Formulation Development of Plai Nanoemulsion Based on the Influence of Surfactant Combinations

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Received: 11 April 2012
Accepted: 8 August 2012

ABSTRACT

Plai (Zingiber cassumuna Roxb.) nanoemulsions were prepared under oil-in-water (O/W) emulsion system by high pressure homogenization. This study investigated the effect of emulsifier complex on the appropriate compositions. The obtained formulation characterized in terms of droplet size, surface charge, storage stability and physical morphology were examined by visual observation and optical microscope. Uniform nanoemulsions can be achieved at appropriate concentration 5-6.5% (w/v) of surfactant and co-surfactant combinations. This optimized formulations demonstrated particle size less than 200 nm with the homogeneous distribution curve at 0.05-0.15 polydispersity index values and presented zeta potential in the range of -35.0 to -37.6 mV. For the stability of nanoemulsions were evaluated by storage at 25°C (room temperature) and 45°C (accelerated condition) for one month, the optimized nanoemulsions were maintained homogeneous emulsion when compared with other formulations. Furthermore, physical morphology photograph supported further results which their small droplet particles were observed. This research has an impact on the development of topical delivery system with the high oil loading in the composition.

Keywords: nanoemulsion, Zingiber cassumuna Roxb., oil-in-water emulsion, topical delivery system

1. INTRODUCTION

Zingiber cassumunara Roxb. (Plai) is a local medicinal herb famous which found in the Asian countries and has strong benefit characteristic. Generally, sabinene, terpinene-4-ol, (E)-1-(3, 4-dimethoxyphenyl) butadiene (DMPBD) and (E)-4-(3′, 4′-dimethoxyphenyl) but-3-en-1-ol (Compound D), isolated Zingiber cassumunara Roxb., are main active components of volatile oil derived from rhizome [1, 2] as shown in Figure 1. DMPBD has been reported about the potent anti-inflammatory activity through the inhibition of cyclooxygenase (CO) and lipoxygenase (LO) pathway and analgesic action. Meanwhile, compound D inhibited the prostaglandin biosynthesis which was a predominantly pro-inflammatory influence [3,4]. For the Thai traditional medicine, volatile...
oil from *Zingiber cassumunar* have long been used directly apply and penetrate on the skin for remedied for muscle stress and joint pain. In the recently, it was already supplied in to the commercial in term of purified oil extract or gel formulation. However, products from Plai volatile oil quiet have problems about their features such as strong color and odor.

Nanoemulsion is one of encapsulation technology which represents an effective approach for encapsulate bioactive compound both of hydrophilic and hydrophilic ingredients. Currently, there are growing interest of essential oil which prepared in term of nanoemulsions such as curcumin [5], eucalyptus oil [6] and lemon oil [7]. Generally, nanoemulsion consist of oil phase, surfactant and aqueous phase which represented the oil droplet size in the range of 50-200 nm dispersed in the aqueous phase using the appropriate surfactant and their concentration. Emulsifiers play the important role in the formation of stability of nanoemulsion system. In this study, we are interested in hydrophilic non-ionic surfactant such as polysorbate 80, Poloxamer 188 and also hydrophobic surfactant (Montanov TM 82) were selected and Plai essential oil represented as an oil phase in this preparation. The purpose of this study is investigated the appropriate formulation of Plai nanoemulsion. Particularly, we focus on the influence of type and concentration of surfactant leading to improve the stability and formation of Plai emulsion based on nanoemulsion structure.

2. **MATERIALS AND METHODS**

2.1 **Materials**

Plai oil (*Zingiber cassumunar* Roxb. extraction) was purchased from Sand M International Co. Ltd. (Bangkok, Thailand). Glycerol, poloxamer 188 (Pluronic F68) and Polyoxyethylene sorbitan monoolate (Tween 80) (Analytical grade) were purchased from Sigma-Aldich Co., (St. Louis, MO). MontanovTM 82 (mixture of cetearyl alcohol and coco-glucoside; MV82) obtained from Adinop Co. Ltd. Butylene glycol cocoate (Cocoate BG) was acquired from P.C. Intertrade Co., Ltd. All of these chemicals were analytical reagent grade.

