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# Investigation of Nanostructured Lipid Carriers for Transdermal Delivery of Flurbiprofen

**Fei Han, Sanming Li, Ran Yin, Xiaolei Shi, and Qiang Jia**

*School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, Liaoning Province, People's Republic of China*

**The aim of this study was to develop nanostructured lipid carriers (NLC) for transdermal delivery of Flurbiprofen (FP). The physical stability of FP-NLC and its in vitro permeation profile were investigated. After three months of storage at 4°C, 20°C, and 40°C, no significant differences between the evaluated parameters, such as pH value, the entrapment efficiency, particle size, and zeta potential were observed. In in vitro permeation studies, the cumulative permeated amounts and the release rate from FP-NLC were 412.53 ± 21.37 μg/cm$^2$ and 35.25 μg/cm$^2$/h after 12 h ($n$ = 6), respectively, while from saturated FP-PBS (pH = 7.4) were 90.83 ± 8.67 μg/cm$^2$ and 6.99 μg/cm$^2$/h, respectively. These results indicated that the FP-NLC were with good physical stability and were able to improve the permeated amounts and the release rate of FP. It could potentially be exploited as a carrier with improved drug entrapment efficiency and permeated amount in the transdermal delivery of FP.**

**Keywords** nanostuctured lipid carriers; flurbiprofen; transdermal; in vitro permeation

## INTRODUCTION

Flurbiprofen (FP, Figure 1) is a chiral non-steroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid class, which is used to treat gout, osteoarthritis, rheumatoid arthritis, and sunburn (Lee, Burt, & Koch, 1992; Poul, West, Buchanan, & Grahame, 1993). Its therapeutic benefits combined with a good gastrointestinal tolerability are well documented in comparison with other NSAIDs. However, its oral administration can produce some side effects such as bellyache and indigestion. In order to avoid the irritation of gastrointestinal tract, one promising method is to administer the drug via skin (McNeill, Potts, & Francoeur, 1992). Transdermal dosage forms such as gels, ointments and creams have been intensively studied for this purpose (Kitagawa et al., 1993; Kyuki et al., 1984; Masumoto et al., 1982).

Recently, more attention has been focused on solid lipid nanoparticles (SLN) for transdermal delivery of drugs. The transdermal delivery of vitamin-A (Jenning, Schäfer-Korting, & Gohla, 2000), glucocorticoids (Santos Maia, Mehnert, & Schäfer-Korting, 2000), retinoid (Jenning & Gohla, 2001), and clotrimazole (Souto, Wissing, Barbosa, & Müller, 2004) have been reported. The SLN for transdermal delivery has several advantageous characteristics for drug delivery: good local tolerability, high inclusion rate for lipophilic substances (Müller et al., 1995), and the small size of the lipid particles ensures close contact to the stratum corneum so as to increase the amount of the drug penetrating into the mucosa or skin. NLC, consisted of solid lipid and liquid lipid, are a new generation of SLN, which can overcome the limitations of SLN such as limited drug loading capacity, drug expulsion during storage, and so forth.

In this present work, NLC were selected to develop as FP transdermal vehicles. We assessed the feasibility of FP loaded NLC to improve the skin permeation of the drug. The physical properties of FP-NLC, such as drug entrapment efficiency, stability in storage, and in vitro permeation behavior were investigated. Reasonable conclusions on designing stable drug loaded NLC system was expected.

## MATERIALS AND METHODS

### Materials

Compritol® ATO 888 (Gattefosse) and Miglyol® 812 (BASF, Germany) were used as solid lipid material and as liquid lipid material for NLC, respectively. Lecithin was purchased from Sigma, Poloxamer 188 (BASF, Germany); SDC (Serva) and Tween-80 (Shanghai Chemical Co., Ltd., China) were chosen as surfactant. FP was supplied by Hangzhou Keben Chemical Co., Ltd. (Zhejiang, China). Water used in experiment was distilled. All other chemicals and solvents were of analytical reagent grade.

Address correspondence to Sanming Li, School of Pharmacy, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, Liaoning Province, P. R. China. E-mail: li_sanming@126.com

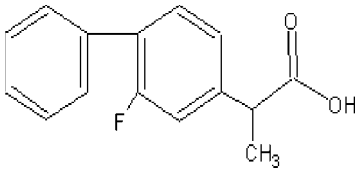


FIGURE 1. The chemical structure of flurbiprofen.

