A new alternative for intravenous lipid emulsion 20% w/w from superolein oil and its effect on lipid and liver profiles in an animal model

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Abstract

Purpose Intravenous lipid emulsion (IVLE) was first used to prevent essential fatty acids deficiency. IVLE with α -tocopherol was reported to provide protection against parenteral nutrition-associated liver disease. This study aims to determine the optimal parameters and conditions in developing a physically stable IVLE from superolein palm oil (SoLE 20%) and its effect on lipid and liver profiles in an animal model.

Methods SoLE 20% was prepared using superolein oil and MCT oil (1:1), stabilized with egg lecithin and homogenized using a high pressure homogenizer. Mean droplet size was used as the response variable and was measured using laser diffraction and dynamic light scattering method. Physical stability at 4 °C, 25 °C and 40 °C storage temperatures were determined based on particle size and distribution, polydispersity index, zeta potential, viscosity, vitamin E contents and pH. Sterility and pyrogenicity were also investigated. Rabbits were administered with 1.0 g/kg SoLE 20% for 5 h and repeated daily for 3 days to investigate its effect on blood lipid and liver enzymes profile.

Results SoLE 20% was successfully prepared using the optimized parameters of 800 psi, 7 cycles and 1.2 g lecithin. The IVLE prepared had a particle size of 252.60 ± 4.88 nm and was physically stable for 4 weeks at different storage temperatures. SoLE 20% had a high content of natural vitamin E, remained sterile and pyrogen free. It was also safe for intravenous administration and did not alter the blood lipid ($p > 0.05$) and liver enzymes profiles ($p > 0.05$) of the rabbits.

Conclusion The optimal parameters to develop a stable superolein based IVLE are 800 psi homogenization pressure, 7 homogenization cycles and using 1.2 g lecithin as the emulsifier. SoLE 20% is safe for intravenous administration and does not significantly alter lipid and liver enzymes profiles of the rabbits.

Keywords Superolein palm oil · Intravenous lipid emulsion · MCT · Egg lecithin · Response surface methodology

Introduction

Intravenous lipid emulsion (IVLE) was first created to prevent the development of essential fatty acids deficiency in patients requiring intravenous nutrition support. Subsequently, IVLE has gained acceptance as an alternative energy source, a delivery vehicle for poorly water-soluble drugs, an antidote in local and non-local anaesthetic drug toxicities and an effective treatment of overdoses of a wide range of drugs [1–4]. Intralipid® was the first IVLE that is safe for human use, which was invented by Arvid Wretling from Switzerland [5]. As the most important component of IVLE, fatty acids (FA) play an important role and have commonly been used as biologically active substrates, cell membrane components and energy source [6].

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Produce from the fruit of the tropical plant *Elaeis guineensis* or African oil palm, are now an important constituent of industrial and food products. Palm oil is the only vegetable oil with palmitic acids as the most abundant FA and having almost equal amount of saturated and unsaturated fatty acids [7]. Other than FA and triglyceride as the main components, a refined liquid fraction of palm oil or palm olein oil (PO) is also rich with vitamin E; namely tocotrienols and tocopherols; beta-carotene and oleic acid. PO has antioxidant effects and can be beneficial in hypercholesterolemia [8]. Latest findings show that α -tocopherol provide a protective effect against parenteral nutrition-associated liver disease when added to lipid emulsion [9]. Despite this, IVLE with vitamin E is not yet commercially available as a product [10] except for Omegaven® that contains a very small amount of α -tocotrienol ($0.67 \pm 0.02 \mu\text{g mL}^{-1}$) and β -tocotrienol ($0.03 \pm 0.00 \mu\text{g mL}^{-1}$) [11]. A recent study has revealed the potential of producing a palm oil-based lipid emulsion for intravenous use [12].

Response surface methodology (RSM) is an approach used to develop good experimental design that involves all of the independent variables [13]. The ability of RSM to determine the relationships and interactions between variables is based on mathematical models and statistical techniques. Basically, there are three steps involved in RSM application: designing the experiment, developing a model equation from statistical and regression analysis, and optimizing the variables. The values derived from the model equation are almost identical to the actual experimental values. The most valuable aspect of RSM is its ability to reduce the number of experiments while providing outcomes with optimal conditions [14, 15].

