Formulation of Fenofibrate-Loaded Lipid Nanoparticles Using High Pressure Homogenisation
การตั้งตำรับอนุภาคไขมันแข็งขนาดนาโนที่บรรจุฟีโนไฟเบรต ด้วยเทคนิคการลดขนาด ด้วยแรงดันอากาศสูง

Wisit Muninnimit (วิสิฐ มุนินทร์นิมิตต์)* Dr.Phanphen Wattanaarsakit (ดร.พรรณเพ็ญ วัฒนาอาษากิจ)**
Waree Tiyaboonchai (วรี ติยะบุญชัย)***

ABSTRACT

In this study, fenofibrate-loaded lipid nanoparticles (LNs) were successfully produced by hot high pressure homogenisation technique. The preliminary studies revealed that processing parameters, for example, homogenised cycle and pressure, and the amount of lipid, were crucial factors affecting the particles size of blank LNs. At the optimal conditions, fenofibrate-loaded LNs with a median particle size of 189 nm, d(0.9) value of 265 nm and span value of 0.6 could be obtained. Processing parameters, such as the types of co-lipid, as well as the incorporation of lecithin played a significant role in controlling the particle size. The nano-sized range formulation could be achieved by using small molecular structure lipid and employing 1.25%w/w of lecithin.

บทคัดย่อ

การศึกษานี้สามารถเตรียมอนุภาคไขมันแข็งขนาดนาโนที่บรรจุฟีโนไฟเบรตได้สำเร็จโดยใช้เทคนิคการลดขนาดด้วยแรงดันอากาศสูงแบบใช้ความร้อน การศึกษาเบื้องต้นแสดงให้เห็นว่าปัจจัยของกระบวนการผลิต เช่น จำนวนรอบและความดันที่ใช้ในการลดขนาดอนุภาค และปริมาณไขมันแข็ง เป็นปัจจัยที่สำคัญที่มีผลกระทบต่อขนาดอนุภาคของอนุภาคไขมันแข็งที่ไม่บรรจุฟีโนไฟเบรต การเตรียมที่สภาวะเหมาะสมจะทำให้อนุภาคไขมันแข็งที่บรรจุฟีโนไฟเบรตมีขนาดอนุภาคเฉลี่ย ค่า d(0.9) และค่าการกระจายขนาดอนุภาคเท่ากับ 189 นาโนเมตร 265 นาโนเมตร และ 0.6 ตามลำดับ นอกจากนี้ปัจจัยของกระบวนการผลิต เช่น ชนิดของไขมันแข็งร่วมรวมถึงการผสมเลซิทินยังมีบทบาทสำคัญในการควบคุมขนาดอนุภาค โดยอนุภาคขนาดนาโนเมตรสามารถเตรียมได้โดยใช้ไขมันแข็งที่มีโครงสร้างโมเลกุลขนาดเล็กและผสมเลซิทินในปริมาณ 1.25 เปอร์เซ็นต์โดยน้ำหนักต่อน้ำหนัก

คำสำคัญ : ฟีโนไฟเบรต ลิพิดนาโนพาร์ทิเคิล การลดขนาดด้วยแรงดันอากาศสูง
Key Words : Fenofibrate, Lipid nanoparticles, High pressure homogenisation

** Graduate student, Faculty of Pharmaceutical Sciences, Chulalongkorn University*
*** Ph.D. of Faculty of Pharmaceutical Sciences, Chulalongkorn University*
**** Assist. Prof., Faculty of Pharmaceutical Sciences, Naresuan University*

Introduction

Lipid nanoparticles (LNs) have been widely studied during the past two decades (Muller *et al.*, 2000, Tiyaboonchai *et al.*, 2007, Westesen *et al.*, 1993). There are two types of LNs; solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). SLNs and NLCs are a nano-sized range of drug carrier systems consisting of lipid and/or oil, emulsifier and water. There are several advantages such as acceptable low toxicity of lipid (Rainer *et al.*, 1997), improvement of drug stability (Heiati *et al.*, 1998), increasing of drug bioavailability (Luo *et al.*, 2006) and targeted drug delivery (Blasi *et al.*, 2007). The LNs is able to be prepared by many techniques such as high pressure homogenisation (HPH) (Kuntsche *et al.*, 2005), ultrasonication (Castelli *et al.*, 2005), supercritical fluid process (Chattopadhyay *et al.*, 2007), and solvent diffusion method (Hu *et al.*, 2005). HPH has an advantage over the other techniques, for example, simple production process, organic solvent-free process, and easy scaling-up. Owing to its advantages, HPH has been interested in preparation of LNs (Bunjes *et al.*, 2001, Scholer *et al.*, 2002).