## Preparation of NLC

The formulations of NLC, listed in Table 1, were prepared by hot high pressure homogenization method. Briefly, the lipid phase (consisted of 60% Compritol® ATO 888 and 40% Miglyol®812 content) was melted at 85°C with Lecithin to obtain a clear lipid phase. Meanwhile an aqueous surfactant solution had been prepared and heated at the same temperature. The hot surfactant solution was then dispersed in the hot lipid phase using a mechanical agitator (DC-40, Hangzhou Electrical Engineering Instruments, China) at 2000 rpm for 15 min. The obtained pre-emulsion was homogenized at 85°C, using a Niro soavins10012k high pressure homogenizer (Italy) under a pressure of 500 bar and three homogenization cycles. The siliconized glass vials was immediately sealed after it was filled with obtained product.

## Transmission Electron Microscopy (TEM)

The Cu grid coated with C film was put into the NLC nanosupensions several times. After being stained by 2% phosphotungstic acid (PTA) solution, the sample was dried at room temperature for about half an hour, and the sample was gained for the TEM investigation.

## Particle Size and Zeta Potential Analysis

The mean particle size of NLC was determined by Laser Particle Size Analyzer (Coulter LS-230, Bekman Coulter Co., Ltd., U.S.A.) at room temperature. Before measurement, NLC dispersions were diluted with filtered water. The particle size analysis data were evaluated using volume distribution to detect even a few large particles. The Zeta potential of NLC was determined using Zeta Potential and Particle Size Analyzer (DELSA 440 SX, Bekman Coulter Co., Ltd., U.S.A.) after diluting samples with distilled water at room temperature.

TABLE 1
Composition of Drug-Free and Drug-Loaded NLC Formulations (%, m/m)

| Formulation Composition | Drug-Free NLC | Drug-Loaded NLC |
|---|---|---|
| Compritol®ATO 888 | 6 | 6 |
| Miglyol ®812 | 4 | 4 |
| Lecithin | 1 | 1 |
| Poloxamer 188 | 10 | 10 |
| SDC | 0.5 | 0.5 |
| FP | — | 1 |
| Water ad | 100 | 100 |

## Drug Entrapment Efficiency Determination

Entrapment efficiency was calculated after separation of the non-entrapped drug using the mini-column centrifugation method (Fry, White, & Goldman, 1978; New, 1990). Sephadex G50 (10g) was swollen in distilled water (120 ml) at room temperature for at least 5 h and stored at 4°C. To prepare the mini-columns, filter papers were inserted in the bottom of the barrels of 2.5 $cm^3$ syringes, which were then filled with gel. Excess water was centrifuged off at 3000 rpm for 3 min, and 0.2 ml of the NLC suspensions were added dropwise to the center of the column, followed by centrifugation as before. To the mini-column, 0.2 ml distilled water was added and centrifuged was repeated 5 times. All of the centrifugate was combined and the amount of drug entrapped in the vesicles was determined by HPLC. The entrapment efficiency was calculated as a certain percentage of the initial drug added. The drug entrapment efficiency (*EE*) of nanoparticles was calculated from Equation 1:

$$EE(\%) = \frac{W_2}{W_1} \times 100\% \quad (1)$$

where $W_2$ and $W_1$ were the weight of drug entrapped in the vesicles and the weight of lipid added in system, respectively.

## In Vitro Permeation Studies

The abdominal hair of male Wistar rats (200–250 g) was removed carefully using electric razors. After the animals were sacrificed, the abdominal skin was excised and the adhering fat was eliminated. The skin used was of thickness 800 ± 50 μm. And it was mounted on vertical Franz-type diffusion cell with the dermis facing the receptor compartment. The donor side was charged with 0.25 g of the investigated FP-NLC or 8 ml the saturated FP-PBS (pH = 7.4) containing 2.5 mg FP. The membrane surface area available for diffusion was 0.785 $cm^2$. The donor chamber was covered with parafilm. 12 ml PBS (pH = 7.4) was used as receptor medium and the sink condition was maintained by replacing the fluid at every sampling interval with fresh receptor medium. The temperature was maintained at 37 ± 0.5°C and the receptor compartment was constantly stirred at 300 rpm. The percentage values of cumulative FP permeated were calculated.

## Stability Test of NLC

The storage stabilities of NLC were determined as follows. A volume of 5 ml of NLC dispersion were filled into glass

vials, and stored at 4, 20, and 40°C for 3 months, the changes of pH value, drug entrapment efficiency, particle size, and zeta potential against storage time were investigated. The experiments were carried out in triplicate for each sample, and the results were presented as an average ± *SD*.

