Isotretinoin (IT) is used orally for the treatment of severe acne that has not responded to other measures. IT has also been tried in a number of other skin disorders and in some forms of neoplastic disease. It has obvious adverse side effects in oral administration. The most common is dryness of the mucous membranes, visual disturbances, skeletal hyperostosis, and musculoskeletal symptoms. Elevation of serum triglycerides, blood glucose and hepatic enzymes has also been reported (1, 2). However, topical preparations of IT are available, such as creams and gels that show systemic absorption. The topical utility of IT is strongly limited by several disadvantages, such as skin irritation, very low water solubility, and high instability in the presence of light, air and heat.

The aim of this study was to develop new solid lipid nanoparticles of isotretinoin (IT-SLNs) and evaluate the ability of IT-SLNs to improve photostability, reduce skin permeation and irritating effects. IT-SLNs were prepared by the hot high pressure homogenization method. Size, zeta potential and morphological characteristics of the preparations were assessed by transmission electron microscopy (TEM) and thermotropic properties with differential scanning calorimetry (DSC). IT-SLNs had a small average diameter of 74.05 ± 8.91 nm and high encapsulation efficiency (EE) of 80.6 ± 1.2 %. The results showed that the entrapment of IT into SLNs reduced significantly its photodegradation. The in vitro permeation data showed that IT-SLNs can accumulate in the different layers of the skin and prevent systemic uptake of IT in mouse skin. IT-SLNs also significantly increased IT accumulation in the different layers of the stratum corneum of human skin. IT-SLN formulation was significantly less irritating compared to commercial IT-GEL, which shows its potential for improving skin tolerability and being a carrier for topical delivery of IT.

Keywords: SLN, isotretinoin, photostability, skin irritation, skin permeation

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Improved photostability, reduced skin permeation and irritation of isotretinoin by solid lipid nanoparticles

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It is therefore necessary to improve skin uptake and reduce systemic absorption and irritation of IT using a carrier with a targeting effect and controlled release in different skin layers (2, 3).

Lipid nanoparticles have been proven valuable for drug application to the skin (4–6). Solid lipid nanoparticles (SLNs) are a type of lipid nanoparticle colloidal dispersions, which have been claimed to combine the advantages of other colloidal carriers and avoid their disadvantages. The advantages include the possibility of controlled drug release and drug targeting, increased drug stability, feasible incorporation of drugs with different lipophilicity (3–5, 7), lack of carrier biotoxicity, avoidance of organic solvents and no difficulties with large-scale production and sterilization (8). Besides controlled release, SLNs combine the advantages of conventional carriers such as creams and emulsions: good in vivo toleration and protection of active compounds (9). Furthermore, SLNs can facilitate drug penetration into the skin (9, 10), maintain a sustained release to avoid systemic absorption, act as a UV sunscreen system, and reduce irritation (11, 12).

Recent investigations have proven that IT incorporated into Precirol®-based SLNs increases accumulative uptake of IT by the skin to a higher extent compared to conventional vehicles. These results indicate that the studied IT-SLNs with skin targeting may be a promising carrier for topical delivery of IT (3).

The present study focused on the preparation and characterization of IT-SLNs using glyceryl monostearate (GMS) and Tween 80 and evaluation of the influence of IT-SLNs on improving the photostability, occlusive factor, irritation and IT penetration into the skin by in vitro Franz diffusion cells and in vivo stripping methods.

EXPERIMENTAL

Materials

Isotretinoin (Across, America), glyceryl behenate (Compritol® 888 ATO) (Gattefossé, France, glyceryl palmito stearate (Precirol® ATO 5) (Gattefossé, France), cetyl palmitate (Sigma Aldrich, Germany), glyceryl monostearate (Gattefossé, France), Poloxamer 188 (Uniqema, Belgium), glyceryl tripalmitate (Dynasan 116 Fluka, Germany) were used as received.

