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# Optimization of Homogenization Conditions for Gac (*Momordica cochinensis* Spreng) Oil–Loaded Solid Lipid Nanoparticles (SLNs)

**Mai Huynh Cang[1] . Nguyen Thi Kim Thanh[2] . Nguyễn Le Minh Hai[2] . Le Thi Hong Nhan[3]**

[1]Chemical Engineering Department, Nong Lam University, Ho Chi Minh City, Viet Nam.
[2]Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Viet Nam.
[3]Organic Chemical Department, Ho Chi Minh University of Technology, Ho Chi Minh City, Viet Nam.

ABSTRACT
In this study, gac oil- loaded solid lipid nanoparticles (SLNs) were successfully prepared by using Emulgade SE-PF™ lipid, high-speed homogenization with the support of surfactant. The suspensions contained 5% active agents (Gac oil, w/w) were dispersed in the presence of surfactant (Tween 80 : Span 80 ratio of 72 : 28 w/w) at content of 5% by using the IKA homogenizer at the speed of 11200 rpm for 60 minutes of hot homogenization at 80°C and 30 minutes of cold homogenization. The resulted mixture had the median size of 200 nm (DLS), solid lipid nanoparticles were sphere with dried size of about 80 nm (TEM). The color changes (ΔE) of the samples at high storage temperatures were more rapidly and distinctly in comparison to the low-temperature condition. The ΔE value of blank sample increased faster than Gac oil-loaded SLNs samples.


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## Introduction

Colloidal drug delivery systems, namely, oil-in-water emulsions, liposomes, micelles, micro- particles, and nanoparticles, offer new opportunities for targeting drugs, pharmaceuticals, and cosmetics [1]. Introduced in 1991, solid lipid nanoparticles (SLNs) have demonstrated superiority in certain features over other types of colloidal carriers. SLNs comprise spherical lipid particles in the nanometer size range of 50-1000 nm [2, 3]. They offer various advantages in drug delivery because of their small particle size, large surface area as well as ability to modify their surface properties easily. SLNs systems also help dissolve solubilize poorly water-soluble compounds and control the release [4]. Moreover, SLNs provide both stability of solid matrix and biological compatibility of lipid carriers while avoiding the shortcomings of liposomes and polymeric particles which are subject to the undesired stability problems and the potential toxicity of the materials, respectively [4-6].

Natural carotenoid compounds especially from Gac fruit (*Momordica Cocochinensis* Spreng.), which are regarded as a herbal medicine for skin [7]. This is due to the ability of anti-aging, skin tanning, skin care, and skin protection [8-10]. The content of β-carotene, a pre-vitamin A compound in Gac fruit aril is 1.8 times higher than that in cod-liver oil and 15 times higher than that in carrots [7, 11]. Together with this, vitamin A supplementation helps to maintain a healthy skin as well as improving skin elasticity and moisture [12-14]. Besides, in Gac aril, lycopene content is 70 times higher than that in tomatoes [15]. Lycopene has a strong anti-aging and is the only carotenoid which can prevent heart attacks and protect gene from the damage [16]. With these advantages, the incorporation carotenoids into cosmetics such as skin lotions can help enhance the skin protection ability [17-19]. However, the biggest drawback of carotenoids is extremely sensitive to temperature, light, and oxygen; thus, this compound is easily decomposed. Therefore, the encapsulation of Gac oil into solid lipid nanoparticles is a new approach to not only maximize the activity of the carotenoids but also improve their durability in cosmetic products [20-24].

Here in, the aim of this study was to optimize the homogenization conditions in SLNs containing gac oil production. Besides, the stability of the Gac oil-loaded SLNs under different conditions of storage was also investigated.

## Experimental

### Materials

Gac oil was obtained from Gac Viet Company, Vietnam. Emulgade SE-PF™ (glyceryl stearate, ceteareth-20, ceteareth-12, cetearyl alcohol, cetyl palmitate) was purchased from Cognis Deutschland GmbH & Co. KG Care Chemicals. All other chemicals including Tween 80 and Span 80 were of analytical grade.

### Preparation of gac oil-loaded SLNs

According to the procedure previously developed by our group [17], SLNs containing gac oil were prepared by high-pressure homogenization including two steps: hot and cold homogenization. The ratio of gac oil : Emulgade : Twen 80 : Span 80 : water was 5 : 2.5 : 3.6 : 1.4 : 87.5. In the first stage, the solid lipid was melted to form a lipid phase and kept at proper temperature. Meanwhile, deionized water containing two surfactants (Tween 80 and Span 80) was also heated to the same temperature as the melting lipid phase before they were mixed and stirred by a stirring instrument for 15 minutes. Then, the mixture was treated with a high-speed homogenizer (IKA, Germany) at speed, time and temperature investigated to form a pre-emulsion.

