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Optimization of beta-carotene self-nanoemulsifying drug delivery system formula based on a low-energy method preparation and simplex lattice design

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ABSTRACT: Beta-carotene is an antioxidant compound that benefits health by boosting immunity and preventing chronic diseases. However, beta-carotene has low solubility (practically insoluble in water) and bioavailability, so it will produce a poor beneficial effect. An alternative candidate that may overcome this problem is a self-nanoemulsifying drug delivery system (SNEDDS). SNEDDS can enhance the oral bioavailability of poorly soluble active compounds by increasing the solubility and stability of active compounds in oil droplets. This study aims to develop beta-carotene SNEDDS by determining the optimum composition of SNEDDS. The low-energy method and simplex lattice design (SLD) were combined to determine the optimum formula. Based on the results, the solubility of beta-carotene in sesame oil, polysorbate 80, and PEG 400 as components of SNEEDS were 0.455 mg/mL, 1.538 mg/mL, and 0.667 mg/mL, respectively. The optimum formula of beta-carotene SNEDDS had a mixing ratio of sesame oil and surfactant mixture in 1:15.2 (v/v), emulsification time of 38.48 ± 0.70 seconds, the droplet size of 13.13 ± 0.50 nm, and size distribution (PdI) of 0.133 ± 0.06. The optimum formula of beta-carotene SNEDDS had good stability in the artificial gastric fluid after 4 hours of incubation. Moreover, the optimum beta-carotene SNEDDS formula had high stability during storage at room temperature and 40°C ± 2°C/75%RH±5%RH.

KEYWORDS: SNEDDS; beta-carotene; low energy; simplex lattice design; nanoemulsion.

1. INTRODUCTION

Beta-carotene is a carotenoid compound that can be used as a natural dye, antioxidant, and supplement for chronic disease prevention [1]. However, beta-carotene has low solubility and bioavailability, so the beneficial effect becomes weak. In order to increase the effectiveness of beta-carotene in the body, it is necessary to increase the bioavailability of beta-carotene. Various approaches have been taken to deliver compounds with low solubility and bioavailability, including the self-nanoemulsifying drug delivery system (SNEDDS).

SNEDDS is an isotropic mixture of oil, surfactant, and cosurfactant, and will form a nanoemulsion in additional water with light agitation. Generally, SNEDDS produces droplets in nano size after the emulsification process so that it can increase the dissolution rate and bioavailability of beta-carotene [2]. SNEDDS has higher stability than emulsions and can be easily prepared on a small or large scale (Jeevana and Sreelakshmi., 2011). Moreover, SNEDDS can deliver active substances directly to the lymphatic transport system, so first-pass metabolism and hepatic clearance of beta-carotene can be reduced [3].

In this study, beta carotene was formulated into SNEDDS using sesame oil as a base oil and a mixture of surfactants (polysorbate 80) and cosurfactants (PEG 400). The composition of each component was optimized using the simplex lattice design (SLD). The optimum formula was determined based on parameters such as emulsification time, nanoemulsion droplet size, and nanoemulsion droplet size distribution. Furthermore, the stability of the optimum formula was evaluated by using artificial gastric fluid (AGF) as incubation media. This research was expected to produce good and stable beta-carotene SNEDDS.

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2. RESULTS & DISCUSSION

2.1 Solubility of beta-carotene

The solubility test was carried out to determine the solubility of beta-carotene in sesame oil, surfactant (Polysorbate 80), and cosurfactant (PEG 400) as the basis of the SNEDDS formula. The solubility of the active substance in a solvent determines the loading capacity of the active substance in the solvent. the loading capacity of the active substance in the solvent will be high if the solubility of the active substance is also high in the solvent [4]. SNEDDS must be able to act as a solvent so that the active substance will not precipitate during dilution [5]. The active substance must also be ensured to dissolve in each component of the SNEDDS to prevent precipitation in the intestinal lumen [6]. The solubility of beta carotene in sesame oil, polysorbate 80, and PEG 400 can be seen in Table 1.