As an immiscible liquid system, palm olein oil lipid emulsion is thermodynamically unstable and has a high risk for phase breakdown [16]. The most common breakdown processes are creaming, flocculation and coalescence [17]. These stability issues might be influenced by processing parameters, types of emulsifiers, homogenization cycle, sterilization process and antioxidants used [18, 19]. Some of the methods to investigate the stability of lipid emulsion are visual observation, zeta potential, dynamic surface tension, peroxide level, pH measurement and globule-size distribution [20]. Globule-size distribution limit for all commercial IVLE has been established to assist in developing a stable pharmaceutical product. These limits include mean droplet size (MDS) of below 500 nm, percentage of fat globules $>5 \mu\text{m}$ (PFAT₅) less than 0.05%, pH range between 6 and 9, and free FA content limited to 0.07 mEq g^{-1} [2, 21].

The aims of this study are to determine the optimal parameters conditions in developing a physically stable IVLE from palm superolein oil using RSM, and its effect on lipid and liver profiles in an animal model.

Methods

Materials

Super-olein oil was obtained from FELDA IFFCO Sdn. Bhd. (Klang, Malaysia). Coconut MCT oil (Enersos®) was purchased from Pharm-D Sdn. Bhd. (Malaysia). Lecithin (Lipoid E80®) was purchased from Lipoid AG (German). Glycerol was purchased from EMD Millipore Corp. (Billerica, MA, USA). Sodium oleate and sodium hydroxide were purchased from Sigma Aldrich Chemie GmbH (German). Distilled water used was prepared in-house.

Preparation of intravenous lipid emulsion

Lecithin was dispersed in the oil phase containing superolein oil and MCT coconut oil (1:1). Sodium oleate and glycerol were added into distilled water. Both phases were stirred with a magnetic stirrer and incubated in a 70°C water bath for 10 min. Subsequently, the oil phase was added to the aqueous phase slowly in a drop-wise manner with constant stirring using an Ultra Turrax T25 Basic homogenizer (IKA, German) at 3000 rpm. The mixture was continuously stirred with increasing speed from 10,000 rpm to 18,000 rpm within 5 min. The stability of the prepared coarse emulsion was observed visually and particle size was measured using a laser diffraction method. Later, the coarse emulsion was passed through an APV High Pressure Homogenizer 2000 (Denmark) with cooling water circulating in the machine. Different homogenization pressures, ranging from 400 to 800 psi, and varying number cycles from 1 to 9 cycles were applied as suggested by RSM (Tables 1 and 2).

The pH for the fine emulsion was adjusted to 8.0 ± 0.5 with 1 mol L^{-1} NaOH. This process was done in a preparation room (grade B). Then, the final emulsion was transported to the production room (grade B) for packing in a laminar air-flow cabinet. The fine lipid emulsion was then transferred into 100 mL sterile, clear glass vials. The vials were purged with nitrogen gas for 5 min, then closed with rubber stoppers and sealed with aluminum vial caps. Finally, the IVLE was sterilized using a Hirayama Hiclave HVE-50 autoclave (Japan) at 121°C for 10 min.

Experimental design

A three-factor, three-level Box-Behnken design was applied to evaluate the main interaction and quadratic effects of three formulation and processing variables on the physical stability of 20% lipid emulsion for intravenous use. The independent variables were homogenization pressure (400 psi, 600 psi, 800 psi), number of cycles (1 cycle, 5 cycles, 9 cycles) and amount of lecithin used (0.6 g, 1.5 g, 2.4 g). The dependent variables were the MDS; Z-average and MDS; $D_{(4,3)}$.

Table 1 Box-Behnken design center points and other points in random order

Formulation	Factors; independent variables			Responses; MDS (nm)	
	Pressure (psi)	Cycle (cycles)	Lecithin (g)	Z-average	Mean D[4,3]
*1	600	5	1.5	257.63 ± 3.21	376.30 ± 0.00
*2	600	5	1.5	259.90 ± 2.62	375.70 ± 0.00
3	400	9	1.5	275.07 ± 1.77	576.00 ± 0.00
4	600	1	0.6	358.27 ± 3.87	1396.00 ± 0.02
5	600	9	2.4	223.67 ± 0.45	340.00 ± 0.00
6	800	5	2.4	220.23 ± 1.66	331.00 ± 0.00
*7	600	5	1.5	293.87 ± 4.08	678.70 ± 0.10
*8	600	5	1.5	302.23 ± 2.90	553.70 ± 0.00
9	800	9	1.5	237.97 ± 23.94	310.30 ± 0.02
10	400	1	1.5	336.47 ± 4.30	1612.00 ± 0.00
11	600	1	2.4	306.20 ± 5.05	1331.00 ± 0.11
12	400	5	2.4	297.83 ± 2.15	851.70 ± 0.00
13	800	5	0.6	322.87 ± 2.55	695.70 ± 0.00
14	600	9	0.6	288.77 ± 4.21	536.00 ± 0.09
15	400	5	0.6	363.93 ± 4.00	1199.30 ± 0.02
16	800	1	1.5	304.80 ± 4.29	787.00 ± 0.00
*17	600	5	1.5	254.23 ± 1.60	381.00 ± 0.00

*Replicated centre points

Seventeen experimental runs based on the Box-Behnken design were generated by the Design-Expert version 6.0.6 (DX6) software (Stat-Ease Inc., Minneapolis, USA). The Box-Behnken design consisted of five replicated center points and twelve other points in random order as shown in Table 1.

