Drug Development and Industrial Pharmacy, 31:161–168, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1081/DDC-200047791

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Triptolide Loaded Solid Lipid Nanoparticle Hydrogel for Topical Application

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ABSTRACT Triptolide (TP) has been shown to have anti-inflammatory, antifertility, antineoplastic, and immunosuppressive activity. However, its clinical usage is limited to some extent due to its poor water solubility and toxicity. In order to use innovative ways to administer TP and to overcome or alleviate its disadvantages, controlled-release delivery systems such as solid lipid nanoparticle(SLN(s)) have been developed. In the present paper we describe the preparation and some characterization of specialized delivery systems for TP. The transdermal delivery and anti-inflammatory activity were also evaluated. The results indicated that SLN could serve as an efficient promoter of TP penetrating into skin. Furthermore, different formulations were optimized in this study. The best formulation of SLN, consisted of tristearin glyceride, soybean lecithin, and PEG400MS, with a particle size of 123 ± 0.9 nm, polydispersity index (PI) of 0.19, and zeta potential of -45 mV. When this SLN dispersion was incorporated into hydrogel, the nanoparticulate structure was maintained, and aggregation and gel phenomena of the particle could be avoided. The cumulative transdermal absorption rate in 12 h was 73.5%, whereas the conventional TP hydrogel was 45.3%. The anti-inflammatory effect is over two-fold higher than that of conventional TP hydrogel. Moreover, this SLN hydrogel consists of pharmaceutically acceptable ingredients, such as soybean lecithin and lipid, and the nanoparticle can improve safety and minimize the toxicity induced by TP.

KEYWORDS Triptolide, Solid lipid nanoparticle, Hydrogel, Anti-inflammatory, Transdermal absorption

INTRODUCTION

The development of topical drug delivery systems for systematic effects has gained more and more interest in the past few years, due to the obvious advantages that transdermal delivery offers over conventional routes for drug administration, such as the possibility of systematic drug therapy, the avoidance of first-pass metabolism, and the minimization of side effects. However, the relative impermeability of the stratum corneum prevents

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TABLE 1 The Compositions of Different Formulations for SLN Dispersion or SLN Hydrogel

Formulation	Carbomer	TSG (%)	SA (%)	SL (%)	P188 (%)	NaOH	TEA	PEG400MS (%)	Particle size	PI	ζ (mV)	DL (%)
A	–	5.0	–	1.2	–	–	–	3.6	123±0.9	0.19	−45	0.057
B	–	5.0	–	–	1.2	–	–	3.6	147±1.5	0.27	−42	0.055
C	–	–	5.0	1.2	–	–	–	3.6	157±1.2	0.29	−40	0.053
D	–	–	5.0	–	1.2	–	–	3.6	173±2.3	0.24	−39	0.052
E	+	5.0	–	1.2	–	–	+	3.6	158±2.2	0.29	−43	0.055
F	+	5.0	–	–	1.2	–	+	3.6	183±3.7	0.32	−40	0.052
G	+	5.0	–	1.2	–	+	–	3.6	420±4.5	0.43	−37	0.050
H	+	5.0	–	–	1.2	+	–	3.6	452±4.0	0.54	−30	0.045

Note: SL is soybean lecithin, P188 is poloxamer 188, TSG is tristearin glyceride, SA is stearic acid, TEA is triethanolamine, PI is polydispersity index, ζ is zeta potential, DL is drug loading.

percutaneous absorption. Solid lipid nanoparticles (SLNs) appear promising as a drug carrier system for topical application. The small particle size ensures close contact of nanoparticles with the stratum corneum and should increase the amount of encapsulated agents penetrating into viable skin.

Triptolide (diterpenoid triepoxide), a purified traditional Chinese medicine component, is extracted from a shrublike vine named *Triterygium wilfondil Hook F* (TWHF). Two extracts of TWHF, methanol/chloroform (T2) and ethyl acetate (EA), have been reported to be effective in treating patients with a variety of inflammatory and autoimmune diseases, especially rheumatoid arthritis. The extracts were found containing TP, which accounts for their immunosuppressive activity. In addition, TP has been shown to have other functions, such as antifertility and antineoplastic activity. However, the clinical use of TP has some other practical disadvantages, mainly due to its low water solubility and toxic effects. The incidence of adverse drug reaction (ADRs) is significantly higher than other drugs. The organic systems affected by ADRs of TP were gastrointestinal, urogenital, cardiovascular, blood circulatory, bone marrow, as well as the skin. Especially in the gastrointestinal tract, symptoms such as nausea, vomiting, bellyache, diarrhea, dyspnoea, duodenal ulcer, and gastrointestinal bleeding have been reported. Therefore, the development of novel types of delivery systems could lead to significant advantages in the clinical use of the drug (Tao & Lipsky, 2000; Tao et al., 1999).

