Heliyon 9 (2023) e20376

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Soft-capsule formulation of a re-esterified triglyceride omega-3 employing self-emulsifying technology and bioavailability evaluation in healthy volunteers

Gi Hyeong Sin [1], Sun Ho Hong [1], Yoon Tae Goo, Hyun Min Jung, Sangkil Lee [**], Young Wook Choi [*]

College of Pharmacy, Chung-Ang University, 84 Heuksuk-ro, Dongjak-gu, Seoul 06974, Republic of Korea

ARTICLE INFO

Keywords:
Re-esterified triglyceride
Self-emulsifying delivery system
Soft capsule
Clinical study
Bioavailability

ABSTRACT

Despite the superior clinical efficacy of the re-esterified triglyceride (rTG) form compared to the ethylester form, few studies have been conducted on improving the bioavailability of the rTG form of omega-3 oil. The aim of study was to evaluate the effect of self emulsifying formulation on the improvement of bioavailability of rTG form of omega-3 oil.

To develop a re-esterified triglyceride (rTG) soft capsule, an rTG-loaded self-emulsifying delivery system (SEDS) was designed using coconut oil, polysorbate 80, and lecithin. Candidate formulations were designed from a phase-diagram study and optimal SEDS formulations containing 85% of high omega-3 (ω−3) oils were screened from the evaluation of droplet size distribution, measurement of oil floating area and emulsion turbidity. The selected, optimized rTG SEDS formulation was filled into a soft capsule (NOVASEDS) and applied to a sequence-randomized, double-blind, single-dose, and two-way crossover clinical study (n = 44), and the the bioavailability of NOVASEDS was compared with that of a 'raw' rTG capsule (rTG OMEGA3) as control. The droplet size (D_{50}) formed from the candidate formulations was approximately 30–45 μm, and the optimal formulation showed a unimodal particle distribution with the smallest oil floating area and small changes in turbidity after 24 h. C_{max} and AUC from 0 to 24 h for NOVASEDS, calculated from docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and as the sum of DHA and EPA, were significantly higher ($P < 0.05$) than corresponding values for rTG OMEGA3. In conclusion, NOVASEDS formulated by SEDS technology enabled the manufacture of a high rTG payload soft capsule with improved bioavailability in human subjects.

1. Introduction

Omega-3 polyunsaturated fatty acids (ω-3) have long been widely used to manage lipid levels in patients with dyslipidemia and healthy individuals. ω-3 in various commercially available nutritional supplements containing docosahexaenoic acid (DHA) and

* Corresponding author. College of Pharmacy, Chung-Ang University, 84 Heuksuk-ro, Dongjak-gu, Seoul 06974, Republic of Korea.
** Corresponding author. College of Pharmacy, Chung-Ang University, 84 Heuksuk-ro, Dongjak-gu, Seoul 06974, Republic of Korea.)
E-mail addresses: jeaus1995@naver.com (G.H. Sin), bcbf2326@naver.com (S.H. Hong), rndbsxo5318@naver.com (Y.T. Goo), hmin1229@naver.com (H.M. Jung), skdavid@cau.ac.kr (S. Lee), ywchoi@cau.ac.kr (Y.W. Choi).
[1] Two authors contributed equally.

https://doi.org/10.1016/j.heliyon.2023.e20376
Received 24 July 2023; Received in revised form 19 September 2023; Accepted 20 September 2023
Available online 21 September 2023

Table 1
Scoring criteria for the evaluation of candidate formulations.

Evaluation item	Score		
	0	1	2
Span	>1.4	1.2–1.4	<1.2
Oil floating area	>75%	25–75%	<25%
T_0	<20%	20–50%	>50%
Turbidity changes during 24h	>30%	15–30%	<15%

Abbreviations: T_0, turbidity (%) measured immediately after centrifugation.

eicosapentaenoic acid (EPA) mainly originated from fish oils containing natural forms of triglycerides (TGs). However, ω-3 bound only to the sn-2 position of the glycerol skeleton of TGs has a lower ω-3 content in oil of the same weight compared to ω-3 bound to the entire TG skeleton (sn1–3 positions). Therefore, natural TGs with ω-3 bound only to the sn-2 position of the glycerol skeleton of TGs are not considered ideal raw materials due to their low ω-3 content [1–5].

To solve this problem, a second-generation ω-3 supplement manufactured by conjugation of ethyl esters (EEs) has recently been developed, but the amount of gastrointestinal (GI) absorption of ω-3 in the form of EEs is significantly lower than that of TGs [2,5,6]. Therefore, to improve oral bioavailability of the EE form, microencapsulation [7] and self-emulsifying drug delivery systems (SEDS) [8] have been developed. In addition, third-generation ω-3 synthetized in the form of TGs by esterifying free ω-3 back into the glycerol skeleton have been introduced to enhance oral absorption of ω-3, and the oral bioavailability of re-esterified TG (rTG) is approximately 1.2 times higher than that of TGs and approximately 1.3–1.7 times higher than that of EEs [2,9–11]. However, studies on improving the GI absorption of ω-3 have concentrated mostly on EEs, whereas for rTG, not much research has been conducted due to high unit prices and complexity in manufacturing processes [8,12]. Nevertheless, further research is needed to explore the nutraceutical and clinical potential benefits of rTG ω-3 supplements because recent studies showed that rTG had better bioavailability and clinical efficacy than EEs [2,5,6].

SEDS, delivery systems that aid in the transport of lipophilic bioactive agents across the epithelial cell membrane of the GI tract [8,13,14], are an appropriate delivery technique to improve the oral bioavailability of rTG. SEDS generate an oil-in-water (o/w) emulsion spontaneously with gentle agitation upon introduction into the GI tract [15,16]. Small emulsion droplets formed by SEDS have large surface areas, which increase solubility and improve the permeability of bioactive materials through biological membranes, resulting in better absorption into the intestinal epithelium. Therefore, SEDS loaded with lipophilic drugs have improved oral bioavailability [15,17].

SEDS comprise primarily oil, surfactant and co-surfactant, and most SEDS contain less than 50% of oil [16,18]. However, in the present study, we set a target loading amount of rTG to 85% v/v, based on the daily limit of DHA and EPA, and the maximum filling amount in a soft capsule. Various mixtures consisting of coconut oil, polysorbate 80 and lecithin were prepared and blended with rTG to prepare 85% rTG-containing SEDS formulations. Because oil floating was inevitable if the formulation contained high amounts of rTG, we developed a novel, customized oil-floating meter, together with evaluation criteria, to find rTG SEDS formulations with good dispersion and low oil-floating properties. Afterward, the optimal formulation was selected for soft capsule filling, based on the size distribution of emulsion droplets, oil floating area and turbidity. Subsequently, an *in vivo* pharmacokinetic (PK) study was performed in healthy volunteers using a raw rTG omega-3 filled capsule as reference.

2. Materials and methods

2.1. Materials

Raw rTG (EPA and DHA 600 mg/g, from fish oil), Coconut Oil (Honest Organic Extra Virgin Coconut Oil), Lecithin (Sunflower Lecithin Liquid), Polysorbate 80 (Non-ionic organic surface-active agent) and soft capsule were supplied by Novawells Co., Ltd. (Cheongju-si, Chungbuk-do, Republic of Korea). EPA, DHA, EPA-d_5, and DHA-d_5 were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography-grade acetonitrile and methanol were purchased from JT Baker (Phillipsburg, NJ, USA). All other chemicals were purchased from commercial sources and were of analytical grade.

