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The Effect of Pharmaceutical Excipients for Applying to Spray-Dried Omega-3 Powder

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Abstract: Omega-3 fatty acid plays a role in protecting cells in the human body, maintaining the structure of the cell, and helping smooth metabolism. Also, it inhibits the formation of blood clotting and is effective in enhancing the formation of bone. However, the instability due to fatty acid oxidation and a fishy smell are the reasons it is avoided by people. In this study, we tried to obtain the omega-3 powder through spray-drying method using a variety of binders and surfactants for improving the limit of omega-3 fatty acid. First of all, an olive oil was used instead of omega-3 for optimization of the preparation of spray-dried omega-3 powder. Through the screening of binders and surfactants, γ-cyclodextrin and hydrogenated lecithin were chosen as a binder and a surfactant, respectively. Omega-3-loaded spray-dried powder was obtained, eventually. The morphology of omega-3-loaded spray-dried powder was spherical of 310 nm and the DHA amount was 98%. This study suggested that the transformation of omega-3 fatty acid into solid state by spray-drying using a binder and a surfactant was successively performed.

Keywords: omega-3 fatty acid; spray drying; powder; pharmaceutical excipients

1. Introduction

Solid formulations are highly preferred in the pharmaceutical market because they easy to administer and have superior stability during storage. On the other hand, the liquid formulation is easily hydrolyzed and oxidized. Oil components such as fatty acid were filled into soft capsules and marketed. However, these soft capsules have still some problems by leaking. In particular, the omega-3 is an unsaturated fatty acid, which is composed of DHA (docosahexaenoic acid) and EPA (eicosapentaenic acid). DHA or EPA is composed of 20 or 22 carbon atoms with five or six double bonds and has a first double bond at the third methyl group from the ω side. EPA was formed from α-linolenic acid (18:3 ω-3) and DHA was obtained from EPA. α-Linolenic acid was synthesized from linoleic acid (18:2 ω-6). Both α-linolenic acid and linoleic acid have been regarded as essential fatty acids because they cannot be synthesized in the body. However, the efficiency of the α-linolenic acid converted to EPA and DHA in adults is low with about 10–15%, about 3–6% in children [1]. It has been reported that intake of EPA and DHA may help to improve blood circulation, stimulate the breakdown of fibrin, and reduce blood pressure and blood triglyceride levels [2–7]. Therefore, customers had better take foods containing a lot of DHA and EPA in order to expect the effect of EPA and DHA. Long chain polyunsaturated fatty acids such as omega-3 are easily oxidized, leading to instability.

This limitation yields their shelf-life only 6 months, when stored at 4 °C in a closed container under nitrogen [8].

So, there are many advantages as follows: firstly, possible reduction in the volume of administration (e.g., converting a liquid emulsions or suspensions containing more than 50% liquid into the dry state that can be filled/processed into single capsule or tablet unit) if omega-3 changes into solid forms. Secondly, enhanced precise dosing (e.g., packing of the whole dose into capsules/tablets provides more accurate and precise dosing in comparison to administering a prescribed volume of a suspension using a syringe or spoon). Thirdly, ease of transfer and storage (i.e., liquid formulations are bulky and typically prone to instability and microbial contamination). Fourthly, better patient compliance (i.e., it is generally preferred by adult patients to administer capsules/tablets rather than a liquid formulation) [2,4–6]. As well as, the herbal extracts, for example, mulberry extracts need to be solidified for the stability. The general approaches for solidification include physical adsorption onto solid carriers, spray drying, freeze-drying or lyophilization, rotary evaporation, melt extrusion-spheronization and melt granulation [9]. Omega-3 fatty acids are essential nutrients that are important in preventing and managing heart disease, which are in a class of medications called antilipidemic or lipid-regulating agents. Therefore, authors focused on the preparation and characterization of spray-dried omega-3 powder for the combination with other medications. First of all, the optimization was performed using a cheap olive oil instead of an expensive omega-3 for the preparation of a spray-dried powder in this study.

