater amount of CoQ10-loaded oil was incorporated. The poorer retention of CoQ10 related to higher oil loads could be attributed to the insufficiency of the wall material, κ-carrageenan, to fully cover the oil droplets, which shortens the penetration path length of the core oil to the air/particle interface, thus diminishing the EE (28).

The negative synergism between the κ-carrageenan and emulsifier concentrations (β_{12}=-5.66) on the powder particle size gave rise to the shape of Fig. 1C. At the low SSL concentration, the microparticle size increased sharply from 26.50 to 37.01 μm with an increase in the encapsulant concentration, which could be attributed to the emulsion viscosity. The higher the feed viscosity, the larger the droplets formed during atomization at constant drying condition, and in turn the greater the size of the finished particles. This finding is supported by Tonon *et al.* (29) and Jinapong *et al.* (30) with spray-dried flaxseed oil and instant soymilk powders, respectively. Both authors associated the increase in product particle size to the increase in carrier agent concentration. Figure 1D illustrates that the mean diameter of the spray-dried microcapsules decreased significantly from 30.03 to 16.05 μm with increasing SSL concentration and then increased slightly thereafter. On the other hand, the mean powder particle size was directly proportional to the oil content in the feed emulsion.

Both moisture content and a_w of the spray-dried powder are important indicators of the drying efficiency and product quality. Table 1 shows that the spray-dried microcapsules had moisture contents within the range of 1.01-2.88%. These values were under the maximum moisture specification set for most dried powders in the food industry, which is between 3 and 4% (31). On the other hand, a_w was found to vary between 0.255 and 0.420. This suggests that the powders produced were relatively stable against almost all microbial activity (32,33).

In this study, the optimum formulation was determined based on the yield, EE, powder particle size, moisture content, and a_w. Although the EE of the spray-dried powder improved with reduced emulsion droplet size, once the droplet size was reduced past a certain level,