RSM was applied to predict the independent variables' variation effects in producing IVLE. In the current study, the best combination of independent variables were chosen based on the desirable responses that were MDS; Z-average and MDS; $D_{(4,3)}$. After several experiments were performed, a polynomial regression equation was used to analyze the response received. The generalized RSM model is shown below:

$$Y_i = \beta_0 + \beta_{1 \times 1} + \beta_{2 \times 2} + \beta_{3 \times 3} + \beta_{11 \times 1}^2 + \beta_{22 \times 2}^2 + \beta_{33 \times 3}^2 + \beta_{12 \times 1 \times 2} + \beta_{13 \times 1 \times 3} + \beta_{23 \times 2 \times 3}$$

Where Y_i is the predicted response; x_i is the independent variables (three variable); β_0 is the model constant; β_i is the linear

coefficients, β_{ii} is the quadratic coefficients and β_{ij} is the interaction coefficients.

Thermal sterilization test

A new batch of IVLE was prepared using the best combination of independent variables. 10 mL of each emulsion were taken and the MDS, PFAT₅, polydispersity index (PDI), zeta potential (ZP), viscosity (Vc) and pH were measured. Then, the emulsions were sterilized using Hirayama Hiclave HVE-50 autoclave (Japan) at 121 °C for 10 min. After the sterilization process was completed, the MDS, PFAT₅, PDI, ZP, Vc and pH of the lipid emulsion were measured again. Sterility test, pyrogenicity test and determination of vitamin E contents (pre and post sterilization) were also performed to monitor the effects of sterilization towards the IVLE.

Determination of vitamin E

Fatty acids methyl esters (FAME) of IVLE was prepared by mixing 50 mg of sample with 1 mL hexane and centrifuged for 30 s. Then, 1 mL of 2.5 M sodium methoxide in methanol anhydrate was added and centrifuged again for 6 s. After 5 min, the mixture was centrifuged for 5 min at 2000 rpm. The supernatant was filtered using 0.22 µm nylon filter (Thermo Fisher Scientific, USA). The filtered solution was used to measure vitamin E contents by reverse phase-high performance liquid chromatography (RP-HPLC). The HPLC

Table 2 Numerical optimization specification for the define particles sizes of SoLE 20%

Parameter	Goal	Low level	High level
Homogenization pressure (psi)	is within range	400	800
Homogenization cycles (cycle)	is within range	1	9
Lecithin amount (g)	target = 1.2	0.6	2.4
Particles sizes (nm)	minimum	310	1612

was equipped with isocratic pump, absorption detector, automatic injector and C18 XBridge column (5.0 μm , 4.6 mm $\times$ 250 mm) (Waters Corporation, USA). The mobile phase used was methanol: water (73:7) at 1.0 mL/h flow rate. The identification and quantification were done at 292 nm wavelength.

Sterility and pyrogenicity test

The sterility testing was conducted using Sabouraud dextrose agar (SDA) and nutrients agar (NA). IVLE samples were applied onto the SDA agar plates and incubated for 14 days at 25 °C and on NA agar plates at 37 °C. Monitoring for micro-organism growth was done on a daily basis. The pyrogenicity test was done using the *Limulus Amebocyte Lysate* (LAL) test kit (Thermo Scientific, USA). Endotoxin standard stock, LAL reagent solution, blank solution and chromogenic solution were prepared according to the manufacturer's recommendation. Approximately 50 μl samples or endotoxin standard solution was pipetted into the microplate vessel and warmed for 10 min at 37 ± 1 °C. LAL reagent solution (50 μl) and chromogenic solution (100 μl) were added to each vessel and continued to be warmed at 37 ± 1 °C for 10 min each time. Acetic acid 25% was added as the stop reagent and the absorption was measured at 405 nm using a plate reader. Endotoxin concentration was obtained based on a linear regression equation of the newly developed standard curve.

