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# Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery

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ABSTRACT

The objective of this investigation was to develop solid lipid nanoparticles (SLNs) of penciclovir and evaluate the potential of SLNs as the carrier of penciclovir for topical delivery. Penciclovir-loaded SLNs were prepared by a double (W/O/W) emulsion technique. The SLNs presented spherical with the mean diameter of 254.9 nm. The entrapment efficiency, drug loading and zeta potential were 92.40%, 4.62% and −25.0 mV, respectively. DSC study showed that penciclovir encapsulated in SLNs was in the amorphous form. The cumulative amount of penciclovir penetrated through excised rat skin from SLNs was more than 2-fold that of the commercial cream as a control at 12 h after administration. There was no significant difference of penciclovir content deposited in epidermis between the cream and SLNs administrated for 2, 6 and 12 h, while SLNs increased the cumulative uptake of penciclovir in dermis significantly at the same intervals. Microscopic pictures showed that the interaction between SLNs and the skin surface changed the apparent morphology of stratum corneum and broke the close conjugation of corneocyte layers, which was the possible reason that SLNs increased the permeation of penciclovir into skin dermis. It can be concluded from our study that SLNs provide a good skin targeting effect and may be a promising carrier for topical delivery of penciclovir.



## 1. Introduction

Penciclovir, 9-[4-hydroxy-3-(hydroxymethyl)butyl] guanine, a synthetic nucleoside analogue, is a potent and highly selective inhibitor of herpes viruses, such as herpes simplex virus type 1 and type 2, varicella-zoster virus, epstein-barr virus, hepatitis virus and cytomegalovirus. However, the oral bioavailability of penciclovir is very poor (5–10%) and its clinical application is greatly restricted (Andrei et al., 2004; Schmid-Wendtner and Korting, 2004; Smith et al., 2001; Zhu et al., 2008). One promising route of penciclovir administration is topical delivery. Various topical formulations including cream and gel have been reported (Ahmed et al., 2004), and appropriate vehicles with high permeation ability have attracted increasing attention in recent years.

Solid lipid nanoparticles (SLNs) have emerged as an alternative carrier system to traditional carriers, such as polymeric nanoparticles, emulsion and liposomes, and they attract great attention as a novel colloidal drug carrier for topical use (Fang et al., 2008). The advantages of the carrier include negligible skin irritation, controlled release, and protection of active substances (Mei et al., 2003). Because they are composed of non-irritative and non-toxic lipids, SLNs seem to be well suited for use on inflamed and damaged skin. Moreover, SLNs have distinct occlusive properties due to the formation of an intact film on the skin surface upon drying, which decreases transepidermal water loss and favors the drug penetrating through the stratum corneum (Jenning et al., 2000a; Wissing and Müller, 2003). Besides the nonspecific occlusion effect, the enhanced drug penetration might be related with SLNs themselves, the highly specific surface area of nanometer sized SLNs facilitates the contact of encapsulated drug with stratum corneum (Jenning et al., 2000a). The nanometer sized particles can make close contact with superficial junctions of corneocyte clusters and furrows between corneocyte islands, which may favor accumulation for several hours allowing for sustained drug release (Cevc, 2004; Schäfer-Korting et al., 2007).

SLNs have been used to improve the skin/dermal uptake of several drugs such as triptolide, isotretinoin, podophyllotoxin and prednicarbate (Chen et al., 2006; Maia et al., 2000, 2002; Liu et al., 2007; Mei et al., 2003; Sivaramakrishnan et al., 2004), which supports that SLNs can be employed as the carrier for the topical delivery of penciclovir. The dermal location of SLNs is desirable to interfere with the establishment of the latent HSV infection. This would prevent the diseases associated with HSV and the transmission of the virus among men (Smith et al., 2001). The present work

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focused on the preparation, characterization, in vitro percutaneous permeation and skin targeting behaviors of penciclovir-loaded SLNs. Additionally, the interaction between SLNs and the skin surface was also investigated.

## 2. Materials and methods

### 2.1. Materials

Poloxamer 188 and Brij 78 were obtained from Sigma Chemical (St. Louis, MO, USA). The powered penciclovir (purity 99%) was purchased from Livzon Pharmaceutical Group Co., Ltd. (Changzhou, China). Egg-phosphatidylcholine (EPC) was obtained from Lipoid (Ludwigshafen, Germany). Glyceryl monostearate (GMS) was provided by Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Dichloromethane, Tween 80 and Tween 20 were purchased from Yongda Chemical Reagent Co. Ltd. (Tianjin, China). All the other chemicals and solvents were of analytical grade or higher, obtained commercially.

### 2.2. Preparation of SLNs

SLNs were prepared by a double (W/O/W) emulsion technique with slight modifications (García-Fuentes et al., 2002). Briefly, 6 mg of penciclovir was dissolved in 200 μl of 0.1 M sodium hydroxide solution (inner aqueous phase). Oil phase was prepared by dissolving different amount of GMS and 40 mg of EPC in 1 ml of dichloromethane. The inner aqueous phase was mixed with the oil phase and then subjected to ultrasonic probe (JY92-II, Ningbo Science Biotechnology Co. Ltd., Ningbo, China) for 60 s at 40 W to obtain the primary W/O emulsion. The double (W/O/W) emulsion was formed after the addition of 3.8 ml of surfactant solution with different concentration (outer aqueous phase) to the primary W/O emulsion followed by sonication for 18 s at 40 W. The resulting emulsion was kept at 25 °C under mechanical agitation at 700 rpm for 5 h to remove the organic solvent and form SLNs. The influences of variables such as GMS concentration, different surfactants in outer phase and their concentrations on the entrapment efficiency of penciclovir-loaded SLNs were investigated to achieve an optimal formulation. 1% (w/v) EPC was adopted according to preliminary experiment and the reported data (García-Fuentes et al., 2002).

The powder of SLNs was prepared by lyophilization using 5.0% (w/v) mannitol as supporting agent in order to facilitate DSC investigation. The samples of SLNs dispersion with mannitol were frozen in an ultra cold freezer (MDF-382E, SANYO, Japan) at −80 °C for 24 h, then freeze-dried using a lyophilizer (LGJ 0.5, Sihuan Scientific Instrument Works, Beijing, China) at −40 °C with the pressure of 0.10 mbar for 48 h to yield dry powder (Gao et al., 2008).