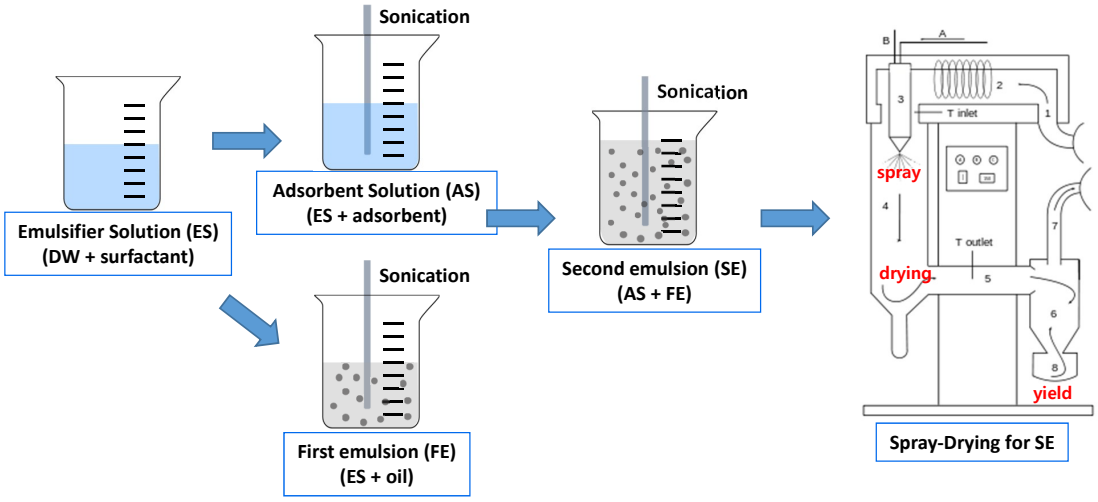
2. Materials and Methods

2.1. Materials

Omega-3 fatty acid containing EPA (460 mg/mL) and DHA (380 mg/mL) were received from Kuhnil Pharmaceutical co., Ltd. (Seoul, Korea). Edible olive oil was purchased from Sempio Co., Ltd. (Seoul, Korea). Methyl cellulose (MC) was purchased Junsei Chemical Co., Ltd. (Tokyo, Japan). Hydropropylmethylcellulose (HPMC, Metolose® 60SH-50) was received from Richwood trading Co., Ltd. (Pyeongtaek, Korea). Gelatin type A (G1890), type B (G9391), hydrogenated lecithin and PEG-40 glyceryl monostearate were purchased from Sigma-Aldrich Co., Ltd. Korea (Yongin, Korea). α, β, and γ-cyclodextrin (α, β, and γ-CD) was provided from Whawon Pharm Co., Ltd. (Seoul, Korea). Polyvinylpyrrolidone (PVP, Kollidone® K30) and poloxamer 188 (Lutrol® F68) was received from BASF Co., Ltd. Korea (Seoul, Korea). All other excipients were used as received without further purification.

2.2. Preparation of Spray-Dried Powder

The emulsifier solution (ES) was prepared by dissolving surfactant in distilled water (DW). The adsorbent solution (AS) was prepared by dispersing the pharmaceutical excipients such as MC, HPMC, PVP, α, β, γ-CD, gelatin A and gelatin B in ES and sonicated for 10 min with an amplitude of 85%, 130 watt, 20 kHz frequency, and pulse cycle of 15 s/8 s to disperse the adsorbent. The first emulsion (FE) was prepared by adding an olive oil to ES, which was sonicated until the oil droplet was disappeared by the same condition with the preparation of AS. The second emulsion (SE) was prepared by mixing AS with FE. The SE containing 50% (*w*/*w*) olive oil was stored in the dark until spray drying. Spray drying was conducted at a flow rate 1.0 L/h, 150 °C inlet temperature and 70~75 °C outlet temperature using spray dryer with a single nozzle atomizer (SD-1000, Eyela, Japan) (Scheme 1). The spray-dried powder was stored at −20 °C until use. MC, HPMC, and PVP were used for amphiphilic polymers. α, β, and γ-CD were used for polysaccharide-based material. Gelatin type A and B were used based on the characteristics of the film formation of gelatin. On the other hand, poloxamer 188, hydrogenated lecithin or PEG-40 glyceryl monostearate as an amphiphilic surfactant.



Scheme 1. The spray drying process for the preparation of oil powder.

2.3. Determination of Particle Size of Spray-Dried Powder

The particle size of SE before spray-drying and the reconstituted emulsion (RE) after spray-drying was determined by ELS-8000 laser scattering particle size analyzer (Otsuka Electronics Co., Osaka, Japan), which measures particle size and particle-size distribution based on the dynamic light-scattering method. The primary results from dynamic light scattering (DLS) systems are typically reported as an intensity distribution. Key values included in DLS-based specifications are the intensity-weighted average (often called the "z average") and the polydispersity index (PI), which quantifies distribution width. The SE was diluted with DW enough to prevent multiple scattering effects. For analysis of RE, 0.1 g of spray-dried powder was dispersed in 10 mL of DW, and vortexed for 5 min. Then, RE was diluted with DW enough to prevent multiple scattering effects.

2.4. Determination of Moisture Amount, Free Oil, Encapsulated Oil, and Total Oil of Spray-Dried Powder

Approximately 3 g of spray-dried powder was placed in an aluminum plate and the moisture amount was determined using moisture determinant balance (FD-600, Kett electric Lab., Tokyo, Japan). All determination was repeated three times. Extraction of free oil, encapsulated oil, and total oil from spray-dried oil powder was followed by modification of publication [10].

Ten mL of hexane was added to 1.0 g of spray-dried oil powder. The mixture was mixed using vortex mixer for 1 min and was centrifuged at 4500 rpm for 10 min. Then, the supernatant was filtered using filter paper, and the filter paper washed with 10 mL of hexane. The residual hexane onto the filter paper was evaporated under nitrogen at 40 °C and the filter paper was dried in 70 °C. The amount of excess free oil was determined by measuring the filter paper, gravimetrically.