2.2 **Preparation of Nanoemulsions**

Plai nanoemulsions were prepared by oil-in-water emulsification method (O/W emulsion) through high pressure homogenization (HPH) technique [8], with different components and optimize the appropriate conditions which have been studied. Plai oil and Butylene glycol cocoate (Cocoate BG) were chosen as an oil phase while polysorbate 80, Montanov™ 82 and...
poloxamer 188 were contained in water phase. For each formulation, oil phase and water phase ration was used at 3:7 with the different volume of surfactant and cosurfactant. The oil phase and water phase were heated at 70°C then pre-emulsions were prepared at 60-70°C by homogenizing the oil phase in the aqueous phase with high speed homogenizer Ultra-Turrax® model T25 digital (IKA, Germany) for 5 min at 10,000 rpm. This pre-emulsion was continues homogenized with high pressure homogenizer (EmulsiFlex-C3, Germany) at pressure 1,000 bar 5 cycles. The different nanoemulsion formulations were all characterized which formulation as shown in Table 1.

Table 1. Effect of nanoemulsions component on the mean droplet diameter and zeta potential.

<table>
<thead>
<tr>
<th>Code</th>
<th>Component in formulation</th>
<th>Mean droplet size ± S.D.¹ (nm)</th>
<th>Mean zeta potential ± S.D.¹ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fx 1</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 1.5, Tween 80: 5, Polox. 188: -</td>
<td>243.57 ± 2.20</td>
<td>-26.47 ± 0.38</td>
</tr>
<tr>
<td>Fx 2</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 1.5, Tween 80: 3, Polox. 188: 2</td>
<td>176.17 ± 1.12</td>
<td>-35.00 ± 0.62</td>
</tr>
<tr>
<td>Fx 3</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 1.5, Tween 80: -5, Polox. 188: 5</td>
<td>180.03 ± 0.90</td>
<td>-37.63 ± 0.74</td>
</tr>
<tr>
<td>Fx 4</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 1.5, Tween 80: -10, Polox. 188:</td>
<td>258.03 ± 4.61</td>
<td>-40.13 ± 0.64</td>
</tr>
<tr>
<td>Fx 5</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 1.5, Tween 80: -5, Polox. 188: 5</td>
<td>257.07 ± 3.22</td>
<td>-37.97 ± 1.42</td>
</tr>
<tr>
<td>Fx 6</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 2.5, Tween 80: -5, Polox. 188:</td>
<td>358.60 ± 12.93</td>
<td>-41.20 ± 0.53</td>
</tr>
<tr>
<td>Fx 7</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 3.0, Tween 80: 1, Polox. 188: 4</td>
<td>321.03 ± 1.55</td>
<td>-68.70 ± 6.59</td>
</tr>
<tr>
<td>Fx 8</td>
<td>Oil: 28.5, Surfactant &amp; Cosurfactant (ratio): MV 82: 5.0, Tween 80: -5, Polox. 188:</td>
<td>554.53 ± 138.88</td>
<td>-51.70 ± 0.98</td>
</tr>
</tbody>
</table>

¹ S.D., Standard deviation of three measurements

2.3 Determination of Droplet Size and Surface Charge
Particle size distribution was determined by photon correlation spectroscopy (PCS). The samples were diluted in distilled water at optimal ratio for measurement purposes, the measurement was done at an angle of 173° using backscattering technique. The zeta potential was determined through dynamic light scattering (DLS) of Zeta Sizer, Nano-ZS (Malvern Instrument, UK). All measurements were performed in triplicate at room temperature 25°C, each consisting of 20 runs.

2.4. Evaluation of Nanoemulsions Stability
Nanoemulsions were evaluated the stability of structure through visual observation particle mean size and zeta potential after 14 and 30 days of storage at 25°C (room temperature) and 45°C (accelerate condition).

2.5. Optical Microscope and Image Analysis
Nanoemulsions were analyzed the microstructure of emulsion base under the phase separation. Sample was dropped on the microscope slide and covered with glass cover slip and observed using conventional optical microscope (Olympus-BX51, UK). The objective lens 20x, 50x and 100x were used to visualize the droplet structure of microemulsions and nanoemulsions.