### 2.3. Physicochemical characterization of SLNs

#### 2.3.1. Particle size and zeta potential

The mean diameter of penciclovir-loaded SLNs suspension was measured by photon correlation spectroscopy (PCS) using a particle sizer (Zetasizer 3000 HAS, Malvern Instruments Ltd., Malvern, Worcestershire, UK) at a fixed angle of 90° at 25 °C. The measurements were obtained using a He–Ne laser of 633 nm and the particle size analysis data were evaluated using the volume distribution. Zeta potential was analyzed by TV microscopic Electrophoresis System (DXD-II, Jiangsu Optics Co. Ltd., Jiangsu, China) at 25 °C. The two measurements were performed in triplicate.

#### 2.3.2. Morphology

The morphology of penciclovir-loaded SLNs suspension was examined by transmission electron microscope (TEM, JEM-1200EX, JEOL, Tokyo, Japan). One drop of diluted penciclovir-loaded SLNs suspension was deposited on a copper grid and then negatively stained with one drop of 2% (w/v) aqueous solution of phosphotungstate acid (PTA) for contrast enhancement. The excess staining solution was removed with filter paper in 60 s and the sample was allowed to dry before examined under the TEM (Zhu et al., 2008).

#### 2.3.3. Entrapment efficiency (EE) and drug loading (DL)

The entrapment efficiency of penciclovir-loaded SLNs was directly determined by ultrafiltration method using centrifugal filter tubes (Amicon® Ultra-4, Millipore, Ireland) with a 10 kDa molecular weight cut-off (Teeranachaideekul et al., 2007). Briefly, 2 ml of penciclovir-loaded SLNs suspension was placed into a centrifugal filter tube which was centrifuged at 10,000 rpm for 1 h at 25 °C (Biofuge primo R, Heraeus, Hanau, Germany). The amount of free drug in the aqueous phase after isolation of the system was detected by high performance liquid chromatograph (HPLC). The amount of incorporated drug was calculated as a result of the initial drug minus free drug. The entrapment efficiency and drug loading of penciclovir in SLNs were calculated according to the following equations (Luo et al., 2006; Souto et al., 2004):

$$\mathrm{EE} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100\% \quad (1)$$

$$\mathrm{DL} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{lipid}}} \times 100\% \quad (2)$$

where "$W_{\text{initial drug}}$" is the amount of drug used for the assay and the "$W_{\text{free drug}}$" is the amount of free drug detected in the aqueous phase after isolation of the suspension. The "$W_{\text{lipid}}$" stands for the weight of the vehicle.

#### 2.3.4. Stability study

The physicochemical stability of penciclovir-loaded SLNs was evaluated at 4 °C for 3 months via clarity, particle size and entrapment efficiency. The centrifuge tests at 13,000 rpm for 30 min were used to assess the physical stability of penciclovir-loaded SLNs based on the previous report (Chen et al., 2006).

#### 2.3.5. Differential scanning calorimeter (DSC) analysis

Thermal behavior of penciclovir-loaded SLNs was analyzed using differential scanning calorimeter (CDR-4P, Shanghai Tianping Instrument Ltd., Shanghai, China). Approximately 10 mg of samples was placed in aluminum crimp cells and heated at the scanning rate of 10 °C/min from 30 to 400 °C in a nitrogen atmosphere. Aluminum oxide was used as the standard reference material to calibrate the temperature and energy scale of the DSC instrument (Hou et al., 2003; Sun et al., 2008).

### 2.4. In vitro percutaneous permeation and skin uptake behaviors

#### 2.4.1. Preparation of skin

Male Wistar rats weighing 200 ± 20 g (SCXK (Lu) 20030004) were purchased from Experimental Anima Center of Shandong University (Shandong, China) for the in vitro permeation studies. Skin from the abdominal region was excised after hair was removed with a depilatory, and examined for integrity using a lamp-inspecting method. The subcutaneous fat and connective tissue were carefully removed. The obtained skin was rinsed with physiological saline (Liu et al., 2007).

#### 2.4.2. In vitro permeation study

The skin samples were mounted carefully on Franz-type diffusion cells with the stratum corneum side up and an effective diffusion area of 3.8 $cm^2$ (Zhu et al., 2008). The receiver compartment was filled with 15 ml of physiological saline to ensure sink

condition (1.3 mg/ml in physiological saline). The diffusion cells were maintained at (37 ± 0.5) °C with stirring at 600 rpm throughout the experiment. The skin samples were equilibrated for 30 min before being dosed (Wissing and Müller, 2002; Tiyaboonchai et al., 2007). Then 800 μl of SLNs dispersion containing 1.2 mg of penciclovir or 120 mg of commercial cream containing the same amount of penciclovir as a control was applied on the skin surface. 1 ml sample of receiver medium was withdrawn at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10 and 12 h) and an equivalent volume of fresh physiological saline maintained at 37 °C was then added in (Mei et al., 2003; Shah et al., 2007). All samples were filtered through an aqueous 0.45 μm pore size cellulose membrane filter and analyzed by HPLC.

*2.4.3. Calculation of the in vitro permeation data*

The cumulative amount of penciclovir ($Q_n$, μg/cm$^2$) permeated through the rat skin was plotted as a function of time ($t$, h), and the cumulative amount of drug permeated through excised skin was calculated based on the following Eq. (3) (Zhu et al., 2008). According to Fick's second law of diffusion, the cumulative amount of drug ($Q_n$) in the receptor solution at time $t$ is expressed as Eq. (4) (Mei et al., 2003):

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i}{A} \quad (3)$$

$$Q_n = AKLC_0 \left[ \left( \frac{D_t}{L^2} \right) - \left( \frac{1}{6} \right) - \left( \frac{2}{\pi^2} \right) - \sum \left( \frac{(-1)^n}{n^2} \right) \times \exp \left( \frac{D^n 2\pi^2 t}{L^2} \right) \right] \quad (4)$$

where $C_n$ stands for the drug concentration of the receiver medium at each sampling time, $C_i$ for the drug concentration of the $i$th sample, $A$ for the effective diffusion area, $V_0$ and $V_i$ stand for the volumes of the receiver solution and the sample, respectively. In Eq. (4), $C_0$ represents the drug concentration which remains constant in the donor vehicle, $D$ is the diffusion coefficient, $L$ is the thickness of the membrane and $K$ is the partition coefficient of drug between membrane and vehicle. In the steady state, Eq. (4) is expressed as follows:

$$\frac{Q_n}{A} = KLC_0 \left[ \left( \frac{D_t}{L^2} \right) - \left( \frac{1}{6} \right) \right] \quad (5)$$

The flux, $J$, is calculated from the slope of the steady state portion of the amount of the drug permeated divided by $A$ versus time (Mei et al., 2003). From Eq. (5), the flux is expressed as

$$J = C_0 \frac{KD}{L} = C_0 K_p \quad (6)$$

where $K_p$ stands for the permeability coefficient.