Table 1. Solubility of beta-carotene in SNEDDS component

Bases	Beta-carotene (mg)	Total volume (mL)	Solubility (mg/mL)
Sesame oil	10	22	0.455
Polysorbate 80	10	6.5	1.538
PEG 400	10	15	0.667

2.2 Pseudoternary Phase Diagram Construction

Pseudoternary phase diagrams are used to determine the upper and lower limits of the oil and surfactant mixture (Smix) ratio (polysorbate 80: PEG 400) which will be used in determining the optimum beta carotene formula for SNEDDS. This study developed a pseudo-ternary phase diagram based on the phase inversion competition (PIC) approach. This method is a technique often used in the preparation of low-energy-based nanoemulsions.

The preparation of nanoemulsion using the PIC approach follows the following steps, the oil and surfactant are mixed first, and then the water is added gradually. The mixed system will go through several phases, namely oil in water (W/O) emulsion, liquid crystalline (LC) gel-like/microemulsion/double emulsion (O/W/O) phase, and O/W nanoemulsion [7]. The visual appearance of the nanoemulsion and the pseudo-ternary phase diagram of each ratio of sesame oil and Smix can be seen in Figure 1-2.

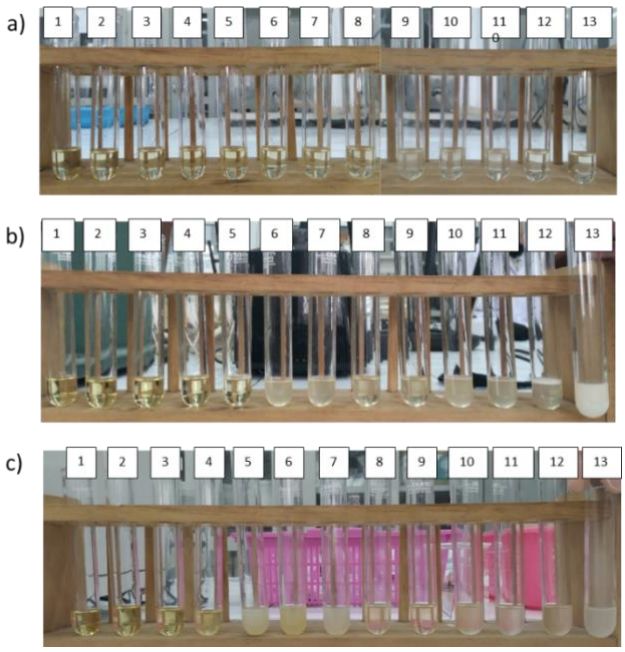

Figure 1. Visual appearance of nanoemulsion by following oil-Smix ratio: a) 0.5: 9.5, b) 0.75: 9.25, c) 1:9. Percent of oil-Smix was gradually decreased from right to left (80-20%).

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Based on Figure 1-2, it was known that samples No. 1-8(a), No. 1-5 and 8-9(b), and No. 1-4 and 8-9(c) had a clear appearance and LC gel-like. However, samples No. 9-11(a), No. 6-7 and 10-12(b), and No. 10-13(c) were also in the LC gel-like phase with a slightly cloudy appearance. In addition, sample No. 12-13(a) had a clear appearance with low viscosity LC gel-like. However, samples No. 13(b) and No. 5-7(c) had a very cloudy appearance with low viscosity LC gel-like.

