Preparation of Salicylic Acid Loaded Nanostructured Lipid Carriers Using Box-Behnken Design: Optimization, Characterization and Physicochemical Stability

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Abstract: Nanostructured lipid carriers loaded salicylic acid (NLCs-SA) were developed and optimized by using the design of experiment (DOE). Box-Behnken experimental design of 3–factor, 3-level was applied for optimization of nanostructured lipid carriers prepared by emulsification method. The independent variables were total lipid concentration ($X_1$), stearic acid to Lexol® GT-865 ratio ($X_2$) and Tween® 80 concentration ($X_3$) while the particle size was a dependent variable ($Y$). Box-Behnken design could create 15 runs by setting response optimizer as minimum particle size. The optimized formulation consists of 10% of total lipid, a mixture of stearic acid and capric/caprylic triglyceride at a 4:1 ratio, and 25% of Tween® 80 which the formulation was applied in order to prepare in both loaded and unloaded salicylic acid. After preparation for 24 hours, the particle size of loaded and unloaded salicylic acid was 189.62±1.82 nm and 369.00±3.37 nm, respectively. Response surface analysis revealed that the amount of total lipid is a main factor which could affect the particle size of lipid carriers. In addition, the stability studies showed a significant change in particle size by time. Compared to unloaded nanoparticles, the addition of salicylic acid into the particles resulted in physically stable dispersion. After 30 days, sedimentation of unloaded lipid carriers was clearly observed. Absolute values of zeta potential of both systems were in the range of 3 to 18 mV since non-ionic surfactant, Tween® 80, providing steric barrier was used. Differential thermograms indicated a shift of endothermic peak from 55°C for α-crystal form in freshly prepared samples to 60°C for β’-crystal form in storage samples. It was found that the presence of capric/caprylic triglyceride oil could enhance encapsulation efficiency up to 80% and facilitate stability of the particles.

Key words: Box-Behnken design, nanostructured lipid carriers, salicylic acid, capric/caprylic triglyceride

1 INTRODUCTION

The nanostructured lipid carriers (NLCs), which could be achieved by mixing solid lipid with partial liquid lipid, have become an interesting delivery system for cosmetic and pharmaceutical products after the development of solid lipid nanoparticles (SLNs). The special structure of the lipid matrix could enhance a high loading capacity, especially for lipophilic drugs and physicochemical observations, which are better than the first generation of solid lipid nanoparticles[1-2].

Many researchers have reported the cosmetic benefits toward SLNs and NLCs are related to the potential to control the release of active ingredients from the lipid matrix. These systems can not only improve solubility of the encapsulated agent but also reduce a chance of skin irritation and others[3-7]. Besides the controlled release property of the encapsulated active agents, NLCs enhance the chemical stability of many cosmetic active ingredients; for instance, phenylethyl resorcinol, ascorbyl palmitate and coenzyme Q10[8, 9]. To apply the lipid particles, which is smaller than 400 nm, led to a film formation of dense packing of particles on the skin resulted in occlusion effect and skin hydration[9]. Furthermore, the NLCs particles are able to enhance Sun Protection Factor (SPF) when combining with inorganic sunscreens; for example, titanium dioxide[10].

Beta hydroxybenzoic acid (BHA), such as salicylic acid, has been successfully used as an anti-acne, anti-microbial, and keratolytic agent in various cosmetic products. The usage of this bioactive substance has been apparently limited by the reason of an appearance of skin irritation, which mostly depended on pH of the vehicles, and its low

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solubility in aqueous system\textsuperscript{11–13}. The ASEAN cosmetic documents recommended that concentration of salicylic acid in topical preparations should not exceed 2\%\textsuperscript{14}. Therefore, encapsulation technology for delivery system could be found in many research papers in order to reduce side effect and improve efficiency of salicylic acid\textsuperscript{15–20}.