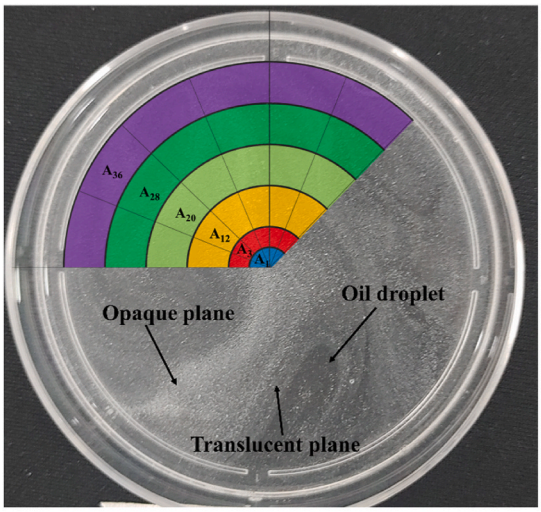
2.2. Construction of phase diagram and preparation of SEDS formulations of high rTG content

The optimal ratio for the oil-surfactant-cosurfactant (OSC) blend showing high self-emulsification properties was identified from construction of a phase diagram by evaluating size distribution, oil floating area, and turbidity. To prepare a SEDS formulation containing rTG, an OSC blend composed of coconut oil (oil), polysorbate 80 (surfactant) and lecithin (co-surfactant) was blended at 60 °C, and a SEDS formulation containing 85% (v/v) of rTG was prepared by adding OSC to raw rTG; the resulting blend was thoroughly blended at a temperature of 60 °C for 10 min with a sonicator (Mujigae SD-120H bath-type sonicator, 50W; Sungdong Ultrasonic, Seoul, Republic of Korea). To construct the phase diagram, four evaluation criteria were developed, and scores were given based on the following criteria (Table 1): (1) span value less than 1.2: +2, 1.2–1.4: +1, and more than 1.4: 0; (2) oil floating area less than 25%: +2, 25–75%: +1, and more than 75%: 0; (3) T_0 less than 20%: 0, 20–50%: +1, and more than 50%: +2; (4) turbidity changes during 24 h less than 15%: +2, 15–30%: +1, and more than 30%: 0. Compositions of all rTG-containing SEDS formulations are

Table 2
Composition of SEDS formulations and results on droplet size after dispersion into water.

Formulation	rTG content (% v/v)	OSC blend composition (% v/v)			Droplet size evaluation				
					Distribution (μm)			Span value	Peak shape
		CO	P80	LC	D_{10}	D_{50}	D_{90}		
F1	85	1.50	3.00	10.50	11.92	34.89	63.25	1.47	Unimodal
F2	85	1.50	4.50	9.00	11.18	40.57	64.68	1.32	Unimodal
F3	85	3.00	3.00	9.00	10.73	32.87	64.00	1.62	Bimodal
F4	85	1.50	6.00	7.50	10.28	41.38	70.30	1.45	Bimodal
F5	85	3.00	4.50	7.50	9.39	41.26	73.15	1.55	Bimodal
F6	85	4.50	3.00	7.50	10.68	43.58	76.75	1.52	Bimodal
F7	85	1.50	7.50	6.00	10.50	43.60	75.71	1.50	Bimodal
F8	85	3.00	6.00	6.00	12.06	40.82	67.76	1.36	Unimodal/bimodal
F9	85	4.50	4.50	6.00	11.96	39.52	68.88	1.44	Unimodal/bimodal
F10	85	3.00	6.75	5.25	17.52	43.69	68.02	1.16	Unimodal
F11	85	1.50	9.00	4.50	11.97	42.00	71.77	1.42	Unimodal/bimodal
F12	85	4.50	6.00	4.50	11.55	41.07	67.76	1.37	Bimodal
F13	85	1.50	10.50	3.00	12.47	39.67	67.35	1.38	Unimodal
F14	85	9.00	3.00	3.00	12.78	38.85	64.8	1.34	Uni/bimodal

Abbreviations: CO, coconut oil; LC, lecithin; OSC blend, blend of oil (coconut oil), surfactant (polysorbate 80) and co-surfactant (lecithin); rTG, re-esterified triglyceride; SEDS, self-emulsifying delivery system; P80, polysorbate 80.



Scores	Evaluation criteria of oil floating area
0	< 10 oil droplets
1	≥ 10 oil droplets
2	≥ 20 oil droplets
3	≥ 30 oil droplets
4	< 25% area of translucent plane
5	≥ 25% area of translucent plane
6	≥ 50% area of translucent plane
7	≥ 75% area of translucent plane or < 25% area of opaque plane
8	≥ 25% area of opaque plane
9	≥ 50% area of opaque plane
10	≥ 75% area of opaque plane

(A) **(B)**

Fig. 1. Oil floating meter (A) and evaluation criteria for oil floating area (B).
Note: A_1, A_3, A_{12}, A_{20}, A_{28}, and A_{36} represent areas of the blue, red, yellow, light green, green and purple zones, respectively. The relative area ratio for A_1, A_3, A_{12}, A_{20}, A_{28}, and A_{36} is 1:3:12:20:28:36. Each area has 16 zones to be awarded points. The total 96 zones independently assign the highest point according to evaluation criteria of the oil floating area. "Plane" means that the oil exists in a cohesive mass or forms a boundary with its surroundings rather than as individual droplets, while "translucent" refers to a feature that allows some light to pass through, showing a grayish color with visible background, and "opaque" refers to a feature that does not allow any light to pass through, appearing white with no visible background.

presented in Table 2.

2.3. Measurement of droplet size and distribution

One milliliter of each rTG-containing SEDS formulation was added to 250 mL of distilled water. The solution was then stirred using a magnetic stirring bar at 200 rpm and maintained at room temperature. After 5 min, the droplet size and distribution of each sample were measured using a laser-scattering particle size analyzer (Partica LA-950V2; Horiba, Japan). To evaluate droplet size distribution, span value, a measure of particle size uniformity and consistency, was calculated using the following equation:

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}} \quad (1)$$

where, D_{10}, D_{50}, and D_{90} are the corresponding droplet sizes when the cumulative percentage reaches 10%, 50%, and 90%, respectively [19,20].

2.4. Evaluation of oil floating area

To evaluate the oil floating properties of rTG-containing SEDS formulations, the same method used in particle size measurement was applied. In brief, each formulation was dispersed in distilled water for 5 min, and 10 mL of the dispersion was transferred to a Petri dish. An oil floating area meter (Fig. 1a) and evaluation criteria (Fig. 1b) were developed to measure the oil floating area of each formulation. The oil floating area was calculated using the following equation:

$$\text{Oil floating area } (\%) = \frac{(S_1 + 3S_3 + 12S_{12} + 20S_{20} + 28S_{28} + 36S_{36})}{1,600} \times 100 \quad (2)$$

where, S_x refers to mean scores assigned to areas, A_x, in the oil floating area meter.