The goal here was to characterise factors affecting particle size of LNs. The interesting processing parameters were homogenised pressure and cycle and the amount and types of lipids and surfactants. Fenofibrate was used as model drug. It is a lipophilic drug that is insoluble in water. The chemical structure of fenofibrate is shown in Figure 1. According to the Biopharmaceutics Classification System (BCS) category, it is classified as BCS class II, high permeability and low solubility.

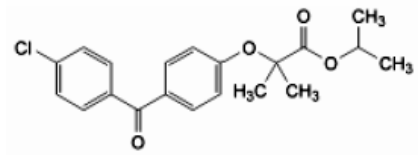

Figure 1 Chemical structure of fenofibrate.

Materials and methods

Materials

Fenofibrate, micronised powder, was selected as model drug and obtained from Alembic (Vadodara, India). Cetyl palmitate (Stepan© 653) was purchased from VITA Company Limited (Bangkok, Thailand). Glyceryl behenate (Compritol(r) 888 ATO) was a gift from GATTEFOSSÉ (France). Trimyristin (Dynasan 114), hydrogenated palm oil (Softisan® 154) and caprylic/capric triglyceride (Miglyol® 812) were purchased from The Sun Chemical Co., Ltd. (Bangkok, Thailand). Tween® 80 was purchased from Merck Ltd. (Bangkok, Thailand). Lutrol® F68 and F127 were a gift from BASF (Thai) Limited (Bangkok, Thailand). Lecithin (3-sn-phosphatidylcholine from soybean, ≥ 30% phosphatidyl choline basis) was obtained from Sigma-Aldrich (Buchs, Switzerland). Ultrapure water was supplied from ELGASTAT MAXIMA UF (Elga Ltd, Bucks, England)

Methods

Preliminary study

The LNs was produced by hot HPH technique. The lipid phase consisting of 10% w/w of trimyristin was heated up to 80°C. The molten lipid was dispersed into the hot surfactant solution (80°C), consisting of 7%w/w poloxamer 407 in

water, using a homogeniser (Polytron PT 3100, Kinematica, Switzerland) at 10,000 rpm for 30 seconds. Afterwards, the resulting coarse premix was finely dispersed under pressure (800 bar and five passes) using a high pressure homogeniser (Emulsiflex C-5, Avestin Inc., Canada) and then allowed it to cool down at ambient temperature. Empty LNs were prepared with different processing parameters to study the effect of a number of variables on their particle size. Process parameters were varied as follows: the homogenised cycle was varied from 3 to 9 passes; the homogenised pressure was varied from 500-1100 bar; the lipid content was varied from 2.5-10.0% w/w while keeping the ratio of trimyristin and poloxamer 407 at 1.25:1.

Preparation of fenofibrate-loaded LNs

The LNs containing 7%w/w of fenofibrate based on the lipid content were produced by hot HPH technique (Table 1). The lipid phase containing cetyl palmitate (CP), co-lipid, oil and fenofibrate was heated up to 80°C. The molten lipid was dispersed into the hot surfactant solution (80°C) using a homogeniser at 10,000 rpm for 30 seconds. Afterwards, the resulting coarse premix was finely dispersed under high pressure, 800 bar, for five passes using a high pressure homogeniser and then allowed it to cool down at ambient temperature. Process parameters were varied as follows: the types of co-lipid were chosen from trimyristin (TM), glyceryl behenate (GB), and hydrogenated palm oil (HPO); and the absence and presence of 1.25% w/w lecithin. The ranges of these variable values were selected based on preliminary experiments.