The present study focused on topical drug release properties of SLN dispersions and SLN hydrogels encapsulating TP. We investigated in vitro the effects of various SLN dispersions and SLN hydrogels on the percutaneous absorption of TP (0.025% w/w). In addition, the in vivo acute anti-inflammatory activity on carrageenan-induced paw edema of the most promising formulation was tested (Elvira et al., 2003). The long-term goal of this work is to develop topical TP formulations for clinical use to increase its therapeutic index.

MATERIAL

Tristearin glyceride (TSG) and stearic acid (SA) were purchased from the Chemical Plant No. 1 of Shanghai. Polyethylene glycol (400) monosterate (PEG 400 MS) was obtained from the Baoan chemical factory. Medicinal grade of soybean lecithin was purchased from Shanghai No. 1 Oils and Fats Factory. Poloxamer 188 was obtained from China Pharmaceutical University. Triptolide (TP) was purchased from Fujian Institute of Medicinal Sciences. Carrageenan was purchased from Sigma. Voltaren (diclofenac emulgel) was a gift of Beijing Novartis. Pharma Ltd. All other chemicals were obtained from Shanghai Chemical Reagent Corporation.

METHODS

Preparation of SLN Dispersions

Lipids (tristearin glyceride, stearic acid) were heated to 80°C, TP was dissolved in the melt lipid, and lecithin or poloxamer 188 was dispersed in the melt until the dispersion appeared clear (Mehnert & Mäder, 2001). PEG400MS was dissolved in double-distilled water containing thiomersal (0.01% w/w) as a

preservative. The aqueous phase was heated to 80°C. Then the heated aqueous phase was added to the melt lipid and emulsified by probe sonication for up to 20 min (Table 1) (Müller & Lucks, 1992).

Preparation of SLN Hydrogels

The hydrogel was composed of 0.5% glycerol, 40% SLN dispersion, 1.5% Carbomer 940, and double-distilled water. Firstly, Carbomer 940 was dispersed in an appropriate amount of double-distilled water and stored overnight, and then neutralized by NaOH or triethanolamine. After adding the glycerol, the sample was mixed with a high-speed stirrer at approximately 1000 rpm for 5 min. Finally, the SLN dispersion was added and stirring continued.

Preparation of Conventional TP Hydrogels (0.025%, Formulation I)

The hydrogel was composed of 5% glycerol, 1.5% Carbomer 940, appropriate TP ethanol solution, 0.01% thiomersal, and double-distilled water. The preparation process was similar to that of the SLN hydrogel.

Particle Size Analysis and Zeta Potential Measurements

The mean particle size and zeta potential of colloidal carriers are important characteristics of SLNs, from which the stability of drug-loaded SLNs can be predicted. In general, particle aggregation is less likely to occur for charged particles with high zeta potential due to electric repulsion (Hu et al., 2002). They were performed by Zetapals (Zetapals Brookhaven, NY, USA). To determine the size of nanoparticles and zeta potential in the hydrogel, 20 mg of the hydrogel was suspended in 10 mL of water and treated for 15 min in an ultrasonic bath to mix the viscous gel with water. The samples for zeta potential measuring were diluted with double-distilled water adjusted to a conductivity of 50 μS/cm with sodium chloride. The measured electrophoretic mobility was convened to zeta potential using the Helmholtz-Smoluchowski equation; this processing was done by the software included within the system. In order to evaluate the stability of the systems and to exclude drug crystallization on the boundary, each formulation was measured five times in triplicate during a period of 15 days.

Determination of Drug Loading

The TP-SLN dispersion was separated by Sephadex G-25 gel chromatography. The concentration of TP in the suspension and in SLN were assayed by reverse-phase high-performance liquid chromatography (HPLC) after dilution with ethanol (see 3.7).