For encapsulated oil, 3 mL of water was added to 0.5 g of free oil-extracted powder and mixed using vortex mixer for 5 min. Then, 10 mL of hexane/isopropanol (3:1 v/v) was added to the mixture. The resulting mixture was shaken for 20 min using an automatic mixer, and then centrifuged for 10 min. The upper organic phase was collected. Lower aqueous phase was re-extracted with 10 mL of hexane/isopropanol (3:1 v/v). The collected solution was filtered using filter paper, and solvent was evaporated under nitrogen at 40 °C. After evaporation, the filter paper was dried at 70 °C oven. The amount of encapsulated oil was calculated by the difference between the free oil-extracted powder and the filter paper, gravimetrically.

For total oil, 3 mL of water was added to 0.5 g of spray-dried oil powder and mixed for 5 min. Then, the procedure of extraction was conducted using the same method as mentioned above for extraction of encapsulated oil.

2.5. Scanning Electron Microscopy (SEM)

A SEM (JEOL JSM-7500F, Thermo, Waltham, MA, USA) was used to observe and compare the morphology of spray-dried powder between different formulations. Samples were mounted on stubs using double stick carbon tape. A gold/palladium was then applied to the surface in a vacuum evaporator to make samples conductive and samples were coated. All samples were examined at an acceleration voltage of 1 to 5 kV. Photographs were taken at 2.0 k, 4.0 k, and 10.0 k magnification.

2.6. Chromatography Condition

The analysis of EPA and DHA was performed on a HP 6890 gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with an autosampler and a flame ionization detector. The samples were analyzed on a HP-5 capillary column (50 mm × 0.32 mm × 0.52 μm). Data collection was performed by the HP chemstation software. The temperature program was as follows: the oven temperature was held at 60 °C for 1 min, ramped to 160 °C at 25 °C/min, held at 160 °C for 28 min, increased to 190 °C at 25 °C/min, held at 190 °C for 17 min, ramped to 220 °C at 25 °C/min and held at 220 °C for 10 min. Direct on-column injection was used. The injector port temperature was ramped instantly from 50 to 250 °C and the detector temperature was 250 °C. The carrier gas was ultrapure helium at a pressure of 82 kPa. Analysis time was 60 min. Analysis method and sample preparation were followed by the method of Araujo et al. [11]. Briefly, 50 μL (or 50 mg) of sample was mixed with 2 mL of BF3/CH_3OH. And the mixture was heated at 100 °C for 1 h and cooled down at room temperature. Then, 1 mL of hexane and 2 mL of DW were added, vortexed for 15 s, centrifuged at 3000 rpm for 2 min and the methyl esters were extracted from the upper hexane phase.

2.7. Statistical Analysis

The obtained data were evaluated by one way-ANOVA, $p < 0.05$ was considered as the significant level.

3. Results

3.1. Preparation of Spray-Dried Emulsion Powder

Recently, powders with 30%, 35% and 40% (w/w) load of triglyceride oil and 25%, 30% and 40% (w/w) load of ethyl ester oil, respectively, were prepared to further explore how much fish oil can be included in a direct compression grade powder. All powders with up to 35% (w/w) triglyceride oil and 30% (w/w) ethyl ester oil, respectively were dried by spray granulation [12].

Amphiphilic polymers such as poloxamers, HPMC, carboxymethylcellulose sodium and PVP had many applications as emulsifiers and solid carriers [8,13,14]. On the other hand, owing to gelatin's film-forming properties, gelatin had been used to encapsulate fish oils and oily vitamin in gelatin beadlets using coacervation technique, which can then be handled as a powdered formulation. Polysaccharide (or carbohydrate)-based excipients had a long history of applications in the food and pharmaceutical industries as sweeteners, coating agents, bulking agents, viscosity-enhancers, tablet/capsule binders, diluents, and direct compression agents, which are low molecular weight mannitol, sorbitol, sucrose, lactose, trehalose, and the higher molecular weight maltodextrins, cyclodextrins, dextrins, gum acacia and starch sodium octenyl succinate, etc. [15]. Twenty-eight spray dried olive oil powder was obtained.

According to the surfactants, 28 formulations were prepared using olive oil as a substitute of omega-3. In detail, various pharmaceutical excipients such as MC, HPMC, PVP, gelatin A, gelatin B, α-CD, β-CD, and γ-CD were used with fixing poloxamer 188 as a surfactant for spray-dried olive oil powder. The formulations were numbered from P1 to P8 according to the order of pharmaceutical excipients. The spray-dried olive oil powder using pharmaceutical excipients such as MC, HPMC, PVP, gelatin A, gelatin B, α-CD, β-CD, and γ-CD was prepared with a fixation of hydrogenated lecithin as a surfactant. The formulations were numbered from P11 to P18 according to the order