Short term physical stability

After preparation of the IVLE based on the best combination of independent variables, the emulsion was observed over a period of 1 month at the storage temperatures of 4 °C, 25 °C and 40 °C. MDS, PFAT₅, polydispersity index (PDI), zeta potential (ZP), viscosity (Vc) and pH were the dependent variables observed.

Visual observation

Visual observation was carried out after the high pressure homogenization process using an APV High Pressure Homogenizer 2000 (Denmark) to identify stability issues and non-homogenized emulsion.

Measurement of MDS ($D_{(4,3)}$) and PFAT₅

Particle sizes and particle sizes distribution for the IVLE were measured using a Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) laser diffraction (LD) analyzer. The Mastersizer was operated at 633 nm, 25 °C and equipped with a backscatter detector angle of 173°. The detection used helium neon laser (red light) and also solid-state light (blue light). The emulsion samples were slowly added in 800 mL recirculating de-ionized water at 1800 rpm until it reached an

ideal obscuration range of 10–20%. The refractive index of super-olein oil used for the measurement was 1.458. Measurements were made in triplicate for each sample. The results were reported as volume mean diameter $D_{(4,3)}$ and PFAT₅. Background calibrations were done prior to sample measurements [19].

Measurement of MDS (Z-average) and polydispersity index

Particle sizes and polydispersity index of the IVLE were measured in ordinary cuvettes using dynamic light scattering (DLS) method. A Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) was used for this purpose and was operated at 633 nm, 25 °C with a scattering angle of 173°. The emulsions used were diluted to 1:100 with de-ionized water. Measurements were made in triplicate for each sample [19].

Measurement of zeta-potential

Zeta-potential for the IVLE was measured in capillary cuvette using dynamic light scattering method utilizing a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The Zetasizer was operated at 633 nm, 25 °C with a scattering angle of 173°. The emulsions used were diluted to 1:100 with de-ionized water to avoid the effect of multiple scattering. Measurements were made in triplicate for each sample [19].

Measurement of viscosity

Viscosity for the IVLE was measured using Brookfield Digital Rheometer DVIII viscometer (Brookfield Engineering Laboratories Inc., USA). The viscometer was equipped with the SC4–13 sample jar and SC4–18 spindle. Approximately 6 mL of IVLE samples were used and placed in the jars for each test. Measurements were made in triplicate for each sample.

Measurement of pH

The pH value of the IVLE was measured using a digital Five Easy Plus pH meter FEP20 (Mettler-Toledo AG Analytical, Switzerland). The pH meter was calibrated using standard buffers at pH 4.01, 7.0 and 9.21. Measurements were made in triplicate for each sample.

Animal study

This study was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (FF/2017/AHMAD FUAD/24-MAY/847-JULY-2017-FEB.-2018). All procedures were performed on the rabbits under anaesthesia. Three male New Zealand white rabbits, weighing between

2.22–2.26 kg were used in this study. Rabbits were purchased from the Laboratory Animal Resource Unit (LARU), Faculty of Medicine, Universiti Kebangsaan Malaysia. The animals were individually caged in the Animal Room, Faculty of Pharmacy, Universiti Kebangsaan Malaysia for 2 weeks for acclimatization prior to the actual study. Weight, heart rates and respiratory rates were monitored on a daily basis, with free access to rabbit pellet and water throughout the acclimatization and study periods. The temperature and humidity of the room were set at 25 °C and 55 ± 10%, respectively, with a light-dark cycle of 12 h each.

A mixture of ketamine, Zoletil-50 and Xylazine (KTX) (LARU, Universiti Kebangsaan Malaysia) were used as the anaesthetic agent to restrain the rabbits. A dose of 0.2 mL/kg were administered as the first dose via the marginal blood vessels of the ear using a 1 mL syringe equipped with 30G × 4/8" injection needle (Terumo Corporation, Japan). Subsequently, additional 0.1 mL/kg doses were supplied every 30–45 min. Each rabbit was infused with 1.0 g/kg SoLE 20% for 5 h and the infusion was repeated daily for 3 days. The infusions were done through the marginal vein of the ear with Surflo® Winged Infusion Set (23G size, 0.65 mm outer diameter, 19 mm long) (Terumo Corporation, Japan) connected to Infusomat® Space P infusion pump (BBraun Melsungen, Germany).