In Vitro Cutaneous Permeation Study

Animal testing was approved by Tongji Medicinal University Ethical Committee. In vitro permeation studies were performed with Franz diffusion cells. The diffusion cells were thermoregulated with a water jacket at 37°C. Full-thickness abdominal skin, including subcutaneous fat tissue, was excised from rats (provided by Central Animal Laboratory of Tongji Medicinal University), whose hair had been removed beforehand by an electric clipper. The excised skin (area: 3.14 cm^2) was used as a permeation membrane mount on Franz diffusion cells with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor. A mixture of saline and ethanol (9:1) was used as the receptor fluid. Then, 1 g hydrogel or 1 mL dispersion was applied to the donor compartment. At 2, 4, 6, 8, 10, 12, and 24 h after the hydrogel or dispersion was applied to the donor compartment, 0.5 mL aliquots were drawn from the receiver compartment. Thereafter, an equivalent volume of receptor fluid was supplied to the receiver compartment. The concentrations of TP in receptor fluids were analyzed with HPLC.

HPLC Analysis of TP

The amount of TP penetrated into the receptor compartment was determined with a slight modification of reverse-phase HPLC described previously (Li et al., 1998). The HPLC apparatus (Waters, Boston, MA, USA) was equipped with a Hypersil C_{18} column, and the mobile phases consisted of a mixture of methanol and water (40:60, v/v) at a flow rate of

1.0 mL/min, with retention time of 14.1 min ultraviolet absorption was read at 218 nm. The assay was linear (r^2>0.996) in the concentration range 50–1000 ng mL^{-1} with the lowest detection limit of 20 ng mL^{-1} of TP. The percentage recoveries ranged from 98.7 to 101.8. Stability studies were performed for TP solution placed on a laboratory bench and in a refrigerator for 50 days. The samples were found to be stable for the study periods.

Suppression of Carrageenan-Induced Inflammation

Male Wistar strain rats, body weight between 150 and 180 g (provided by Central Animal Laboratory of Tongji Medicinal University), were randomly divided into five groups of six rats for receiving topical treatment. The rats of the standard group were treated with the Voltaren. The three experimental groups received Formulation A, E, and I, respectively, while the control group received saline solution. Voltaren (10.0 mg kg^{-1}), different formulations of TP (0.6 mg kg^{-1}), and the control were applied to the shaved abdominal skin of rats. After 30 min, 0.05 mL of 1% carrageenan was injected into the right hind foot of each rat under the planter aponeurosis. Measurements of foot volume were performed by the displacement technique using a calibrated glass tube immediately before and 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after the injection of carrageenan (Chin & Chun, 1999). The edema rate and inhibit rates of each group were calculated as follows (Ghamdi, 2001):

$$\text{Edema rate (E)}\% = \frac{\text{Vt} - \text{Vo}}{\text{Vo}}$$

$$\text{Inhibition rate (I)}\% = \frac{\text{Ec} - \text{Et}}{\text{Ec}}$$

where Vo is the mean paw volume before carrageenan injection (mL), Vt is the mean paw volume after carrageenan injection (mL). Ec is the edema rate of the control group, and Et is the edema rate of the treated group.

Statistical Analysis

Data were expressed as mean±S.D. and statistically assessed by one-way analysis of variance (ANOVA). Differences between drug-treated groups and the control group were evaluated by Dunnett's *t*-test. $P<0.05$ was considered significant. Further analysis between the drug-treated groups was evaluated by the Newman-Keuls test.

RESULTS AND DISCUSSION

Incorparation of SLNs in Hydrogels

The SLN dispersion possesses low viscosity and a yield value of practically zero, so it isn't convenient for use in skin. Hydrogel preparations are normally preferred because of their controlled release characterization, good tissue compatibility, easy manipulation of swelling level, and may improved targeting to the viable epidemis. In addition, aqueous dispersions of solid lipid nanoparticles are basically stable for up to 3 years. However, some systems show particle growth followed by gelation (Freitas & Muller, 1999a,b), but when the SLN dispersions were incorporated into hydrogels, the nanoparticulate structure was still maintained, and aggregation and gel phenomena of the particles could be avoided.