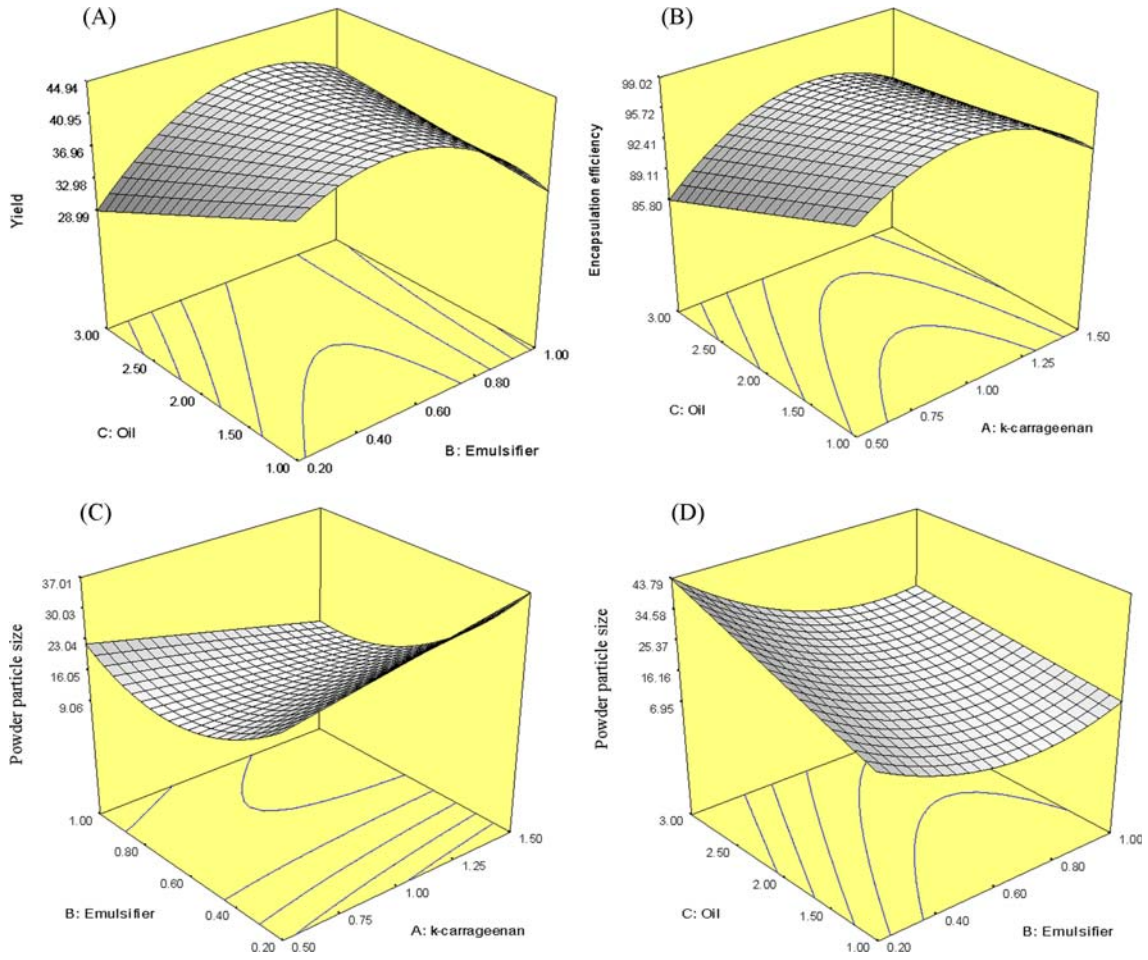

Fig. 1. Response surface plot for (A) yield; (B) encapsulation efficiency; and (C, D) powder particle size of spray-dried CoQ10-loaded κ-carrageenan microcapsules as the combined effect of κ-carrageenan, emulsifier, and oil concentration. The spray drying conditions were fixed throughout the experiment.

the EE did not improve any further. Therefore, emulsion droplet size was excluded in the optimization process. Based on the RSM results, the combination of 0.91% κ-carrageenan, 0.64% emulsifier, and 1.00% oil with the high desirability of 0.924 produced the best quality spray-dried CoQ10 powder. Under the optimum feed formulation, the experimental results were in close agreement with the predicted values with no significant difference (p>0.05) according to the one-sample t-test. This result confirms the suitability of the response surface model in predicting the quality attributes of CoQ10 encapsulated in κ-carrageenan microcapsules via spray drying.

Characterization of optimized spray-dried formulation

***Morphology*:** The SEM images of the κ-carrageenan (Fig. 2A), spray-dried κ-carrageenan (Fig. 2B), pure CoQ10 powder (Fig. 2C), and spray-dried CoQ10 in κ-carrageenan microspheres (Fig. 2D) are shown in Fig. 2. Under SEM observation, the pure CoQ10 was found to consist of a mixture of irregular shaped crystalline masses with rough surfaces and projections, which might be due to the micronization or any other size reduction process during the manufacturing time (Fig. 2C) (34). In the presence of the wall material, the SEM observation revealed a large number of optimized spray-dried CoQ10 microcapsule particles in spherical shapes with no obvious surface cracks, indicating a good protection of the core material (Fig. 2D).

***DSC*:** The thermal analyses of the raw materials and the spray-dried κ-carrageenan microcapsules encapsulated in CoQ10 were examined by DSC. A single sharp and intense endothermic peak was seen at 51.57°C, with an enthalpy of fusion (ΔH) of 117.81 J/g, due to the melting of crystalline CoQ10. Interestingly, the DSC scans found that the endothermic peak of spray-dried CoQ10 in the κ-carrageenan microcapsules was negligible. The absence of a melting peak in the spray-dried κ-carrageenan microcapsules confirmed that CoQ10 was well embedded in the polysaccharide matrices and was no longer present as a crystalline material, existing instead in an amorphous (non-crystal) form. High pressure homogenization prior to spray drying is effective in dispersing the crystalline CoQ10 in κ-carrageenan/SSL liquid emulsion, reducing the size of crystalline particles.