Table 1 Composition of fenofibrate-loaded LNs; trimyristin(TM), glyceryl behenate (GB), and hydrogenated palm oil (HPO) were used as co-lipid.

	%w/w
Fenofibrate	0.40
Cetyl Palmitate	2.50
Co-lipid	2.50
Miglyol 812	0.75
Poloxamer 188	1.50
Polysorbate 80	0.50
Ultrapure water	91.85

Particle size analysis

To detect the particle size of fenofibrate-loaded LNs, laser diffractometry (LD) was conducted by Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK). The particle size was evaluated using d(0.5) and d(0.9). The d(0.5) is the size in microns at which 50% of the sample is smaller and 50% is larger and the d(0.9) is the size of particle below which 90% of the sample lies. The span was calculated to measure the width of the distribution. The narrower the distribution, the smaller the span becomes. The span is calculated as:

$$\frac{d(0.9)-d(0.1)}{d(0.5)}$$

The calculation of LD data was performed by using the Mie theory. During the operation, the ultrasonic was applied to deagglomerate the particle. Three replicated samples were measured. For this technique, the particle refractive index of 1.45 and imaginary refractive index of 0.1 were used across the samples.

Statistics

All data were presented as mean ± standard deviation (SD). One-way analysis of variance (One-way ANOVA) was used to test the significance of the estimates with 0.05 significant levels.

Results and discussion

An aqueous LNs dispersions consisting of 10% w/w trimyristin and 7% w/w poloxamer 407 were prepared using a hot HPH technique in order to study the effect of process parameters on the particle size of LNs. The median particle size and size distribution (span) of LNs were determined by LD. The results showed that the median particle size was affected by various processing parameters. The cycle of homogenisation played a crucial role in controlling the size of LNs (Figure 2). The particle size was significantly decreased with increasing the homogenised cycle ($p < 0.05$). At the homogenisation over 5 passes, the median particle size of LNs were below 185 nm. In the similar way, the d(0.9) value was also smaller (below 254 nm). As presented in Figure 2, the downward trend in the width of the distribution was also detected. This result is due to large particles are broken at higher passes. Furthermore, all formulations exhibited monodisperse pattern (data not shown) that indicated a good formulation stability.

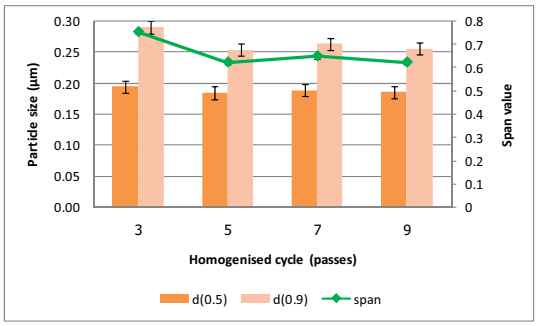

Figure 2 The particle size of LNs after homogenisation at variable homogenised cycles prepared by HPH of 10% w/w trimyristin and 7% w/w poloxamer 407. The error bars (I) represent S.D. of the data.

The particle size of LNs was not only affected by the homogenised cycles but also strongly influenced by the pressure of homogenisation. As presented in Figure 3, the increase in homogenised pressure from 500 to 700 bar showed no statistically significant difference in the median particle sizes of around 192 nm ($p > 0.05$), whereas the pressure over 700 bar showed statistically significant difference in such median ($p < 0.05$). In addition, the d(0.9) value and span value were significantly smaller when prepare at high pressure, > 700 bar ($p < 0.05$).

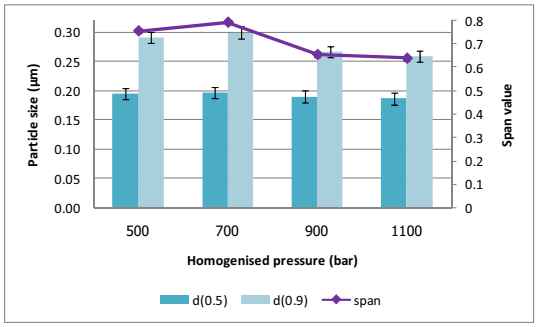

Figure 3 The particle size of LNs after homogenisation at variable homogenised pressures prepared by HPH of 10% w/w trimyristin and 7% w/w poloxamer 407. The error bars (I) represent S.D. of the data.