*2.4.4. Determination of drug content in epidermis and dermis*

The drug amount uptaken in epidermis and dermis was determined at 2, 6, and 12 h after treating with the same method described in Section 2.4.2. The excessive SLNs dispersion or cream was removed and the skin samples with a penetration area of 3.8 cm$^2$ were rinsed with alcohol and water to eliminate the penciclovir remained on the surface, then gently dried with a cotton wool (Hassonville et al., 2004). Heat-separated membrane separation was carried out based on the reported method (Christophers and Kligman, 1963). The skin samples were immersed in distill water at (60 ± 1) °C for 2 min, then the epidermis was removed from the dermis using a dull scalpel blade (Puglia et al., 2001, 2008). The epidermis sample or dermis cut into small pieces was soaked in 5 ml of 0.4% perchloric acid solution for 24 h and subjected to homogenization with a Diax 900 homogenizer (Heidolph Electro, Kelhaim, Germany) for 1 min and ultrasonication for 60 min in an ultrasound bath (CX-250, Peking Medical Equipments Ltd., Peking, China), followed by centrifugal separation (0412-1 Shanghai Surgical Instruments Co. Ltd., Shanghai, China). The drug extracted from epidermis or dermis was analyzed by HPLC, respectively (Bianca et al., 2000; Puglia et al., 2008).

*2.5. HPLC analysis of penciclovir*

Penciclovir was analyzed by HPLC using a LC-10 ATvp pump, a SPD-10Avp UV–vis detector (Shimadzu Co., Tokyo, Japan) with a Hypersil ODS-2 $C_{18}$ column (5 μm, 4.6 mm × 250 mm) (Elite Analytical Instrument Co. Ltd., Dalian, China). The mobile phase consisted of 0.1% acetic acid solution and acetonitrile (98:2, v/v) with a flow rate at 1 ml/min. The detection wavelength was set at 253 nm. Aliquots of 20 μl of each sample were injected into the column. The assay was linear in the concentration range of 1–50 μg/ml with the lowest detection limit at 0.1 μg/ml. No interference from the composition of the formulation or the blank skin sample was observed. All samples were filtered through a 0.45 μm pore size cellulose membrane filter to protect the column (Zhu et al., 2008).

*2.6. Investigation on the mechanisms of penetration enhancement*

*2.6.1. Effect of SLNs on the surface of rat skin*

The experiments were carried out to find out the topical effect of penciclovir-loaded SLNs dispersion on rat skins according to the established method (Fang et al., 2008). SLNs and commercial cream were applied to the hair-removed excised rat skins for 12 h, respectively. The experiments were conducted with the same method described in Section 2.4.2. The treated skin pieces were collected in 10% formalin for histopathological studies. Paraffin tissue sections of 3–4 μm were cut, and subjected to hematoxylin/eosin staining. Microscopic pictures were taken using a Nikon Alphaphot-2 YS2 microscope (Tokyo, Japan).

*2.6.2. DSC investigation*

The rat epidermis was achieved after permeation experiment as shown in Section 2.4.4. The obtained epidermis was placed on a filter paper soaked with 0.1% (m/v) trypsin solution in PBS pH 7.4 and stored at 4 °C for 24 h and afterwards at 37 °C for 1 h. The stratum corneum sheet was peeled off, shaken in 0.1% (w/v) trypsin inhibitor solution for about 30 s and then rinsed twice with purified water. The stratum corneum was placed in a desiccator with the relative humidity of about 25% for 48 h to get a hydration level of about 20% (Bouwstra et al., 1995; Kuntsche et al., 2008). The sample was punched into small pieces and subjected to DSC study, and the determination was performed in the same condition described in Section 2.3.5.

*2.7. Statistical analysis*

Statistical data were analyzed using the Student's $t$-test with $p < 0.05$ as the minimal level of significance.

## 3. Results and discussion

*3.1. Formulation optimization*

In order to optimize penciclovir-loaded SLNs prepared by the double emulsion method, different variables in the formulation were evaluated. The results of entrapment efficiency were depicted in Tables 1 and 2. The penciclovir-loaded SLNs were comprised of a high melting point GMS core with a protective hydrophilic coating of EPC and Poloxamer 188 (García-Fuentes et al., 2002; Heiati

**Table 1**
Influence of the concentration of GMS on the EE of SLNs.

| GMS (%, w/v) | Poloxamer 188 (%, w/v) | Lecithin (%, w/v) | Volume of the outer phase (ml) | EE[a] (%) |
|---|---|---|---|---|
| 1.0 | 2.0 | 1.0 | 3.8 | 61.88 ± 1.3 |
| 1.5 | 2.0 | 1.0 | 3.8 | 76.41 ± 0.6 |
| 2.0 | 2.0 | 1.0 | 3.8 | 84.14 ± 1.2 |
| 2.5 | 2.0 | 1.0 | 3.8 | 88.31 ± 0.5 |
| 3.0 | 2.0 | 1.0 | 3.8 | 88.47 ± 0.3 |

[a] Mean ± S.D. ($n$ = 3).

et al., 1996). It is known that three drug incorporation models can be deduced: (1) solid solution model; (2) core–shell model, drug-enriched shell; (3) core–shell model, drug-enriched core (Müller et al., 2000). The drug incorporation model relates to specific nature of the solid lipid matrix and the lipophilicity of drug. Only the drug with moderate lipophilicity can be loaded at high rate (Schäfer-Korting et al., 2007). Penciclovir has a poor lipophilicity whose partition coefficient in n-octanol/water at pH 7.5 was 0.024 ($\log P = -1.62$). Because of the low lipophilicity of penciclovir and the limited space in the GMS crystal lattice (Mühlen and Mehnert, 1998; Schäfer-Korting et al., 2007), penciclovir should disperse or dissolve in the coating of EPC and Poloxamer, which leaded to the formation of core–shell model, drug-enriched shell with a small portion of drug dispersing in the lipid matrix of GMS. It was evident in Table 1 that the entrapment efficiency increased with GMS content increasing in the final dispersion. The possible reason is that the increase in GMS content can afford more space to encapsulate drug under the condition of given surfactant concentration in outer phase. The effects of different surfactants on entrapment efficiency were investigated as shown in Table 2. Poloxamer 188, Brij 78, Tween 80 and Tween 20 were chosen as the tested surfactants in outer phase based on the reported studies (Fang et al., 2008; Hsu et al., 2003; Liu et al., 2007a; Singh et al., 2008). The data clearly showed that the formulation using Poloxamer 188 as surfactant had the highest entrapment efficiency. The results might be related to the effects of the hydrophile–lipophile balance (HLB) value and the structure of the surfactant. Moreover, the entrapment efficiency was affected by the concentration of surfactant in outer phase and it increased by 44% when the concentration of Poloxamer changed from 1 to 2.5% (w/v). That is due to the high concentration of Poloxamer resulting in the increasing thickness of the hydrophilic coating, and then more drugs can disperse and dissolve in it. Therefore, the optimized formulation was obtained as follows, 6 mg of penciclovir, 120 mg of GMS, 40 mg of EPC and 2.5% Poloxamer 188 as surfactant in 4 ml final dispersion.