A 10 mL syringe with 25G × 1" needle was used to take blood samples from the rabbits' marginal ear artery. The blood sampling was performed before the start of infusion on day 1 and then were repeated on day 2 and day 3. Blood samples were also taken on day 4 to monitor the effect of the last dose. The blood samples were placed in an EDTA-containing test tube. Biochemical analysis of rabbit blood was performed to monitor plasma lipid content: including cholesterol (CHOL); low density lipoprotein (LDL); high density lipoprotein (HDL); and triglyceride (TG), and the profile of liver enzyme content: alanine aminotransferase (ALT); alkaline phosphatase (ALP); and aspartate aminotransferase (AST). Samples analysis were performed using ADVIA 2400 machine (Siemens Healthineers GmbH, Germany) within 24 h of blood sample collection and stored at 4 °C throughout the storage period.

Statistical analysis

GraphPad Prism® version 5 (GraphPad Software Inc., USA) was used to analyze experimental data. The independent variables differences were analyzed using analysis of variance (ANOVA). Independent variables with non-significant difference ($p > 0.05$) were eliminated. Comparisons of two samples were done using t-test and comparisons for pre and post effect were done using paired t-test. P values smaller or similar to 0.05 were considered statistically significant.

Results

Selection of processing condition

White colored and fine lipid emulsions were produced for every formula suggested by RSM. To select the best of these three independent parameters, a 17-run Box-Behnken design was generated with 12 factorial points and 5 repeat center points. Particles sizes (Z-average and $D_{(4,3)}$) were measured for every run as the response for this experiment and are reported in Table 1. Mean droplet sizes (MDS) were used to generate equations that showed the relationship between dependent (responses) and independent variables (factors).

In this study, a second-order polynomial model was used to predict the response to the independent variables. $D_{(4,3)}$ (Y) had a significant regression between variables and the response ($p = 0.0068$). On the other hand, the Z-average (X) had an insignificant regression between variables and response ($p = 0.9858$). Both MDS had a non-significant lack of fit with $p > 0.05$ ($D_{(4,3)}$, $p = 0.5975$ and Z-average, $p = 0.1129$). The generalized response surface models are listed below:

Equation for response X:

$$\begin{aligned} MDS(X) = & +295.86 - 5.46A \\ & + 4.38B - 1.89C - 6.30A^2 - 5.32B^2 - 4.09C^2 \\ & + 6.01AB - 45.41AC - 4.52BC \end{aligned}$$

Equation for response Y:

$$\begin{aligned} MDS(Y) = & +473.20 - 264.37A - 420.50B - 121.63C \\ & + 108.40A^2 + 239.65B^2 + 187.90C^2 \\ & + 139.75AB - 4.50AC - 32.75BC \end{aligned}$$

We used the numerical optimization choice by DX6 software to find the best point that maximizes the factors function. Our aim is to obtain the smallest particles sizes with targeted lecithin amount of 1.2 g. Table 2 shows the specific optimum condition for the defined particles sizes of IVLE. Thus, the selected combination of lipid emulsion preparation parameter were 800 psi of high pressure homogenization, 7 cycles of high pressure homogenization and 1.2 g of lecithin.

Verification of model

The adequacy of the selected model and response surface equations were examined by comparing the predicted outcomes and the experimental outcomes. The preparation of the experimental sample was done using the selected combination parameter. The result of the experimental outcomes values and predicted outcome values are presented in Table 3. The experimental sample prepared using the selected

Table 3 Experimental outcomes versus predicted outcomes of the SoLE 20% MDS

Response	Predicted outcomes	Experimental outcomes	<i>p</i>
MDS; Z-Average (nm)	288.47	252.60 ± 4.88	< 0.05
MDS; D [4,3] (nm)	305.14	306.33 ± 3.22	0.59

combination parameter produced an almost similar MDS compared to predicted outcomes values for both Z-Average (288.47 nm versus 252.60 ± 4.88 nm) and D_(4,3) (305.14 nm versus 306.33 ± 3.22 nm). Even though the Z-average shows a statistically significant different outcome compared to predicted, the selected parameters produced a smaller particle size of the IVLE.

Thermal sterilization

Based on our findings, all of the tested parameters were good before and after sterilization except for pH value (Table 4). The pH decreased from 8.75 ± 0.01 to 7.84 ± 0.05 after sterilization for 15 min (*p* < 0.05). The effect of sterilization on vitamin E content was also monitored. Table 5 shows that vitamin E content was affected by the thermal sterilization process but no difference was detected statistically. Only γ-tocotrienol showed a significant difference of content percentage after sterilization with *p* = 0.0165.

NA plates were used to monitor the growth of bacteria, while SDA plates were used to monitor fungal growth. In this study, both media showed no growth of microorganisms from day 1 until day 14. The pyrogenicity test showed that the endotoxin level of the IVLE was less than 1.5 EU/mL. The value was low compared to the product release limit which is less than the calculated 16.0 UE/mL.