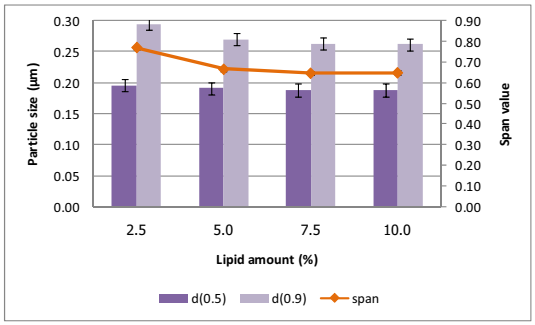

Figure 4 The particle size of LNs after homogenisation at variable lipid contents prepared by maintaining trimyristin and poloxamer 407 ratio at 1.25:1. The error bars (I) represent S.D. of the data.

Furthermore, the effect of the amount of lipid on the particle size of LNs was also investigated. In order to understand the role of lipid content on the particle size of LNs, the aqueous LNs dispersions were prepared by maintaining the ratio of lipid and surfactant at 1.25:1. The results showed that the particle size of LNs decreased with increasing lipid amounts ($p < 0.05$) (Figure 4). The smallest median particle size (188 nm) can be achieved by preparing sample at 7.5% lipid content. Furthermore, the span trend line decreased with increasing lipid amount. These results suggested that larger amount of lipid increase the chance of particle collision leading to breaking large particle into small particle.

From the obtained data, the optimal condition appeared to be a homogenisation at 1100 bar and 5 passes. Note that the homogenisation at 1100 bar was the most efficient of particle size reduction. According to the statistical analysis, the increase in homogenised cycles from 5 to 9 passes presented statistically insignificant in median, d(0.9) and span values ($p > 0.05$); then, this study applies 5 passes in order to optimise time for production. Thus, we further used these conditions to formulate fenofibrate-loaded LNs using different lipid types. In order to achieve high drug loading efficacy, a combination of waxes, glycerides, and medium chain triglycerides was used as a lipid phase (Hu *et al.*, 2006, Teeranachaideekul *et al.*, 2007). The results showed that the particle size and size distribution were affected by the types of co-lipids. Even though, the median particle size of all formulations exhibits value in the range of 232-276 nm. Nevertheless, the large particle size can be found in every formulation. The fenofibrate-loaded LNs containing HPO showed the largest particle size and

broadest size distribution, d(0.9) and span value of 11.121 μm and 39.667, respectively. In the contrary, the d(0.9) value of fenofibrate-loaded LNs prepared with TM and GB were 2.565 and 1.025 μm, respectively, with the span value of 9.986 and 3.754, respectively (Figure 5). HPO was considered as a large molecular structure than TM and GB as it comprises blend of natural, saturated, and even-numbered unbranched a chain length of C_{10}-C_{18} triglycerides. Thus, the large particles and broad size distribution observed may be a result from particle agglomeration which may cause by a large molecular structure of HPO cannot be covered sufficiently by surfactant.

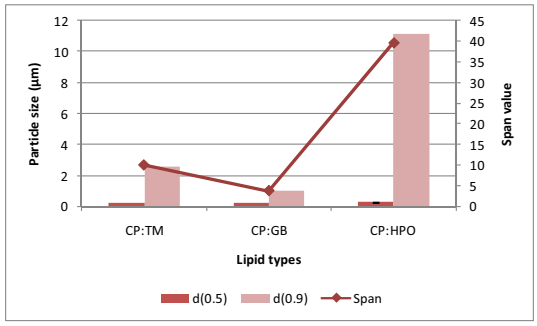

Figure 5 The particle size of fenofibrate-loaded LNs containing variable lipid types; cetyl palmitate (CP), trimyristin(TM), glyceryl behenate (GB), and hydrogenated palm oil (HPO). The error bars (I) represent S.D. of the data.