### 3.2. The characterization of optimized SLNs

TEM was conducted to investigate the morphology of the SLNs. The optimized nanoparticles were spherical as shown in Fig. 1 with the particle size ranging from 124.1 to 752.1 nm. The wide size range may be related to the formation of multiple phospho-

**Table 2**
Influence of surfactants and the concentration of Poloxamer 188 on the EE of SLNs.

| Surfactant in the outer phase (%, w/v) | Lecithin (%, w/v) | GMS (%, w/v) | Volume of the outer phase (ml) | EE[a] (%) |
|---|---|---|---|---|
| Tween 80 (2%) | 1.0 | 3.0 | 3.8 | 55.96 ± 4.8 |
| Tween 20 (2%) | 1.0 | 3.0 | 3.8 | 48.00 ± 2.7 |
| Brij 78 (2%) | 1.0 | 3.0 | 3.8 | 51.96 ± 1.7 |
| Poloxamer 188 (1%) | 1.0 | 3.0 | 3.8 | 48.00 ± 1.2 |
| Poloxamer 188 (1.5%) | 1.0 | 3.0 | 3.8 | 60.32 ± 0.7 |
| Poloxamer 188 (2%) | 1.0 | 3.0 | 3.8 | 88.47 ± 0.3 |
| Poloxamer 188 (2.5%) | 1.0 | 3.0 | 3.8 | 92.40 ± 1.4 |

[a] Mean ± S.D. ($n$ = 3).

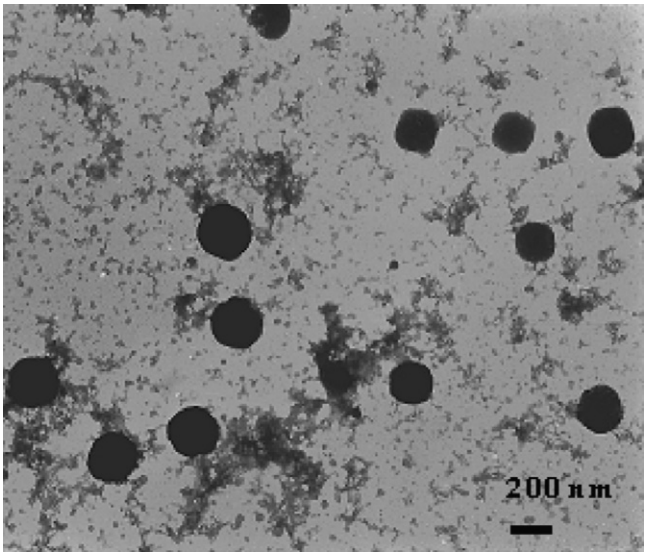


**Fig. 1.** TEM photograph (72,000×) of penciclovir-loaded SLNs.

lipid layers or the formation of other structures such as liposomes, micelles, and drug nanosuspensions (García-Fuentes et al., 2002; Mehnert and Mäder, 2001). The mean diameter and zeta potential were 254.9 ± 8.2 nm and −25 ± 0.05 mV, respectively. The average entrapment efficiency and drug loading were 92.40 ± 1.4% and 4.62 ± 0.2%, respectively.

### 3.3. Stability study

Penciclovir-loaded SLNs exhibited a good stability during the period of 3 months. No obvious changes of clarity and degradation were found. The mean diameter of penciclovir-loaded SLNs was 264.5 ± 9.3 nm and the entrapment efficiency was decreased by 3.4%. No demixing phenomenon was observed after centrifuge test. This may imply that the transition of GMS in SLNs from metastable forms to stable forms occurred slowly on storage due to small particle size and the presence of emulsifier, and the transition of GMS form in SLNs leaded to drug expulsion from SLNs (Chen et al., 2006; Venkateswarlu and Manjunath, 2004).

### 3.4. DSC investigation

DSC is a tool to investigate the melting and recrystallization behavior of crystalline material like SLNs (Liu et al., 2005). Fig. 2 shows the DSC thermograms of pure penciclovir, EPC, GMS, mannitol, Poloxamer 188, the physical mixture with the same formulation compositions as SLNs, and penciclovir-loaded SLNs. The pure penciclovir showed a single sharp endothermic peak at about 279 °C. A depressed endothermic peak could be found almost at the same temperature from the curve of physical mixture. In addition, no endothermic peak was found at the range of 200–300 °C in the thermogram of the penciclovir-loaded SLNs. The results showed that the penciclovir incorporated in SLNs was not in a crystalline state but in an amorphous form. It was the possible reason that EPC and GMS inhibited the crystallization of penciclovir during the nanoparticle formation. Similar results were reported by other groups (Liu et al., 2005; Venkateswarlu and Manjunath, 2004). The amorphous form was thought to have higher energy with increased surface area, subsequently higher solubility, dissolution rates and bioavailability (Corrigan et al., 2003; Morissettea et al., 2004).