Preparation of IT-SLNs

Different lipids (Compritol 888 ATO, Precirol® ATO 5, Dynasan®, cetyl palmitate and glyceryl monostearate (GMS)) and surfactants (Tween 80 and Poloxamer 188) were used to find out the best composition for SLN formulations (Table I). SLNs were prepared by the hot high pressure homogenization method. IT-SLNs were prepared using GMS (5 %) as the lipid phase and Tween 80 (2.5 %) as surfactant. GMS and IT (0.05 %) were dissolved in ethanol. Afterwards, ethanol was completely removed using rotary evaporation at 80 °C. The melted residue was added to the hot water containing Tween 80. A pre-emulsion was then prepared using a T 25 Ultra Turrax (Janke und Kunkel GmbH and Co KG Staufen, Germany) for 1 min at 20500 rpm. The pre-emulsion was processed at 7 × 10^7, 10^8 and 1.4 × 10^8 Pa, at 80 °C, for ten cycles using a high pressure
homogenizer (EmulsiFlex-C5® (Avestin Inc., Canada). Homogenized samples were then cooled at room temperature and SLNs were obtained (3, 13–15).

Characterization of SLNs

**Particle size and zeta potential.** – Dynamic light scattering (ZetaSizer Nano-ZS; Malvern Instruments Ltd., United Kingdom) was used to assess the mean particle size (z-average diameter), polydispersity index and zeta potential of SLN dispersions. All measurements were performed in triplicate at a temperature of 25 ± 2 °C and an angle of 90 ° to the incident beam. No multi-scattering phenomenon was observed (3, 16).

**Transmission electron microscopy (TEM).** – TEM (CEM 902A; Zeiss, Germany) was performed to characterize the morphology of IT-SLNs. SLNs were diluted 50 times with water and placed on a carbon-coated copper grid and after 30 seconds the excess water was wiped off with filter paper. Then, 20 µL of uranyl acetate 2 % in water was spread on SLNs and after 30 seconds wiped off with filter paper. The grid was dried at room temperature and then observed by TEM (3).

**Differential scanning calorimetry (DSC).** – DSC was performed to investigate the melting and recrystallization behavior of crystalline materials after solidification. DSC scans of IT, empty and IT-SLNs were carried out in a Mettler DSC 821e (Mettler Toledo, Germany). Approximately 2-mg samples were filled in aluminum pans, sealed and analyzed. An empty aluminum pan served as reference. Samples were scanned from 25 to 250 °C (5 °C min–1) and the melting point of SLN dispersions was compared to the bulk lipid. Melting points corresponded to the heating curve maximum. Analysis was carried out under nitrogen purge (11, 15).

**Entrapment efficiency (EE).** – The entrapment efficiency (%) was determined by measuring the concentration of free and entrapped IT (17). A known dilution of the SLN dispersion was prepared and 500 µL was transferred to the upper chamber of centrifuge tubes fitted with an ultrafilter (Amicon Ultra-15, PLHK Ultracel-PL Membrane, 100 kDa, Millipore). Amicon tubes were centrifuged at 10,000 rpm for 30 min. The filtrate was analyzed for unencapsulated IT at 349 nm using a validated UV-spectrophotometric method after suitable dilution. Entrapment efficiency was calculated (18, 19).

**Stability of IT-SLNs.** – Chemical and physical stability of IT-SLNs were evaluated at 2–8 °C for 3 months based on clarity, particle size and zeta potential of IT (3).

**UV analysis of IT.** – A standard solution of IT was prepared by dissolving 1 mg of accurately weighed quantity of IT in 10 mL acidified isopropyl alcohol. Other concentrations were prepared by dilution of this standard solution with acidified isopropyl alcohol and were used to set up the calibration curves. Absorbance of the solutions was recorded at 349 nm. The assay was linear \( (R^2 = 0.9976) \) in the concentration range of 0.2–10 µg mL⁻¹. All standard solutions were protected from light (18, 19).

**Evaluation of photostability.** – The photostability of IT-SLNs and methanolic solution of IT were compared. Briefly, 10 mL of methanolic solution and IT-SLNs (concentrations: 6, 8, 10 µg mL⁻¹) was exposed to natural sunlight and the UV spectra of both samples were recorded just after preparation \( (t = 0) \) and at the time intervals of 5, 30, 60, 90, 120
and 180 min. Photodegradation of IT was monitored by recording its absorption spectra at the wavelength of 349 nm. A placebo dispersion was diluted suitably with methanol to omit any possible absorption arising from the solid lipid (19, 20).