Due to various factors play a critical role in the development towards optimized formulation, it is necessary to study the one factor at a time which causes excessive trails and wasteful ingredients. Furthermore, the character of nanostructured lipid carriers is controlled by types of lipids and surfactants as well as their relative concentration which these variables strongly influence the quality of nanoparticle dispersion. To formulate the pre-emulsions, which are suitable for the preparation of lipid nanoparticles, is considered to be difficult since the ingredients significantly affect the final physicochemical properties of the lipid particles. The statistical experimental design is a useful alternative way to understand the relationship among factors in formulations\textsuperscript{21}.

The Box-Behnken design of response surface methodology (RSM) is an advantageous method to study when variable interactions are extremely complicated. It is one of the most efficient designs of response surface methodology (RSM) based on 2-level factorial designs and incomplete block designs which requires fewer runs than all other RSM design\textsuperscript{21, 22}. In order to analyze the result, the second-order polynomial model is generated to estimate the level of variables for the optimization process. Box-Behnken design is able to study in both quantitative and qualitative variables and also used widely for studying 3-factor, 3-level to find the optimal formulation, which can enhance the quality of drug delivery system\textsuperscript{23–26}.

In this present research work, the 3-factor, 3-level Box-Behnken design was chosen to examine the proper amount of total lipid, surfactant and solid lipid to liquid lipid ratio in order to prepare the pre-emulsion containing salicylic acid for preparation of NLCs which has a minimum particle size. The NLCs obtained from the optimized formulation were characterized and evaluated for physicochemical stability during long-term storage.

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variables</td>
<td>Low (-1)</td>
</tr>
<tr>
<td>$X_1$ Total lipid (%)</td>
<td>5</td>
</tr>
<tr>
<td>$X_2$ Stearic acid : Lexol\textsuperscript{®} GT-865</td>
<td>1.50:1</td>
</tr>
<tr>
<td>$X_3$ Tween\textsuperscript{®} 80 (%)</td>
<td>15</td>
</tr>
<tr>
<td>Dependent variables</td>
<td>Optimized response</td>
</tr>
<tr>
<td>$Y$ Particle size</td>
<td></td>
</tr>
</tbody>
</table>

### 2 EXPERIMENTAL

#### 2.1 Materials

The active agent used in the experiment is salicylic acid which the chemical purity is 99\% from Ajax Finechem (Australia). Lipid carriers comprising of stearic acid which the chemical purity is 98\% from Lab Pan Reac (Spain) and a mixture of capric/caprylic triglyceride (Lexol\textsuperscript{®} GT865) purchased from Inoxel Chemical Company (USA). Tween\textsuperscript{®} 80 which has extra pure grade from Q-Rec (New Zealand) and absolute ethanol which the chemical purity is 99.9\% from Merck (Germany).

#### 2.2 Preparation of salicylic acid loaded nanostructured lipid carriers

Salicylic acid loaded nanostructured lipid carriers (NLCs-SA) were prepared by emulsification method using high-speed homogenization. According to preliminary screening test, the lipid phase which was well solubilizing for salicylic acid consisted of stearic acid (m.p. 69.6\textdegree C) and Lexol\textsuperscript{®} GT-865. The lipid phase was melted at above 70-75\textdegree C and the molten lipid phase was mixed into preheated aqueous phase comprising of Tween\textsuperscript{®} 80 and 95\% ethanol at 1:1 ratio. The pre-emulsion was homogenized by high-speed homogenizer (Heidolph Silent Crusher M, Germany) at speed of 12,000 rpm. for 10 minutes and was added drop-wise using needle syringe into cold distilled water (2-3\textdegree C) at 1:20 ratio. The mixture was simultaneously stirred at 500 rpm for 10 minutes to achieve NLCs dispersion. In addition, the obtained dispersions were kept at 4 ± 1\textdegree C for stability evaluations. Salicylic acid loaded nanoparticles were prepared by adding known amount of salicylic acid in lipid phase then repeated the above mentioned process.