Bulk GMS showed a sharp endothermic peak around 63.2 °C, ascribing to the melting. When the material was formulated as SLNs, the endothermic peak was broader and the temperature was

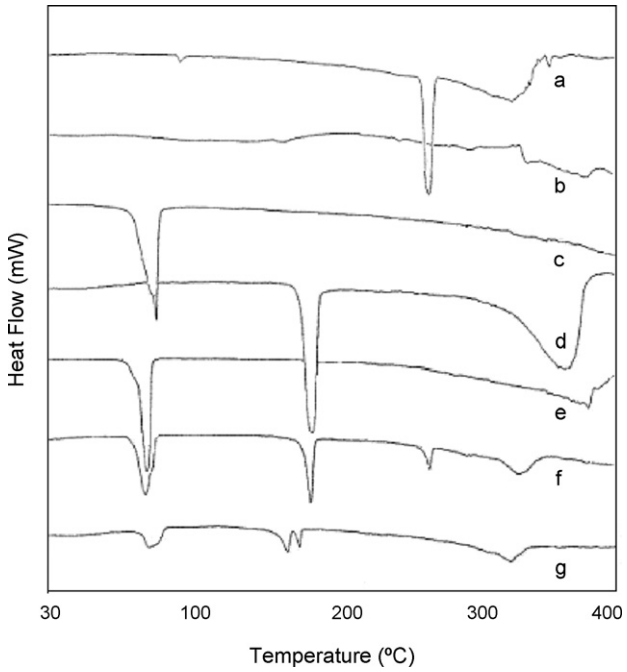


**Fig. 2.** DSC thermograms of (a) pure penciclovir, (b) EPC, (c) GMS, (d) mannitol, (e) Poloxamer 188, (f) physical mixture, and (g) penciclovir-loaded SLNs.

lower. However, there was no obvious change when the raw material was mixed in physical mixture with the endothermic peak around 57.3 °C. The depressed and broader endothermic peak for SLNs may be due to the nanometric size of the particles which had a huge surface area besides a certain effect of surfactant (Jenning et al., 2000; Westesen and Bunjes, 1995). The loading of penciclovir and mannitol provoked no considerable effect on the thermal behavior of lipid matrix under the experimental conditions.

### 3.5. Percutaneous permeation and skin uptake behaviors

The cumulative amounts of penciclovir at different time in the receptor solution were shown in Fig. 3, and the steady-state flux ($J_s$), permeation coefficient ($K_p$) for 12 h according to Eqs. (4)–(6) were shown in Table 3. It indicated that the steady-state flux of cream and SLNs were $3.31 \pm 0.37$ and $7.67 \pm 0.19$ μg/(cm$^2$ h), respectively. The permeation of cream and SLNs followed zero order release kinetics, and the equations were expressed in Table 3. The cumulative amounts of penciclovir from cream and SLNs at 12 h after dosing were $41.07 \pm 3.07$ and $88.44 \pm 4.19$ μg/cm$^2$, respectively. In other words, the cumulative amount of penciclovir from SLNs penetrating through the rat skins was more than 2-fold that of the cream at 12 h, which was coincident with previous reports (Mei et al., 2003). The cumulative amounts of penciclovir in epidermis from the commercial cream and the SLNs at 2, 6 and 12 h after administration were shown in Fig. 4. There was no significant difference in cumulative amounts of penciclovir in epidermis between the cream and SLNs ($p > 0.05$), while the SLNs significantly increased the cumulative uptake of penciclovir in dermis ($p < 0.05$). The amount of penciclovir

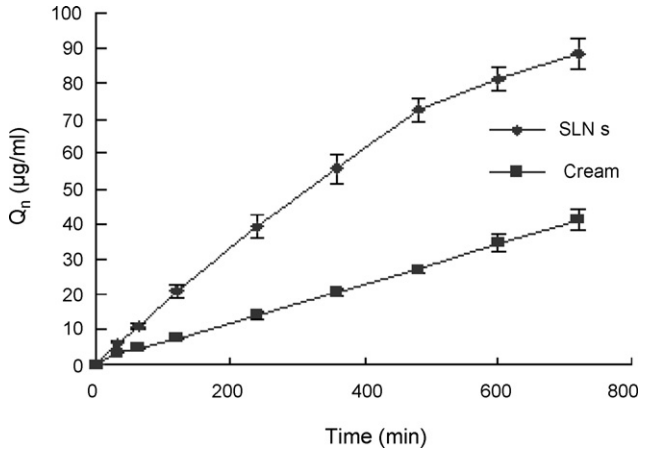


**Fig. 3.** Percutaneous permeation profiles of penciclovir from the SLNs and the commercial cream through the excised rat skins (mean ± S.D.; $n = 5$).

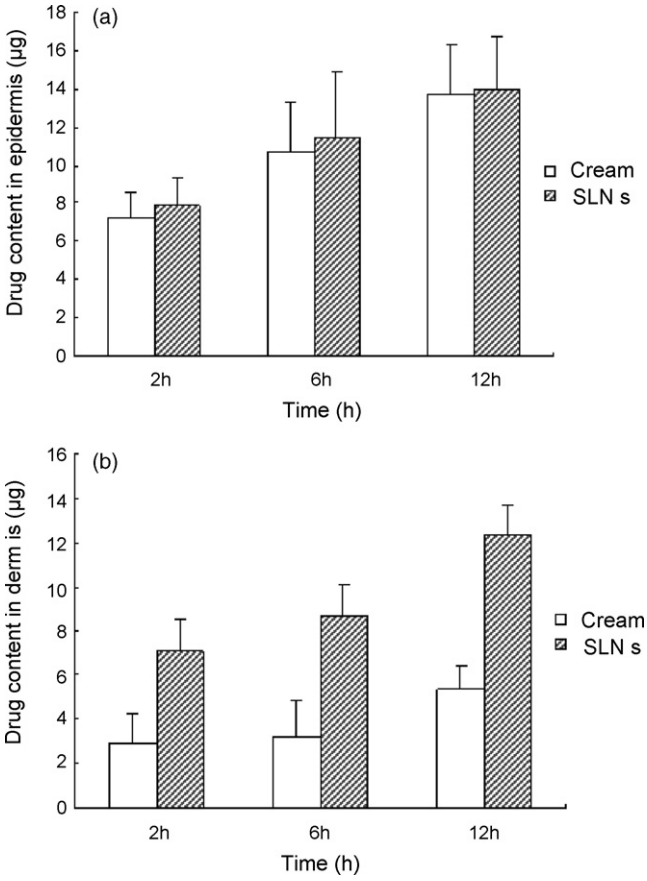


**Fig. 4.** The amount of penciclovir in epidermis (a) and dermis (b) after 2, 6, and 12 h, respectively.

**Table 3**
Skin permeation parameters of cream and SLNs.

| Preparation | Permeation equation | $J_s$[a] (μg/(cm$^2$ h)) | $K_p$[a] (cm/h) | $R$ |
|---|---|---|---|---|
| Cream | $Q_n = 3.31\,t - 1.04$ | 3.31 ± 0.37 | 0.0003 ± 0.00004 | 0.999 |
| SLNs | $Q_n = 7.67\,t + 0.37$ | 7.67 ± 0.19 | 0.0051 ± 0.0001 | 0.989 |