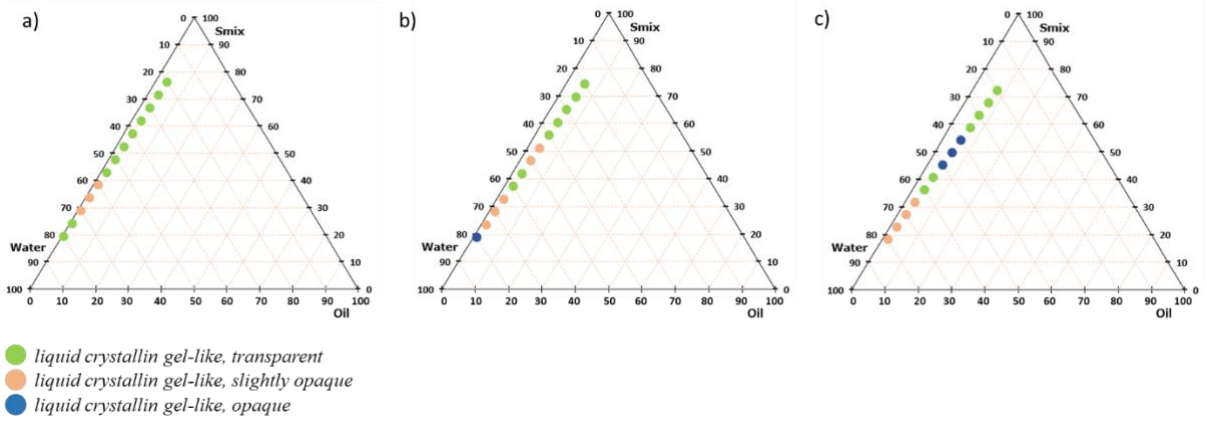

Figure 2. Pseudo-ternary phase diagram *of rasio* minyak-Smix a) 0.5: 9.5, b) 0.75: 9.25, c) 1:9.

All LC gel-like samples (150 µL) with a clear appearance were diluted by adding 10 mL of distilled water in total volume, then the droplet size and size distribution were analyzed. Samples that produced small droplet sizes and PdI were used to determine the upper and lower limits oil and Smix ratio to determine the optimum beta-carotene SNEDDS formula. The results of the sample characterization are shown in Table 2. The range of oil and Smix ratios that produced nanoemulsions were 2.25-4.5 and 36-61.75, respectively. The ratio ranges of oil and Smix that were used in optimizing the beta-carotene SNEDDS formula with the SLD approach were 2.5-4.5 and 36-38.

Table 2. Size and polydispersity index (PdI) of LC gel-like sample

Ratio Oil: Smix	Sample code	Composition ratio		Size of the droplet (nm)	PdI
		Oil	Smix		
0.5:9.5	4	3.25	61.75	16.31	0.147
	5	3	57	68.49	0.26
	8	2.25	42.75	12.74	0.125
0.75:9.25	5	4.5	55.5	15.91	0.163
	9	3	37	43.19	0.44
1:9	8	4.5	40.35	137.96	0.163
	9	4	36	76.77	0.242

2.3 Preparation and characterization of beta-carotene SNEDDS formula

Beta-carotene SNEDDS was prepared based on the generated composition by the SLD method shown in Table 8. Each SNEDDS sample was incubated for 24 hours (at room temperature) to ensure that beta-carotene was completely dissolved in the system. The solubility of beta carotene in the SNEDDS system was indicated by the absence of separation of the SNEDDS components. The results showed that all samples produced clear SNEDDS and there was no separation and precipitation of beta-carotene. Furthermore, beta-carotene SNEDDS were characterized to determine emulsification time, droplet size, and droplet size distribution of nanoemulsions.

Emulsification time testing was carried out to see the ability of SNEDDS to form nanoemulsions spontaneously in artificial gastric fluid (AGF). A good SNEDDS has less than one minute of emulsification

time in the stomach [8]. Emulsification time is a parameter determining the ability of surfactants and cosurfactants to form an oil-water interface layer under low energy conditions (mild agitation) in the gastrointestinal tract. The results of beta-carotene SNEDDS emulsification time in AGF media can be seen in Table 4.

Based on the data in Table 3, the fastest emulsification time was produced by Run 3 with an emulsification time of 38.07 ± 1.02 seconds, and the longest emulsification time was shown by Run 6 with an emulsification time of 48.53 ± 1.95 seconds. However, the 8 Runs had an emulsification time of less than one minute. The short emulsification time was affected by the action of surfactants and cosurfactants which were able to immediately form an oil-water interface layer [9].