In order to exhibit gel-forming properties, the carboxylic acid groups (such as Carbomer 940) have to be neutralized. However, sodium hydroxide can reduce the zeta potential of the particle, and the ions caused by destabilization of SLN dispersions contributed to the particle growth and subsequent formation of semi-solid gels. The extent of solidification was highly dependent on the crystallinity of the lipid phase. The recrystallization indices of the 'gels were distinctly higher compared with those of the liquid systems. Additionally, unstable modifications, being present in liquid dispersions, were transformed into stable ones with increasing solidification. The mechanisms of the destabilizing effect of the electrolytes are reduced electrostatic repulsion and transformation of the lipid TSG to the beta modification promoting gel formation (Freitas & Muller, 1999a), Therefore, in vitro cutaneous permeation and anti-inflammatory effect studies for Formulations G and H were meaningless. Trimethanolamine, another type of neutralization agent and an organic base, didn't induce particle growth and subsequent formation of semi-solid gels, but was used only when the molecular weight of nonionic surfactant polyethylene glycol

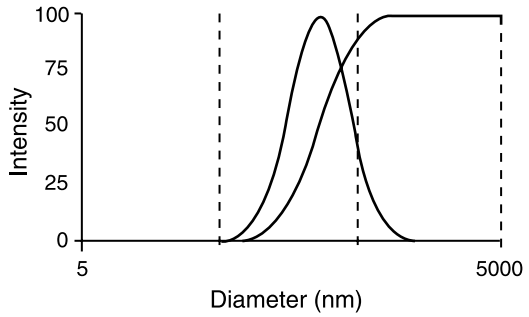

FIGURE 1 **The Nanoparticle Size of Formulation E.**

(PEG) wasn't too high, otherwise induced polymer precipitation. Table 1 illustrates the diameter size and zeta potential of TP-SLN prepared from various formulations. Some data have been published in our previous article (Mei et al., 2003a).

From Table 1, it can be seen that the particle size of TSG-SLN dispersions was smaller than that of SA-SLN dispersions, and the particle size when using Poloxamer 188 as emulsifier was larger than that of soybean lecithin. When the TP-SLN dispersion was incorporated into a hydrogel, Carbomer 940 was used as the gelling agent, triethanolamine as neutralization agent; the nanoparticulate structure could be maintained and the formulation was very stable (Figs. 1 and 2). Stratification was not observed when this hydrogel was centrifuged at 3000 rpm/min for 30 min. When the samples were stored at 40°C, 25°C, and 5°C, respectively, for 3 months, abnormalities were not observed. The nanoparticulate structure still maintained stability after storage at room temperature for 3 years. In addition, during the experiment, the contents of TP in TP-SLN didn't change obviously, according to HPLC analysis (data not given).

Drug Release from Hydrogel

The cumulative amounts of TP in the receptor fluids at different time periods are shown in Table 2. The highest cumulative amounts of drug and the sustained effects were obtained from the smaller particle size of TSG-SLN hydrogel. However, the SLN dispersions, regardless of particle size differences, did not have sustained effects. Compared to the hydrogel, the drug release rate was higher at the first 8 h, due to the burst release of the SLN dispersion. Under this experimental setting, water was vaporized from the SLN dispersion and the fluid SLN dispersion turned slowly into a semisolid gel. Gel formation of SLNs can be correlated with polymorphic transitions of the lipid matrix as the drug is extruded from the lipid matrix. In other experiments, the cumulative amounts of drug in the receptor department almost did not change (Jenning et al., 2000).

Small particle size ensures close contact with the stratum corneum and should increase the amount of encapsulated agents penetrating into the viable skin. On the other hand, during the preparation of SLNs, surfactants such as soybean lethicin, Poloxamer 188, and PEG400MS were used as emulsifier/coemulsifier and as physical stablilizer. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently (Mehnert & Mäder, 2001). It is well known that surfactants have effects on the permeability characteristics of several biological membranes, including skin (Lopez et al., 2000). They can enhance the permeation of the drug through the rat skin, as the nature of surfactants seems to exert an important influence on cutaneous barrier impairment. As to nonionic surfactants, there are two possible mechanisms of transportation. Firstly, the surfactants may penetrate into the intercellular regions of the stratum corneum, increase fluidity, and eventually solubilize and extract lipid components. Secondly, penetration of the surfactants into the intercellular matrix followed by interaction and binding with keratin filaments may result in a disruption within the corneocyte (Schäler et al., 2001). According to another article (Shokri et al., 2001), because PEG400MS contains ethylene oxide and a long hydrocarbon chain, it was assumed to enhance the penetration of TP via both the lipophilic and the hydrophilic molecular mechanisms and to disrupt the lipid arrangements in the stratum corneum and to increase the water content of the proteins in the barrier.