$J_s$ stands for the steady-state flux, $K_p$ is partition coefficient and $R$ stands for correlative coefficient.
[a] Mean ± S.D. ($n = 5$).

penetrated into dermis from SLNs at 12 h was 2.3-fold that of commercial cream. The small size and close interaction between SLNs and the stratum corneum are the possible reasons why SLNs can increase the drug amount penetrating into the viable skin. The similar findings were reported by Maia et al. and Sivaramakrishnan et al. According to Maia's research, the amount of prednicarbate (PC) from SLNs penetrated into human skin increased by 30% as compared to the commercial cream, and PC concentration in the dermis increased considerably (Maia et al., 2000). Lately, Sivaramakrishnan et al. reported the entrapment of glucocorticoid (betamethasone 17-valerate, BMV) into SLN and its influence on dermal uptake. The SLNs with a mean diameter of 214 nm increased the dermal uptake of BMV about 4-fold compared with the commercial cream, however, failed to achieve epidermal targeting (Sivaramakrishnan et al., 2004). This is one of the reasons why SLNs were chosen as the carrier for topical delivery of penciclovir. The dermal location of SLNs is desirable to interfere with the establishment of the latent HSV infection, and the effect of SLNs would prevent the diseases associated with HSV and inhibit human-to-human transmission of the virus (Smith et al., 2001).

The increase amount of drug in the dermis is also related to the occlusion properties. Following the water evaporation from the SLNs dispersion applied to the skin surface, an adhesive layer occluding the skin surface is formed. Then the hydration of stratum corneum can increase, which can facilitate drug penetration into deeper skin strata by reducing corneocyte packing and widening the inter-corneocytes gaps (Cevc, 2004; Schäfer-Korting et al., 2007). Occlusive effects appear strongly related to particle size. Nanoparticles were turned out 15-fold more occlusive than microparticles, and particles smaller than 400 nm were more favorable (Wissing and Müller, 2001). So penciclovir-loaded SLNs with the mean diameter of 254.9 nm should have the ability to form an occlusive film on the surface of skin, increase the hydration of stratum corneum, and improve the permeation of penciclovir into skin.

### 3.6. Investigation on the mechanisms of penetration enhancement

#### 3.6.1. Effect of SLNs on the surface of rat skin

The microscopic pictures of untreated skin, skin treated with commercial cream, and penciclovir-loaded SLNs were shown in Fig. 5. Untreated skin (Fig. 5a) had a compact stratum corneum with corneocytes layer closely conjugated. Application of the commercial cream only slightly changed the structure (Fig. 5b). The stratum corneum appeared swollen and overall thickness had increased for the skin treated by SLNs dispersion (Fig. 5c). This may be due to the SLNs' effects of reducing or preventing the skin dehydration, as a result, the increase of stratum corneum hydration can facilitate drug penetration into deeper skin strata (Cevc, 2004; Schäfer-Korting et al., 2007). In addition, the components of SLNs, particularly EPC, may fuse and mix with skin lipids to loosen their structure and increase the thickness of stratum corneum by disturbing the lamellar arrangement of the lipids (El Maghraby et al., 2008; Yokomizo and Sagitani, 1996; Zellmer et al., 1995). In a word, besides the effects of increasing skin hydration, the SLNs can increase the thickness of stratum corneum by disturbing the lamellar arrangement of the lipids (Jenning et al., 2000a), which may compromise the stratum corneum barrier and improve drug penetration.

#### 3.6.2. DSC analysis

The skin treated by SLNs showed a decreased endothermic peak at 67.8 °C whereas untreated skin stratum corneum exhibited the expected transition at 69.7 °C (Fig. 6). The decreased melting point can be explained by the presence of SLNs (melting point 58.6 °C in our studies). Clues for the presence of SLNs were derived from

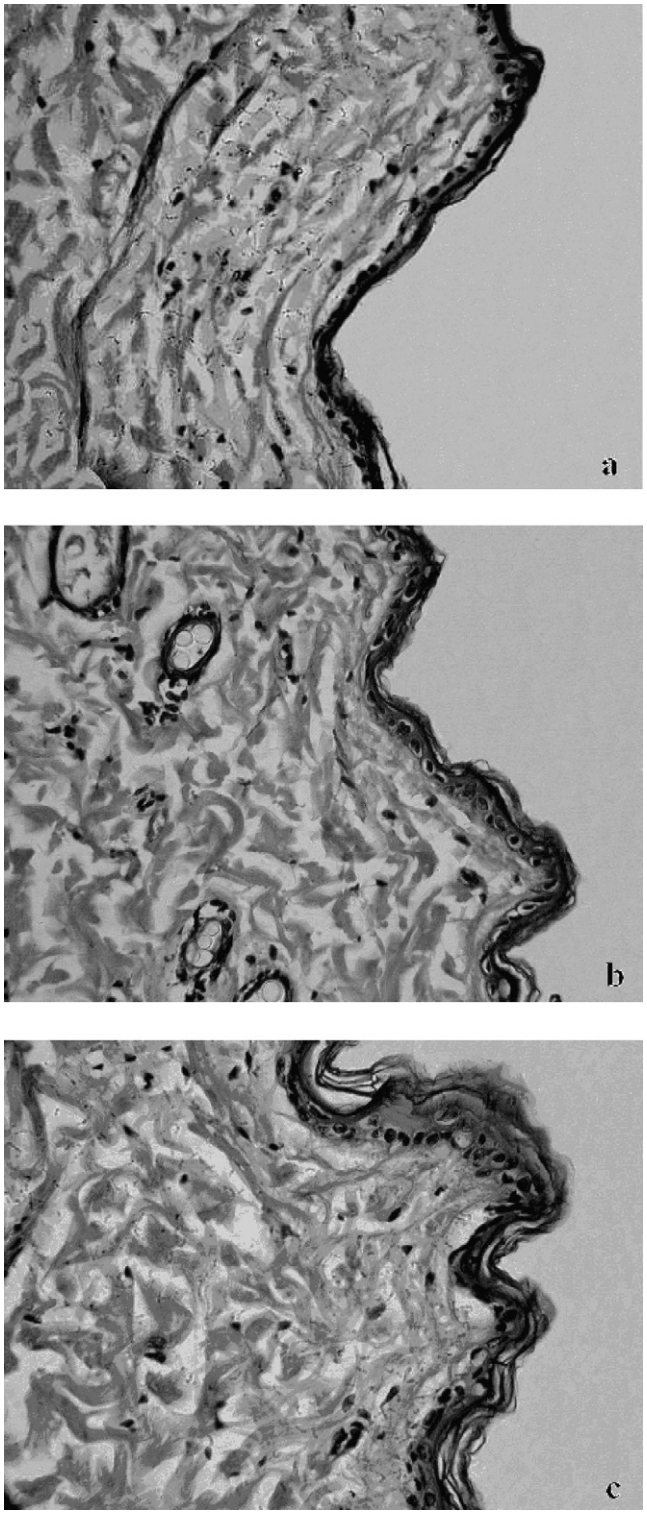


**Fig. 5.** Microphotograph (400×) of vertical section of rat skin (H/E staining): (a) untreated skin, (b) skin treated with commercial cream, and (c) skin treated with penciclovir-loaded SLNs.