2.5. Measurement of emulsion turbidity

Five minutes after dispersion of each formulation into distilled water, 10 mL of dispersed solution was transferred into a 15-mL centrifuge tube and centrifuged for 5 min at 500 rpm and 25 °C. After collecting 100 μL of each sample from the bottom of the tube, each solution was transferred to a 96-well plate, and the transmittance of each sample was measured at a wavelength of 650 nm. The percentage transmittance was measured twice using a microplate reader (FlexStation 3; Molecular Devices, Sunnyvale, CA, USA) immediately and 24 h later from centrifugation using distilled water as a blank. Subsequently, dispersion turbidity was measured [21, 22] and changes in turbidity during 24 h were calculated:

$$\text{Turbidity } (\%) = 100 - \text{Transmittance } (\%) \quad (3)$$

$$\text{Turbidity changes during 24 h } (\%) = \frac{(T_0 - T_{24})}{T_0} \times 100 \quad (4)$$

where, T_0 and T_{24} are turbidity (%) measured immediately and 24 h after centrifugation, respectively.

2.6. Preparation of rTG SEDS for soft capsule filling

Lecithin and coconut oil were mixed at an optimized ratio using a homogenizer (Homogenizing Mixer Mark II Model 2.5) at 4000 rpm for 10 min. Then, the mixture was cooled below 45 °C and polysorbate 80 was added. The final mixture was homogenized at 4000 rpm for 10 min. Finally, raw rTG was added and homogenized at 4,000 rpm for 20 min. The resulting mixture was passed through an 80-mesh sieve (180 μm) for filtration, and then filled into a gelatin capsule using a filling machine (780SR, Changsung softgel system CO., Ltd, Pocheon, Republic of Korea).

2.7. Enrollment of study participants and safety assessments

2.7.1. Enrollment of study participants

Adults aged ≥19 years without congenital chronic diseases or pathologic symptoms, and with body mass index of 18–30 kg/m^2 and bodyweight ≥50 kg (males) or ≥45 kg (females), were candidates for study inclusion. All participants were assessed as healthy, based on a detailed medical examination that included medical history, blood pressure, pulse rate, hematology, blood chemistry, urinalysis, serology, and clinical laboratory examinations.

The exclusion criteria were as follows: any use of drugs that significantly induce (e.g., barbiturates) or inhibit drug-metabolizing enzymes, within 30 days before study start; any use of drugs that may affect the study, within 10 days before the first study dose; participation in a bioequivalence test or other clinical trial within 6 months before the first study dose; donation of blood within 2 months, or blood components within 2 weeks, before study start (the first administration date); a history of regular alcohol intake (>21 drinks/week for men, >14 drinks/week for women) within 6 months before study start; a history of significant psychiatric illness; pregnancy, lactation or suspicion of pregnancy; a history of hypersensitivity to this drug; a history of hypersensitivity or allergy to fish; or adults who, for reasons other than the selection and exclusion criteria, were deemed unsuitable for the clinical trial by its director (or delegated physician).

2.7.2. Safety assessments

Throughout the study, safety observations including assessments of adverse events (AEs), concomitant medications, vital signs, and clinical laboratory tests were conducted. All AEs were classed by system organ class, preferred term, severity, and causality. The types and frequencies of AEs between the reference and test capsules are presented as percentages. The severity of AEs was classed as mild,

moderate, or severe. As part of the safety assessments, vital signs and clinical laboratory tests were conducted at screening. Serum transaminase levels were measured 2 days before hospitalization and at the last blood-sampling time (at 24 h). Left forearm blood pressure and pulse rate were measured within 24 h after study drug administration. In addition, according to the recent Korean Good Clinical Practice (KGCP) Annex No. 77 form, AE causality was categorized by relevance or non-relevance.

2.8. Pharmacokinetic study

2.8.1. Study design

This was a double-blind, sequence-randomized, single-dose, and two-way crossover study. The participants were accommodated at Bumin Hospital (Seoul, Republic of Korea) from the evening before dosing in each study period. The study was conducted in compliance with the NOVASEDS-01 study protocol (BMH 2022-11-012-007) approved by the Institutional Review Board of the Clinical Trial Research Center (Bumin Hospital), and with ethical principles outlined in the Declaration of Helsinki for biomedical research involving human subjects [23]. Each study participant was given a detailed explanation of the study, and written informed consent was obtained before screening. The volunteers agreed to avoid strenuous physical activity, restrict the intake of grapefruit or grapefruit-containing foods, and alcohol consumption for 48 h before the study and until the final PK sampling. A total of 44 participants were randomly assigned to receive either a SEDS formulation-loaded capsule (experimental group) or a raw rTG omega-3 filled capsule (control group). Each participant took 600 mg of rTG omega-3 and blood was collected at specified time points over a 24 h period. The blood samples were used to plot time-dependent changes in baseline-adjusted plasma DHA, EPA, and total omega-3 (DHA + EPA) concentrations.

2.8.2. Drug administration and plasma sampling

The study participants were randomly assigned to one of two groups, each of which was designated to take either the reference or test capsule during the first administration period. Study participants were admitted to the study center two days before dosing and were hospitalized there for two nights. Starting 30 min before oral study drug administration, all participants completely ingested a low-fat meal ($\leq$700 kcal and $\leq$20% fat) within 20 min; then, the soft capsule was administered at a dose of 600 mg (sum of DHA + EPA) with 150 mL of water. No participants received concomitant medications.

Blood samples (6 mL) were collected into potassium ethylenediaminetetraacetic acid-treated tubes at predetermined time points (20, 16, and 12 h predose, and 0, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h postdose). Saline 1 mL was injected into the catheter to prevent blood clotting. The collected blood samples were immediately centrifuged at 3000 rpm and 4 °C for 10 min, and the obtained plasma samples were stored in an Eppendorf® tube at −80 °C until analysis. A 7-day washout period followed the first study drug administration. The second administration period was the same as the first, but the administration formulation was changed to the reference or test formulation not taken previously.

2.8.3. Calculation of pharmacokinetic parameters

Maximum plasma drug concentration (C_{max}), time to peak plasma concentration (T_{max}), and areas under the plasma drug concentration–time curves from 0 to 24 h (AUC_{0-24h}) and infinity (AUC_{inf}) were obtained calculated by trapezoidal method. The C_{max} and T_{max} values were determined directly from the obtained concentration–time data.

2.9. Blood sample preparation and DHA and EPA analyses

2.9.1. Blood sample preparation

Stock solutions of DHA and EPA 20,000 μg/mL were prepared using ethanol. The following steps were performed to prepare standard calibration curves and analyze samples. DHA and EPA stock solutions were mixed in a 5:2 (v/v) ratio, and sequentially diluted with ethanol. To prepare standard calibration curves, the stock solutions were serially diluted in the following ratios: 100/40, 200/80, 500/200, 1000/400, 2000/800, 5000/2,000, and 10,000/4000 μg/mL. DHA-d_5 and EPA-d_5 500 μg/mL stock solutions were prepared using ethanol as the internal standard; the solutions were mixed in a 5:2 (v/v) ratio, and sequentially diluted with ethanol to prepare a 100/200 μg/mL concentration. Standard calibration curves for DHA and EPA were prepared in a surrogate matrix (5% human serum albumin in Dulbecco's phosphate-buffered saline and Ham's F-12 nutrient mixture [1X], liquid + L-glutamine mixed buffer) by adding DHA/EPA working solution to achieve concentrations of 5/2, 10/4, 25/10, 50/20, 100/40, 250/100, and 500/200 μg/mL. Human plasma samples were mixed with 10 μL of DHA-d_5/EPA-d_5 100/20 and 190 μL of acetonitrile. Therefore, 200 μL of acetonitrile:5 M hydrochloric acid (90:10, v/v) was added to the mixture, and then vortexed for 30 s at 2000 rpm. Afterward, the mixture was stored in an oven at 84 °C for 30 min. Then, after adding 200 μL of methanol:10 M sodium hydroxide (90:10, v/v), the mixture was vortexed for 10 s, and incubated for 30 min at 84 °C in an oven, and then centrifuged at 12,000 rpm for 5 min at 4 °C. Subsequently, 50 μL of the supernatant was transferred to a micro tube and 50 μL of 5 M hydrochloric acid and 1 mL of hexane were added. After vortexing for 30 s at 2200 rpm, it was centrifuged for 5 min at 12,000 rpm at 4 °C. Then, 200 μL of the supernatant was transferred to a glass tube and dried completely with nitrogen gas for 5 min at 50 °C and then reconstituted with 500 μL of 70% acetonitrile, vortexed at 2200 rpm for 30 s, and 1 μL of sample was transferred to a vial for analysis.