Short term physical stability of the IVLE

The physical stability of lipid emulsion in different storage temperatures are presented in Table 6. The results show that there were no changes in particle size diameter stored at 4 °C and 25 °C for 4 weeks. Lipid emulsion stored at 40 °C showed the largest particle size after 4 weeks (*p* = 0.03). Other than

that, storing IVLE at 25 °C and 40 °C, decreased the pH value significantly (*p* = 0.00 and *p* = 0.04). The other parameters showed no significant difference after 4 weeks.

Animal study

Rabbits were administered with SoLE 20% for 3 days and survived during and after the administration without complications. Throughout the acclimatization and study period, weight, rectal temperature (38.17–39.23 °C), heart rate (142.67–148.00 cpm) and respiratory rate (41.67–50.89 cpm) did not differ statistically. Figure 1 shows the results of the NZW rabbit plasma lipid profile prior to the administration of SoLE 20%. The contents of plasma lipid were not significantly different before and after SoLE 20% administration (*p* > 0.05).

Figure 2 shows the results of NZW rabbit plasma liver enzymes profile prior to the administration of SoLE 20%. The contents of the enzymes were not statistically different before and after the SoLE 20% administration (*p* > 0.05).

Discussion

Production of SoLE 20%

Previous studies have shown the significant effect of homogenization pressure, homogenization cycles and amount of lecithin on mean droplet size of an IVLE [22, 23]. Based on our findings, the preparation of IVLE should include the use of high pressure homogenizer and at 800 psi pressure for 7 cycles.

The preparation of superolein based IVLE uses 1.2 g lecithin for every 100 mL of IVLE. Most of the widely available

Table 4 Effect of sterilization on SoLE 20% physical stability (*n* = 6)

Parameter	Pre-sterilization	Post-sterilization	<i>p</i>
MDS; Z-average	253.17 ± 1.31	253.63 ± 3.98	0.88
PFAT ₅	0.00	0.00	
Polydispersity index	0.069 ± 0.041	0.047 ± 0.041	0.35
Zeta potential	−27.47 ± 2.84	−35.9 ± 2.26	0.10
Viscosity	1.85 ± 0.24	1.74 ± 0.04	0.42
pH	8.75 ± 0.01	7.84 ± 0.05	< 0.05

Table 5 Effect of sterilization on vitamin E contents

Vitamin E isomers		Contents (%)		<i>p</i>
		Pre-sterilization	Post-sterilization	
α - Tocopherol	α - T	18.43 ± 0.10	18.51 ± 0.20	0.3001
α - Tocotrienol	α - T ₃	9.22 ± 9.59	7.74 ± 0.25	0.2674
β - Tocotrienol	β - T ₃	13.15 ± 2.06	11.61 ± 0.10	0.3066
γ - Tocotrienol	γ - T ₃	43.01 ± 0.00	45.87 ± 0.64	0.0165
δ - Tocotrienol	δ - T ₃	16.20 ± 0.04	16.27 ± 0.01	0.0561

Table 6 Effect of short term storage on SoLE 20% physical stability ($n = 9$)

Parameter	Temperature	0 week	4 week	* <i>p</i>
MDS; Z-average	4 °C	229.07 ± 1.57	235.90 ± 5.30	0.18
	25 °C		228.20 ± 0.46	0.41
	40 °C		243.90 ± 3.03	0.03
PFAT ₅	4 °C	0.00	0.00	
	25 °C		0.00	
	40 °C		0.00	
Polydispersity index	4 °C	0.09 ± 0.02	0.08 ± 0.03	0.67
	25 °C		0.08 ± 0.03	0.60
	40 °C		0.07 ± 0.05	0.60
Zeta potential	4 °C	−45.23 ± 8.45	−49.33 ± 5.92	0.30
	25 °C		−48.30 ± 6.30	0.56
	40 °C		−50.67 ± 5.11	0.50
Viscosity	4 °C	1.72 ± 0.04	1.93 ± 0.01	0.66
	25 °C		1.86 ± 0.05	0.60
	40 °C		1.83 ± 0.07	0.59
pH	4 °C	7.60 ± 0.05	7.89 ± 0.08	0.004
	25 °C		7.99 ± 0.02	0.002
	40 °C		7.68 ± 0.01	0.094

10% and 20% IVLE also use 1.2 g egg lecithin as their emulsifier due to its ability to stabilize both types of IVLE [10]. Beside that, 20% IVLE with 1.2 g lecithin would also cause more efficient clearance of triglycerides based on their lower phospholipid-triglyceride ratio [24].