#### 2.3 Design of experiments (DOE)

Box-Behnken design with 3-factor, 3-level was employed to investigate the effects of the independent variables on a dependent variable of particle size ($Y$) using MINITAB software (Trial Version 17, USA). The independent variables including total lipid concentration ($X_1$), solid lipid to liquid lipid ratio ($X_2$) and surfactant concentration ($X_3$) were studied at 3 different levels as shown in Table 1. The experimental design of 3-factor, 3-level, Box-Behnken design theoretically generates a few treatment combinations of 15
Preparation of Salicylic Acid Loaded Nanostructured Lipid Carriers Using Box-Behnken Design


∆

Particle size, the optimized formulation of NLCs-SA was re-selected on the minimizing another variable was kept constantly. As the criterion for plots were created to determine the interaction effects between two independent variables and response when between two independent variables and response when.

\[ Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1^2 + \beta_5X_2^2 + \beta_6X_1X_2 + \beta_7X_1X_3 + \beta_8X_2X_3 \]

where \( Y \) was predicted response, \( \beta_0 \) was a constant intercept, \( \beta_1, \beta_2, \beta_3 \) were linear coefficients, \( \beta_4, \beta_5, \beta_6 \) were squared coefficients and quadratic terms, \( \beta_7, \beta_8 \) were interaction coefficients and \( X_1, X_2, X_3 \) were independent variables, which were selected re-solved on the preliminary study. Predicted \( R^2 \) was calculated to evaluate fitness of model.

2.4 Optimization and validation of response surface methodology (RSM)

The NLCs dispersions of which the particle size was determined were prepared according to the experimental design. All observed responses were substituted to software, which could obtain the polynomial equation. The selected model was evaluated based on statistically significant coefficients and \( R^2 \) values. The 3D surface response plots were created to determine the interaction effects between two independent variables and response when another variable was kept constantly. As the criterion for selection of optimized formulation based on the minimizing particle size, the optimized formulation of NLCs-SA was repeatedly prepared and evaluated for size, zeta potential and entrapment efficiency as a function of time.

2.5 Characterization of the particles

2.5.1 Determination of particle size and zeta potential

The DelsaTM Nano C (Beckman Coulter Inc., USA) instrument was used to examine the physical characteristics of the samples by applying 2 detectors. The Dynamic Light Scattering (DLS) was applied to determine the mean of particle size and Polydispersity Index (PI) by measuring the rate of fluctuations in laser light intensity through the particles as they diffuse throughout a fluid. The samples were diluted in 1:1 ratio with deionized water in quartz cell and kept standing for 20 minutes prior to measurement at 25°C. The measurements were conducted in triplicate and repeated three times. The Electrophoretic Light Scattering (ELS) was applied to determine the electrophoretic movement of surface charged particles under the influence of an applied electric field based on the Doppler shift of scattered light[27]. The sample, about 1 mL, which was diluted in 1:1 ratio with deionized water was carefully filled in flow cell avoiding generation of gas bubble and kept standing for 20 minutes prior to measurement at 25°C. The determination of zeta potential was also conducted in triplicate and repeated three times.

2.5.2 Determination of particle morphology

The morphology of the nanostructured lipid carriers was investigated by using a scanning electron microscopy (SEM, LEO 1450VP, UK). One drop of the dispersion sample was placed on the carbon-coated grid and kept dry in a desiccator approximately 1 hour before observation.