In cold homogenization, the temperature of pre-emulsion was cold down to 0 – 5°C by placing into an ice bath. Then, the mixture was continually homogenized with speed and time which are similar to hot homogenization. The gac oil-

Corresponding Author: Mai Huynh Cang, Tel: 0084 938228017
Email: maihuynhcang@hcmuaf.edu.vn
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loaded SLNs were then characterized in terms of their particle size distribution, particle morphology, entrapment efficiency as well as stability during different storage conditions.

### Optimization of hot homogenization conditions and identification of cold homogenization time

After prospective experiments, the Response Surface Method (RSM) coupled with central composite design (CCD) was used for experiment design. The software JMP version 9.0 (SAS, Cary, NC, USA) was used to generate the experiment design, statistical analysis, and regression model. The independent variables of hot homogenization process were: speed ($X_1$), time ($X_2$), and temperature ($X_3$). Each variable had five regularly spaced levels (Table 1). A total of thirty-two combinations of independent variables were recognized. From the obtaining results, the correlation between speed, time and temperature of hot homogenization was determined. Also, the response surface equation was identified.

**Table 1:** The central composite experiment design and their levels

| Independent variables | Coded variable level | | | | |
|---|---|---|---|---|---|
| | -2 | -1 | 0 | 1 | 2 |
| $X_1$: speed (rpm) | 8000 | 9000 | 10000 | 11000 | 12000 |
| $X_2$: time (minutes) | 30 | 45 | 60 | 75 | 90 |
| $X_3$: temperature (°C) | 40 | 50 | 60 | 70 | 80 |

In cold homogenization, after reaching to 0 – 5°C the emulsion was homogenized at different times including 10, 20, 30 and 90 minutes. Based on particle size distribution and stability, the proper time of cold homogenization was identified.

### Particle size determination

The size distribution and median size of gac oil-loaded SLNs were measured using a dynamic light scattering instrument (DLS) (Horiba LA920, Japan). All analyses were performed in auto-measuring mode at 25°C and the results were presented as the average value of triplicate samplings and measurement for each formulation.

The SLNs suspension was dispersed on a metal grid and dried. The shape and internal matrix of individual nanoparticles were characterized using a transmission electron microscope with a camera system (JEM 1010, JEOL).

### Durability evaluation of gac oil-loaded SLNs during different storage conditions

Gac oil encapsulated SLNs was stored under various conditions: UV light, 45°C, and 10°C avoiding light. The solid lipid suspension without gac oil as the blank sample was also preserved under similar conditions for comparison.

## Results and Discussion

### Optimization of hot homogenization conditions

From the preliminary results, the matrix of optimal experiments was established by the Center Composite Design (CCD) type of RSM with three variable factors: hot homogenization speed, time and temperature. The total of 32 experiments is presented in Table 2. The response of experiment was evaluated by the measurement of size of particles in dispersion (by DLS measurement). Results show that size of particles in dispersions were ranged from 87 nm to 664 nm.

**Table 2:** Encoding the experimental planning matrix and the obtained size

| No. | Independent variables | | | Response of Model |
|---|---|---|---|---|
| | $X_1$ (speed) | $X_2$ (time) | $X_3$ (temperature) | Y (size) |
| 1 | -2 (8000) | 0 (60) | 0 (60) | 479 |
| 2 | +1 (11000) | +1 (75) | -1 (50) | 98 |
| 3 | 0 (10000) | +2 (90) | 0 (60) | 99 |
| 4 | +1 (11000) | -1 (45) | -1 (50) | 146 |
| 5 | 0 (10000) | 0 (60) | 0 (60) | 157 |
| 6 | -1 (9000) | +1 (75) | -1 (50) | 190 |
| 7 | 0 (10000) | -2 (30) | 0 (60) | 149 |
| 8 | 0 (10000) | 0 (60) | -2 (40) | 98 |
| 9 | +1 (11000) | -1 (45) | +1 (70) | 151 |
| 10 | 0 (10000) | 0 (60) | +2 (80) | 210 |
| 11 | -1 (9000) | -1 (45) | +1 (70) | 224 |
| 12 | 0 (10000) | 0 (60) | -2 (40) | 206 |
| 13 | -1 (9000) | -1 (45) | +1 (70) | 309 |
| 14 | +1 (11000) | -1 (45) | -1 (50) | 147 |
| 15 | 0 (10000) | 0 (60) | 0 (60) | 215 |
| 16 | +2 (12000) | 0 (60) | 0 (60) | 87 |
| 17 | +1 (11000) | +1 (75) | -1 (50) | 142 |
| 18 | +1 (11000) | +1 (75) | +1 (70) | 158 |
| 19 | -2 (8000) | 0 (60) | 0 (60) | 664 |
| 20 | -1 (9000) | -1 (45) | -1 (50) | 218 |
| 21 | 0 (10000) | -2 (30) | 0 (60) | 228 |
| 22 | 0 (10000) | 0 (60) | 0 (60) | 184 |
| 23 | -1 (9000) | +1 (75) | +1 (70) | 233 |
| 24 | 0 (10000) | 0 (60) | +2 (80) | 171 |
| 25 | -1 (9000) | +1 (75) | -1 (50) | 190 |
| 26 | +1 (11000) | -1 (45) | +1 (70) | 185 |
| 27 | 0 (10000) | +2 (90) | 0 (60) | 207 |
| 28 | -1 (9000) | +1 (75) | +1 (70) | 195 |
| 29 | +1 (11000) | +1 (75) | +1 (70) | 147 |
| 30 | 0 (10000) | 0 (60) | 0 (60) | 175 |
| 31 | +2 (12000) | 0 (60) | 0 (60) | 100 |
| 32 | -1 (9000) | -1 (45) | -1 (50) | 277 |