2.9.2. Analytic method validation

Linearity of the calibration curve for DHA and EPA, with a coefficient of determination (r^2) value $>$ 0.995, was established using least-square linear regression in the range 5–500 μg/mL and 2–200 μg/mL, respectively. The validation method was conducted

Table 3
Summary of the evaluation results for rTG-containing SEDS formulations.

Formulation	Evaluation item				Total score
	Span	Oil floating area (%)	T_0 (%)	Turbidity changes during 24 h (%)	
F1	1.47	89.01	16.90	34.79	0
F2	1.32	87.80	35.50	39.82	2
F3	1.62	86.56	15.20	12.11	2
F4	1.45	94.40	27.60	33.12	1
F5	1.55	80.18	20.90	17.87	2
F6	1.52	89.68	14.40	5.98	2
F7	1.50	85.89	29.30	49.00	1
F8	1.36	84.68	25.10	29.25	3
F9	1.44	67.83	10.30	24.51	2
F10	1.16	38.97	28.80	12.55	6
F11	1.42	87.70	20.60	44.31	1
F12	1.37	80.23	21.10	52.73	2
F13	1.38	87.81	32.60	31.91	2
F14	1.34	81.00	7.70	26.14	2

Abbreviations: rTG, re-esterified triglyceride; T_0, turbidity (%) measured immediately after centrifugation.

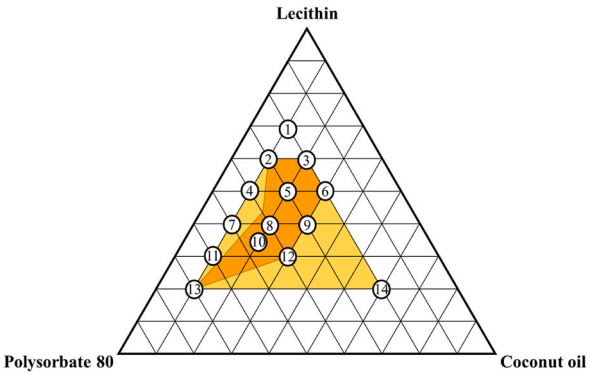


Fig. 2. Phase diagram of rTG-SEDS formulations with 85% rTG content using an OSC blend comprising coconut oil (oil), polysorbate 80 (surfactant), and lecithin (co-surfactant).
Abbreviations: rTG, re-esterified triglyceride; SEDS, self-emulsifying delivery system; OSC, blend of oil (coconut oil), surfactant (polysorbate 80), and co-surfactant (lecithin).
Note: The yellow and orange areas surround formulations scoring 2 points or ≥3 points, respectively, in the evaluation of self-emulsification efficiency.

according to Bioanalytical Method Validation—Guidance for Industry [24], and Guideline on Bioanalytical Method Validation [25]. For DHA, the within-run precision and accuracy were found to be 0.98–1.56% and 98.05–100.30%, respectively. The between-run precision and accuracy for DHA were 0.40–2.49% and 98.73–99.41%, respectively. Regarding EPA, the within-run precision and accuracy were determined to be 0.78–2.33% and 96.54–98.92%, respectively. The between-run precision and accuracy for EPA were 0.38–1.27% and 96.54–98.53%, respectively.

2.9.3. Analytic condition for DHA and EPA plasma concentrations

The concentrations of DHA and EPA in human plasma were determined by ultra-performance liquid chromatography with tandem mass spectrometry. A liquid chromatography system (Waters Acquity UPLC® System/Waters Xevo® TQ-XS) was used for analysis. Data were analyzed with MassLynx™ SCN1012. Chromatographic separation was performed on a column (Waters ACQUITY UPLC® BEH C_{18}; 1.7 μm; 2.1 mm i.d. × 150 mm), with the mobile phase consisting of 2 mM ammonium acetate (A) and acetonitrile (B) used in gradient elution in the following order: duration/mobile phase B, 3.5 min/60%, 1.6 min/80%, and 2.9 min/60% at a flow rate of 0.3 mL/min. The liquid chromatography system was coupled with an electrospray tandem quadrupole mass spectrometer for detection. The multiple-reaction monitoring mode of a negative ion electrospray was used for quantification: EPA m/z 301.10 → 257.00, DHA m/z 327.10 → 283.00, EPA-d_5 (internal standard) m/z 306.00 → 262.10, and DHA-d_5 (internal standard) m/z 332.00 → 288.00.

2.10. Statistical analyses

All data are presented as mean ± standard deviation (SD). Data analyses were performed with one-way, independent-groups analysis of variance (ANOVA) using Minitab software (version 18.0; Minitab Inc., State College, PA, USA) for log-transformed data. For

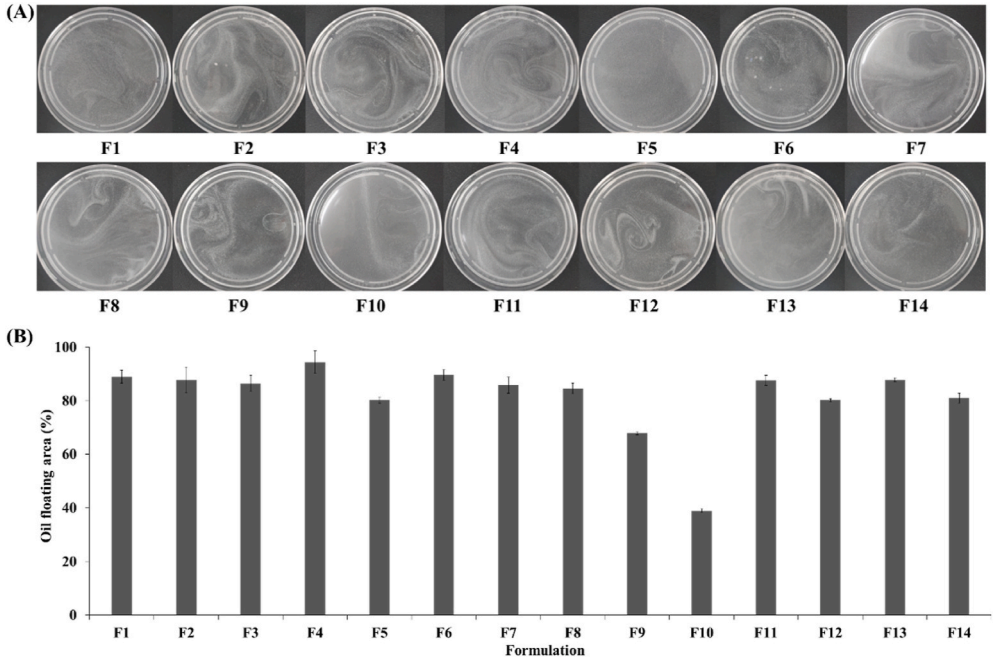


Fig. 3. Evaluation of oil floating area in rTG-SEDS formulations with 85% rTG content. Appearance of oil floated surface (A) and oil floating area (B).
Abbreviations: rTG, re-esterified triglyceride; SD, standard deviation; SEDS, self-emulsifying delivery system.
Note: Values are presented as mean ± SD (n = 3).

all analyses, differences were considered significant when *P* values were less than 0.05, unless otherwise indicated.