Under the chosen experimental conditions, the SLN hydrogel possessed a sustained drug release over a

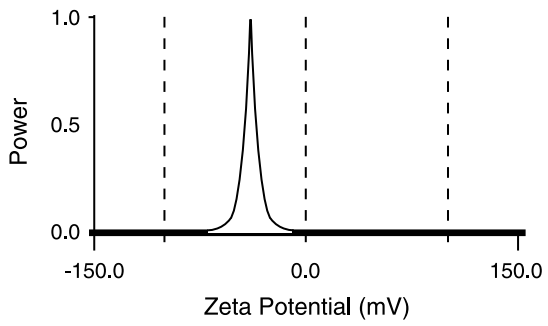

FIGURE 2 **The Zeta Potential of Formulation E.**

TABLE 2 The Cumulative Amount of TP in the Receptor Fluid from Excised Skin ($\mu g\ cm^{-2}$) at Different Time Intervals (n=3)

Formulation	2 h	4 h	6 h	8 h	10 h	12 h	24 h
A	1.52±1.04	2.54±1.61	2.82±0.43	3.24±0.92	3.54±0.88	3.74±1.34	4.05±1.01
B	1.02±0.77	1.24±0.99	1.83±1.11	2.22±0.81	2.89±1.24	3.22±1.04	3.95±0.85
C	1.00±0.79	1.70±1.20	1.22±0.59	2.42±0.52	2.56±0.93	3.36±1.37	3.73±0.97
D	0.88±0.62	1.22±0.79	1.60±0.77	2.00±0.83	2.62±1.11	2.94±0.57	3.74±0.81
E	1.17±0.67	1.94±0.94	2.60±0.82	2.91±1.10	3.15±0.82	3.77±0.77	7.75±1.14
F	1.10±0.55	1.85±0.67	2.50±0.94	2.77±1.00	3.10±1.04	3.55±0.88	7.11±0.89
I	0.68±0.54	0.70±0.93	0.97±0.18	1.87±1.31	2.79±1.10	3.20±1.28	5.80±1.42

period of 24 h. In the in vitro cutaneous permeation study, the cumulative transdermal absorption rate during 12 h was 73.5%, whereas the conventional TP hydrogel was 45.3%. Compared to the conventional TP hydrogel, the release rate was determined by the diffusion of TP from the solid matrix into the hydrogel vehicle and skin (Table 2) (Mühlen et al., 1998).

Anti-Inflammatory Effect

It has been reported by our group (Mei et al., 2003a) that the smaller the particle size of SLNs, the higher the cumulative amounts of TP and the stronger the antiacute inflammatory activity. So in this article, the representative formulations of A, E, and I were chosen to compare the antiacute inflammatory activity. The rat's footpad became edemateous soon after injection of carrageenan. The edema rate of the left footpad reached its peak at 4 h (63.2%). It has been reported that carrageenan-induced edema can be divided into two phases. The first phase occurs during 1 h after carrageenan injection, and derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area. The second phase occurs 3–5 h after carrageenan injection; in this phase, the macrophages in the carrageenan-insulted dermal tissue release Interleukin-1 (IL-1) to induce accumulation of polymorphic nuclear cells (PMNs) into the inflammatory area. This then releases the lysomal enzymes and active oxygen to destroy connective tissues and induce paw edema (Zhao et al., 2000). The carrageenan-induced edema could be significantly suppressed by TP. The results obtained with different formulations of TP and Voltaren in the carrageenan-induced edema test at specific time intervals are shown in Table 3. Results indicate the antiacute inflammatory activity of TP containing SLN dispersions and SLN hydrogel delivery systems as well as TP itself. It is of interest to see that SLN hydrogel reduction in carrageenan induced paw edema when comparable to the SLN dispersion group and control group; this anti-inflammatory effect is over two-fold higher than that of conventional TP hydrogels, especially better than Voltaren (Table 3, Fig. 3). The cutaneous permeation rate in the first 6 h of Formulation A was a little bit higher than that of Formulation E, but the viscosity was lower than Formulation E, so the anti-inflammatory effect is much stronger than Formulation E. Similarly, the cutaneous permeation rate in the first 6 h of Formulation I was much lower than Formulation A, so the anti-inflammatory effect is weaker than Formulation A. The histological workup of inflammatory markers in the skin of the footpad