Table 3. Characterization of beta-carotene SNEDDS (N = 3)

Run	Component ratio		Emulsification time (second)	Droplet size (nm)	Size distribution (PdI)
	Oil	Smix			
1	3	37.5	14.24 ± 0.80	41.66 ± 1.23	0.203 ± 0.05
2	3.5	37	15.22 ± 1.19	41.89 ± 1.55	0.235 ± 0.07
3	2.5	38	12.96 ± 0.37	38.07 ± 1.02	0.114 ± 0.04
4	3.5	37	16.15 ± 2.21	43.51 ± 2.21	0.306 ± 0.12
5	4	36.5	20.78 ± 4.01	45.45 ± 1.94	0.462 ± 0.11
6	4.5	36	28.48 ± 15.30	48.53 ± 1.95	0.541 ± 0.15
7	2.5	38	13.53 ± 0.31	39.26 ± 2.28	0.184 ± 0.02
8	4.5	36	37.32 ± 15.56	46.81 ± 1.26	0.476 ± 0.22

The droplet size of the nanoemulsion was also characterized because the droplet size will determine absorption and the rate of active substance release. The small droplet size produces a larger interfacial surface area so that it can increase the bioavailability of the active substance. Based on the droplet size data in Table 4, the smallest size was produced by Run 3 with a droplet size of 12.96 ± 0.37 nm, and the largest droplet size was produced by Run 8 with a size of 37.32 ± 15.56 nm. The results of the nanoemulsion droplet size test from all runs had an average of 19.84 ± 8.74 nm.

Evaluation of the droplet size distribution was also carried out to obtain the uniformity and reliability parameters of the nanoemulsion preparation method. The polydispersity index (PdI) value indicates the homogeneity of the nanoemulsion droplet diameter. The PdI value ranges from 0.0 to 1.0, and if the PdI value is close to 0, the particle/droplet size is close to homogeneous [10]. Based on the data in Table 4, the smallest PdI value was produced by Run 3 with a value of 0.114 ± 0.04, while the highest PdI was produced by Run 6 with a value of 0.541 ± 0.15.

Table 4. SLD equation for each characteristic parameter of nanoemulsion

Parameter	SLD equation
Emulsification time	Y = 47.571A + 38.724B… (1)
Droplet size	Y = 32.533A + 13.609B – 29.284AB… (2)
Size distribution	Y = 0.5037A + 0.1266B… (3)

Y = response of parameter
A = oil
B = Smix (Polysorbate 80-PEG 400)

All characteristic data of nanoemulsion were analyzed using the SLD method to determine the effect of each component or the interaction between components on each parameter. The result of the SLD analysis was some equations which can be seen in Table 4. Moreover, the effect profile of the SNEDDS component on the characteristics of the beta-carotene nanoemulsion can be seen in Figure 3.

Based on equations (1) and (3), the emulsification time and droplet size distribution of beta-carotene SNEDDS were influenced by sesame oil and Smix because both components have positive coefficient values. However, the component that most influenced the responses was sesame oil because it had the highest positive coefficient value [11].

The droplet size was influenced by sesame oil, Smix, and the oil-Smix interaction regarding Equation 2. Sesame oil and Smix provided positive coefficient values, and the one that had the greatest influence on droplet size was sesame oil. However, the interaction of sesame oil and Smix gave a negative coefficient, which means that with increasing interaction of sesame oil and Smix, the droplet size will become smaller. If the ratio of the oil component is increased in the formula, the droplet size will increase because the proportion of surfactants simultaneously decreases in the formula [12].