Table 2 Composition of fenofibrate-loaded LNs containing lecithin.

	%w/w
Fenofibrate	0.40
CP	2.50
HPO	2.50
Miglyol 812	0.75
Lecithin	-
Poloxamer 188	1.50
Polysorbate 80	0.50
Ultrapure water qs to	100.00

As the results showed that fenofibrate-loaded LNs in nano-sized range could not be achieved when prepared with poloxamer 188 and polysorbate 80. Thus, the addition of lecithin in water phase was performed to investigate the influence of lecithin on particle size of fenofibrate-loaded LNs. The combination of CP and HPO was used as lipid phase. The composition of fenofibrate-loaded LNs containing lecithin was displayed in Table 2. The preparations of fenofibrate-loaded LNs containing lecithin were carried out by mixing lecithin into surfactant solution at 0.50, 1.00, 1.25, 1.50, 1.75, and 2.00%. From the data obtained it was found that the median size and the width of size distribution of LNs dispersions decreased with increasing lecithin contents (Figure 6). The formulation prepared with 1.25% of lecithin showed small median particle size of 189 nm with d(0.9) value of 265 nm. This formulation also exhibited monodisperse pattern (data not shown) with a span value of 0.654.

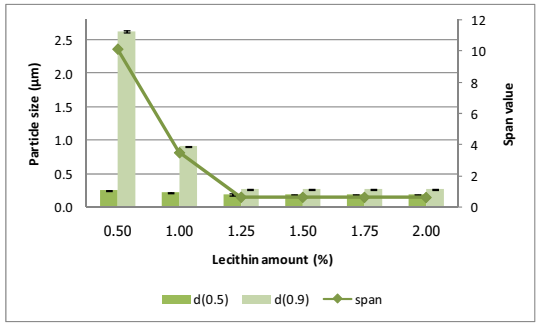

Figure 6 The particle size of LNs after homogenisation; prepared by mixing lecithin into surfactant solution at variable lecithin contents. The error bars (I) represent S.D. of the data.

The results suggested that the incorporation of lecithin in LNs system offered a great advantage.

However, at the lecithin content over 1.25 percent, the particle size of LNs remained unchanged. Thus, the optimal amount of lecithin was 1.25%. Lecithin contains the L-α-glycerophosphoylcholine skeleton esterified to two long-chain fatty acids, and a polar head group. As a result from this structure, lecithin exhibits highly efficient to decrease interfacial tension between oil and water interface leading to decrease in particle size of LNs. On the other hand, when the particle curvature reaches the critical value, lecithin does not affect the particle size of LNs (Schubert and Muller-Goymann, 2005). However, Schubert, *et al.* (2006) suggested that the large amount of lecithin may results in destabilisation of colloidal system. In aqueous media, lecithin is capable of assembling into concentric bilayer structures known as liposomes (Bummer, 2000). As the two particles containing lecithin layers come close enough, the fusion of lecithin bilayer leading to the particle agglomeration and growth.

Conclusions

In this study, fenofibrate-loaded LNs were successfully produced by hot HPH technique. The preliminary studies revealed that process parameters, for example, homogenised cycle and pressure, and the amount of lipid, were crucial factors affecting the median particles size of LNs. The increasing of homogenised cycle and pressure, and amounts of lipid resulted in the decreasing of particle size of LNs. Nevertheless, the use of poloxamer 188 and polysorbate 80 cannot achieve the preparation of fenofibrate-loaded LNs in nano-sized range. Nevertheless, the results demonstrated that the incorporation of 1.25% w/w lecithin is capable of reducing the particle size.

Suggestions

These initial findings pointed out to a need for further study. It is necessary to assess the relationships between small particle size of fenofibrate-loaded LNs and its physicochemical properties, such as dissolution profiles, in term of its pharmaceutical utility. Preliminary results of dissolution studies suggested that the LNs could enhance the dissolution rate of fenofibrate that could be potential in improving drug bioavailability.

Acknowledgements

The authors wish to acknowledge the financial support of the National Science and Technology Development Agency (NSTDA) and SilomMedical Co, Ltd. (Thailand).