TABLE 3 The Anti-Inflammatory Effect of Carrageenan-Induced Paw Edema

	Edema rate (%)					
Formulation	1 h	2 h	3 h	4 h	5 h	6 h
Control	37.1±5.6	57.8±10.1	62.0±9.2	63.2±12.4	50.5±9.3	67.0±7.3
A	11.2±2.3^{b}	20.0±5.6^{b}	22.5±3.6^{b}	27.5±8.9	24.6±1.5	36.2±8.6^{b}
E	9.2±1.9^{b,c}	11.0±3.1^{b,d}	13.7±4.2^{b,d}	18.7±7.7^{b,d}	18.9±5.6^{b,d}	28.8±6.6^{d}
I	25.9±3.2^{b}	34.4±7.1^{b}	42.9±7.3^{b}	51.7±7.4^{a}	42.6±8.6^{b}	50.8±6.1^{b}
Voltaren	24.2±3.5^{b}	33.7±3.6^{b}	40.2±5.3	45.6±6.4^{b}	39.6±3.9^{b}	47.8±2.6^{b}

Values represent the mean±S.D. of six animals for each group. Statistically significant from control $^{a}P < 0.05$ and $^{b}P < 0.01$. Statistically significant from SLN dispersion $^{c}P < 0.05$ and $^{d}P < 0.01$ (Dunnett's T-test).

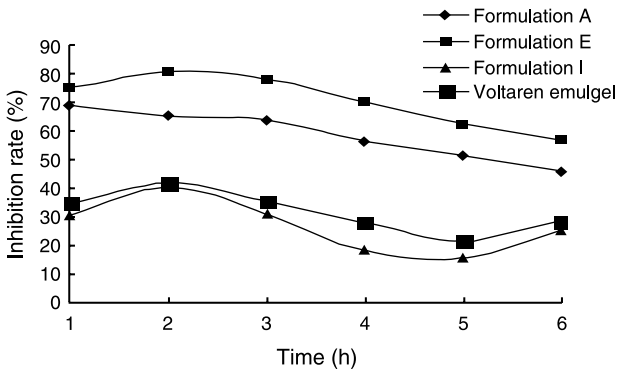

FIGURE 3 **The Inhibition Rate of Different Formulations.**

induced by different TP formulations is under further investigation.

Adverse Side Effects of the Drug

It was assumed that the SLN hydrogel, when administered directly onto skin in a small but sufficient quantity, would cause fewer side effects, if any, than the currently available formulations. The obvious advantages of transdermal delivery are systemic drug therapy, the avoidance of first-pass metabolism and gastrointestinal side effects, and the more favorable drug biodistribution from SLNs that allows the agent to be concentrated in the tissue to be treated. Soybean lethicin is also known to improve the safety of it is co-applied agents, when the latter is surfactant-like. Furthermore, the co-applied lipids are likely to minimize the danger of allergic contact dermatitis that might be induced by the drug (Cevc & Blume, 2001). In our other study, the results suggest that the SLN delivery system could enhance the anti-inflammatory activity of TP while decreasing tissue toxicity (Mei et al., 2003b).

CONCLUSION

The present study clearly shows the potential of SLN hydrogel; as a drug carrier for topical application. It offers systemic drug therapy, and avoids first-pass metabolism and the gastrointestinal side effects. Moreover, the ingredients of SLN, soybean lecithin, and lipids can improve the safety and minimize the toxicity induced by the TP. The drug easily transports through the skin barrier. The highest permeation rate, the sustained effects, and the anti-inflammatory activities of the drug are obtained with the smallest particle size SLN hydrogel.

Triptolide-TP loaded SLN dispersion; have two kinds of emulsifiers, and the SLN dispersion was incorporated into hydrogels, in which case the nanoparticulate structure was maintained, and aggregation and gel phenomena of the particle was avoided.

ACKNOWLEDGMENTS

The authors would like to thank States Development Plan of High Technology ("863" Plan) for its financial support and Prof. K. Mäder, Free University of Berlin, Germany, and Dr. H. Bunjes, Friedrich Schiller University, Germany for their helpful discussions.