IVLE can be sterilized by autoclaving, membrane filtration, gamma irradiation, ethylene oxide sterilization and high hydrostatic pressure sterilization methods [25]. However, sterilization may cause issues such as increase in particle size and changes of the emulsion pH. Based on our study, the bpH level was influenced by the sterilization process. Even though the pH level was still within the recommended range, the value decreased significantly. This issue might be due to lecithin being hydrolyzed at a high temperature and producing free fatty acids [23]. The IVLE was stable after sterilization and the characteristics fulfilled the suggested criteria and are

comparable to commercially available products in the market such as Lipidem 20% [20, 26, 27].

Based on our findings, the method used in the present study was able to produce sterile IVLE. This was proven by no growth of bacteria and fungi after 14 days of exposure to the specific environment. Other than the sterilization process, aseptic handling, the use of laminar air-flow cabinet, the use of personal protective equipment and non-pyrogenic materials also influenced the sterile production of IVLE [28–30].

Based on the LAL test, the IVLE only produced <1.5 EU/mL endotoxin compared to the calculated endotoxin level that was <16.0 EU/mL for the product release limit. In the LAL test, endotoxins will activate clot enzymes and stimulate the production of yellow color fluid by p-nitroaniline. The color production will be proportionate to the amount of endotoxin occurring in the samples [31]. This result could be influenced

Fig. 1 Plasma lipid profile prior to the administration of SoLE 20% for 3 days

Fig. 2 Plasma liver enzymes profile prior to the administration of SoLE 20% for 3 days

by the use of non-pyrogenic materials, the autoclave of materials for 1 h at 250 °C and the use of distilled water [32].

Short term physical stability of the IVLE

Lipid emulsion is prone to become physically and chemically unstable. The main issues include creaming, flocculation, disproportionation (Ostwald Ripening), coalescence and lipid peroxidation. This lipid emulsion instability issues are commonly related to formation of large lipid globules, low pH, concentration of positive-charged ions, presence of oxygen and, UV-light and high temperature [16, 17]. The instability issues will result in technical and clinical complications after intravenous administration of the lipid emulsion. Lipid embolism is an example of the clinical complication [10]. It is related to the fact that the diameter of the smallest blood vessel in the human body is 5 µm which is located in the lung. This is among the justification for USP <729> requirement for PFAT₅ < 0.05% [33]. Once the PFAT₅ > 0.4% the phase separation can be observed visually [22].

The early signs of lipid emulsion destabilization can be detected through the increment of particle size diameter and the increasing trend of the particle size distribution toward larger ranges [20]. Particle size and their distribution therefore become crucial factors in the development of coalescence and Ostwald ripening processes [34].

Particle size for SoLE 20% ranges from 228.20 ± 0.46 to 243.90 ± 3.03 nm after 4 weeks. Even though the storage at 40 °C caused changes to the particle size ($p < 0.05$), it was still within the suggested range for IVLE.

As the parameter used to measure an emulsion droplets distribution homogeneity and width, PDI can range from 0 to 1. Values ranging from 0.0 to 0.4 indicate monodispersity and a range of 0.5 to 1.0 indicates a relatively broad distribution [10, 14]. Values ranging from 0.00 to 0.25 are acceptable for intravenous lipid emulsion [13]. In the current study, PDI for lipid emulsion ranged from 0.007 ± 0.005 to 0.008 ± 0.003 after 4 weeks of storage at 4 °C, 25 °C and 40 °C. All of the

formulations stored in different temperatures were within the monodisperse range, indicating a narrow droplet size distribution. The small PDI value could be due to the low addition rate of oil phase into the aqueous phase during pre-homogenization process [14]. This optimized the oil droplet breaking down process during emulsification. A low concentration of emulsifier used may also produce a lipid emulsion with low viscosity and affect the emulsification efficiency. This situation contributed to the small PDI value as the particle size of the lipid emulsion became narrow.

Zeta potential (ZP) value is a parameter used to measure long term stability of a colloidal system such as lipid emulsion [5, 13]. The zeta potential value of our lipid emulsions range from -49.40 ± 2.43 to -50.43 ± 2.17 mV after 4 weeks of storage at 4 °C, 25 °C and 40 °C. All of the emulsions were in good condition visually without any physical separation. The zeta potential values for intravenous lipid emulsion normally range between -30 to -50 mV. The value of -30 mV is required to have a sufficient electrostatic repulsion between droplets to maintain physical stability and to prevent coalescence from occurring [35]. Lipid emulsions with values higher than -60 mV will show good stability for long term [5]. This is influenced by the negative charge of the phospholipids that create a mutual repulsion among the emulsion droplet. This environment keeps the droplets separated which stabilizes the emulsion. Addition of cations, drugs, water and changes of lipid emulsion charge, may cause changes in the emulsion environment and finally change the zeta potential value [20].