3. Results and discussion

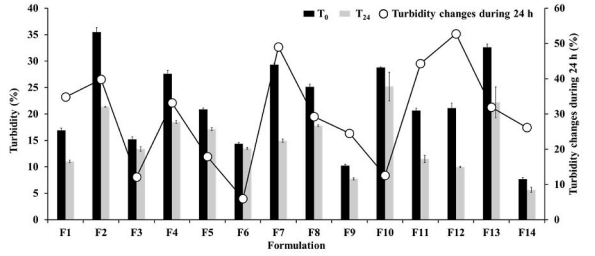
3.1. Construction of the phase diagram

Table 3 summarizes the total scores assigned to F1–14 evaluated by span value, oil floating area, and turbidity changes during 24 h. Based on these results, a phase diagram was constructed: formulations scoring 2 points were marked in yellow, and formulations scoring ≥3 points were marked in orange (Fig. 2). The lowest score of 0 was assigned to F1, which is due to high lecithin content and low polysorbate 80 and coconut oil content. Conversely, the OSC blend consisting of coconut oil: polysorbate 80:lecithin 2:4.5:3.5 (v/v/v) received the highest score and was selected as an optimized OSC blend (B_{opt}) for further study. From the above results, when the lecithin content was high or the polysorbate 80 or coconut oil content was low, emulsification was difficult to achieve. This is thought to be due to the high viscosity of lecithin (98 P at 25 °C), which makes it difficult for spontaneous emulsification to occur, and if the polysorbate 80 or coconut oil content is low, it is difficult to lower the interfacial tension required for emulsification. This idea is supported by Züge et al. In their work, an emulsion emulsified with lecithin required higher mixing energy than polysorbate 80, and the oscillatory test revealed that the final emulsion prepared by lecithin showed strong gel behavior, while the polysorbate 80-based emulsion exhibited concentrated solution behavior [26].

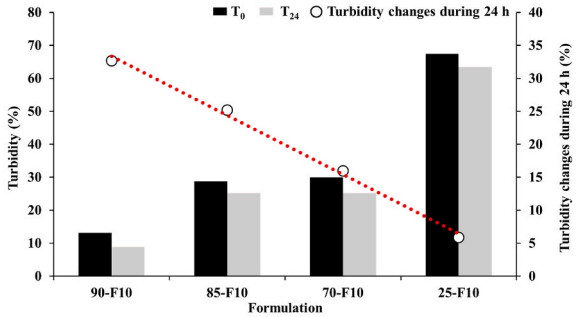
3.2. Evaluation of droplet size and distribution

Droplet size and distribution for the formulations are summarized in Table 2, where D_{50} for all formulations was in the range of approximately 30–45 μm. Among candidate formulations, F10 showed the most unimodal size distribution with a narrow span value of 1.16, which supports that F10 forms the most uniform droplets when it is emulsified. Conversely, the other candidate formulations showed bimodality of dispersion and span values ranged from 1.32 to 1.62. Typically, SEDS formulations form droplets with a diameter of less than 1 μm [8]. The mean size of the candidate formulations was greater than traditional SEDS formulations, which results from the low amounts of surfactant and co-surfactant used in the rTG-SEDS formulations. In detail, less than 15% (w/v) emulsifying agent was used in the present formulations; however, common SEDS formulations comprise 40–80% of emulsifying agent [8,18].

It has been reported that emulsifying fish oil prior to oral administration improves bioavailability, even though the particles are large [18,27]. For example, Bremell et al. reported that the oral bioavailability of ω-3 was increased compared to the control group when ω-3 in the form of EE was 15%. Therefore, although the particle size is larger than that of conventional SEDS formulations, it is



(A)



(B)

Fig. 4. Turbidity over time and turbidity changes during 24 h: in rTG-SEDS with 85% of rTG (A); and in rTG-SEDS using B_{opt} (B).
Abbreviations: rTG, re-esterified triglyceride; SEDS, self-emulsifying delivery system; T_0 and T_{24} are % turbidity measured immediately and 24 h after centrifugation, respectively; SD, standard deviation; B_{opt}, experimental optimized OSC blend; OSC, blend of oil (coconut oil), surfactant (polysorbate 80), and co-surfactant (lecithin).
Note: 90-F10, 70-F10, 60-F10, and 20-F10 formulations contain 90, 70, 60 and 20% of rTG. The red dotted line represents the regression line of turbidity changes during 24 h. Values are presented as mean ± SD (n = 3).

thought that there will be no difficulty in absorbing micro-sized droplets in the intestines.

3.3. Evaluation of oil floating area

The second criterion for self-emulsifying efficiency is oil floating area, indicating how much oil is emulsified. Oil floating areas for each formulation are depicted in Fig. 3. Dispersing F1–14 formulations into water, all formulations showed the results of oil floating in water, which was caused by an excessive amount of oil not emulsified by the OSC blend (Fig. 3A). Visually, F5, F10, and F13 showed relatively lower oil floating areas than the other formulations. For a more detailed evaluation, we used a custom oil float meter to numerically express the amount of oil floating on the water surface as the area where the oil was floating (Fig. 3B). The lowest oil floating area was 38.97% at F10, while most formulations showed more than 80% oil floating area. The oil floating meter was first used in this study. There are few studies on SEDS formulations containing rTG ω-3 oils, and generally, droplet size and zeta potentials have been used to evaluate SEDS or simply compare bioavailability [28]. Therefore, the oil floating meter and evaluation criteria are useful tools for evaluating the emulsification potential of soft-capsule products with high oil content.

3.4. Turbidity evaluation

To evaluate SEDS stability over time, time-dependent changes in turbidity were observed, because turbidity changes in emulsions over 24 h result from changes in concentration and droplet size, which reflect changes in the physical stability of emulsions [29]. For example, as shown in Fig. 4A, F2 showed the highest turbidity when measured immediately, but after 24 h, the reduction rate of turbidity was relatively high, indicating low physical stability of SEDS after dispersion. Like F2, formulations F1, F4, F7, F11, F12, and F13 showed a high turbidity change rate of >30%. A common feature of these formulations, except F12, was that the coconut oil concentration in the OSC blend was relatively low (10%) compared to other formulations. Coconut oil contains various fatty acids that increase the dispersion stability of emulsions because the fatty acids act as surfactants [30–32]. Therefore, formulations with low coconut oil concentrations are thought to have low dispersion stability for the emulsions. Meanwhile, comparing F1, F3, F6, F9, and F14 at T_0, these formulations showed relatively low turbidity (<20%), where the concentration of polysorbate 80 was 20–30% of the OSC blend, and it is thought that polysorbate 80 plays a major role as an emulsifying agent [15,33]. From these results, if the coconut oil concentration is low, the dispersion stability is poor, even if turbidity is high in the early stages of emulsion formation; and, if the concentration of polysorbate 80 is low, the initial turbidity is considered low.