***XRD*:** To further confirm the physical state of CoQ10, XRD analyses were carried out on different samples and formulations. DSC is a quick method if rapid results are required, while XRD provides a

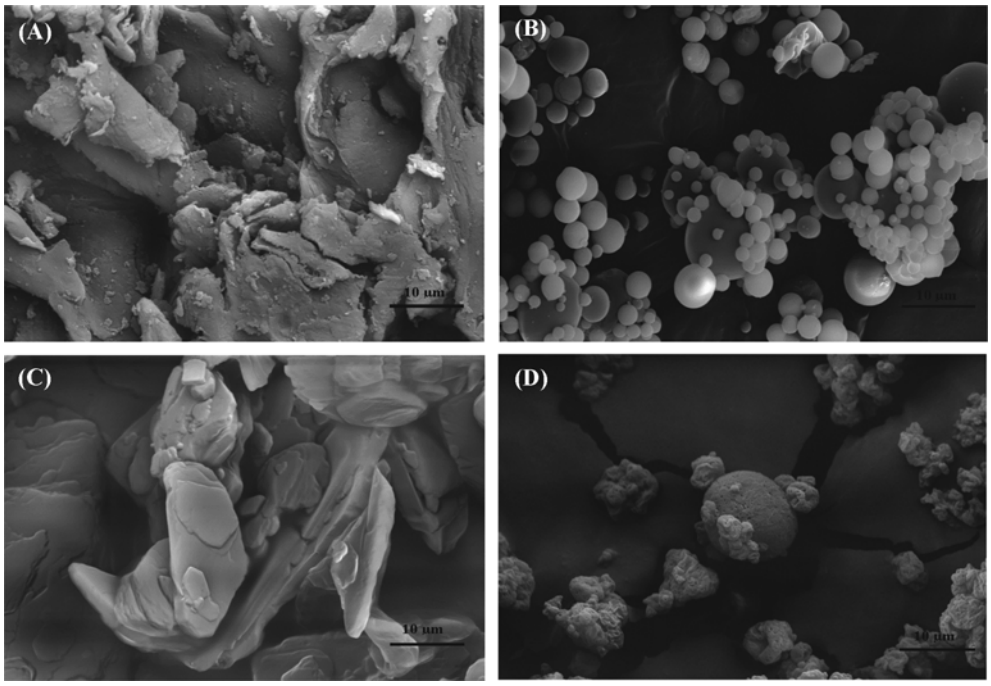

Fig. 2. SEM micrographs (x1,500) of control powder and κ-carrageenan encapsulated CoQ10 powder: (A) κ-carrageenan, (B) spray-dried κ-carrageenan, (C) CoQ10 crystals, and (D) optimized spray-dried CoQ10-loaded κ-carrageenan microcapsules. Scale bar in all images represents 10 μm.