2.5.3 Determination of crystallization and thermal behavior

Differential scanning calorimetry (DSC Perkin Elmer, Japan and Phoenix, UK) was applied to investigate thermal behavior of the nanostructured lipid carriers in terms of polymorphism changes and melting behavior. Pure stearic acid and bulk lipid mixture (4:1 ratio of stearic acid: Lexol® GT-865) were used as a comparable reference which its sample, about 7-8 mg, was weighed in an aluminum pan and was hermetically sealed. The measurement was operated under inert gaseous nitrogen at a flow rate of 70 mL/min. The heating runs performed from 10 to 200°C and cooled down to 10°C with a heating-cooling rate of 10°C or 5 K/min. An empty aluminum pan was used as the reference. Therefore, the results (enthalpy) obtained through the graphical analysis and degree of crystallization was calculated according to the following equation.

\[ \text{Degree of crystallization, DC} (%) = \frac{\Delta H_{\text{sample}}}{\Delta H_{\text{bulk lipid}}} \times 100\% \]

Where \( \Delta H_{\text{sample}} \) and \( \Delta H_{\text{bulk lipid}} \) were the enthalpy of the samples and the 4:1 ratio of stearic acid: Lexol® GT-865 respectively.

2.6 Determination of encapsulation efficiency

Encapsulation efficiency of salicylic acid loaded NLCs was determined by ultratillation method[26]. NLCs dispersion, approximately 4 g, was placed in an upper chamber of a centrifugal filter tube then centrifuged at 10,000 rpm for 15 min (25°C). One millimeter of filtered aqueous phase was diluted with a mixture of ethanol/water (1:9) before spectrophotometrical measurement (Cary 1E, UV-Vis Spectrometer, Varian, Australia) at wavelength of 296 nm. The concentration of free salicylic acid in each sample was calculated from a standard concentration curve of salicylic acid ranging from 1 to 25 μg/mL. The encapsulation efficiency was calculated according to equation as follows:

\[ \text{Encapsulation efficacy} (%) = \left( \frac{W_T - W_F}{W_T} \right) \times 100\% \]

Where \( W_T \) was the amount of salicylic acid (g) added during NLCs preparation and \( W_F \) was the amount of free salicylic acid (g) in the filtered aqueous phase.
2.7 Statistical analysis

The significant differences on physicochemical characterization among formulations were evaluated by paired t-test and the differences within the same formulations as a function of time were evaluated by one-way ANOVA at p-value was less than 0.05.

3 RESULTS AND DISCUSSION

3.1 Effects of independent variables on particle size

A total number of 17 experiments were generated from Box-Behnken design as shown in Table 2 consisting of 12 experimental setups of factorial design and 5 sets of central point (run order 1, 4, 5, 7, 14). The 17 setups were experimentally prepared and all observed responses were simultaneously fitted to the second-order polynomial model. The positive sign in front of each variable represents a synergistic effect on a response while a negative sign indicates an antagonist relationship. The NLCs dispersions, which were prepared according to the experimental design, provided a particle size range from 1,418.47 nm (run order 2) to 196.07 nm (run order 11) which corresponded to the equation as follows:

\[
Y = -2,295 - 44X_1 - 1X_2 + 288X_3 + 22.9X_1^2 + 156.6X_2^2 + 0.35X_3^2 - 20.9X_1X_2 - 18.48X_1X_3 - 40.5X_2X_3
\]

Based on the analysis of variance (ANOVA), the dependent variable (\(Y\)) is outstandingly fitted to the model with the highest coefficient of determination (\(R^2\)) which its value was 0.8867. The model f-value of 6.09 implied that this model was statistically significant at a p-value, which was less than 0.05. As shown in Table 3, there is a significant effect on particle size (p < 0.05) of the independent variables of \(X_1, X_3, X_2^2\) and \(X_1X_3\). Hence, it is clear that the independent variables of \(X_1\) and \(X_3\) indicate the antagonistic effect with negative coefficients and \(X_2^2\) indicates synergistic effect with positive coefficients.