of pharmaceutical excipients. PEG40 glyceryl monostearate as surfactant was used for spray-dried olive oil powder using pharmaceutical excipients such as MC, HPMC, PVP, gelatin A, gelatin B, α-CD, β-CD, and γ-CD. The formulations were numbered from P21 to P28 according to the order of pharmaceutical excipients. The amount of pharmaceutical excipients or surfactant was 6.8 g or 2.5 g over all formulations, respectively. The olive oil was set to 50% in spray-dried powder. Interestingly, in P13, P14, P15, P23, P24, and P25, spray-dried powder was not obtained. So, the physicochemical characterizations of P13, 14, 15, 23, 24 and 25 were not carried out. There was a publication that the adsorption of polyvinylalcohol spontaneously occurs from aqueous suspensions onto the surface of hydrophobic drug and had been affected by % hydrolysis and molecular mass [16]. It could be suggested that spray-dried powder was not obtained through the strong adsorption when more viscous excipients such as PVP, gelatin A and gelatin B interact with more hydrophobic surfactants such as hydrogenated lecithin or PEG40 glyceryl monostearate during spray-drying step.

3.2. The Particle Size of Spray-Dried Powder

The particle size of SE before spray-drying and RE after spray-drying was shown in Table 1. The droplet sizes of SE and RE were several hundred nanometers. Most formulations showed higher droplet size of RE than that of SE except for P4, P16 and P18. P4, P8, P16, P18, and P21 showed 80~120%.

Table 1. The particle size of second emulsion (SE) before spray drying and reconstituted emulsion (RE) of olive oil powder after spray drying (n = 3).

Formular	SE (nm)	RE (nm)
P1	488.85 ± 30.8	600.85 ± 76.6
P2	396.85 ± 3.0	483.95 ± 65.3
P3	252.40 ± 11.5	469.55 ± 76.2
P4	711.70 ± 168.1	660.85 ± 171.8
P5	214.35 ± 3.5	473.50 ± 141.8
P6	250.80 ± 8.9	360.40 ± 50.3
P7	268.95 ± 13.4	374.75 ± 155.6
P8	258.95 ± 7.3	293.35 ± 19.0
P11	296.47 ± 22.8	472.73 ± 17.0
P12	336.93 ± 37.8	472.97 ± 16.0
P16	257.00 ± 8.3	216.57 ± 2.5
P17	237.40 ± 10.1	330.20 ± 38.2
P18	280.57 ± 17.8	246.20 ± 19.9
P21	284.87 ± 5.1	342.13 ± 10.9
P22	285.97 ± 2.0	405.60 ± 9.1
P26	540.10 ± 39.5	847.70 ± 58.3
P27	354.87 ± 3.3	695.83 ± 108.0
P28	339.23 ± 24.1	712.20 ± 72.6

3.3. Determination of Moisture Content, Free Oil, Encapsulated Oil, and Total Oil

The moisture content, free oil, encapsulated oil, and total oil of formulations according to binder and surfactant were presented in Table 2. When poloxamer 188 was used as a surfactant, the moisture (%) of spray-dried oil powder (P1~P8) was from 3.50 to 8.30%. When hydrogenated lecithin was used as a surfactant, the moisture (%) of spray-dried oil powder (P11~P18) was from 2.85 to 4.00%. When PEG40 glyceryl monostearate was used as a surfactant, the moisture (%) of spray-dried oil powder (P21~P28) was from 3.80 to 6.45%. Most formulations showed about 10% of encapsulated oil. Generally, small difference between droplet size of SE and RE indicated well re-dispersibility and stability. The moisture (%) of spray-dried oil powder is 2.85~8.30% over the formulations. When hydrogenated lecithin was used as a surfactant, the encapsulated oil in spray-dried oil powder was more detected than in the group of poloxamer 188 or PEG40 glyceryl stearate as a surfactant.

Table 2. The comparison of spray-dried olive oil powder by determining the moisture amount and oil amount (free oil, encapsulated oil and total oil) (n = 3).

Formular	Moisture (%)	Free Oil (g/100 g)	Encapsulated Oil (g/100 g)	Total Oil (g/100 g)
P1	5.10 ± 0.70	48.28 ± 1.42	10.85 ± 3.82	66.42 ± 1.33
P2	3.50 ± 0.14	36.66 ± 3.26	10.30 ± 0.66	56.41 ± 2.47
P3	5.05 ± 0.49	39.44 ± 3.48	5.92 ± 1.05	58.30 ± 0.30
P4	8.30 ± 2.12	40.00 ± 2.99	7.79 ± 1.18	60.75 ± 0.83
P5	6.20 ± 1.13	39.36 ± 0.90	9.24 ± 2.65	62.42 ± 0.23
P6	3.55 ± 0.21	38.87 ± 2.21	13.74 ± 1.98	58.33 ± 3.01
P7	4.10 ± 0.99	39.35 ± 1.60	9.66 ± 1.36	59.37 ± 2.29
P8	4.98 ± 1.20	37.96 ± 2.97	8.66 ± 3.28	56.02 ± 5.89
P11	2.90 ± 0.14	58.04 ± 1.53	17.33 ± 3.86	56.04 ± 2.90
P12	2.85 ± 0.07	47.80 ± 0.37	11.36 ± 3.64	58.49 ± 7.90
P16	3.75 ± 0.21	52.59 ± 0.97	10.27 ± 2.64	54.45 ± 1.79
P17	3.75 ± 0.49	48.04 ± 1.44	10.86 ± 2.02	54.78 ± 2.93
P18	4.00 ± 0.14	49.55 ± 0.63	10.18 ± 2.95	56.96 ± 4.26
P21	5.90 ± 1.70	57.78 ± 0.42	11.77 ± 0.83	67.43 ± 4.52
P22	4.75 ± 1.91	52.36 ± 0.44	10.22 ± 0.62	56.26 ± 1.94
P26	6.45 ± 1.20	56.63 ± 5.43	9.18 ± 0.26	59.69 ± 3.84
P27	3.80 ± 0.57	62.55 ± 2.06	8.57 ± 1.58	65.79 ± 6.57
P28	5.50 ± 0.85	65.68 ± 1.69	7.73 ± 0.53	67.51 ± 2.03