### HPLC Analysis

The drug entrapment efficiency and the amount in the receptor phase were measured by HPLC method using a JASCO 1500 series HPLC system. The detection wavelength was set at 247 nm and oven at 30°C. A Diamond $C_{18}$ column (5 μm, 4.6 mm × 200 mm) was used. The mobile phase consisted of methanol: 0.05 mol/L phosphate buffer (80:20, v/v) was delivered at a flow rate of 1.0 ml/min. All samples were filtered through a 0.45 μm pore size membrane filter before injection.

### Data Analysis of Skin Permeation

The cumulative drug permeation per unit of skin surface area ($Q_t$) was calculated from Equation 2

$$Q_t = \frac{V_r C_t + \sum_{i=0}^{t-1} V_s C_i}{A} \quad (2)$$

where $C_t$ is the drug concentration of the receiver solution at each sampling time, $C_i$ is the drug concentration of the $i$ th sample, and $V_r$ and $V_s$ are the volumes of the receiver solution and the sample, respectively. $A$ represents the skin surface area. The skin permeation rate at steady-state ($J_s$, μg/cm$^2$/h) was calculated from the slope of the linear portion of the plots of $Q_t$ against time. All skin permeation experiments were repeated 6 times, and the results were presented as an average ± *SD*.

## RESULTS AND DISCUSSION

### Preparation of NLC

The type of surfactant and its concentration might affect stability of the lipid matrix (Radomska & Müller, 2006; Souto & Müller, 2006a). Cui and Mumper have reported on a novel nanoparticle system engineered from oil-in-water (O/W) microemulsion precursors using mixed surfactants (Cui & Mumper, 2002), and we developed the drug-free NLC with the blend of surfactants (Fei et al., 2007). In this study, three types of surfactants were combined to improve the stability of NLC in the presence of FP. NLC samples showed favorable physical properties for stabilization more than 3 months. It indicates that the mixture of different surfactants can better the physical characters of NLC.

When NLC was prepared, the surface quality would change a lot in the process of the formation of nanoparticles by solidification (cooling crystallization) of colloid nanodroplets. Except for the improvement of Lecithin on nanoparticles interface quality, the main reason for this should likely be attributed to the synergistic effect of electrostatic repulsion caused by the ionic surfactant SDC and the additional steric stabilization resulting from the non-ionic surfactant Poloxamer 188. This is consistent with the previous study (Reich, 1997; Souto. & Müller, 2006b).

### TEM Investigation

TEM (JEM-1200, JEOL Co., Ltd., Tokyo, Japan) was a method of probing the microstructure of rather delicate systems such as micelles, liquid crystalline phases, vesicles, emulsions, and also nanoparticles (Danino, Talmon, & Zana, 1997). Figure 2 shows the shape of the nanoparticles entrapping with the model drug. It was evident that the particles investigated revealed round and homogeneous shading, the particle size ranged from approximately 50 to 200 nm.

### Storage Stability of NLC

It is known that the particle size distribution is one of the most important characteristics for the evaluation of the stability of colloidal systems and also influences the penetration mechanism of drugs into the skin (Constantinides & Yiv, 1995; Müller & Müller, 1984). Therefore, the particle size and the zeta potential (ZP) have been evaluated immediately after production of the systems, and during 3 months of storage at three different temperatures (4°C, 20°C, and 40°C).

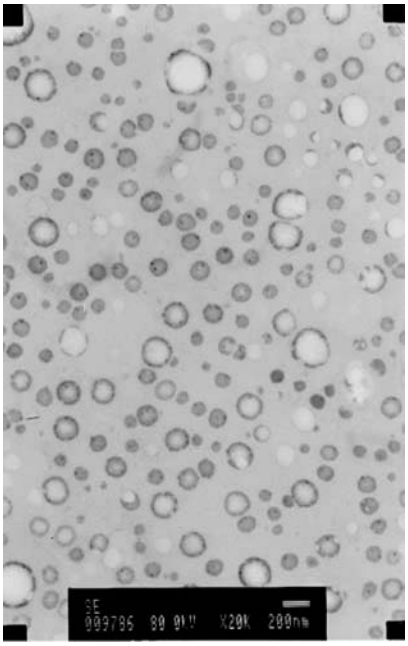


FIGURE 2. TEM photography of drug-loaded NLC.