The suitability of model was calculated by the sum of square of error in the 5 times repeated experiment which the finding shown as the lack-of-fit (Table 3). The lack-of-fit f-value of 330.00 was significant at a p-value of less than 0.0001 implying the occurrence of a large residual. This probably caused by the multicollinearity of the independent variables. Multicollinearity is a condition that occurs when some predictor variables in the model are correlated with other predictor variables. As the finding shown in Table 3, there is a significant effect on the particle size at a p-value of 0.044 of the interaction between

<table>
<thead>
<tr>
<th>Run order</th>
<th>(X_1)</th>
<th>(X_2)</th>
<th>(X_3)</th>
<th>(Y^*) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>2.75 : 1</td>
<td>20</td>
<td>321.57 ± 5.82</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>1.50 : 1</td>
<td>25</td>
<td>1,418.47 ± 47.51</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1.50 : 1</td>
<td>20</td>
<td>329.23 ± 2.87</td>
</tr>
<tr>
<td>4</td>
<td>7.5</td>
<td>2.75 : 1</td>
<td>20</td>
<td>338.00 ± 2.90</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>2.75 : 1</td>
<td>20</td>
<td>294.61 ± 13.93</td>
</tr>
<tr>
<td>6</td>
<td>7.5</td>
<td>1.50 : 1</td>
<td>15</td>
<td>239.89 ± 2.19</td>
</tr>
<tr>
<td>7</td>
<td>7.5</td>
<td>2.75 : 1</td>
<td>20</td>
<td>307.07 ± 13.51</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>4.00 : 1</td>
<td>20</td>
<td>1,202.70 ± 41.91</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>2.75 : 1</td>
<td>25</td>
<td>217.87 ± 1.84</td>
</tr>
<tr>
<td>10</td>
<td>7.5</td>
<td>4.00 : 1</td>
<td>25</td>
<td>386.43 ± 0.97</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>4.00 : 1</td>
<td>20</td>
<td>196.07 ± 5.12</td>
</tr>
<tr>
<td>12</td>
<td>7.5</td>
<td>4.00 : 1</td>
<td>15</td>
<td>219.47 ± 1.10</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>1.50 : 1</td>
<td>20</td>
<td>1,074.57 ± 192.83</td>
</tr>
<tr>
<td>14</td>
<td>7.5</td>
<td>2.75 : 1</td>
<td>20</td>
<td>301.60 ± 4.57</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>2.75 : 1</td>
<td>15</td>
<td>312.60 ± 8.53</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>2.75 : 1</td>
<td>25</td>
<td>1,078.97 ± 28.83</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>2.75 : 1</td>
<td>15</td>
<td>249.47 ± 1.16</td>
</tr>
</tbody>
</table>

* n=3, mean ± SD

\(X_1\); total lipid concentration, \(X_2\); stearic acid to Lexol® GT-865 ratio, \(X_3\); Tween® 80 concentration, \(Y\); particle size
terms $X_2$ and $X_3$. Thus, it is considered that the concentration of surfactant tended to affect the lipid ratio resulting in a dramatic effect on pre-emulsion and a response particle size of NLCs.

The RSM plots in Fig. 1(a-c) explained the mutual interaction effects of two independent variables on particle size of NLCs at one time. In addition, the total amount of lipid indicated the most significant and negative effect on particle size (Fig. 1(a)). Increasing the concentration of stearic acid could lead to smaller particle size when keeping the amount of Tween® 80 at 20% w/v because an increase in the amount of solid lipid resulted in faster solidification of lipid carriers with smaller in particle size. On the other hand, the increase of concentration towards Tween® 80 resulted in a significant increase in size of particles as shown in Fig. 1(b) and (c), which could be the result of the bridging flocculation of nearby lipid particles involved in a dense packing of surfactant molecules as a protective layer on the lipid surface. In contrast, as a higher concentration, a range packing of surfactant molecules as a protective layer on the surface of NLCs particles might cause progressive aggregation, which resulted in instability of the system.