REFERENCES

Cevc, G., & Blume, G. (2001). New, highly efficient formulation of diclofenac for the topical transdermal administration in ultradeformable drug carriers, transfersomes. *Biochimica et Biophysica Acta*, 1514:191–205.

Chin, C. T., & Chun, C. L. (1999). Anti-inflammatory effects of Taiwan folk medicine 'Teng-Khia-U' on carrageenan and adjuvant-induced paw edema in rats. *Journal of Ethnopharmacology*, 64:85–89.

Elvira, E., Ana, C. C., Josep, Q., Rossend, O., & Jose, D. (2003). Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. *European Journal of Pharmaceutical Sciences*, 19:203–210.

Freitas, C., & Muller, R. H. (1999a). Stability determination of solid lipid nanoparticles (SLN) in aqueous dispersion after addition of electrolyte. *Journal of Microencapsulation*, 16:59–71.

Freitas, C., & Muller, R. H. (1999b). Correlation between long-term stability of solid lipid nanoparticles (SLNTM) and crystallinity of the lipid phase. *European Journal of Pharmaceutics and Biopharmaceutics*, 47:125–132.

Ghamdi, A. (2001). The anti-inflammatory, analgesic and antipyretic activity of Nigella Sativa. *Journal of Ethnopharmacology*, 76:45–48.

Hu, F. Q., Yuan, H., Zhang, H. H., & Fang, M. (2002). Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. *International Journal of Pharmaceutics*, 239:121–128.

Jenning, V., Gysler, A., Schäfer, K. M., & Gohla, S. V. (2000). Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *European Journal of Pharmaceutics and Biopharmaceutics*, 49:211–218.

Li, K., Yuan, Y., Dai, X., & Qao, X. (1998). Determination of TP in extract from leigongteng (Tripterygium Wilfordii Hook F) by RP-HPLC. *Se Puede*, 16:356–357.

Lopez, A., Llinares, F., Correll, C., & Herraez, M. (2000). Comparative enhancer effects of span 20 with Tween 20 and Azone on the in vitro percutaneous penetration of compounds with different lipophilicities. *International Journal of Pharmaceutics*, 202: 133–140.

Mühlen, Z. A., Schwarz, C., & Mehnert, W. (1998). Solid lipid nanoparticles (SLN) for controlled drug delivery-drug release and

release mechanism. *European Journal of Pharmaceutics and Biopharmaceutics*, 45:149–155.

Müller, R. H., & Lucks, J. S. Arzeistoffträger aus Festen Lipid Teilchen-Feste Lipid Nanosphären (SLN). European Application 92,02,132, 1992.

Mehnert, W., & Mäder, K. (2001). Solid lipid nanoparticles: production, characterization and applications. *Advanced Drug Delivery Reviews*, 47:165–196.

Mei, Z. N., Chen, H. B., Weng, T., Yang, Y. J., & Yang, X. L. (2003a). Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. *European Journal of Pharmaceutics and Biopharmaceutics*, 56:189–196.

Mei, Z. N., Yang, Y. J., Xu, H. B., & Yang, X. L. (2003b). Experimental research on Triptolide solid lipid nanoparticle for decreasing liver toxicity. *Chinese Traditional and Herbal Drugs*, 34:817–819.

Schäler, N., Olbrich, C., Tabatt, K., Müller, R. H., Hahn, H., & Liesenfeld, O. (2001). Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages. *International Journal of Pharmaceutics*, 221:57–67.

Shokri, J., Nokhodchi, A., Dashbolaghi, A., Dashbolaaghi, A., Hassan, D., Ghafourian, T., & Barzegar, J. M. (2001). The effect of surfactants on the skin penetration of diazepam. *International Journal of Pharmaceutics*, 228:99–107.

Tao, X., & Lipsky, P. E. (2000). The Chinese anti-inflammatory and immunosuppressive herbal remedy Tripterygium Wilfordii Hook F. *Rheumatic Diseases Clinics of North America*, 26:29–50.

Tao, X., Ma, L., Mao, Y., & Lipsky, P. E. (1999). Suppression of carrageenan induced the inflammation in vivo by an extract of the Chinese herbal remedy Tripterygium Wilfordii Hook F. *Inflammation Research*, 48:148–179.

Zhao, G., Vaszar, L. T., & Qiu, D. (2000). Anti-inflammatory effects of triptolide in human bronchial epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 279:L958–L966.