Stability Test of Nanostructured Lipid Carriers-Loaded Mefenamic Acid prepared by Microemulsion Technique

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Abstract. The objectives of this study were to prepare the nanostructured lipid carriers (NLCs)-loaded mefenamic acid and to study the particle size and zeta potential of the NLCs-loaded mefenamic acid after storage in refrigerator (approximately 2 – 4°C) for 7 days. The preparation method of NLCs-loaded mefenamic acid used the microemulsion technique. All compositions were vigorously mixed with vertex method to obtained the clear microemulsion solution. Tween®80 (surfactant) and 1 – butanol (co – surfactant) were mixed at the ratio of 3:2 to use as the surfactant mixture. Then, it was dispersed in cold water (approximately 2 – 4°C) at different ratio, and the droplet size was reduced by a homogenizer at 6,000 rpm for 5 minutes and sonicator for 30 minutes to form the NLCs-loaded mefenamic acid. The prepared NLCs-loaded mefenamic acid were kept in refrigerator (approximately 2 – 4°C) for 7 days. After that, the solutions were tested the particle size and zeta potential. The particle size and zeta potential of NLCs-loaded mefenamic acid after storage were in the range of 160-310 nm and -4.00 to -19.00 mV, respectively. It was found that the F4 formula was the best to prepare the NLCs-loaded mefenamic acid. F4 formula composed of the 1% w/w mefenamic acid, 10% w/w water, 80%w/w surfactant, and 10%w/w capric triglyceride, and the dilution ratio was 1:25.

1. Introduction
Nanostructured lipid carriers (NLCs) are the lipid nanoparticles that produce by blending of a solid lipid and a liquid lipid, this blend also being solid at body temperature. The solid lipid and liquid lipid blend are melted and hot aqueous surfactant or stabilizer solution is added. The obtained pre – emulsion is produced to form the NLCs by various preparation techniques such as hot and cold high-pressure homogenization, high shear homogenization and ultrasound, microemulsion technique, and solvent emulsification/evaporation process. When the solution is cool down, the emulsion droplets crystallize forming lipid nanoparticles with solid particle matrix [1-3]. The advantage of the NLCs is the increased loading of active compound compared to solid lipid nanoparticles and firmer inclusion of the active compound inside the particle matrix during the shelf life. The use of a lipid mixture between solid lipid and a liquid lipid distorts the formation of very differently structured molecules that present a perfect crystal with high space for accommodate the active compound in molecular form or as amorphous clusters [3].

Mefenamic acid is classified in a Biopharmaceutics Classification System II, low solubility and high permeability drug. which can dissolve in water at 0.21 mg/mL; in addition, it can the solubility is greatly increased when co-solvent is added such as ethanol, propylene glycol, ethylene glycol,
polyethyleneglycol-400, glycerin, and 10% surfactant solutions (Tween®80, sodium lauryl sulfate, or polyethylene glycol dodecyl ether). Ethanol and polyethyleneglycol-400 are reported to be the most effective to enhance the solubility to more than 11 mg/mL [4]. Mefenamic acid is a non-steroidal anti-inflammatory drug indicated for relief of mild-to-moderate pain and primary dysmenorrhea by inhibition of cyclo-oxygenase-2 and prostaglandin synthesis. It is available in the as tablets, capsules, and pediatric suspensions. It has a short elimination half-life which 2 hours. Thus, oral administration requires frequent dosing every 6 hours in order to maintain the steady-state plasma concentration. Recent effort to increase the drug level in plasma more than 2 hours employed controlled release beads [5], sustained release matrix tablets [6, 7], and controlled release tablets [8]. However, crystallization of mefenamic acid has been found after preparing it for use in transdermal patches [9]. Thus, the aim of this study was to prepare the NLCs-loaded mefenamic acid from microemulsion method. The clear microemulsion was prepared and was dispersed in cold water and its droplet size was reduced by homogenizer and sonicator to form the NLCs. Capric triglyceride was used as a liquid lipid phase. The distilled water was used as water phase. Tween®80 and 1-butanol were used as a surfactant and a co-surfactant, respectively. The characterization of NLCs-loaded mefenamic acid were analysed after storage in refrigerator (approximately 2 – 4°C) for 7 days.

2. Experimental works

2.1 Materials
Mefenamic acid and 1-Butanol were obtained from Sigma-Aldrich (USA). Capric triglyceride and Tween®80 were obtained from P.C. Drug Center Co. ltd (Thailand).