Table 4
Demographic data for the study participants (N = 44).

	Inspection item (unit)	Range	Mean ± SD
Anthropometric measurement	Age (years)	19–45	25.86 ± 5.83
	Height (cm)	158.8–182.3	173.50 ± 4.84
	Weight (kg)	53.7–98.0	72.84 ± 8.96
	Body mass index (kg/m^2)	19.7–29.8	24.15 ± 2.41
Vital signs	Systolic blood pressure (mmHg)	100.00–143.00	120.80 ± 10.66
	Diastolic blood pressure (mmHg)	60.00–91.00	73.73 ± 7.27
	Pulse rate (beats/min)	59.00–103.00	82.57 ± 11.31
Hematology	WBC count (× 10^3/μL)	4.04–9.19	6.51 ± 1.33
	RBC count (× 10^3/μL)	4.66–5.84	5.16 ± 0.30
	Hemoglobin (g/dL)	14.7–17.7	15.89 ± 0.74
	Hematocrit (%)	44.7–53.6	48.48 ± 2.14
	Platelet count (× 10^3/μL)	158–393	241.36 ± 46.72
	Segmented neutrophils (%)	41.2–80.0	56.86 ± 9.08
	Lymphocytes (%)	12.7–46.8	32.19 ± 8.09
	Eosinophils (%)	0.3–7.8	2.63 ± 1.86
	ANC (mm^{-3})	1941–7352	3749.52 ± 1163.39
	BUN (mg/dL)	7.1–20.7	14.02 ± 3.11
	Creatinine (mg/dL)	0.6–1.1	0.86 ± 0.12
Blood chemistry	eGFR (mL/min/1.73m^2)	72.4–165.5	111.18 ± 19.76
	Total protein (g/dL)	6.5–8.1	7.45 ± 0.33
	Albumin (g/dL)	4.4–5.0	4.71 ± 0.18
	AST (U/L)	14–44	22.84 ± 6.35
	ALT (U/L)	9–57	26.50 ± 11.88
	γ-GTP (U/L)	7–51	20.48 ± 10.14
	Total bilirubin (mg/dL)	0.4–2.1	1.00 ± 0.39
	Glucose (mg/dL)	73–100	86.95 ± 6.27

Abbreviations: γ-GTP, gamma-glutamyltransferase; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; RBC, red blood cell; SD, standard deviation; WBC, white blood cell.

The change in turbidity and the rate of turbidity change after 24 h were observed while changing oil content of the optimized formulation F10 from 25% to 90%. Thus, when 90% of oil was loaded, the turbidity was the lowest, and the turbidity change rate (%) was the highest (Fig. 4B). Conversely, when 25% of oil was loaded, the turbidity was the highest, and the turbidity change rate was the lowest; the droplet size of the formed emulsion was on a nano scale (data not shown). Therefore, the optimized formulation, B_{opt}, formed a very stable and small emulsion when the oil content was low. After 24 h, the turbidity change rate tended to increase as oil content increased, and for compositions with relatively high oil content (from 60 to 85%, but not 90%), a relatively stable emulsion was formed.

3.5. Participant enrollment and observation for adverse effects

Based on guidelines from the Korean Food and Drug Administration, intrasubject variability in AUC and C_{max} for DHA and EPA is estimated to be 19.24–42.31% when 4 g of ω-3 is orally administered to healthy adults. Assuming intrasubject variability of approximately 28% [34,35], T/R ratio of 1.05, power of 80%, and a significance level of 0.05, the projected number of participants was calculated as 38 [36]. Assuming a dropout rate of approximately 13% during the study period, the total number of study participants was set at 44. A total of 71 subjects were screened, of whom 27 were excluded due to medical examinations and withdrawal of consent. Thus, 44 individuals were included as enrolled study participants and completed the trial. Overall demographic data are summarized in Table 4. All study participants were evaluated as healthy and without clinically significant conditions. One study participant had a mild treatment-emergent AE, but this did not require withdrawal from the study. Altogether, NOVASEDS was well tolerated by healthy volunteers during the study.

3.6. Comparative pharmacokinetic evaluation

To evaluate the effect of SEDS formulation on rTG oral absorption, a clinical trial was conducted on humans. Basal levels of DHA and EPA were measured and no significant differences were found between the control and experimental groups (data not shown). PK profiles of DHA, EPA and sum of DHA and EPA are shown in Fig. 5A, B and C, respectively. In addition, PK parameters such as $AUC_{0–24h}$, C_{max}, and T_{max} values for DHA, EPA, and the sum of DHA and EPA are summarized in Table 5. C_{max} values for DHA, EPA, and the sum of DHA and EPA were 1.64, 1.38, and 1.61 times higher, respectively, in the NOVASEDS versus rTG OMEGA3 group. $AUC_{0–24}$ values for DHA, EPA, and the sum of DHA and EPA were 1.67, 2.11, and 1.70 times higher, respectively, in the NOVASEDS versus rTG OMEGA3 group. From the one-way ANOVA test, the *P* values on the differences of C_{max} and AUC between two formulations were below 0.05, which supporting that NOVASEDS showed statistically significant differences in pharmacokinetic properties compared to rTG OMEGA3 group (Table 6). The significant differences in C_{max} and $AUC_{0–24}$ indicate that NOVASEDS, formulated by SEDS technology, enabled greater absorption of rTG ω-3 than from the control formulation. The greater absorption of omega-3 could be attributed to

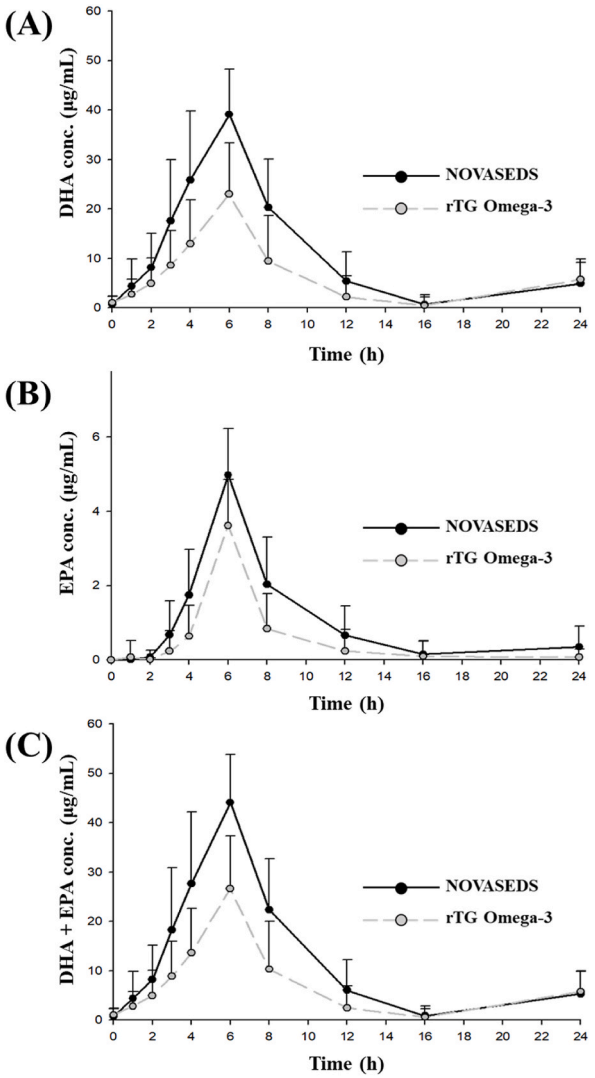


Fig. 5. Plasma concentration–time profiles for DHA (A), EPA (B), and DHA + EPA (C) after oral administration of formulations containing ω-3.
Abbreviations: ω-3, omega-3 fatty acids; rTG, re-esterified triglyceride; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SE, standard error.
Note: Values represent changes from baseline and are presented as mean ± SE (N = 44).