3.4. SEM Image

Figure 1 showed the morphology of P8, P16, P18 and P21, respectively. P8 and P16 looked like forming aggregates. Maybe, it was due to incomplete evaporation or oil leakage. P18 showed the spherical shape with many pores but its surface was smooth. P21 seemed like a wrinkled sphere and had many pores at surface, which made the particles stick together. These results are consistent with publications, which detected wrinkles on the surface of the particles in spray-dried powder [10,16]. SEM image of spray-dried oil powder looked like forming aggregates due to incomplete evaporation or oil leakage. It was reported that wrinkles were attributed to the results of mechanical stresses induced by uneven drying at different parts of the droplets during the early stages of drying [17], to the movement of the moisture during the surface drying period [18], and to the effect of a surface tension-driven viscous flow [17]. Wrinkles of the particle followed by an excipient expansion may induce changes in the size of particles and causes the wall material to break [19]. Therefore, P18 was chosen for further study, which was confirmed using a software of Design Expert® 11 (Tables S1–S3). The key parameters to obtain the optimized formulation are SE, RE and morphology (Figure S1).

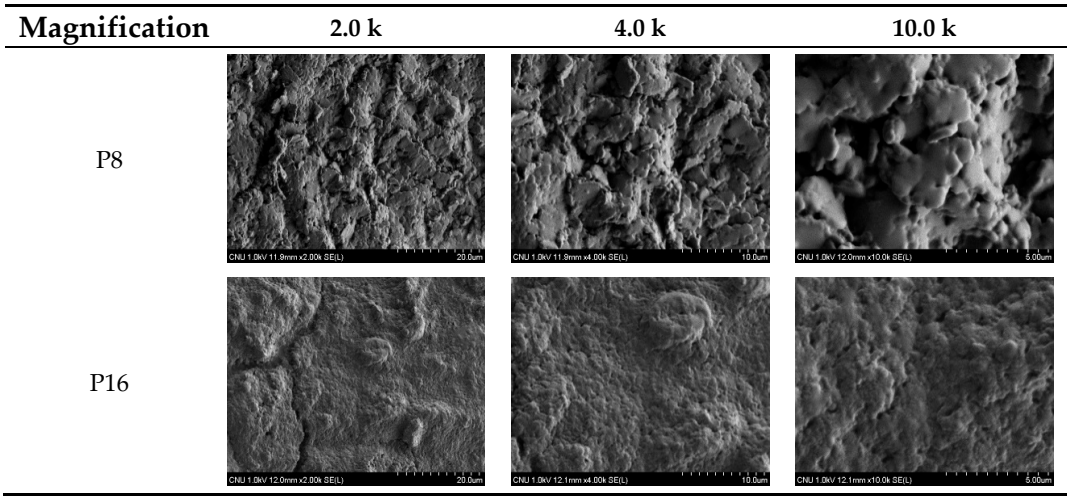


Figure 1. *Cont.*

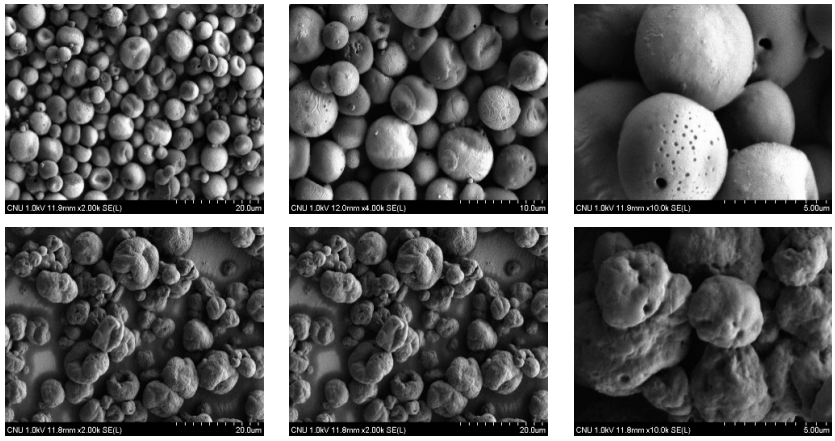


Figure 1. SEM image of spray-dried olive oil powder, P8, P16, P18, and P21. P18 showed the spherical shape with many pores but its surface was smooth.