### 3.3 Characterization and stability study

#### 3.3.1 Determination of particle size and morphology

The optimized formulations of NLCs-SA and NLCs were prepared and evaluated the stability for 90 days under 4 ± 1°C. The observed data of particle size, polydispersity index and zeta potential of NLCs-SA and NLCs samples as a function of time were shown in Table 4. It was found that the size of salicylic acid loaded NLCs particles periodically measured was smaller than NLCs particles. The SEM photograhps obviously indicated a spherical shape of the particles (Fig. 2). The particle size of NLCs-SA was found to be significantly increased after 30-day storage; however, polydispersity index was not greater than 0.25 and sedimentation could not be observed throughout the study period. On the other hand, it was found that unloaded NLCs particles were significantly increased in size on the 7th day and completely precipitated after 90-day storage. This observation revealed that a decrease in size and good dispersion of NLCs-SA particles might be a result of an existence of salicylic acid in a mixture of lipid particles.

#### 3.3.2 Determination of zeta potential

As shown in Table 4, the zeta potential value of NLCs-SA was higher than NLCs; however, the values of both lipid particles were higher than -20 mV and the values were unchangeable significantly throughout the study period. The present of salicylic acid in the dispersion might attribute to the increase in zeta potential of NLCs-SA. In general, the suspended particles are considered to be physically stable when the absolute value of zeta potential is above 30 mV because the system is dominantly stabilized by electrostatic repulsion. Since non-ionic surfactant, Tween® 80, containing poly(ethylene oxide) moiety was employed as a stabilizer in this study, its electrostatic repulsion effect was negligible as comparing to steric repulsion effect. Therefore, the smaller size and sufficient steric repulsion might be the reason that NLCs-SA particles still homogeneously suspended in water even though its absolute zeta potential value was obviously far from 30 mV and lower than the value of NLCs. Comparing to NLCs which its initial size was larger than NLCs-SA, it is considered that excessive protective layers on the surface of NLCs particles might cause progressive aggregation, which resulted in instability of the system.

#### 3.3.3 Determination of crystallization and thermal behavior

The DSC curve of NLCs-SA and NLCs showed one endo-

### Table 3 ANOVA analysis of the responses generated by Box-Behnken design.

<table>
<thead>
<tr>
<th>Term</th>
<th>Particle size (Y)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>-4.36</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td>$X_2$</td>
<td>-1.81</td>
<td>0.113</td>
<td></td>
</tr>
<tr>
<td>$X_3$</td>
<td>3.56</td>
<td>0.009*</td>
<td></td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>1.42</td>
<td>0.197</td>
<td></td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>2.43</td>
<td>0.045*</td>
<td></td>
</tr>
<tr>
<td>$X_3^2$</td>
<td>0.09</td>
<td>0.933</td>
<td></td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>-0.63</td>
<td>0.547</td>
<td></td>
</tr>
<tr>
<td>$X_1X_3$</td>
<td>-2.24</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>$X_2X_3$</td>
<td>-2.45</td>
<td>0.044*</td>
<td></td>
</tr>
</tbody>
</table>

Lack-of-fit

- f-value: 330.00
- p-value <0.0001

* Significance at p-value <0.05

The optimized formulation of pre-emulsion was predicted by software based on the criteria of minimizing particle size consisting of 10% of total lipid (4:1 ratio of stearic acid and Lexol® GT-865) and 25% of Tween® 80. The actual size of NLCs-SA particles was 189.62 ± 1.82 nm which was considered to be in a good agreement with the calculated size. Furthermore, the physicochemical properties of the above-mentioned preparation as a function of time were determined by comparing with unloaded NLCs.
Fig. 1 Three-dimensional response surface plot for particle size (PS) analysis: (a) PS vs total lipid and stearic acid : Lexol® GT-865, (b) PS vs total lipid and Tween® 80, (c) PS vs stearic acid : Lexol® GT-865 and Tween® 80.
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thermic peak (Fig. 3) between 50-60°C while similar peaks obtained from pure stearic acid and bulk lipid (mixture of stearic acid and Lexol® GT-865) were observed between 60-70°C. In addition, the decrease in melting temperature of stearic acid in particles as compared with pure stearic acid or bulk lipid was attributed to their small size and the