DSC experiments. Therefore, a conclusion could be drawn that SLNs might penetrate into follicular opening of the skin or stick tightly to the stratum corneum, which prevented removal by the washing procedure (Jenning et al., 2000a). In addition, the enthalpy of lipid-related transitions of the stratum corneum increased from 37.9 to 39.02 J/g after treated with SLNs. This accorded with the reported research of Zellmer et al. who revealed that the individual phos-

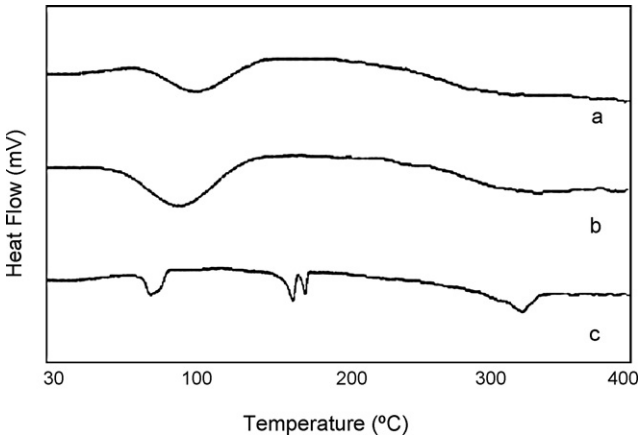


**Fig. 6.** DSC thermograms of (a) untreated skin, (b) skin treated with penciclovir-loaded SLNs, and (c) penciclovir-loaded SLNs.

phatidylcholine molecule could interact with the lipid barrier of the stratum corneum and penetrate into the latter, which resulted in an increase of the enthalpy related to the lipid components of the stratum corneum (Zellmer et al., 1995). Therefore, SLNs can produce an enhancing effect on skin penetration relating to the effect of EPC which may penetrate deeply into the stratum corneum or may fuse and mix with skin lipids to loosen their structure (El Maghraby et al., 2008; Yokomizo and Sagitani, 1996).

## 4. Conclusion

In this paper, the penciclovir-loaded SLNs were prepared by a double (W/O/W) emulsion technique for topical delivery of penciclovir. The cumulative amount of penciclovir penetrated through excised rat skins from SLNs was more than 2-fold that of the commercial cream as a control at 12 h after administration. Compared with commercial cream, the amount of penciclovir penetrated into dermis from SLNs increased by 130%. The interaction between SLNs and the skin surface changed the apparent morphology of stratum corneum and broke the close conjugation of corneocyte layers, which resulted in the increased permeation of penciclovir into skin dermis. Our studies provided evidence that SLNs were valuable as a topical delivery carrier to enhance the penetration of penciclovir.