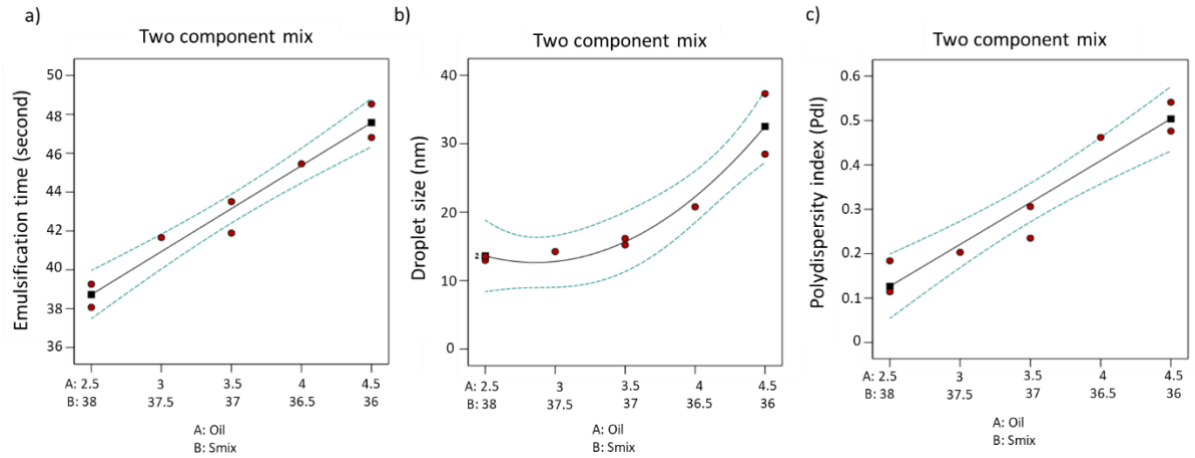

Figure 3. Profiling of (a) emulsification time, (b) droplet size, (c) droplet size distribution generated by SLD

2.4 Determination of Optimum beta-carotene SNEDDS formula

Optimization of the SNEDDS formula based on SLD was determined regarding desirability values close to 1. Each parameter was set to the expected target response such as in range, maximize, minimize, and target (Table 5). The lower limit of each variable was determined based on the lowest value of the nanoemulsion characterization data, for the emulsification time of 38.07 seconds, nanoemulsion droplet size of 12.96 nm, and polydispersity index of 0.114. The upper limit was set for the emulsification time of 60 seconds, the droplet size of 100 nm, and the polydispersity index of 0.5.

Table 5. The upper and lower limits of each parameter

Variable	Target	Lower limit	Upper limit
Oil	*in range*	2.5	45
Smix	*in range*	36	38
Emulsification time	*minimize*	38.07	60
Droplet size	*minimize*	12.96	100
Polydispersity index	*minimize*	0.114	0.5

The results of the SLD analysis provided a recommendation for the best formula composition which had a desirability value of 0.98 (Figure 4a). The desirability value is the similarity of the optimization process with the expected target. The desirability value is a number from numerical optimization in the range 0 to 1. If the desirability value is close to 1, then the expected results will be closer to the number predicted by SLD. The desirability value of 0.98 means that the recommended formula will produce the most optimum characteristics as expected at 98%. The recommended optimum formula had a composition ratio of sesame oil and Smix of 2.5:38. The formula was predicted to have a characteristic emulsification time of 38.72 seconds, droplet size of 13.61 nm, and droplet size distribution of 0.127 (Figure 4b-d).