3. RESULTS AND DISCUSSION
3.1 Droplet Size and Zeta Potential of Nanoemulsions
In this study, surfactant at various types
and concentrations are effects on the physical properties of nanoemulsions were evaluated by droplet size and zeta potential as shown in Table 1. The maximum amount oil that could be emulsified was applied to be 28.50% (w/w). At the same total amount of surfactant and co-surfactant (6.5% w/w) in formulation 1 to 3 presented different influence on the emulsion system as showed droplet size at $243.57 \pm 2.20$, $176.17 \pm 1.12$, and $180.03 \pm 0.90$ nm, respectively.

On further increasing the proportion of surfactant until 10% w/w, it was observed that droplet size of emulsion was increased more than 200 nm as a result in formulation 4 to 8. Even though using the MV82 alone which is an innovative combination of fatty alcohols and alkyl glucosides, at 1.5 and high concentration at 5% wt. without cosurfactant, it was still shown larger particle size more than surfactant-mixed and induced particle drop size to be bulky up to 550 nm and in broader size distribution. Moreover, the concentration of poloxamer 188 also had effect on their size, at the high poloxamer 188 concentration leads to an increase in the particle size and zeta potential of formulation (Fx.2-4, Table 1) cause from emulsified properties. The results in Table 1, Fx.2 (surfactant mixer between MV82: Tween80: Poloxamer 188 at 1.5: 3: 2) was producing the smallest particle size and gave high surface charge more than -30 mV. This result could be explained due to the poloxamer triblock copolymer structure and high HLB value of poloxamer 188 was suitable compromise with the HLB of MV82 and Tween80. Meanwhile, their zeta potential in all formulations was displayed in the range of -20 to -70 mV which values of zeta potential was related with the surfactant concentration. The charge of droplet emulsion was increased according to poloxamer concentration extended.

### 3.2 Physical Morphology and Stability Determination of Optimized Nanoemulsions

The storage stability of Plai nanoemulsion was determined from droplet size and zeta potential of nanoemulsion at 0, 14, and 30 days (Figure 2). From the results, it can be seen that only formulation 2 and 3 still showed small size of nanoemulsion droplet less than 200 nm while others formulation after times passed to 14 and 30 days, their particles size were increased until 1 μm. Furthermore, physical visualization also was observed at the conventional (25°C) and accelerated condition (45°C) for 1 month.

Formulation 2 and 3, at surfactant concentration 5% w/v, presented homogeneous formation even though it was storage at accelerated temperature for 1 month. Whereas, further formulations showed separated phase after 1 month of storage for unstable nanoemulsions. It can be clearly observed the oil phase and water phase leading to the creaming behavior as shown in Figure 3

Microstructure of nanoemulsions was analyzed from images of O/W emulsion. Nanoemulsion droplet of formulation 2, particle size about 176.17 nm, was showed small and homogeneous in particle size (Figure 4A). Conversely, the structure of nanoemulsion in formulation 8 was observed large size of oil droplet and some of them generated droplet coalescence. This observation can be confirmed the droplet size which was determined large size more than 500 nm (Figure 4B).
Figure 2. Stability of Plai nanoemulsions after storage (at 25°C) during 0, 14, and 30 days, respectively.

Figure 3. Physical appearance of nanoemulsion at different storage times after preparation at day 0 (A), after storage at room temperature (B) and accelerated aging at 45°C (C) for 1 month.
Figure 4. Micrographs of nanoemulsion at different component of surfactant concentration. A: Formulation 2 with 5% w/v surfactant (MV82:Tween80: Poloxamer 188 at 1.5:3:2), B: Formulation 8 with 5% w/v MV82 surfactant.

4. CONCLUSIONS

From all experimental results, we can obtain optimal surfactant component in formulation for fabricate Plai nanoemulsion with the small droplet size and had impact in long last stability. This research can be lead to a better Plai nanoemulsion formulation, thus present influence for design in pharmaceutical and food emulsion.

ACKNOWLEDGEMENTS

The materials and equipments are based up on work supported by Nano Delivery System laboratory, National Nanotechnology Center, NSTDA, Thailand.

REFERENCES