**Determination of occlusive properties.** – For the occlusion test, 100-mL beakers were filled with 50 mL water and covered with filter paper (cellulose acetate filter, cutoff size: 4–7 µm) and sealed. Samples were spread evenly on the filter surface (10.6 mg cm\(^{-2}\)) and stored at 32 °C and 50–55 % RH for 48 h. Beakers covered with filter paper without samples were considered as reference. The occlusion factor (\(F\)) was calculated according to the following equation after 6, 24 and 48 h, where \(A\) is water loss without sample (reference), and \(B\) is water loss with sample. Every experiment was carried out in triplicate (21, 22).

\[
F = 100 \times \frac{(A-B)}{A}
\]

**Skin-irritation testing (Draize patch test).** – The irritation potential of IT-SLNs in comparison with commercial IT-GEL (Isotrex\(^\circledR\)) was evaluated by carrying out the Draize patch test on rabbits. Animals of 2.5–3 kg were divided into four groups (\(n = 3\)) as follows: Group 1: No application (Control); Group 2: IT-free SLN (Placebo); Group 3: IT-SLN (0.05 %, m/m); Group 4: Commercial IT-GEL (Isotrex\(^\circledR\) gel). Backs of the rabbits were shaved 24 h prior to the application of formulations. An amount of 0.5 g of each formulation was applied to the hair-free skin of rabbits by uniform spreading within an area of 4 cm\(^2\). The skin was observed for any visible change such as erythema (redness) at 24, 48 and 72 h after the application of various formulations. The mean erythema scores were recorded (ranging from 0 to 4) depending on the degree of erythema as follows: no erythema = 0, slight erythema (barely perceptible – light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4 (16, 23).

**In vitro skin permeation studies.** – Jacketed Franz cells with a receiver volume of 25 mL were used and every experiment was conducted in triplicate at 37 °C. Receptor chambers were filled with a freshly prepared mixture of phosphate buffer of pH 7.4 and 95 % ethanol (7:3, V/V). A suitable part of full-thickness skin of a BALB/c mouse was cut and mounted in a Franz cell, with the stratum corneum side facing upward. The mouse was properly shaved on the day before the experiment. Membranes were initially left in Franz cells for 30 min in order to facilitate hydration. Subsequently, 1 g of the formulation (either commercial IT-GEL or IT-SLN) was deposited onto each membrane surface in triplicate. Diffusion cells were covered with aluminium foil to prevent light exposure. The temperature was maintained at 37.0 ± 0.1 °C. At each time point (0.5, 1, 2, 8 and 24 h intervals), 5 mL aliquots were drawn from the receiver compartment. Thereafter, an equivalent volume of receptor fluid was replaced to the receiver compartment. The concentration of IT in receptor fluid was analyzed using the UV spectrophotometric method. The derived concentration values were corrected using the following equation:

\[
M_t(n) = V_r C_n + V_r \sum C_m
\]

where \(M_t(n)\) is the current cumulative mass of drug transported across the skin at time \(t\), \(n\) is the number of sampling, \(C_n\) is the current concentration in receiver medium, \(\sum C_m\) is
the summed total of previously measured concentrations, $V_r$ is the volume of receiver medium, and $V_s$ corresponds to the volume of the sample removed for analysis (24, 25).

To determine the amount of IT in the formulations retained in the skin at the end of the experiment, the amount of the formulation remaining on the surface of the membrane was collected and assayed for IT. The skins were soaked in 5 mL of methanol-chloroform for IT-SLN or in 5 mL of ethanol/water for marketed IT-GEL for 24 h, followed by centrifugal separation. The skin accumulative amount, namely the total amount of IT extracted from the skin at the end of the permeation study (at 24 h) was obtained (26). The amount of IT retained in the skin was then calculated by subtracting the sum of the amount of IT that remained on the surface and the amount of IT that was released (penetrating through the skin) from the whole amount applied (24–26).