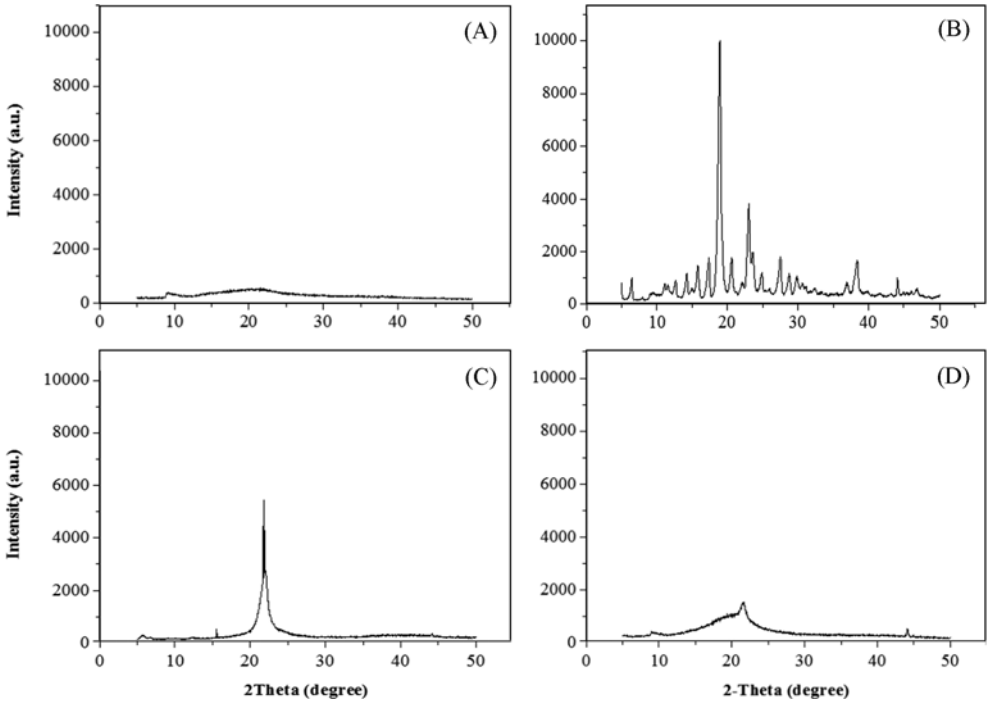

Fig. 3. XRD analysis of (A) κ-carrageenan, (B) pure CoQ10, (C) SSL, and (D) spray-dried CoQ10 in κ-carrageenan microcapsules.

slower, but more accurate result. The XRD patterns of CoQ10 and SSL exhibited a sharp and well-defined peak at 18.92° (2θ) and 21.94° (2θ), respectively (Fig. 3B and 3C). This result indicates that both pure CoQ10 and SSL were crystalline materials, which is consistent with the measurements obtained from DSC. As seen in Fig. 3A and 3D, κ-carrageenan and spray-dried CoQ10 in κ-carrageenan microcapsules were in a completely amorphous state, as confirmed by the presence of broad and non-defined peaks with abundant noise. Furthermore, the characteristic peak of spray-dried CoQ10 in κ-carrageenan microcapsules was much lower than the respective peak of the pure