Table 5
Pharmacokinetic parameters for rTG OMEGA3 and NOVASEDS.

	PK parameter	rTG OMEGA3	NOVASEDS
DHA	C_{max} (μg/mL)	24.66 ± 8.96	40.41 ± 9.63
	T_{max} (h)	6	6
	AUC_{0-24h} (μg•h/mL)	165.34 ± 82.57	276.15 ± 91.60
EPA	C_{max} (μg/mL)	3.66 ± 1.20	5.01 ± 1.22
	T_{max} (h)	6	6
	AUC_{0-24h} (μg•h/mL)	11.24 ± 9.14	23.68 ± 13.08
DHA + EPA	C_{max} (μg/mL)	28.29 ± 9.32	45.42 ± 10.21
	T_{max} (h)	6	6
	AUC_{0-24h} (μg•h/mL)	176.58 ± 85.32	299.83 ± 97.27

Abbreviations: AUC_{0-24h}, area under the plasma concentration–time curve from 0 to 24 h; C_{max}, maximum plasma concentration; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SE, standard error; T_{max}, time to reach C_{max}.
Note: Values are presented as mean ± SE (N = 44).

Table 6
Analysis of variance results of pharmacokinetic parameters.

Parameters	DHA			EPA			Total omega-3		
	SS[a]	F-value	*P*-value	SS	F-value	*P*-value	SS	F-value	*P*-value
AUC									
Administration	270105.2	35.52	<0.05	3408.1	26.78	<0.05	334194.6	39.92	<0.05
Residual	653991.9	–	–	10943.7	–	–	719881.6	–	–
C_{max}									
Administration	5453.8	63.07	<0.05	42.3	28.88	<0.05	6456.8	67.56	<0.05
Residual	7436.9	–	–	126.0	–	–	8218.6	–	–

[a] Sum-of-squares value.

SEDS formulation, which enhances absorption of rTG. The SEDS formulation could form relatively small emulsion droplets in the GI tract, thereby increasing surface area and enhancing solubility in body fluid and permeability of rTG through biologic membranes. In addition, no significant difference in T_{max} values was found between the NOVASEDS and rTG OMEGA3 group (mean 6.00 h in both groups).

4. Conclusion

To date, most studies designed to enhance the oral absorption of ω-3 oils using SEDS have used EE-type ω-3 oils. Few studies have reported on improving the oral absorption of ω-3 oils in the form of rTG, and no studies have reported on high-content rTG formulations for this purpose. Therefore, this study devised a customized floating meter and applied it to the optimization of SEDS formulations and secured a high absorption rate based on the SEDS composition. NOVASEDS allowed loading of a high-content rTG form of ω-3 oils, and the optimized rTG ω-3 SEDS formulation significantly enhanced the oral bioavailability of DHA, EPA, and the sum of DHA + EPA compared to rTG ω-3 oils. The limitation of this study was that the amount of surfactant and co-surfactant used in this study was not enough for full solubilization of a large amount of omega-3 oil. In detail screening studies with more various types of surfactants and co-surfactant can be considered to maximize the bioavailability of omega-3 oils. In conclusion, the NOVASEDS formulation could be a highly effective approach for delivering rTG-form ω-3 oils.

Sources of support

The rTG omega-3 raw materials used in this study were kindly provided through NOVAWELLS Co., Ltd. (Cheongju, Chungcheonbuk-do, Republic of Korea).

Author contribution statement

Performed the experiments: G.H Sin, S.H. Hong, Y.T. Goo, H.M. Jung; Analyzed and interpreted the data: G.H Sin, S.H. Hong, Y.T. Goo, H.M. Jung; YW. Choi, S. Lee; Wrote the paper: Y.W. Choi, S. Lee, G.H Sin, S.H. Hong.

Data availability statement

The data that has been used is confidential.

Declaration of competing interest

No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been previously published and was not contemplated for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

References

[1] T.C. Adarme-Vega, S.R. Thomas-Hall, P.M. Schenk, Towards sustainable sources for omega-3 fatty acids production, Curr. Opin. Biotechnol. 26 (2014) 14–18, https://doi.org/10.1016/j.copbio.2013.08.003.
[2] M. Cholewski, M. Tomczykowa, M. Tomczyk, A comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids, Nutrients 10 (2018) 1662, https://doi.org/10.3390/nu10111662.
[3] M.L. Panse, S.D. Phalke, World market of omega-3 fatty acids, in: Omega-3 Fatty Acids: Keys to Nutritional Health, 2016, pp. 79–88, https://doi.org/10.1007/978-3-319-40458-5_7.
[4] N. Rubio-Rodríguez, S. Beltrán, I. Jaime, S.M. de Diego, M.T. Sanz, J.R. Carballido, Production of omega-3 polyunsaturated fatty acid concentrates: a review, Innov Food Sci Emerg Technol 11 (2010) 1–12, https://doi.org/10.1016/j.ifset.2009.10.006.
[5] J.P. Schuchardt, A. Hahn, Bioavailability of long-chain omega-3 fatty acids, Prostaglandins Leukot. Essent. Fatty Acids 89 (2013) 1–8, https://doi.org/10.1016/j.plefa.2013.03.010.

[6] P. Lembke, Omega-3 Bioavailability Depends on the Chemical Form, Concentration and Fed-State, Omega, 2021. https://vitalremedymd.com/blogs/professionals/omega-3-bioavailability-dependson-the-chemical-form-concentration-and-fed-state.

[7] A. Wakil, G. Mackenzie, A. Diego-Taboada, J.G. Bell, S.L. Atkin, Enhanced bioavailability of eicosapentaenoic acid from fish oil after encapsulation within plant spore exines as microcapsules, Lipids 45 (2010) 645–649, https://doi.org/10.1007/s11745-010-3427-y.

[8] K.E. Bremmell, D. Briskey, T.R. Meola, A. Mallard, C.A. Prestidge, A. Rao, A self-emulsifying Omega-3 ethyl ester formulation (AquaCelle) significantly improves eicosapentaenoic and docosahexaenoic acid bioavailability in healthy adults, Eur. J. Nutr. 59 (2020) 2729–2737, https://doi.org/10.1007/s00394-019-02118-x.

[9] J. Dyerberg, P. Madsen, J.M. Møller, I. Aardestrup, E.B. Schmidt, Bioavailability of marine n-3 fatty acid formulations, Prostaglandins Leukot. Essent. Fatty Acids 83 (2010) 137–141, https://doi.org/10.1016/j.plefa.2010.06.007.

[10] J.P. Schuchardt, I. Schneider, H. Meyer, J. Neubronner, C. von Schacky, A. Hahn, Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations-a comparative bioavailability study of fish oil vs. krill oil, Lipids Health Dis. 10 (2011) 1–7, https://doi.org/10.1186/1476-511X-10-145.