In vivo skin permeation studies (Tape-stripping). – The concentration of the chemical in the stratum corneum of human volunteers was determined by the stripping method. After completing an informed consent, 6 healthy volunteers (women or men), aged 30 ± 7.7 years, participated in the study. They were kept for 15 min in a stripping room where the temperature was maintained at 20 °C. A few tested skin areas of 4 cm² were situated at the level of the forearm. Each product (IT-SLN or commercial IT-GEL) was applied at a dose of 2 mg cm⁻² with a glass spatula, weighed before and after the deposit and spread uniformly over the whole area. Tapes (4 cm²) were applied to the treated areas by applying consistent pressure generated by stroking the thumb 10 times along the tape. Stripping was carried out at 0.5, 3, 5 and 7 h after treatment under controlled conditions over 10 s on the same volunteers. The stratum corneum was removed by 22 stripplings with transparent adhesive tape (transpose tape TM, 3M). Strips 1–2, 3–7, 8–12, 13–17, 18–22 were pooled separately in different groups. These strips were named group 1 (strips 1–2), group 2 (strips 3–7), group 3 (strips 8–12), group 4 (strips 13–17) and group 5 (strips 18–22), indicating the different layers of stratum corneum. Methanol (1 mL) was added to the strips, and each sample was shaken 10 s with a vortex, followed by centrifugal separation. Then the amount of IT was determined in these solutions by the UV spectrophotometric method (27–30).

Data analysis
All the experiments of each preparation were repeated three times and data were expressed as the mean value ± S.D. Statistical data were analyzed using nonparametric techniques with a Tukey-Kramer test. Results with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Preparation of IT-SLN
The composition of SLN formulations, time of stirring and visual observation of the preparations are presented in Table I. Dynasan-based SLN (F7, F8, F9 and F10) were separated into two phases after a period of time. Comperitol®, Precirol® ATO 5 and cetyl
Palmitate-based SLNs (F1, F2, F5 and F6) had large particle sizes of 1150 ± 25.1, 1652 ± 35 and 1165 ± 25.6 nm, respectively. However, the GMS based SLNs (F3 and F4) showed a good size distribution with the zeta potential –30.8 ± 2.2 and –35.9 ± 3.5, respectively. According to physical stability, F4 was selected for IT loaded SLNs. Furthermore, the influence of homogenization pressure was investigated on the selected formulation. The IT-SLN particle size at 7 × 10⁷, 10⁸, 1.4 × 10⁸ Pa with 10 cycles of homogenization was determined. IT-SLN homogenized at 7 × 10⁷ Pa showed a relatively large particle size and also an irregular change of particle diameters in comparison with IT-SLN formulations at 10⁸ and 1.4 × 10⁸ Pa. No significant difference in particle size between 1.4 × 10⁸ and 10⁸ Pa was observed. Therefore, IT-SLNs homogenized at 10⁸ Pa with 10 cycles showed narrow distribution and small diameters and hence this pressure was selected for the production of IT loaded SLNs.

**Characterization of IT-SLNs**

Particle size, zeta potential and entrapment efficiency. – The particle size and zeta potential of IT-free SLNs and IT-SLN formulations were, respectively, measured with a particle size analyzer (PSA) and are shown in Table II. Incorporation of IT into IT-free SLNs resulted only in a slight change of average size. In addition, no significant differences in the distribution between IT-SLNs and IT-free SLNs were obtained. Zeta potentials of all the formulations were above –35 mV. In the present study, IT-SLN formulations with high entrapment efficiency (EE) were prepared (80.6 ± 1.2 %). Since the IT-SLN encapsulation efficiency was high (around 80 %), SLNs containing IT were used for the experiments without separation of un-encapsulated IT.

TEM imaging. – TEM images of IT-SLNs and SLNs free of IT are shown in Fig. 1. It was observed that both SLNs with and without drug exhibited a nanometric size, spher-

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**Table I. Composition and physical appearance of IT-free SLNs containing 5 % of different types of lipids and surfactants**