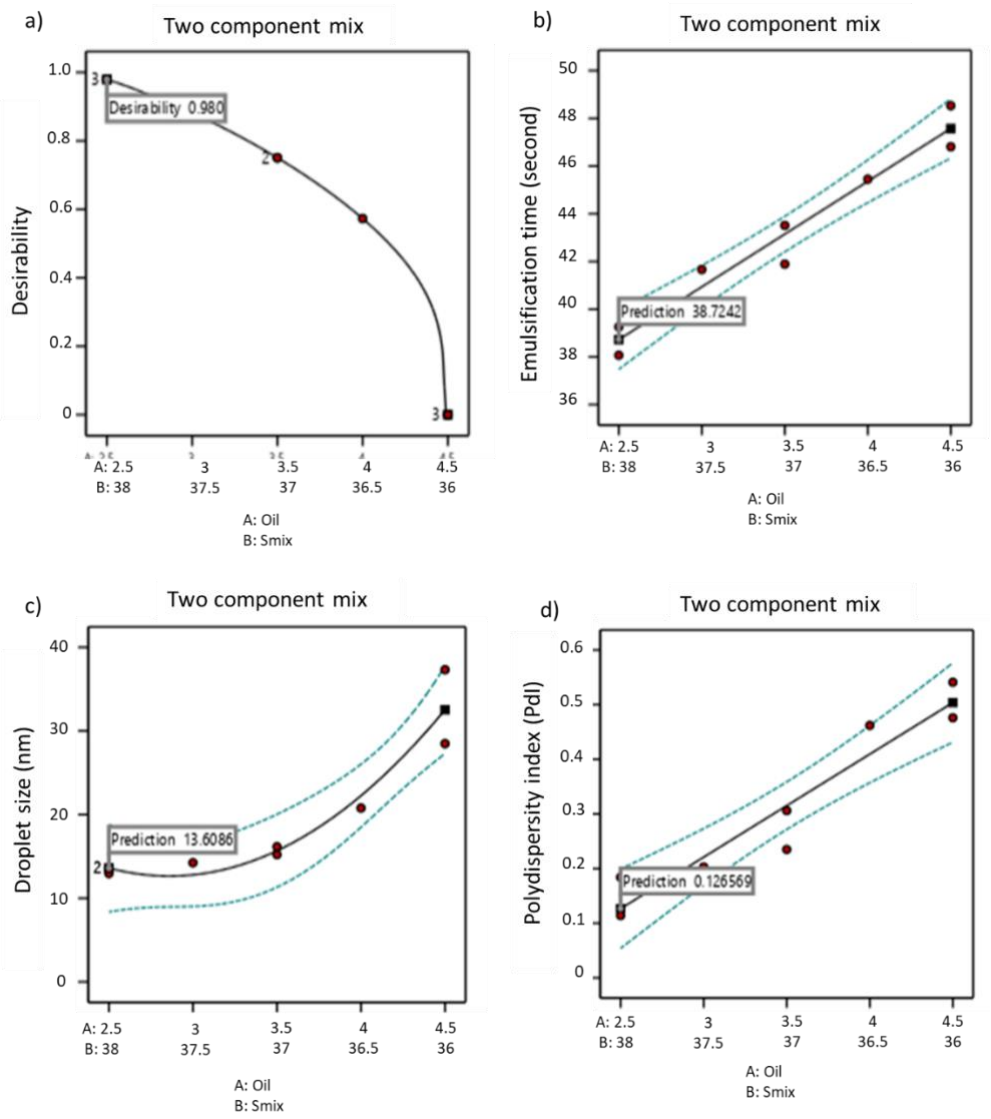

Figure 4. Predicted profile of the optimum beta-carotene SNEDDS formula using simplex lattice design for a) desirability, b) emulsification time, c) droplet size, and d) droplet size distribution.

2.5 Verification of Optimum beta-carotene SNEDDS formula

Optimum formula verification aimed to compare experimental results with SLD prediction results and to ensure that the predicted formula was correct and reproducible. There was no significant difference between the experimental result and the SLD prediction results based on the one-sample t-test with a 95% confidence level (Table 6). This result indicated that SLD had provided correct predictions based on the targeted parameters.

Table 6. Comparison of predicted parameter value with experimental result (N = 3)

Parameter	Predicted value	Experimental value	Sig. 2-*sided* p	Significance
Emulsification time	38.72	38.48 ± 0.70	0.608	Not significant
Droplet size	13.61	13.13 ± 0.50	0.233	Not significant
Polydispersity Index	0.127	0.133 ± 0.06	0.865	Not significant

2.6 Stability study of the optimum beta-carotene nanoemulsion in artificial gastric fluid (AGF)

Based on Table 7, there were no significant changes in size and droplet size distribution of nanoemulsion during incubation in AGF at 37°C for 4 hours. This study proved that the nanoemulsion from the optimum beta-carotene SNEDDS formula had good stability in gastric fluid. The optimum combination of nonionic surfactants can maintain the stability of the nanoemulsion by forming an interfacial layer with high elasticity, thus it can prevent the fusion and coalescence of droplets when the droplets collide with each other [13].

Table 7. Stability of nanoemulsion in AGF (N = 3)

Time (minutes)	Droplet size	Polydispersity Index
0	13.13 ± 0.5	0.133 ± 0.06
15	13.32 ± 0.75	0.120 ± 0.06
30	12.83 ± 0.47	0.084 ± 0.05
60	15.62 ± 0.64	0.227 ± 0.02
120	13.72 ± 0.85	0.156 ± 0.06
180	13.33 ± 0.52	0.141 ± 0.06
240	13.22 ± 0.78	0.118 ± 0.06

2.7 Stability study of the optimum beta-carotene SNEDDS formula

In this study, an accelerated stability test was carried out referring to the storage conditions determined by the ICH guidelines and ASEAN Harmonization. The optimum beta-carotene SNEDDS formula was stored in a climatic chamber at a temperature of 40°C ± 2°C/75%RH±5%RH and at room temperature. Emulsification time, nanoemulsion droplet size, and nanoemulsion droplet size distribution (PdI) were evaluated at weeks 1, 2, 3, and 4. The results of the nanoemulsion characteristics after storage can be seen in Table 8.