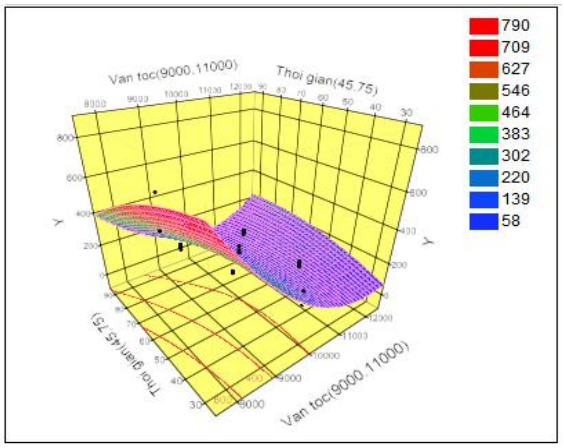


**Figure 1:** Response surface for SLNs size showing the effect of different factors: hot homogenization speed, time and temperature

Figure 1 shows the effect of three factors: hot homogenization speed, time and temperature on the size of gac oil-loaded SLNs (measured by DLS). The variance analysis shows that all of three factors had a considerable effect on the size of gac oil-loaded SLNs. In addition, the determination coefficient ($R^2$= 0.83) indicates a good fitting quality and the reliability was 91%. According to the Fisher standard, at significance level $p$ = 0.05, the regression equation which was compatible with experiment, is following expression:

$$Y = 181,70163 - 88,33451X_1 - 30,00625X_2 + 11,238636X_3 + 24,419022X_1X_2 + 37,375X_1^2 - 11,10924X_2^2$$

The optimization of hot homogenization conditions using RSM presented that optimal size (142 nm) was achieved under the following conditions: homogenization speed of 11200 rpm, homogenization time of 60 minutes and homogenization temperature of 80°C. The best experimental size obtained was 147 nm and the calculated size of SLNs with these parameters using regression model was 142 nm. This confirmed that these conditions were optimal for excellent particle size.

## Effects of cold homogenization time

During cold homogenization, the lipid oil was solidified and then was separated to smaller fragments. Thus, the homogenization time affects significantly on the SLNs size. The results in Fig. 2 show that increasing the homogenization time from 10 to 20 minutes resulted in a decrease of SLNs mean between 1509 nm and 1066 nm. At these values, it can be concluded that 20 minutes was not enough to homogenize the solid lipid particles. In next 10 homogenization minutes, the particle size of SLNs dramatically reduced (approximately 5 times) and reached to a mean value of 201 nm. Continue homogenization between 30 and 90 minutes did not change the particle size significantly. Because of its orientation in cosmetic applications, a particle size of about 200 nm was appropriate and did not need to be further reduced. Also, increasing the time required more energy consumption. Therefore, the cold homogenization time of 30 minutes was appropriate.

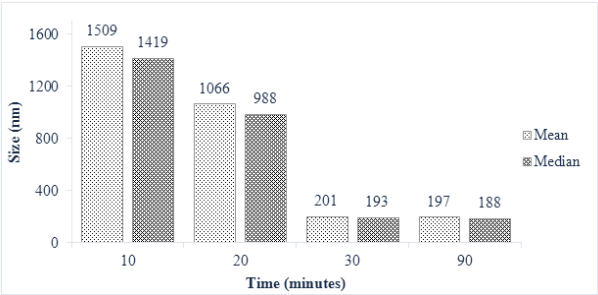


**Figure 2:** Size distribution of gac oil-loaded SLNs at different cold homogenization times

## Transmission electron microscopy (TEM) and Gac oil entrapment efficiency

Figure 3 shows the shape and size of solid lipid nanoparticles containing gac oil recognized by TEM method. In general, Gac oil-loaded SLNs were spherical and had the dried size of about 80 nm. In the DLS method, the nano particles in the suspension had hydration layer around, so the size always was larger (about 200 nm). Moreover, the structure of particles was relatively uniform and matrix. The image also reveals the internal space between particles which allows the estimation of a shell-life structure with a core of gac oil and a solid lipid as well. The internal space owned a large volume corresponding to the high ratio of liquid oil used in the original formula (2 : 1 of liquid : solid).