Table 4  Particle size, Polydispersity Index (PI) and zeta potential (ZP) of NLCs-SA and NLCs.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Period</th>
<th>Particle size (nm)</th>
<th>PI</th>
<th>ZP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLCs-SA</td>
<td>Day 1</td>
<td>189.62 ± 1.82</td>
<td>0.130 ± 0.01</td>
<td>-5.57 ± 2.85</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>217.22 ± 1.58</td>
<td>0.118 ± 0.01</td>
<td>-7.89 ± 4.02</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>283.54 ± 0.89</td>
<td>0.147 ± 0.01</td>
<td>-6.36 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>388.90 ± 4.92*</td>
<td>0.193 ± 0.01</td>
<td>-6.18 ± 2.60</td>
</tr>
<tr>
<td></td>
<td>Day 60</td>
<td>657.77 ± 4.00*</td>
<td>0.266 ± 0.01</td>
<td>-5.98 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>Day 90</td>
<td>829.31 ± 5.99*</td>
<td>0.254 ± 0.01</td>
<td>-3.81 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>369.00 ± 3.37</td>
<td>0.199 ± 0.02</td>
<td>-18.83 ± 3.90</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>741.59 ± 36.96*</td>
<td>0.268 ± 0.01</td>
<td>-16.65 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>843.26 ± 48.14*</td>
<td>0.286 ± 0.01</td>
<td>-15.61 ± 2.37</td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>1,426.56 ± 51.06*</td>
<td>0.437 ± 0.02*</td>
<td>-15.63 ± 3.12</td>
</tr>
<tr>
<td></td>
<td>Day 60</td>
<td>Unstable</td>
<td></td>
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<tr>
<td></td>
<td>Day 90</td>
<td>Unstable</td>
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</table>

* Significant difference (p < 0.05), n=3 (mean ± SD)

Fig. 2  SEM photographs of NLCs-SA and NLCs on magnification 1,000x (left) and 5,000x (right) at 24 hours after productions.
presence of surfactant. Taking the enthalpy of stearic acid at 220.45 J/g as 100‰, the degree of crystallinity of NLCs was dramatically reduced about 80‰ when 20‰ of Lexol® GT-865 was added. The similar observation was also obtained from NLCs-SA sample (Table 5). It is found that the melting point of both NLCs and NLCs-SA shifted towards higher temperature after 60-day storage indicating the transformations of the crystal lattice from high surface energy of α-crystal to the more stable form of β′-crystal which was considered to be able to stabilize the encapsulated substances.  

3.3.4 Encapsulation efficiency

The encapsulation efficiency of salicylic acid in NLCs about 80‰ was prepared by emulsification method. There was no significant difference of encapsulation efficiency among each testing period up to 90-day storage as shown in Fig. 4. The encapsulation efficiency was generally related to the crystallinity degree of lipid nanoparticles. Furthermore, the experimental results implied that crystallinity of solid particles might not markedly change during study period; hence, this might be the reason that an incorporation of liquid oil in the solid lipid matrix could increase the amorphous proportion in the initial samples leading to a decrease in particle crystallinity with time, thereby improving encapsulation stability. Additionally, incorporation of liquid oil, which was a good solubilizer of the encapsulated active substances, rather enhanced encapsulation capacity.

4 CONCLUSION

The application of Box-Behnken design to study the preparation of NLCs loaded salicylic acid showed that it is a suitable instrument establishing the relationship among factors and expected attributes. The formulation of NLCs particles containing steric acid as solid lipid, Lexol® GT-865 as liquid lipid and Tween® 80 as surfactant were optimized by using 3-factor, 3-level design. The optimized formulation of NLCs loaded salicylic acid indicated high entrapment efficiency and good stability. Moreover, the DSC analysis showed that the addition of capric/caprylic triglyceride oil in the particles could increase the amorphous portion in the lipid matrix resulting in high encapsulation efficiency and stability.

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