2.2 Preparation of NLCs-loaded mefenamic acid prepared by microemulsion technique
The compositions of NLCs-loaded mefenamic acid are shown in Table 1. Briefly, the water, capric triglyceride, and surfactant, were vigorously mixed with vertex method to obtained the clear microemulsion solution. Then, it was dispersed in cold water (approximately 2 – 4°C) at different ratio, and the droplet size was reduced using a homogenizer at 6,000 rpm for 5 minutes and a sonicator for 30 minutes to form the NLCs-loaded mefenamic acid. Tween®80 (surfactant) and 1 – butanol (co – surfactant) were mixed at the ratio of 3:2 to obtain the mixture surfactant. The stability testing of NLCs-loaded mefenamic acid, the prepared NLCs-loaded mefenamic acid were kept in refrigerator (approximately 2 – 4°C) for 7 days. After that, the solutions were tested the particle size and zeta potential.

| Formulas | Mefenamic acid | Water | Surfactant (Tween®80:1-Butanol=3:2) | Capric triglyceride | Dilution ratio |
|----------|----------------|-------|-------------------------------------|---------------------|---------------|
| F1       | 1%w/w          | 10%w/w| 70%w/w                              | 20%w/w              | 1:25          |
| F2       | 1%w/w          | 10%w/w| 70%w/w                              | 20%w/w              | 1:50          |
| F3       | 1%w/w          | 10%w/w| 70%w/w                              | 80%w/w              | 1:100         |
| F4       | 1%w/w          | 10%w/w| 10%w/w                              | 10%w/w              | 1:25          |
| F5       | 1%w/w          | 10%w/w| 80%w/w                              | 10%w/w              | 1:50          |
| F6       | 1%w/w          | 10%w/w| 80%w/w                              | 10%w/w              | 1:100         |

Table 1. The formulas of NLC-loaded mefenamic acid prepared by microemulsion technique.

Table 2. Particle sizes, polydisperse index, and zeta potential of NLCs-loaded mefenamic acid.

| Formulas | Particle size (nm) | Polydisperse index | Zeta potential (mV) |
|----------|--------------------|--------------------|---------------------|
| F1       | 201.1±18.5         | 0.358±0.024        | -13.85±1.85         |
| F2       | 191.3±23.0         | 0.364±0.046        | -18.05±1.23         |
| F3       | 304.5±71.2         | 0.336±0.052        | -12.75±0.59         |
| F4       | 216.9±48.8         | 0.291±0.042        | -8.30±0.56          |
| F5       | 306.7±105.1        | 0.279±0.048        | -9.12±0.83          |
| F6       | 160.8±23.9         | 0.563±0.028        | -4.24±0.70          |
Figure 1. Particle distribution of the NLCs-loaded mefenamic acid prepared by microemulsion technique; F1 (A), F2 (B), F3 (C), F4 (D), F5 (E), and F6 (F)
Figure 2. Zeta potential of the NLCs-loaded mefenamic acid prepared by microemulsion technique; F1 (A), F2 (B), F3 (C), F4 (D), F5 (E), and F6 (F)
2.3 Particle size and zeta potential analysis

The distilled water was used as a diluant at an appropriate concentration prior to NLCs-loaded mefenamic acid determination. The solution of prepared NLC-loaded mefenamic acid was diluted in 5 times. The Nanoplus 3 (Particulate system, USA) was used to determine the particle size, size distribution, and zeta potential of the NLCs-loaded mefenamic acid at 25±2°C. The testing was recorded as a mean±SD for ten sub-runs.

3. Results and discussion

The distribution of particle size and zeta potential of NLCs-loaded mefenamic acid prepared by microemulsion technique after storage in refrigerator (approximately 2 – 4°C) for 7 days are shown in Figure 1 and 2, respectively. These values are concluded that in Table 2. It was found that the particle size was in the range of 160-310 nm with polydispersity index less than 0.6. After storage in refrigerator (approximately 2 – 4°C) for 7 days, all formulas had the high distribution of particle size. Their particles might be accumulated in the solution; however, the size of particles showed less than 500 nm, it was accepted to requirement of nanoparticle preparation. The F4 formula showed low distribution of particle size compared to other formulas. The zeta potential was in the range of -18 to -4 mV. When the amount of surfactant increased, the zeta potential of the formulation was increased. The best formula of NLCs-loaded mefenamic acid prepared by microemulsion technique after storage in refrigerator (approximately 2 – 4°C) for 7 days was F4 due to low particle size (216.9±48.8 nm), low polydispersity index (0.291±0.042), and the zeta potential as -8.30±0.56 mV. Therefore, F4 formulas will be improve and develop in the future to increase the stability.

4. Conclusions

The NLCs-loaded mefenamic acid was prepared from the microemulsion technique using the water as liquid phase, capric triglyceride as the oil phase, and mixture surfactant between Tween®80 and 1 – butanol. Its droplet size was reduced by homogenizer and sonicator after the microemulsion solution was dispersed in cold water. The prepared NLCs-loaded mefenamic acid were kept in refrigerator (approximately 2 – 4°C) for 7 days to study the stability. The particle size and zeta potential were shown in the range of 160-310 nm and -4.00 to -19.00 mV, respectively. The F4 formula was the best to prepare the NLCs-loaded mefenamic acid. F4 formula which composed of the 1%w/w mefenamic acid, 10%w/w water, 80%w/w surfactant, and 10%w/w capric triglyceride, and the dilution ratio was 1:25. Therefore, F4 formulas will be improve and develop in the future to increase the stability and it will be characterized and studied the in vitro release.

5. References

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Acknowledgment

The financial supporting of this work was the Undergraduate Scholarships from College of Pharmacy, Rangsit University.