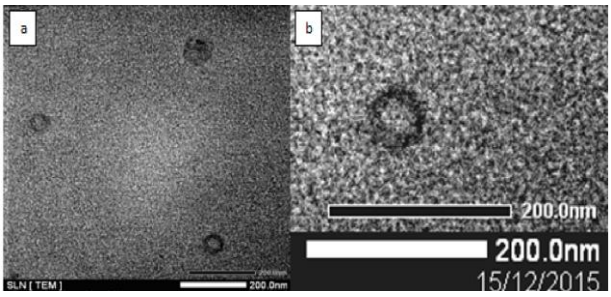


**Figure 3:** TEM images of gac oil-loaded SLNs

## Effects of UV light on gac oil-loaded SLNs stability

According to Fig. 4, UV radiation had a significant effect on the color change (through the value of ΔE) of both Gac oil-loaded SLNs and blank samples. The longer time of UV radiation applied, the greater change of sample color. However, the blank samples were more affected by the UV radiation than each other, presented by a rapid increase of ΔE. After 300 minutes treated with UV, the ΔE value was 62.25 for blank samples while only slightly increased for the Gac oil-loaded SLNs. This is suitable for the encapsulation of carotenoids since these compounds in gac oil were easily decomposed under UV irradiation. In Gac oil-loaded SLNs, the carotenoids were covered and thus were less affected by UV rather than in the blank samples.

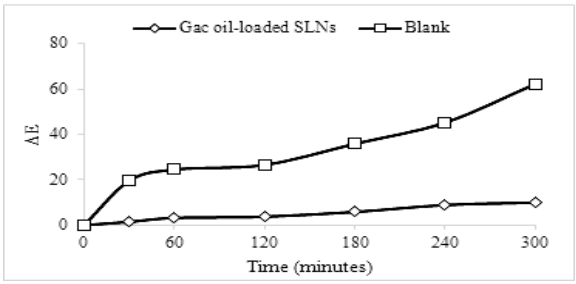


**Figure 4:** Effects of UV light on ΔE values of samples in accordance with storage periods

## Effects of storage temperatures on gac oil-loaded SLNs stability

From the results of LCh measurements (Figure 5), the color changes of the samples at low and high temperatures in accordance with storage time were observed.

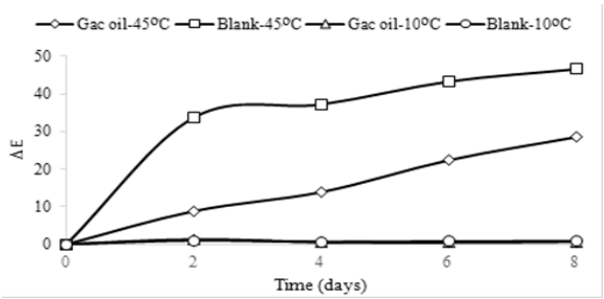


**Figure 5:** Effects of temperatures on ΔE values of samples in accordance with storage periods

It is clearly seen that at high storage temperature, both Gac oil-loaded SLNs and blank samples changed color, but ΔE value of blank samples increased faster. After the first 2

storage days, this parameter of the blank sample increased to 33.7 and reached 46.56 at 6 days later. In the case of Gac oil-loaded SLNs, ΔE value rose steadily and only reached 28.53 after 8 storage days. In comparison to the low-temperature condition, at high storage temperature, color changes occurred more rapidly and distinctly. Also, the structure of the SLNs system was broken while at low temperatures, the system had a better stability.

## Conclusions

The optimum conditions for the formation of Gac oil-loaded SLNs were hot homogenization as the first process, with time of 60 minutes, speed of 11200 rpm and temperature of 80°C, and cold homogenization as the second process, with time of 30 minutes, temperature of 0-5°C. The surfactant content was 5% (Tween 80: Span 80 of 72: 28 w/w), Emulgade SE-PF™ content was 2.5%, and Gac oil content was 5%. The Gac oil-loaded SLNs owned significant characteristics: the suspension form with median size of 200 nm (measured by DLS) while the spherical form with the actual size of 80 nm (measured by TEM).

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