<table>
<thead>
<tr>
<th>SLN formulations</th>
<th>Lipid</th>
<th>Lipid (%, m/V)</th>
<th>Emulsifier</th>
<th>Emulsifier (%, m/V)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Compritol</td>
<td>5</td>
<td>Poloxamer</td>
<td>2.5</td>
<td>1 phase</td>
</tr>
<tr>
<td>F2</td>
<td>Precirol</td>
<td>5</td>
<td>Poloxamer</td>
<td>2.5</td>
<td>1 phase</td>
</tr>
<tr>
<td>F3</td>
<td>GMSᵇ</td>
<td>5</td>
<td>Tween 80</td>
<td>2.5</td>
<td>1 phase</td>
</tr>
<tr>
<td>F4</td>
<td>GMS</td>
<td>5</td>
<td>Tween 80</td>
<td>5</td>
<td>1 phase</td>
</tr>
<tr>
<td>F5</td>
<td>Cetyl Pᶜ</td>
<td>5</td>
<td>Tween 80</td>
<td>2.5</td>
<td>1 phase</td>
</tr>
<tr>
<td>F6</td>
<td>Cetyl P</td>
<td>5</td>
<td>Poloxamer</td>
<td>2.5</td>
<td>2 phases</td>
</tr>
<tr>
<td>F7</td>
<td>Dynasan</td>
<td>5</td>
<td>Tween 80</td>
<td>2.5</td>
<td>2 phases</td>
</tr>
<tr>
<td>F8</td>
<td>Dynasan</td>
<td>5</td>
<td>Poloxamer</td>
<td>2.5</td>
<td>2 phases</td>
</tr>
<tr>
<td>F9</td>
<td>Dynasan</td>
<td>5</td>
<td>Poloxamer</td>
<td>5</td>
<td>2 phases</td>
</tr>
<tr>
<td>F10</td>
<td>Dynasan</td>
<td>5</td>
<td>Poloxamer</td>
<td>2</td>
<td>2 phases</td>
</tr>
</tbody>
</table>

ᵃ Stirring conditions: 1 min at 20500 rpm (high speed stirrer Ultra Turrax 25).
ᵇ GMS – glyceryl monostearate
ᶜ Cetyl P – cetyl palmitate
ical shape and had narrow size distribution. There was no difference between the particle size of IT-SLNs from TEM images (72.42 ± 8.844 nm) and PSA (74.05 ± 8.918 nm).

Determination of crystallinity. – The thermal behaviour of SLN formulations and the corresponding bulk lipid was investigated using DSC (Fig. 2). The IT-SLN thermogram showed the melting peak of crystalline GMS around 50 and IT around 170 °C, which indicates that they are in SLNs in crystalline state. However, the melting peak of lipid in SLN form was decreased when the drug was loaded in SLN.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>1 day</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT-free SLN</td>
<td>54.66 ± 9.17</td>
<td>58.34 ± 10.1</td>
<td>59.56 ± 10.6</td>
<td>63.97 ± 10.3</td>
</tr>
<tr>
<td>IT-SLN&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.05 ± 9.91</td>
<td>77.98 ± 10.1</td>
<td>78.1 ± 10.8</td>
<td>83.5 ± 10.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> F4, homogenized at 7 × 10<sup>7</sup> Pa.
<sup>b</sup> Data expressed as mean ± SD (n = 3).

Fig. 1. The TEM imaging of a) IT-SLN and b) SLN without IT.

Fig. 2. a) DSC thermogram of: a) the GMS bulk, b) SLN without IT and c) IT-SLN.
SLN stability. – IT-SLN showed good stability during the period of three-month storage. No changes were observed in clarity and phase separation. The particle size of IT-SLNs stored for 3 months was 83.5 ± 10.2 nm, with a slight increase that occurred in comparison with 74.05 ± 8.91 on the day of preparation, with no significant difference (p < 0.05) (Table II).

Evaluation of photostability. – Concentrations of the methanolic solution of IT (6, 8, 10 µg mL⁻¹) and IT-SLN with the same concentrations at various times of sunlight exposure were investigated and are shown in Fig. 3. Light exposure caused a sharp degradation of IT in the first few minutes. The rate of IT photodegradation slowed down after 5 min but the photodegradation process continued, leaving a small residual IT concentration of cca 50 % at the end of 180 min. At the end of 180 min, IT concentrations in all irradiated SLNs were significantly higher compared to IT concentration in methanolic solution. Our findings showed that the entrapment of IT into SLNs strongly reduced its photodegradation in comparison with photodegradation of the methanolic solution of IT.

Determination of occlusive properties. – Occlusion factors of SLNs and IT-SLNs were investigated after 6, 24 and 48 h. IT-SLNs showed a high occlusive factor after 6 h, which decreased over a period of 48 h. IT had no significant effect on the SLN-IT occlusive factor in comparison with SLNs without IT.