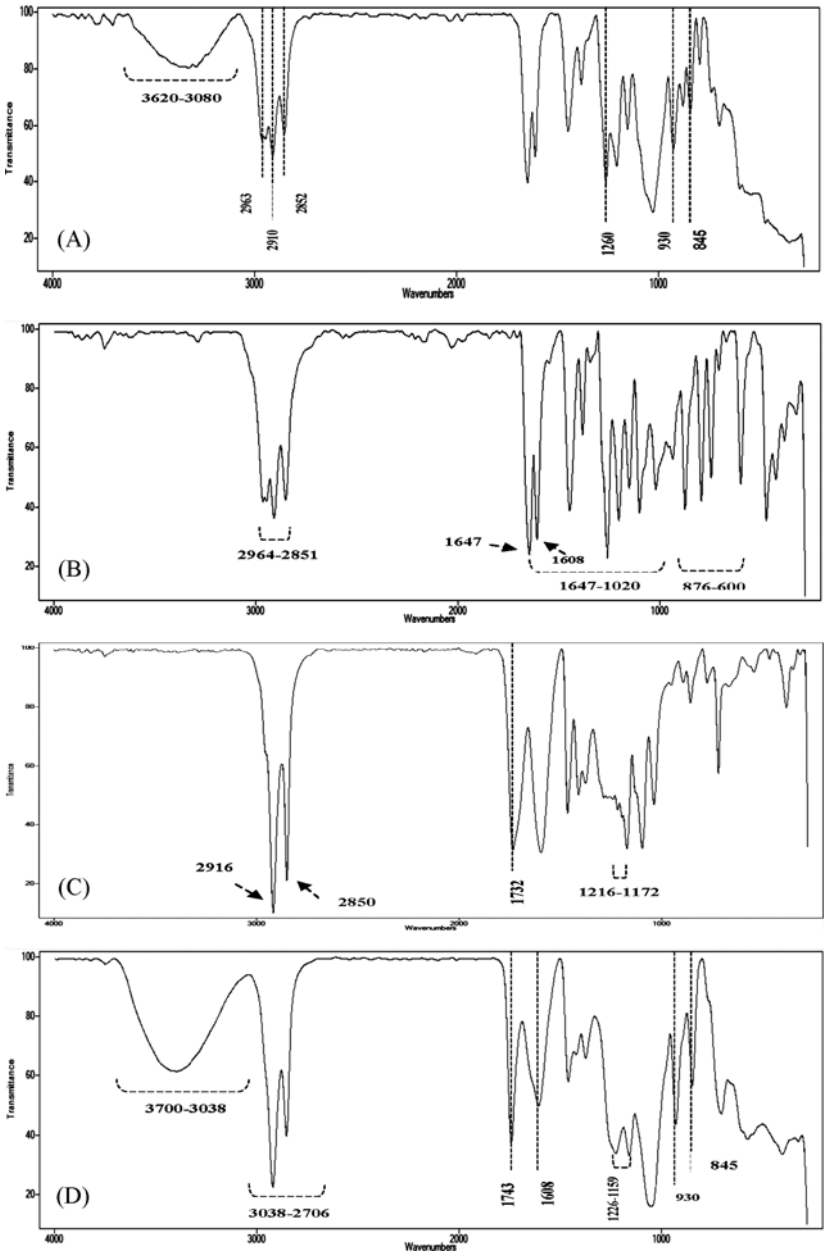

Fig. 4. FTIR spectra of (A) κ-carrageenan, (B) pure CoQ10, (C) SSL, and (D) spray-dried CoQ10 in κ-carrageenan microcapsules.

CoQ10 powder. These findings are consistent with the results from the DSC analysis, suggesting that the crystallinity of CoQ10 molecules was greatly reduced by the spray drying process during the preparation of the water-soluble powder formulation.

***FT-IR*:** FTIR was performed to characterize the molecular structures of the raw ingredients and to understand any possible intermolecular interaction between CoQ10 and the carrier agent, κ-carrageenan. Figure 4A presents the typical FTIR spectra of a commercial κ-carrageenan. A particularly strong band was recorded in κ-carrageenan at approximately 845 cm^{-1}, which was assigned to G4S (35,36). Another intense signal at approximately 930 cm^{-1} indicates the presence of 3,6-AG (35,36). As demonstrated in Fig. 4B, the spectrum of the pure CoQ10 model drug exhibited strong absorption bands in the range of 2,964-2,851, 1,647-1,020 and 876-600 cm^{-1} (37). Two characteristic sharp peaks of CoQ10 were obtained at 1,608 cm^{-1} for the benzoquinone ring and at 1,647 cm^{-1} for the monosubstituted isoprenoid units (38). Figure 4C shows the FTIR spectra of the SSL emulsifier. The absorption bands of SSL at 2,916 and 2,850 cm^{-1} corresponded to C-H stretching in the alkane and aldehyde groups, respectively, in the structure. Furthermore, the appearance of a sharp peak in the range of 1,216-1,172 cm^{-1} was contributed by the C-C(O)-C in the SSL chemical skeleton. Comparing the spectra of the spray-dried CoQ10 in κ-carrageenan microparticles (Fig. 4D) to the spectra of the individual raw ingredients (κ-carrageenan, CoQ10,

palm kernel oil, and SSL), none of the characteristic peaks were significantly shifted. However, peak broadening was observed in some of the components due to their amorphous character. The disappearance of the absorption band of CoQ10 at 1,647 cm^{-1} (from the long, lipophilic isoprenoid side-chain) could be attributed to possible interactions between the CoQ10 and κ-carrageenan or the palm kernel oil, which may enhance the water solubility of CoQ10. ***Solubility*****:** The result showed that CoQ10 (in either the pure or oil form) exhibited a negligible solubility (≈0 mg/L) in water regardless of the incubation temperature used (25 and 37°C). Pure CoQ10 powder and CoQ10 in oil either floated on the surface of the water or stuck to the wall of the centrifuge tube after agitation. Compared to pure CoQ10 and CoQ10 in oil, the physical mixture displayed a significantly (p<0.05) higher solubility of 804.34 and 929.00 mg/L at 25 and 35°C, respectively. Of all the formulations assessed, the CoQ10 encapsulated in κ-carrageenan microparticles showed the highest water solubility of 2,234.55 and 2,242.53 mg/L at 25 and 37°C, respectively. The decreased crystallinity of the spray-dried CoQ10, as assessed by DSC and XRD analysis, might be responsible for the enhanced solubility of this formulation (39). Furthermore, it was observed that CoQ10 solubility enhanced by mild heating.

In conclusion, this study proved that κ-carrageenan microcapsules developed using a spray drying method could be utilized as an effective carrier to solubilize the poorly water soluble CoQ10. The water-soluble CoQ10 offers the food industry the possibility to create newly innovative water-based products, such as beverages, by using it as either the main ingredient or an enriched additive.

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