Table 8. Characteristic nanoemulsion during accelerated stability study (N = 5)

Parameter	Week-			
	1	2	3	4
40°C ± 2°C/75%RH±5%RH				
Emulsification time (second)	39.08 ± 1.15	38.68 ± 1.08	39.01 ± 1.12	39.21 ± 0.78
Droplet size (nm)	12.70 ± 0.19	13.37 ± 1.00	13.22 ± 0.38	13.06 ± 0.39
Polydispersity index (PdI)	0.08 ± 0.03	0.145 ± 0.09	0.134 ± 0.04	0.122 ± 0.03
Room temperature				
Emulsification time (second)	39.10 ± 1.00	38.49 ± 0.91	38.86 ± 1.03	38.78 ± 1.20
Droplet size (nm)	13.25 ± 0.45	13.22 ± 0.47	13.56 ± 0.60	13.24 ± 0.53
Polydispersity index (PdI)	0.137 ± 0.05	0.148 ± 0.05	0.151 ± 0.05	0.133 ± 0.04

Based on Table 8, the emulsification time, droplet size, and PdI of the optimum beta-carotene SNEDDS formula did not change significantly during four weeks of storage. This shows that the optimum beta-carotene SNEDDS formula was stable during storage in both conditions.

3. CONCLUSION

We have succeeded in the formulation of beta-carotene SNEDDS with good characteristics and stability. The optimum beta-carotene SNEDDS formula was obtained by combining sesame oil and Smix in a ratio of 2.5: 38. The optimum beta-carotene SNEDDS formula was able to form nanoemulsions with the following characteristics, emulsification time of 38.48 ± 0.70 seconds, droplet size of 13.13 ± 0.50 nm, droplet size distribution of 0.133 ± 0.06. The optimum formula can also maintain the physical stability of the

nanoemulsion during incubation in artificial gastric fluid. Moreover, the optimum beta-carotene SNEDDS formula has high stability during storage at room temperature and 40°C ± 2°C/75%RH±5%RH (referring to ICH guidelines). The optimum beta-carotene SNEDDS formula has a high potential to be produced in higher-scale production as a pharmaceutical product.

4. MATERIALS AND METHODS

4.1 Materials

Beta-carotene was purchased from Sigma-Aldrich. Sesame oil was purchased from Lee Kum Kee. Polysorbate 80 was purchased from Industria Chimica Panzeri. PEG 400 was purchased from Fred Homberg & Co. NaCl was purchased from Xilong Scientific. Chloride acid and sodium hydroxide were purchased from Merck.

4.2 Solubility of beta-carotene

10 mg of beta-carotene was put into 3 vials which contained 2 mL of sesame oil, polysorbate 80, and PEG 400 as different sample solutions. Then each sample was vortexed at a speed of 3000 rpm and sonicated for 10 minutes. If the beta-carotene was not completely dissolved, then sesame oil, polysorbate 80, and PEG 400 were added to each sample in a certain volume and continued with the same steps. Then, the solution was incubated for 24 hours at room temperature to ensure that beta-carotene crystals did not form.

4.3 Pseudo-ternary Phase Diagram Construction

A mixture of sesame oil, Smix (surfactant-cosurfactant), and water was prepared in 2 mL of total volume. The ratio of surfactants and cosurfactants was used in 2:1. Sesame oil and Smix were mixed with a variation of the ratio 0.5:9.5; 0.75:9.25; and 1:9. The mixing process was carried out using a vortex at 3000 rpm for 2 minutes. Then, water was added to the mixture with variations in the ratio of water and oil-Smix (OS) of 20:80, 25:75, 30:70, 35:65, 40:60, 45:65, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, and 80:20 under 3000 rpm vortex for 2 min. Samples were observed visually to identify samples that produced a clear gel-like liquid crystalline (LC) mixture. Furthermore, the droplet size of clear gel-like liquid crystalline (LC) samples was evaluated using dynamic light scattering (DLS). The samples were diluted 3 times using distilled water before measuring droplet size.