References

Blasi, P., Giovagnoli, S., Schoubben, A., Ricci, M., and Rossi, C. 2007. Solid lipid nanoparticles for targeted brain drug delivery. Advanced Drug Delivery Reviews, 59, 454-477.

Bummer, PM. 2000. Interfacial phenomena. in Gennaro, A. R. (Ed.) Remington: The science and practice of pharmacy. 20th ed. Philadelphia, Lippincott Williams & Wilkins.

Bunjes, H., Drechsler, M., Koch, MHJ., and Westesen, K. 2001. Incorporation of the model drug ubidecarenone into solid lipid nanoparticles. Pharmaceutical Research, 18, 287-293.

Castelli, F., Puglia, C., Sarpietro, MG., Rizza, L., and Bonina, F. 2005 Characterization of indomethacin-loaded lipid nanoparticles by differential scanning calorimetry. International Journal of Pharmaceutics, 304, 231-238.

Chattopadhyay, P., Shekunov, BY., Yim, D., Cipolla, D., Boyd, B., and Farr, S. 2007 Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system. Advanced Drug Delivery Reviews, 59, 444-453.

Heiati, H., Tawashi, R.,and Phillips, NC. 1998. Drug retention and stability of solid lipid nanoparticles containing azidothymidine palmitate after autoclaving, storage and lyophilization. Journal of Microencap sulation, 15, 173 - 184.

Hu, F.-Q., Jiang, S.-P., Du, Y.-Z., Yuan, H., Ye, Y.-Q., and Zeng, S. 2005. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids and Surfaces B: Biointerfaces, 45, 167-173.

Hu, F.-Q., Jiang, S.-P., Du, Y.-Z., Yuan, H., Ye, Y.-Q., and Zeng, S. 2006. Preparation and characteristics of monostearin nanostructured lipid carriers. International Journal of Pharmaceutics, 314, 83-89.

Kuntsche, J., Koch, MHJ., Drechsler, M., and Bunjes, H. 2005. Crystallization behavior of supercooled smectic cholesteryl myristate nanoparticles containing phospholipids as stabilizers. Colloids and Surfaces B: Biointerfaces, 44, 25-35.

Luo, Y., Chen, D., Ren, L., Zhao, X., and Qin, J. 2006. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. Journal of Controlled Release, 114, 53-59.

Muller, RH., Mader, K., and Gohla, S. 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. European Journal of Pharmaceutics and Biopharmaceutics, 50, 161-177.

Rainer, HM., Dörte, R., Stephan, R., Kai, S.-F., and Wolfgang, M. 1997. Cytotoxicity of Solid Lipid Nanoparticles as a Function of the Lipid Matrix and the Surfactant. Pharmaceutical Research, V14, 458-462.

Scholer, N., Hahn, H., Muller, RH., and Liesenfeld, O. 2002. Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on the viability and cytokine production of macrophages. International Journal of Pharmaceutics, 231, 167-176.

Schubert, MA., Harms, M., and Muller-Goymann, CC. 2006. Structural investigations on lipid nanoparticles containing high amounts of lecithin. European Journal of Pharmaceutical Sciences, 27, 226-236.

Schubert, MA., and Muller-Goymann, CC. 2005. Characterisation of surface-modified solid lipid nanoparticles (SLN): Influence of lecithin and nonionic emulsifier. European Journal of Pharmaceutics and Biopharmaceutics, 61, 77-86.

Teeranachaideekul, V., Souto, EB., Junyaprasert, VB., and Muller, RH. 2007. Cetyl palmitate-based NLC for topical delivery of Coenzyme Q10 - Development, physicochemical characterization and in vitro release studies. European Journal of Pharmaceutics and Biopharmaceutics, 67, 141-148.

Tiyaboonchai, W., Tungpradit, W., and Plianbangchang, P. 2007. Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. International Journal of Pharmaceutics, 337, 299-306.

Westesen, K., Siekmann, B., and Koch, MHJ. 1993. Investigations on the physical state of lipid nanoparticles by synchrotron radiation X-ray diffraction. International Journal of Pharmaceutics, 93, 189-199.