Table III. Mean erythemal scores observed using the Draize patch test on rabbits

<table>
<thead>
<tr>
<th>Formulation</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IT-free SLNs (Group 2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IT-SLNs (Group 3)</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Commercial IT-GEL (Group 4)</td>
<td>2.5</td>
<td>3.3</td>
<td>4</td>
</tr>
</tbody>
</table>

Group 1: No application, Group 2: IT-free SLNs, Group 3: IT-SLNs (0.05 %, m/m). Group 4: Commercial IT-GEL obtained at the end of 24, 48 and 72 h.

Mean erythemal scores were: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4.
Primary skin irritation studies. – The results obtained from primary skin irritation studies are listed in Table III and the photographs are shown in Fig. 4. Skin-irritation studies indicated that SLN based IT caused considerably less irritation compared to the commercial IT-GEL (Isotrex® gel) after 72 h of application. Thus, SLN based IT demonstrated a remarkable advantage over the commercial IT-GEL in improving the skin tolerability of IT, indicating SLN potential for improving patient acceptance.

In vitro skin permeation. – The in vitro permeation of IT through BALB/c skin from SLNs and commercial IT-Gel (Isotrex®) was calculated in terms of the mean cumulative amount diffused at each sampling time point over a time period of 24 h. The amounts of IT in IT-SLNs and IT-Gel that penetrated the skin were 5.27 ± 0.65 and 7.04 ± 0.21 %, respectively, while the amounts of IT in IT-SLNs and IT-Gel retained were 63.92 ± 1.5 and 12.15 ± 1.2 %, respectively (Fig. 5). There was a significant difference at \( p < 0.001 \) in the percentage of IT that penetrated the skin and the percentage of IT that was retained between the two formulations.

Determination of human percutaneous absorption of IT after IT-SLN and commercial IT-GEL application using the stripping method. – The results presented in Fig. 6 show the amount of IT in the stratum corneum layers as a function of stripping numbers (groups of strips), from IT-SLNs and IT-Gel. This figure shows the amount of IT in the different layers of stratum corneum. Higher amounts of IT were recovered from the upper layers of stratum corneum at 30 min, 3 and 5 h post application of gel in comparison with IT in SLNs. This phenomenon is not desirable as it can be washed off without penetrating into deeper layers of the skin. Our findings show that after 30 min and 3 h, the amount

Fig. 4. Photographs of skin irritation studies carried out on rabbits: a) control (no application), b) SLN without IT (Group 2), c) IT-SLN (Group 3), d) Marketed IT-GEL (Group 4). Photographs have been taken after 24 h. Marketed IT-GEL clearly shows erythemal lesions, which are not visible in IT-SLN.
of IT in the first strip groups (Groups 1 and 2) in the IT-Gel formulation was higher than in the following strip groups. However, this amount in other groups of strips (Groups 3, 4 and 5) was lower than in IT-SLNs. Higher amount of IT available in the first stripping group in IT-GEL is not desirable as it can be easily washed off from skin surface. This means that SLNs penetrate deeper and then accumulate in deeper layers of the stratum corneum. The overall results obtained in this study showed that SLNs can offer improved topical delivery of IT compared to the commercial IT-Gel in terms of better skin tolerability and higher skin accumulation.