3.5. Characteristics of Spray-Dried Powder According to Ratio of Encapsulant To Surfactant

The formulations were varied from P18-1 to P18-5 according to ratio of γ-CD to hydrogenated lecithin. On the other hand, the operating conditions for spray-drying was varied. P18-6 was diluted with twice volume of water compared with P18-4. P18-6 and P18-7 was prepared to confirm the effects of inlet and outlet temperature. P18-8 was prepared to check the effect of atomization pressure (Table 3).

Table 3. Various spray drying condition for spray-dried olive oil powder using γ-CD and hydrogenated lecithin.

Formular	Inlet Temp. (°C)	Outlet Temp. (°C)	Atomization Pressure	Olive Oil (g)	DW (mL)	γ-CD (g)	Hydrogenated Lecithin (g)	Total (g)
P18-1	180	85–90	15	9.3	200	4.8	4.5	18.6
P18-2	180	85–90	15	9.3	200	5.8	3.5	18.6
P18-3	180	85–90	15	9.3	200	6.8	2.5	18.6
P18-4	180	85–90	15	9.3	200	7.8	1.5	18.6
P18-5	180	85–90	15	9.3	200	8.8	0.5	18.6
P18-6	180	85–90	15	9.3	400	7.8	1.5	18.6
P18-7	150	70–75	15	9.3	400	7.8	1.5	18.6
P18-8	150	70–75	10	9.3	400	7.8	1.5	18.6

3.6. Determination of Particle Size, Moisture and Free Oil, Encapsulated Oil, and Total Oil

The moisture (%) of formulations was about 4% and there is no significant difference between formulations. The diluted emulsion formulation (P18-6) had lower moisture content than that of P18-4. The droplet size of SE and RE was ranged from 300 to 400 nm. Droplet size of RE increased than droplet of SE. But, there was no difference significantly between SE and RE under the various spray-drying operating condition. The content of free oil was ranged from 30.33 to 42.23% (Table 4).

Table 4. The effect of various spray drying condition on moisture amount, particle size and oil amount (free oil, encapsulated oil and total oil) of spray-dried olive oil powder using γ-CD and hydrogenated lecithin (n = 3).

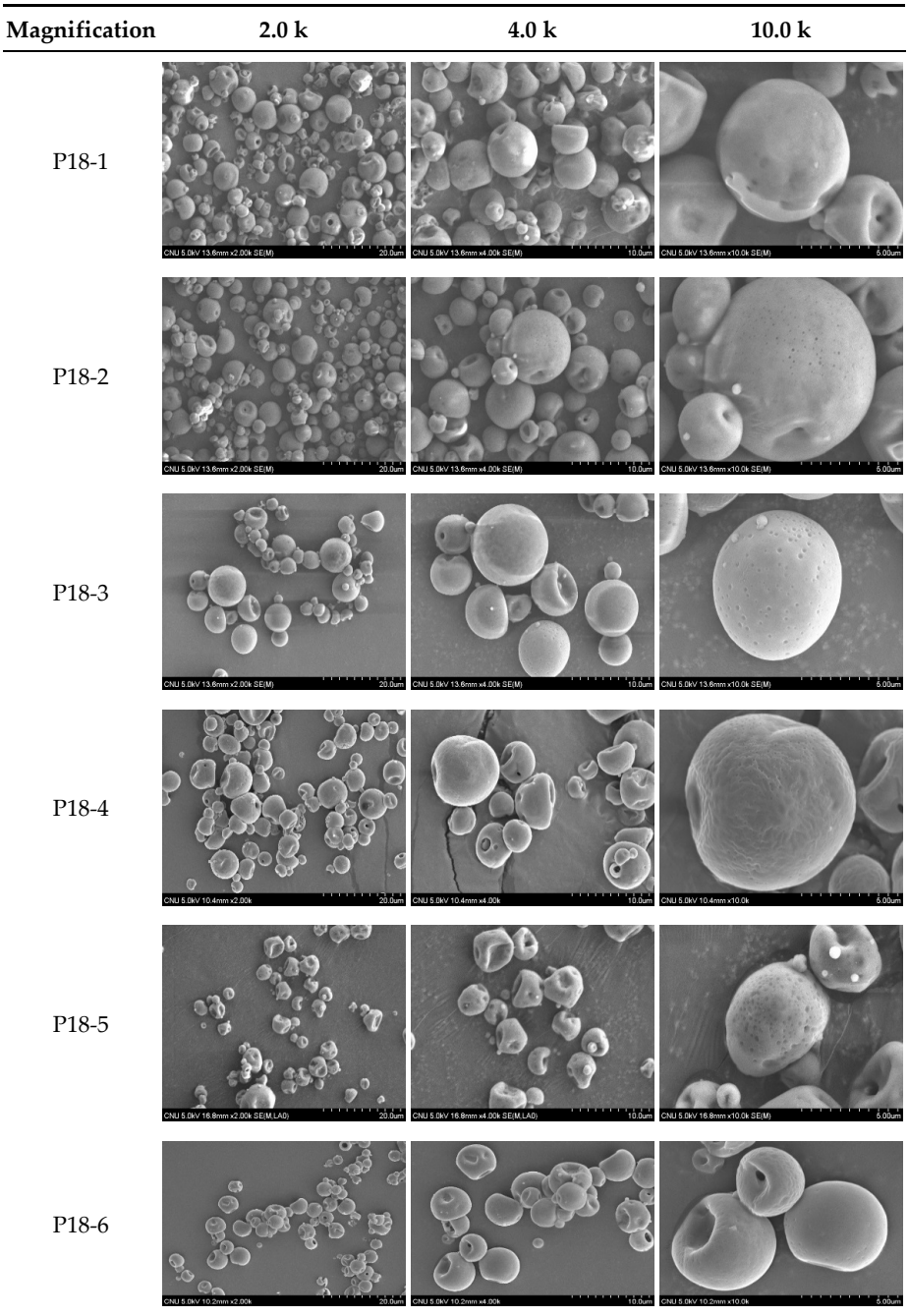
Formular	Moisture (%)	SE (nm)	RE (nm)	Free Oil (g/100 g)	Encapsulated Oil (g/100 g)	Total Oil (g/100 g)
P18-1	4.17 ± 0.50	337.17 ± 8.32	351.77 ± 9.13	42.23 ± 0.03	10.20 ± 0.64	56.95 ± 0.70
P18-2	3.67 ± 0.40	281.70 ± 1.93	388.13 ± 13.88	39.30 ± 4.60	11.14 ± 0.19	54.63 ± 2.11
P18-3	3.87 ± 0.31	305.13 ± 3.45	323.20 ± 7.37	37.98 ± 4.67	12.35 ± 1.74	52.84 ± 3.52
P18-4	3.40 ± 0.30	341.80 ± 4.95	344.73 ± 11.86	35.33 ± 1.21	14.12 ± 0.88	52.10 ± 1.52
P18-5	3.67 ± 0.25	352.77 ± 24.8	378.33 ± 10.82	33.67 ± 0.07	14.22 ± 2.78	51.58 ± 1.85