4.4 Optimization of beta-carotene SNEDDS formula

The design of the optimization beta-carotene SNEDDS formula was determined based on the pseudoternary diagram that had been obtained. The selected ratio of sesame oil and Smix which produced nanoemulsions was applied to determine the upper and lower limits of independent variables. The simplex lattice design (SLD) produced 8 Runs as shown in Table 9. Each run was prepared in 10 mL volume.

Table 9. Generated composition of beta-carotene SNEDDS

Run	Composition ratio (v/v)		Beta-carotene (mg)
	Oil	Smix	
1	3	37.5	10
2	3.5	37	10
3	2.5	38	10
4	3.5	37	10
5	4	36.5	10
6	4.5	36	10
7	2.5	38	10
8	4.5	36	10

4.5 Beta-carotene SNEDDS preparation

Oil and Smix were prepared according to the generated composition for 8 Runs. All components were mixed using a vortex at 3000 rpm for 10 minutes. Then, 10 mg of beta-carotene was dissolved in the oil-Smix mixture using a vortex at 3000 rpm for 10 minutes. The mixture was sonicated using a water bath sonicator for 60 minutes, under cold conditions. The SNEDDS samples were incubated for 24 hours at room temperature to ensure that the beta-carotene was completely dissolved.

4.6 Characterization of beta-carotene SNEDDS

4.6.1 Emulsification time

The emulsification time study was carried out by using artificial gastric fluid (AGF) without pepsin as medium incubation. The AGF preparation followed these steps, 2 g of NaCl was dissolved in distilled water, then 2.59 mL of HCl was added to the NaCl solution, the pH of the solution was adjusted to pH 1.2 by adding 2N NaOH, and the final step was adding water to a total volume of 1000 mL. 3 mL of SNEDDS sample was put into 200 mL of AGF at 37°C and stirred continuously by using a magnetic stirrer at 100 rpm. Then, the emulsification time was recorded when a homogeneous mixture was formed.

4.6.2 Droplet size and size distribution analysis

The droplet size of the nanoemulsion was analyzed using a Zetasizer (Nano-ZS, Malvern). 30 µL of beta carotene SNEDDS was emulsified into 2 mL of AGF, then the 200 µL of nanoemulsion was analyzed to obtain size and droplet size distribution value.

4.7 Determination of the optimum beta-carotene SNEDDS formula

The optimum beta-carotene SNEDDS formula was determined by the simplex lattice design (SLD). Emulsification time, nanoemulsion droplet size, and nanoemulsion droplet size distribution were used as response parameters to determine the optimum point. The optimum formula was expected to produce beta-carotene SNEDDS with an emulsification time value of less than 1 minute, nanoemulsion sizes in the range of 1-100 nm, and a polydispersity index (PdI) value of less than 0.3.

4.8 Verification of the optimum beta-carotene SNEDDS formula

Verification of the optimum beta-carotene SNEDDS formula was carried out by preparing beta-carotene SNEDDS by referring to the composition of the predicted optimum formula by SLD. Samples were characterized to determine emulsification time, droplet size, and nanoemulsion droplet size distribution. Then, the experimental results were compared to the predicted results of SLD using a single sample t-test analysis with a 95% confidence level.

4.9 Stability study of beta-carotene SNEDDS in AGF media

3 mL of optimum beta-carotene SNEDDS formula was put into 200 mL of AGF at 37°C under stirring by using a magnetic stirrer, at 100 rpm. The formation of nanoemulsion was indicated by the formation of a homogeneous mixture. Nanoemulsion droplet size and nanoemulsion droplet size distribution (PdI) were evaluated after 4 hours of incubation.

4.10 Stability study of the optimum beta-carotene SNEDDS formula

The optimum beta-carotene SNEDDS formula was stored in a climatic chamber at a temperature of 40°C ± 2°C/75% RH ± 5% RH and room temperature referring to the ICH guidelines and ASEAN Harmonization. Emulsification time, nanoemulsion droplet size, and nanoemulsion droplet size distribution were evaluated after storage at 1, 2, 3, and 4 weeks.

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