The findings of this study have shown the ability of IT-SLNs to improve the topical delivery of IT and reduce its irritating effects. IT-SLNs could significantly increase the accumulative uptake of IT in mouse and human skin. In this study, ethanol was used as solvent to obtain IT homogeneously distributed in the melted lipid. F3 and F4 showed a suitable size, size distribution and physical stability. GMS was used as the lipid matrix as well as surfactant. This surface active partial glyceride facilitated emulsification and formed more rigid surfactant films and therefore improved long-term stability of SLNs.
In another study, Precirol ATO 5 was used for SLN preparation (3). Also, the influence of homogenization pressure was investigated on the selected formulation. The particle size of IT-SLNs at $7 \times 10^7$, $10^8$, and $1.4 \times 10^8$ Pa, with 10 cycles of homogenization, was determined for GMS as the lipid phase. Increase in homogenization pressures resulted in a decrease in the mean particle diameter of SLN dispersions. High homogenization pressure at $1.4 \times 10^8$ Pa may result in the formation of small particles, and could be aggregated to form a large nanoparticle due to the absence of sufficient surfactants. IT-SLNs homogenized at $10^8$ Pa, with 10 cycles, showed a narrow distribution and small diameters and therefore this pressure was selected for producing IT loaded SLNs. It was observed in a study of Liu et al. that the pressure of $10^8$ Pa resulted in low particle size, but there was a slight increase in the IT-SLNP particle size with the increase of homogenization cycles. The results of another study showed that the optimum homogenization pressure was found to be $1.03 \times 10^8$ Pa for SLN-tamoxifen (32). No significant differences in distribution between IT-SLNs and IT-free SLNs were observed, which was due to the extremely low amount of the drug incorporated in SLNs. Zeta potential values of SLN higher than $-30$ mV indicate high physical stability. Incorporation of IT into SLNs showed its influence on the zeta potentials of nanoparticles due to hydroxyl groups. High zeta potential can provide electric repulsion and higher physical stability of the dispersion and avoid aggregation of particles. Tween 80 also provides steric stability for maintaining the stability of SLNs. In a recent study, it was found that the average size of all formulations containing Precirol ATO 5 SLN incorporated IT was between 30 and 60 nm. The particle size decreased with an increase in the concentration of Tween 80 and the incorporation of IT into IT-free SLNs only resulted in a slight change of the average size (3). Some other studies also reported the spherical shape of SLNs (3, 26). In the present study, the IT-SLN formulations with high EE were prepared. High solubility of IT in the lipid and its low concentration in SLNs can produce high EE. This data was also obtained by Liu et al. In their study, the concentration of surfactants (Tween 80 and SL) had a significant influence on EE. IT-SLNs with a high concentration of precirol as solid lipid exhibited the highest EE of 99 % (3).

DSC was a tool to investigate the melting and recrystallization behavior of crystalline materials. In the current study, the melting peak of the lipid in SLN formulations was decreased when the drug was loaded in SLN. Melting point depression might be due to small particle sizes of the SLN, their high specific surface area and the presence of a surfactant. The enthalpies of GMS in SLN were significantly reduced due to partial recrystallization and the decreased concentration of the dispersed lipid in the lyophilized SLN. It was demonstrated that when the drug was added into SLNs, the lipid crystals in an orderly situation were further disrupted, which could reduce the crystallization property of nanoparticles. Therefore, the lipid melting point in SLN-IT was slightly shifted towards lower temperatures as was shown in Li et al. research for puerarin loaded solid lipid nanoparticles. If the lipid melting point is higher than room temperature, the lipid is actually in the solid state (33, 34).

IT-SLNs showed good stability during three-month storage. Good stability might result from the slow transition of lipid into SLN, low particle size, high zeta potential, and the steric effect of Tween 80 and the presence of GMS (3, 31).

Concentrations of the methanolic solution of IT and IT-SLNs with the same concentrations at various times of sunlight exposure were also investigated. Our findings show
that the entrapment of IT into SLNs strongly reduced its photodegradation in comparison with photodegradation of the methanolic solution of IT. This protection effect is probably due to the light-scattering properties of nanoparticles and the physical UV blocking of SLNs (11, 19). Such photoprotection was also demonstrated by another study. The authors concluded that SLNs had a good potential to improve photostability of tretinoin and could be employed as a valuable delivery strategy (19).

IT-SLNs showed a high occlusive factor after 6 h due to the SLN formulations, which decreased in a period of 48 h because of lipid degradation. Adhesive effect has been claimed for small sized particles forming a film on the skin after application (5). The occlusion factor depends strongly on the crystallinity of the lipid matrix and on size. SLNs show a tendency to fuse, forming a dense film after application to the skin (10, 21). Formation of a dense structure will produce occlusive effects on the skin. Films made from melts of bulk lipid do not form close films as dried SLN dispersions do (35). Another in vivo study showed that both formulations (pure cream and SLN enriched cream) were able to significantly increase skin hydration within 4 weeks of treatment (10).