*Immediately After Production*

Table 2 lists the obtained results immediately after production. The macroscopic appearance of the systems resembled a milky dispersion immediately after production and also after the cooling step. According to the investigation of Souto and Müller (2006b), the macroscopic appearance would be milky due to the higher content of lipid phase. The pH values of all samples measured immediately after production ranged between 5.5 and 6.0, showing no significant differences between the drug-free and drug-loaded NLC. The drug entrapment efficiency was 89.5 ± 0.4%, which indicates that most drug was entrapped into nanoparticles due to the reason that NLC system could supply more position in lipid materials for drug molecule (FuQiang, 2005). Under optimized production conditions very small lipid nanoparticles with a negatively charged surface could be obtained. The presence of drug molecules did not significantly change the particle size (Figure 3) and zeta potential.

Previous studies have shown that the mixed surfactants could yield stable suspensions of drug-free nanoparticles and prevent gel formation (Siekmann & Westesen, 1994). In this study, even for drug-loaded NLC dispersions, the formation of semi-solid gels was not observed. It indicated that these surfactants were able to better the stability of the lipid particles. Hiemenz and Rajagopalan (1997) considered that the surfactant could extend the shear plane of the lipid particles, and consequently the ZP so as to improve the stability of the system. The incorporation of FP decreased the electrical charge at the surface of NLC. These results could be taken as an indication that FP is entrapped in the lipid matrix of NLC.

TABLE 2
Physical Stability Parameters of NLC Obtained Immediately after Production

| Sample | ZP (mv) | PS (nm) | EE% | pH |
|---|---|---|---|---|
| Drug-Free NLC | −22.4 ± 0.2 | 128 ± 1.5 | – | 5.8 |
| Drug-Loaded NLC | −19.5 ± 0.3 | 133 ± 2.0 | 89.5 ± 0.4 | 5.6 |

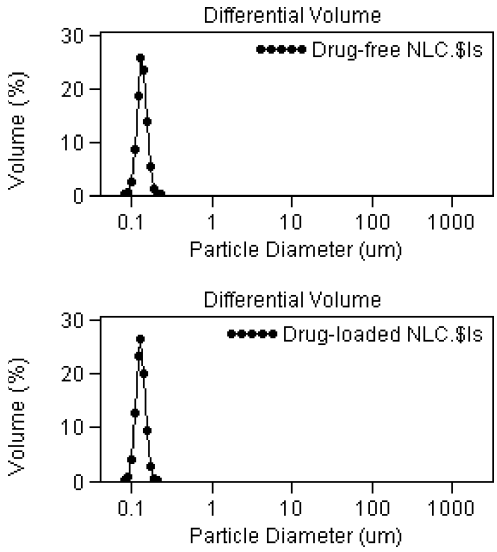


FIGURE 3. Particle size of drug-free and drug-loaded NLC.

*At Different Temperatures of Storage*

The developed formulations have been stored at three different temperatures to investigate the stability of the systems under stress conditions. The obtained results about the storage at different temperatures 3 months after production are shown in Table 3. The macroscopic appearance of all the samples still resembled a milky dispersion just like immediately after production. The pH values of all aqueous dispersions measured remained the same, ranging from 5.5 to 6.0, showing stress conditions could not affect the stability of the formulations. And the drug entrapment efficiency was still higher than 80%, which indicated that the drug entrapped into nanoparticles was not exclusion during the storage at different temperatures. It can be concluded that in contrast to the more or less highly ordered SLN, the incorporation of liquid lipids to solid lipids leads to massive crystal order disturbance. NLC shows great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules, thus, leading to the improved drug entrapment efficiency. The obtained results indicate that NLC could not only improve the drug entrapment efficiency and drug loading capacity, but also prevent the drug exclusion during storage.

In all storage temperatures, the mean size was maintained lower than 200 nm, with a particle size in the same magnitude as the values obtained immediately after production (Table 3). After 3 months of storage, all lipid nanoparticles showed a negative charge at their surface. No phase separation and gel formation have been observed .The differences between the evaluated parameters were not significant, neither under different storage temperatures nor with the presence of drug molecules, meaning that the NLC systems are stable under stress conditions.

## In Vitro Permeation Studies

In transdermal delivery, the goal of dosage design is to maximize the flux through the skin. A useful method for improving percutaneous flux is to choose an appropriate vehicle for the transdermal delivery of drug. The penetration profiles of cumulative amounts of FP from NLC and saturated FP-PBS (pH = 7.4) versus time are shown in Figure 4. A steady increase of FP in the receptor chambers with time was observed. It can be seen in Table 4 that the permeated amounts of FP from NLC and solution were 412.53 ± 21.37 μg/cm$^2$ and 90.83 ± 8.67 μg/cm$^2$ after 12 h, respectively. The cumulative amount permeated after 12 h resulted in about 4.54 times enhancement over the saturated FP-PBS (pH = 7.4). The $J_s$ of FP from NLC and solution were 35.25 μg/cm$^2$/h and 6.99 μg/cm$^2$/h, respectively.