Table 4. *Cont.*

Formular	Moisture (%)	SE (nm)	RE (nm)	Free Oil (g/100 g)	Encapsulated Oil (g/100 g)	Total Oil (g/100 g)
P18-6	2.03 ± 0.15	355.83 ± 21.1	366.80 ± 16.51	32.53 ± 2.20	18.55 ± 0.67	52.77 ± 0.94
P18-7	2.33 ± 0.06	354.93 ± 6.21	337.37 ± 15.46	30.33 ± 0.83	19.14 ± 1.07	51.89 ± 0.93
P18-8	2.43 ± 0.15	328.37 ± 19.5	355.97 ± 14.28	29.21 ± 0.66	19.38 ± 0.81	52.44 ± 1.30

3.7. Characteristics of Omega-3 Fatty Acid Powder

The morphology of P18-1 to P18-4 was dented sphere and they had many pores. However, P18-5 had excessively many dent with an irregular shape. There is no significant difference in morphology according to inlet temperature, outlet temperature and atomization pressure (Figure 2).



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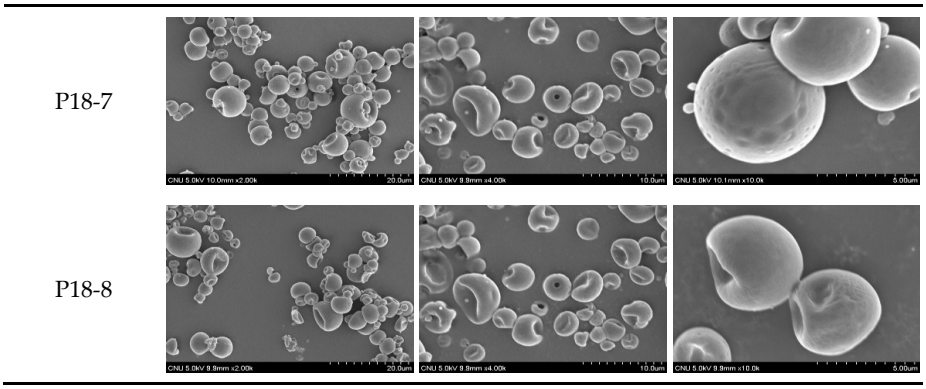

Figure 2. The effect of various spray drying condition on the morphology of spray-dried olive oil powder.

P18-8 was chosen as the best formulation, which had the smallest particle size before spray drying, showed the least amount of free oil and the highest amount of encapsulated oil, and showed relatively uniform morphology without dent. The morphology of omega-3 powder was shown in Figure 3. Even though unsaturated fatty acids do not solidify and exist in liquid form at low temperatures due to the structurally unstable nature [20], the morphology of omega-3 powder differed from powder containing olive oil. Comparing with formulation P18-8, only free-extractable oil content was increased significantly at P-O formulation, and there was no significant difference on the others properties, contrastively having difference in morphology. The free-extractable oil content of P-O formulation was about 33%. And the encapsulated-oil content was approximately 18%. The droplet size was about 310 nm to 320 nm.