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## References

Ahmed, A., Barry, B.W., Williams, A.C., Davis, A.F., 2004. Penciclovir solubility in Eudragit films: a comparison of X-ray, thermal, microscopic and release rate techniques. J. Pharm. Biomed. Anal. 34, 945–956.
Andrei, G., Clercq, E.D., Snoeck, R., 2004. In vitro selection of drug-resistant varicella zoster virus (VZV) mutants (OKA strain): differences between acyclovir and penciclovir. Antiviral Res. 61, 181–187.
Bianca, B., Quintela, M.A.L., Charro, M.A.D., Fadda, M.B., Fadda, A.M., Mendez, J.B., 2000. Microemulsions for topical delivery of 8-methoxsalen. J. Control. Release 69, 209–218.
Bouwstra, J.A., Gooris, G.S., Bras, W., Downing, D.T., 1995. Lipid organization in pig stratum corneum. J. Lipid Res. 36, 685–695.
Cevc, G., 2004. Lipid vesicles and other colloids as drug carriers on the skin. Adv. Drug Deliv. Rev. 56, 675–711.
Chen, H., Chang, X., Du, D., Liu, W., Liu, J., Weng, T., Yang, Y., Xu, H., Yang, X., 2006. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J. Control. Release 110, 296–306.
Christophers, E., Kligman, A.M., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 702–705.
Corrigan, D.O., Healy, A.M., Corrigan, O.I., 2003. The effect of spray drying solutions of bendroflumethiazide/polyethylene glycol on the physicochemical properties of the resultant materials. Int. J. Pharm. 262, 125–137.
El Maghraby, G.M., Barry, B.W., Williams, A.C., 2008. Liposomes and skin: from drug delivery to model membranes. Eur. J. Pharm. Sci. 34, 203–222.
Fang, J.Y., Fang, C.L., Liu, C.H., Su, Y.H., 2008. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur. J. Pharm. Biopharm. 70, 633–640.
Gao, L., Zhang, D., Chen, M., Duan, C., Dai, W., Jia, L., Zhao, W., 2008. Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions. Int. J. Pharm. 355, 321–327.
García-Fuentes, M., Torres, D., Alonso, M.J., 2002. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloid Surf. B 27, 159–168.
Hassonville, S.H., Chiap, P., Liégeois, J., Evrard, B., Delattre, L., Crommen, J., Piel, G., Hubert, P., 2004. Development and validation of a high-performance liquid chromatographic method for the determination of cyproterone acetate in human skin. J. Pharm. Biomed. Anal. 36, 133–143.
Heiati, H., Phillips, N.C., Tawashi, R., 1996. Evidence for phospholipid bilayer formation in solid lipid nanoparticles formulated with phospholipid and triglyceride. Pharm. Res. 13, 1406–1410.
Hou, D.Z., Xie, C.S., Huang, K.J., Zhu, C.H., 2003. The production and characteristics of solid lipid nanoparticles (SLNs). Biomaterials 24, 1781–1785.
Hsu, C.H., Cui, Z., Mumper, R.J., Jay, M., 2003. Preparation and characterization of novel coenzyme $Q_{10}$ nanoparticles engineered from microemulsion precursors. AAPS Pharm. Sci. Technol. 4, 1–12.
Jenning, V., Gysler, A., Schäfer-Korting, M., Gohla, S., 2000a. Vitamin A-loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur. J. Pharm. Biopharm. 49, 211–218.
Jenning, V., Thünemann, A.F., Gohla, S.H., 2000. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int. J. Pharm. 199, 167–177.
Kuntsche, J., Bunjes, H., Fahr, A., Pappinen, S., Ronkko, S., Suhonen, M., Urtti, A., 2008. Interaction of lipid nanoparticles with human epidermis and an organotypic cell culture model. Int. J. Pharm. 354, 180–195.
Liu, M., Dong, J., Yang, Y., Yang, X., Xu, H., 2005. Characterization and release of triptolide-loaded poly (D, L-lactic acid) nanoparticles. Eur. Polym. J. 41, 375–382.
Liu, J., Gong, T., Wang, C., Zhong, Z., Zhang, Z., 2007a. Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: preparation and characterization. Int. J. Pharm. 340, 153–162.
Liu, J., Hu, W., Chen, H., Ni, Q., Xu, H., Yang, X., 2007. Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. Int. J. Pharm. 328, 191–195.
Luo, Y.F., Chen, D.W., Ren, L.X., Zhao, X.L., Qin, J., 2006. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J. Control. Release 114, 53–59.
Maia, C.S., Mehnert, W., Schäfer-Korting, M., 2000. Solid lipid nanoparticles as drug carriers for topical glucocorticoids. Int. J. Pharm. 196, 165–167.
Maia, C.S., Mehnert, W., Schaller, M., Korting, H.C., Gysler, A., Haberland, A., Schäfer-Korting, M., 2002. Drug targeting by solid lipid nanoparticles for dermal use. J. Drug Target. 10, 489–495.
Mehnert, W., Mäder, K., 2001. Solid lipid nanoparticles-production, characterization and applications. Adv. Drug Deliv. Rev. 47, 165–196.
Mei, Z., Chen, H., Wang, T., Yang, Y., Yang, X., 2003. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur. J. Pharm. Biopharm. 56, 189–196.
Morissettea, S.L., Almarsson, O., Peterson, M.L., Remenar, J.F., Read, M.J., Lemmo, A.V., Ellis, S., Cima, M.J., Colin, R.G., 2004. High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. Adv. Drug Deliv. Rev. 56, 275–300.
Mühlen, A., Mehnert, W., 1998. Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 53, 552–555.
Müller, R.H., Mäder, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.
Puglia, C., Bonina, F., Trapani, G., Franco, M., Ricci, M., 2001. Evaluation of in vitro percutaneous absorption of lorazepam and clonazepam from hydro-alcoholic gel formulations. Int. J. Pharm. 228, 79–87.
Puglia, C., Blasi, P., Rizza, L., Schoubben, A., Bonina, F., Rossi, C., Ricci, M., 2008. Lipid nanoparticles for prolonged topical delivery: an *in vitro* and *in vivo* investigation. Int. J. Pharm. 357, 295–304.
Schäfer-Korting, M., Mehnert, W., Korting, H., 2007. Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv. Drug Deliv. Rev. 59, 427–443.
Schmid-Wendtner, M.H., Korting, H.C., 2004. Penciclovir cream-improved topical treatment for herpes simplex infections. Skin Pharmacol. Physiol. 17, 214–218.
Shah, K.A., Date, A.A., Joshi, M.D., Patravale, V.B., 2007. Solid lipid nanoparticles (SLN) of tretinoin: potential in topical delivery. Int. J. Pharm. 345, 163–171.
Singh, S., Bhardwaj, P., Singh, V., Aggarwal, S., Mandal, U.K., 2008. Synthesis of nanocrystalline calcium phosphate in microemulsion-effect of nature of surfactants. J. Colloid Interface Sci. 319, 322–329.
Sivaramakrishnan, R., Nakamura, C., Mehnert, W., Korting, H.C., Kramer, K.D., Schäfer-Korting, M., 2004. Glucocorticoid entrapment into lipid carriers-characterization by parelectric spectroscopy and influence on dermal uptake. J. Control. Release 97, 493–502.
Smith, R.L., Morroni, J., Wilcox, C.L., 2001. Lack of effect of treatment with penciclovir or acyclovir on the establishment of latent HSV-1 in primary sensory neurons in culture. Antiviral Res. 52, 19–24.

Souto, E.B., Wissing, S.A., Barbosa, C.M., Müller, R.H., 2004. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm. 278, 71–77.

Sun, W., Zhang, N., Li, A., Zou, W., Xu, W., 2008. Preparation and evaluation of N3-*O*-toluyl-fluorouracil-loaded liposomes. Int. J. Pharm. 353, 243–250.

Teeranachaideekul, V., Souto, E.B., Junyaprasert, V.B., Müller, R.H., 2007. Cetyl palmitate-based NLC for topical delivery of coenzyme $Q_{10}$-development, physicochemical characterization and in vitro release studies. Eur. J. Pharm. Biopharm. 67, 141–148.

Tiyaboonchai, W., Tungpradit, W., Plianbangchang, P., 2007. Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. Int. J. Pharm. 337, 299–306.

Venkateswarlu, V., Manjunath, K., 2004. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J. Control. Release 95, 627–638.

Westesen, K., Bunjes, H., 1995. Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? Int. J. Pharm. 115, 129–131.

Wissing, S.A., Müller, R.H., 2001. Solid lipid nanoparticles (SLN)—a novel carrier for UV blockers. Pharmazie 56, 783–786.

Wissing, S.A., Müller, R.H., 2002. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J. Control. Release 81, 225–233.

Wissing, S.A., Müller, R.H., 2003. The influence of solid lipid nanoparticles on skin hydration and viscoelasticity—in vivo study. Eur. J. Pharm. Biopharm. 56, 67–72.

Yokomizo, Y., Sagitani, H., 1996. The effects of phospholipids on the percutaneous penetration of indomethacin through the dorsal skin of guinea pig in vitro. 2. The effects of the hydrophobic group in phospholipids and a comparison with general enhancers. J. Control. Release 42, 37–46.

Zellmer, S., Pfeil, W., Lasch, J., 1995. Interaction of phosphatidylcholine liposomes with the human stratum corneum. Biochim. Biophys. Acta 1237, 176–182.

Zhu, W.W., Yu, A.H., Wang, W.H., Dong, R.Q., Wu, J., Zhai, G.X., 2008. Formulation design of microemulsion for dermal delivery of penciclovir. Int. J. Pharm. 360, 184–190.