TABLE 3
Physical Stability Parameters of NLC Obtained after 3 Months of Storage at Different Temperatures ($n$ = 3)

| Sample | Storage Temperature (°C ) | ZP (mv) | PS (nm) | EE% | pH |
|---|---|---|---|---|---|
| Drug-Free NLC | 4 | −22.1 ± 0.0 | 126 ± 1.2 | – | 5.7 |
| | 20 | −23.4 ± 0.5 | 128 ± 0.6 | – | 5.7 |
| | 40 | −22.7 ± 0.7 | 128 ± 2.0 | – | 5.9 |
| Drug-Loaded NLC | 4 | −17.3 ± 0.2 | 133 ± 2.5 | 89.3 ± 0.5 | 5.8 |
| | 20 | −16.1 ± 0.4 | 133 ± 0.6 | 89.8 ± 1.1 | 5.7 |
| | 40 | −20.6 ± 0.4 | 136 ± 2.0 | 89.6 ± 0.9 | 5.8 |

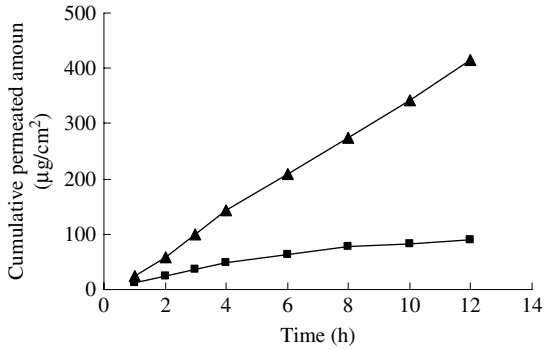


FIGURE 4. Penetration profiles of cumulative amounts of FP from NLC and saturated FP-PBS (pH = 7.4) ($n$ = 6).

TABLE 4
Percutaneous Permeation Parameters of NLC and Solution ($n$ = 6)

| System | Cumulative Amount (μg/cm$^2$) | Release Rate (μg/cm$^2$/ h) |
|---|---|---|
| NLC | 412.53 ± 21.37 | 35.25 |
| FP-PBS | 90.83 ± 8.67 | 6.99 |

The $J_s$ of FP from NLC were 5.04 times higher than solution. And the permeated percent of FP from NLC and solution were 16.5% ± 0.8 and 3.6% ± 0.3 after 12 h, respectively. It shows that the NLC system not only can improve the permeated amounts of FP but also can improve the release rate of FP.

Comparing with saturated FP-PBS (pH = 7.4), NLC was suitable for the transdermal delivery of FP. The main reason was NLC possess occlusive properties due to film formation on the skin surface. They reduce the transepidermal water loss and therefore enhance the penetration of drugs through the stratum corneum by increased hydration. It also has been reported that the occlusion factor of SLN and NLC is related to their particle size, that is, it increases with the decrease of the mean particle diameter (Wissing, Lippacher, & Müller, 2001). Apart from the fact that NLC ensures close contact with stratum corneum and increases the amount of encapsulated drug penetrating into the skin, another reason for this may be the effects of surfactants. During the preparation of NLC, Lecithin and Tween 80 were used as emulsifier and physical stabilizer. It is well known that surfactants have effects on the permeability characteristics of biological membranes, including skin. There are maybe two possible mechanisms of the effects. First, the surfactant may penetrate into the intercellular regions of stratum corneum and increase their fluidity, eventually solubilize and extract lipid components. Second, because Tween 80 contains the ethylene oxide and a long hydrocarbon chain, it can enhance the penetration of FP via both the lipophilic and the hydrophilic molecular mechanisms, disrupt the lipid arrangements in the stratum corneum and increase the water content of the proteins in the barrier.

## CONCLUSION

In this study, a new NLC system for transdermal delivery of FP was studied. It indicates that the mixture of different surfactants can better the physical characters of NLC in the presence of drug. The physical properties of FP-NLC were proved to be stable for at least 3 months at three different temperatures. The investigation showed that the FP-NLC was with good physical stability and was able to improve the permeated amounts and the $J_s$ of FP. NLC might be promising as a active delivery system intended for topical delivery of FP with improved drug entrapment efficiency and permeation.

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