[11] A. Wakil, M. Mir, D.D. Mellor, S.F. Mellor, S.L. Atkin, The bioavailability of eicosapentaenoic acid from reconstituted triglyceride fish oil is higher than that obtained from the triglyceride and monoglyceride forms, Asia Pac. J. Clin. Nutr. 19 (2010) 499–505, https://doi.org/10.3316/ielapa.631807771037066.

[12] Y.-M. Kim, G.-H. Jang, C.-H. Seok, B.H. Kim, J.-W. Bae, B.-H. Kim, M.S. Yoon, A self-emulsifying omega-3 fatty acids delivery system for enhanced gastro-intestinal absorption in rats, Food Sci. Biotechnol. (2022) 1–8, https://doi.org/10.1007/s10068-022-01151-7.

[13] P. Tran, J.-S. Park, Recent trends of self-emulsifying drug delivery system for enhancing the oral bioavailability of poorly water-soluble drugs, J Pharm Investig 51 (2021) 439–463, https://doi.org/10.1007/s40005-021-00516-0.

[14] G. Noh, T. Keum, S. Bashyal, J.-E. Seo, L. Shrawani, J.H. Kim, S. Lee, Recent progress in hydrophobic ion-pairing and lipid-based drug delivery systems for enhanced oral delivery of biopharmaceuticals, J Pharm Investig (2022) 1–19, https://doi.org/10.1007/s40005-021-00549-5.

[15] M.A. Rahman, A. Hussain, M.S. Hussain, M.A. Mirza, Z. Iqbal, Role of excipients in successful development of self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS), Drug Dev. Ind. Pharm. 39 (2013) 1–19, https://doi.org/10.3109/03639045.2012.660949.

[16] F. Xia, Z. Chen, Q. Zhu, J. Qi, X. Dong, W. Zhao, W. Wu, Y. Lu, Gastrointestinal lipolysis and trans-epithelial transport of SMEDDS via oral route, Acta Pharm. Sin. B 11 (2021) 1010–1020, https://doi.org/10.1016/j.apsb.2021.03.006.

[17] S. Dokania, A.K. Joshi, Self-microemulsifying drug delivery system (SMEDDS)–challenges and road ahead, Drug Deliv. 22 (2015) 675–690, https://doi.org/10.3109/10717544.2014.896058.

[18] A. Mahmood, A. Bernkop-Schnürch, SEDDS, A game changing approach for the oral administration of hydrophilic macromolecular drugs, Adv. Drug Deliv. Rev. 142 (2019) 91–101, https://doi.org/10.1016/j.addr.2018.07.001.

[19] C.P. Tan, M. Nakajima, β-Carotene nanodispersions: preparation, characterization and stability evaluation, Food Chem. 92 (2005) 661–671, https://doi.org/10.1016/j.foodchem.2004.08.044.

[20] J. Elversson, A. Millqvist-Fureby, G. Alderborn, U. Elofsson, Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying, J Pharm Sci 92 (2003) 900–910, https://doi.org/10.1002/jps.10352.

[21] C.J. Souza, E.E. Garcia-Rojas, Effects of salt and protein concentrations on the association and dissociation of ovalbumin-pectin complexes, Food Hydrocoll 47 (2015) 124–129, https://doi.org/10.1016/j.foodhyd.2015.01.010.

[22] X. Chang, W. Feng, L. He, X. Chen, L. Liang, Fabrication and characterisation of whey protein isolate–propolis–alginate complex particles for stabilising α-tocopherol-contained emulsions, Int. Dairy J. 109 (2020), 104756, https://doi.org/10.1016/j.idairyj.2020.104756.

[23] World Medical Association, Declaration of Helsinki: ethical principles for medical research involving human subjects, JAMA 310 (2013) 2191–2194, https://doi.org/10.1001/jama.2013.281053.

[24] FDA, in: C.f.V.M. Center for Drug Evaluation (Ed.), Bioanalytical Method Validation-Guidance for Industry, 2018.

[25] C.f.M.P.f.H.U. (CHMP), Guideline on Bioanalytical Method Validation, 2011.

[26] L.C.B. Züge, C.W.I. Haminiuk, G.M. Maciel, J.L.M. Silveira, A.d.P. Scheer, Catastrophic inversion and rheological behavior in soy lecithin and Tween 80 based food emulsions, J. Food Eng. 116 (2013) 72–77, https://doi.org/10.1016/j.jfoodeng.2012.12.008.

[27] I. Garaiova, I.A. Guschina, S.F. Plummer, J. Tang, D. Wang, N.T. Plummer, A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification, Nutr. J. 6 (2007) 1–9, https://doi.org/10.1186/1475-2891-6-4.

[28] S.K. Raatz, L.K. Johnson, M.R. Bukowski, Enhanced bioavailability of EPA from emulsified fish oil preparations versus capsular triacylglycerol, Lipids 51 (2016) 643–651, https://doi.org/10.1007/s11745-015-4100-2.

[29] S. Reddy, H.S. Fogler, Emulsion stability: determination from turbidity, J. Colloid Interface Sci. 79 (1981) 101–104, https://doi.org/10.1016/0021-9797(81)90052-7.

[30] T. Jeyarani, T. Banerjee, R. Ravi, A.G. Krishna, Omega-3 fatty acids enriched chocolate spreads using soybean and coconut oils, J. Food Sci. Technol. 52 (2015) 1082–1088, https://doi.org/10.1007/s13197-013-1053-4.

[31] R.d.S. Lima, J.M. Block, Coconut Oil: what Do We Really Know about it So Far? Food Quality and Safety, 2019 https://doi.org/10.1093/fqsafe/fyz004.

[32] S. Pengon, N. Chinatangkul, C. Limmatvapirat, S. Limmatvapirat, The effect of surfactant on the physical properties of coconut oil nanoemulsions, Asian J. Pharm. Sci. 13 (2018) 409–414, https://doi.org/10.1016/j.ajps.2018.02.005.

[33] A. Gurram, P.B. Deshpande, S.S. Kar, U.Y. Nayak, N. Udupa, M. Reddy, Role of components in the formation of self-microemulsifying drug delivery systems, Indian J Pharm Sci 77 (2015) 249, https://doi.org/10.4103/0250-474x.159596.

[34] C. Galli, F.M. Maggi, P. Risé, C.R. Sirtori, Bioequivalence of two omega-3 fatty acid ethyl ester formulations: a case of clinical pharmacology of dietary supplements, Br. J. Clin. Pharmacol. 74 (2012) 60–65, https://doi.org/10.1111/j.1365-2125.2012.04174.x.

[35] I. Chung, J. Oh, S. Lee, I.J. Jang, Y. Lee, J.Y. Chung, A post hoc analysis of intra-subject coefficients of variation in pharmacokinetic measures to calculate optimal sample sizes for bioequivalence studies, Transl Clin Pharmacol 26 (2018) 6–9, https://doi.org/10.12793/tcp.2018.26.1.6.

[36] Y.-J. Lee, H.J. Yi, H.G. Kim, J.H. Oh, Y.-J. Shin, Y.-G. Kim, S.-N. Kim, One-step sample size determination for 2×2 bioequivalence study, J Pharm Investig 39 (2009) 217–219, https://doi.org/10.4333/KPS.2009.39.3.217.