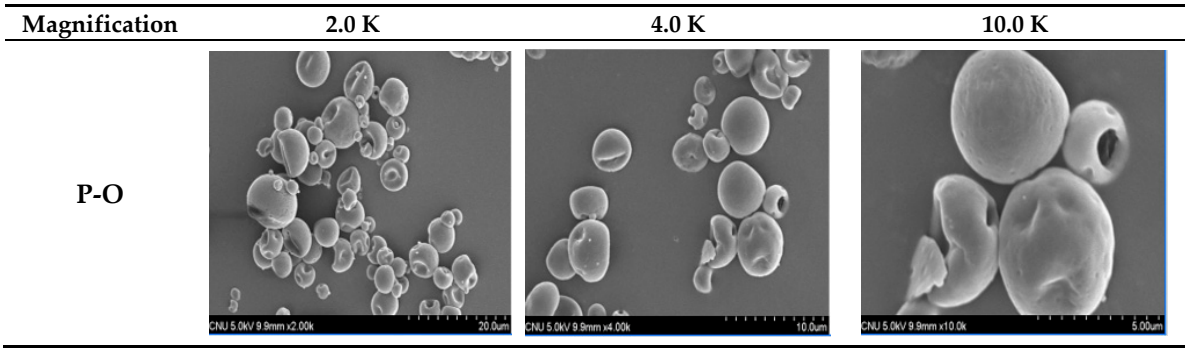

Figure 3. The SEM image of spray-dried omega-3 powder using γ-CD and hydrogenated lecithin with the optimal spray drying condition.

On the other hand, there are some publications about the structural characteristics of oil powders such as sardine oil and echium oil demonstrating a loss in crystallinity [21,22]. Taken together, P18 composed of γ-CD and hydrogenated lecithin was chosen for a further study. As γ-CD was increased, content of free oil was decreased and that of encapsulated oil was increased. In case of diluted emulsion (P18-6), the free oil content was lower than that of P18-4. The free oil content was decreased as spray-drying temperature was decreased (Table 5).

Table 5. The effect of the optimal spray drying condition on moisture amount, particle size and oil amount (free oil, encapsulated oil and total oil) of spray-dried omega-3 powder using γ-CD and hydrogenated lecithin (n = 3).

Formular	Moisture (%)	SE (nm)	RE (nm)	Free Oil (g/100 g)	Encapsulated Oil (g/100 g)	Total Oil (g/100 g)
P-O	2.63 ± 0.21	309.37 ± 10.17	326.70 ± 9.53	33.46 ± 1.22	18.57 ± 0.69	53.29 ± 1.50

It is interesting that as atomization pressure was reduced (P18-8), the free oil content was the least and the encapsulated oil was the most. There was no significant difference between droplet size of secondary emulsion and reconstituted emulsion of spray dried omega-3 powder. This small difference between droplet size of SE and RE suggested that emulsion maintained enough stable and dried-particle was well dispersed. The concentration of EPA and DHA was reported in Table 6. Almost of DHA was remained after spray-drying, but just 80% of EPA was maintained. Maybe high temperature of spray-drying procedure affected EPA stability negatively and enhanced oxidation of EPA. It was correlated that high temperatures and the presence of oxygen may lead to an increased oxidation of long-chain polyunsaturated fatty acids so that a drying process at low temperatures (freeze-drying) is expected to be an alternative for the microencapsulation of fish oil [23,24]. So, the further study on reducing the lipid oxidation during spray-drying is needed. We prepared spray-dried emulsion powder containing olive oil, as an alternative to omega-3 fatty acids. The types of pharmaceutical excipients were investigated for spray-dried olive oil powder and γ-CD and hydrogenated lecithin were chosen. Finally, spray-dried powder containing omega-3 fatty acids was successfully prepared.

Table 6. The content of eicosapentaenic acid (EPA) and docosahexaenoic acid (DHA) of spray-dried omega-3 powder using γ-CD and hydrogenated lecithin with the optimal spray drying condition (n = 3).

	EPA	DHA
Concentration (mg/mg powder)	0.19 ± 0.02	0.22 ± 0.01
Remaining percent (%)	78.68 ± 7.39	98.23 ± 4.38

4. Conclusions

The pharmaceutical excipients such as PVP, gelatin A, and gelatin B in the presence of hydrogenated lecithin or PEG40 glyceryl monostearate affected the formation of spray-dried powder containing olive oil due to the strong adsorption into hydrophobic material. We successfully prepared spray-dried omega-3 powder with γ-CD and hydrogenated lecithin and proposed the spray-drying conditions for scale-up, subsequently, this spray-dried omega-3 powder could be a new ingredient for new functional foods. In successive study, the change of crystallinity and polymorphism of spray-dried omega-3 powders will be examined. In addition, omega-3 powder will be prepared using a mixture of silica-based adsorbents and γ-CD for considering the cost for commercialization.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/9/6/1177/s1.

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