Injectability or infusibility of lipid emulsion to the blood circulation can be checked via the viscosity value. It is a function of the concentration and the particle size of the disperse phase [5]. Long term stability of the lipid emulsion will also be influenced by the low values of viscosity. The particle size, particle charge, colloidal interaction and dispersed phase volume fraction are the main factors that influence lipid emulsion viscosity [34]. The viscosity of our lipid emulsion were between 1.83 ± 0.07 cP to 1.93 ± 0.01 cP after 4 weeks of storage at 4 °C, 25 °C and 40 °C. No difference was observed in

comparison to freshly prepared lipid emulsion ($p > 0.05$). The use of MCT as part of the oil phase and also low percentage of oil phase were the main contributors to the low value of viscosity for our lipid emulsion. A high viscosity value of lipid emulsion can affect emulsion efficacy and result in a higher risk for coalescence due to its large particle size [13].

The physical stability of lipid emulsion can also be affected by the pH value [34]. Free fatty acids produced by the hydrolyzed lecithin in this study might have decreased the pH value of the IVLE [23]. The average pH value of our newly developed lipid emulsion was 7.60 ± 0.05 . Even though changes were detected for our lipid emulsion stored at 4 °C and 25 °C, they were still within the accepted pH range for intravenous lipid emulsion that is within pH 6 and 9 [21]. Neither coalescence nor oiling out were seen in both groups of lipid emulsion. The lower the pH value of lipid emulsion, the higher the chance that it will show a narrow particle size distribution [20]. In other words, it will give a lower PFAT₅ value and low risk of lipid emulsion instability issues.

Animal study

Findings from our animal study have shown that the administration of 1.0 g/kg/day SoLE 20% did not cause abrupt changes to the lipid profiles of the rabbits. The level of cholesterol, triglyceride, high density lipoprotein and low density lipoprotein were statistically unchanged ($p > 0.05$). Other studies reported that the normal range for cholesterol is 0.99–2.05 mmol/L, triglyceride is 0.82–1.51 mmol/L, high density lipoprotein is 0.45–0.76 mmol/L and low density lipoprotein is 0.18–0.52 mmol/L [36–38]. The liver enzymes profile of the rabbits also showed a similar trend. Similarly, the differences between the level of alkaline phosphatase, alanine transaminase, gamma-glutamyl transferase and aspartate transaminase levels were also statistically unchanged ($p > 0.05$). Previous studies on NZW rabbits have reported that the common range for ALP is 12–96 uL⁻¹, ALT is 55–260 uL⁻¹, GGT is 0.00–31.00 uL⁻¹ and AST is 33.00–99.00 uL⁻¹ [39–43].

In comparison, Jufri et al. (2012) [44] has also developed a palm oil based intravenous lipid emulsion named NEMS™ MCT/LCT 20%. They have administered 2–4 g/kg of the emulsion to male NZW rabbits for 6 h through the marginal vein of the rabbit ear. The obvious differences between their study and ours are the dose administered and the timing for blood sampling. Jufri et al. (2012) blood samples were taken for every 2 hours whereas in the current study, blood samples were taken every 24 h. This difference could explain the lower lipid profile level seen for SoLE 20% compared to NEMS 20% due to the maximum elimination rate for the reported ILE being 3.8 g/kg/24 h that is faster for the MCT oil-containing IVLE as it does not require the carnitine carrier medium to be transported into the mitochondria for metabolism [45].

These results suggest that the supply of 1.0 g/kg/d SoLE 20% for 3 days is safe and will not induce hypercholesterolemia, hypertriglyceridemia or liver injury to the recipient. Although at this stage it is too early to conclude the benefits of this newly developed IVLE, this study has shown the potential of superolein based intravenous lipid emulsion as an alternative for soy-based IVLEs.

Conclusion

Based on this study, the optimal parameters to develop a physically stable superolein oil based intravenous lipid emulsion were 800 psi homogenization pressure, 7 homogenization cycles and using 1.2 g lecithin as the emulsifier. SoLE 20% is safe for intravenous administration and does not significantly alter lipid and liver enzymes profiles in an animal model.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Animal study was approved by Universiti Kebangsaan Malaysia Animal Ethic Committee with approval number of FF/2017/AHMAD FUAD/24-MAY/847-JULY-2017-FEB.-2018.

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