Skin irritation of IT (erythema) strongly limits its utility and acceptability to patients. An ideal ability of a drug delivery system is to reduce erythematic events due to the acidic function (COOH) of IT (36). The results obtained in this study indicate that SLN based IT resulted in considerably less irritation compared to the commercial IT-GEL after 72 h of application. Therefore, SLN based IT demonstrated a remarkable advantage over the commercial IT-GEL (Isotrex, 0.05 % IT) in improving the skin tolerability of IT, indicating SLN potential to improve patient acceptance. High EE might be the reason for reduced skin irritation by the drug due to avoiding direct contact between the drug and skin surface. Another study also confirmed our observation that the SLN based gel had a remarkable advantage over the marketed formulation in improving the skin tolerability of tretinoin (TRE), indicating SLN potential for improving patient acceptance and topical delivery of TRE (19).

The in vitro permeation of IT through BALB/c skin from SLNs and commercial IT-Gel (Isotrex®) during a time period of 24 h was calculated. There was a significant difference between the two formulations in the percentage of IT that penetrated the skin and the percentage of IT that was retained. It is noteworthy that IT was not released from SLNs before permeating through the skin, when we would have observed significant irritation like that observed in the case of commercial IT-Gel. While being transported across the skin, SLNs probably expel IT from the SLN matrix. This phenomenon has been hypothesized in some investigations (37). SLNs were shown to improve the dermal localization of several topical therapeutic agents (38). This was one of the reasons for employing the SLN approach for topical delivery of IT, since its epidermal localization is highly desirable for enhancing the treatment of skin diseases such as psoriasis, acne, photoaging and epithelial skin cancer.

SLNs allowed drug release for several hours, as described for liposomes, by establishing close contact with superficial junctions of corneocyte clusters and furrows between corneocyte islands. Moreover, the drug dissolved or finely dispersed within the lipid matrix of the carrier or attached to the carrier surface should facilitate drug dissolution within epidermal lipids. Finally, a lipid film covering the skin surface may further enhance dermal absorption because of an additional occlusive effect (39). Penetration
profiles depend on the chemical nature of drug molecules, size and structure of the nanoparticles (22).

The results presented in Fig. 6 show the amount of IT in the stratum corneum layers as a function of stripping numbers (groups of strips), from IT-SLNs and IT-Gel. Higher amounts of IT were recovered from the upper layers of stratum corneum at 30 min, 3 and 5 h post application of commercial IT-GEL (Isotrex, 0.05 %) in comparison with IT in SLNs. This phenomenon is not desirable, since IT can be washed off and fail to penetrate into deeper layers of the skin. Improved drug uptake has also been observed for topical corticosteroid prednicarbate when delivered by means of SLNs (38).

Penetration of active compounds into human skin was also studied using the tape stripping test. Investigated SLN compounds included coenzyme Q10 and retinol. For acne therapy, it was desirable for the active compounds to remain in the skin, penetrate sufficiently deep into epidermal layers, but not too deep, leading to systemic effects. This was achieved with SLN formulations (6).

Our findings show that after 30 min and 3 h, the amount of IT in the first strip groups (Groups 1 and 2) in the IT-Gel formulation was higher than in the following strip groups. However, this amount other groups of strips (Groups 3, 4 and 5) was lower compared to IT-SLNs. This could be explained by the small size of SLNs and their composition. Higher amount of IT available in the first stripping group in IT-GEL is not desirable, since it can be easily washed off from the skin surface. This means that SLNs penetrate deeper and then accumulate in deeper layers of the stratum corneum. The occlusive properties of SLNs can increase skin penetration. Some studies demonstrate that particles with small sizes can penetrate deeper into the skin (30, 40). On the other hand, some other reports claim a lack of this effect (41). The degree of penetration depends on the chemical composition of the formulation, film formation properties, and the interaction of SLN lipids and surfactants with skin lipids (6). However, further work on more volunteers and clinical studies will be helpful to evaluate the effectiveness of this nanoparticulate delivery system.

In this study, we have prepared IT loaded GMS/SLNs using a high-pressure homogenizer. The effects of IT-SLNs as topical delivery of IT, irritating effects, skin penetration, photostability and the occlusive factor of IT were evaluated. The results of this study show the ability of IT-SLNs to improve topical delivery of IT and reduce its irritating effects. The in vitro permeation data show that IT-SLNs can prevent systemic uptake of IT in mouse skin. The IT-SLN formulation was significantly less irritating compared to commercial IT-GEL, which indicates its potential for improving skin tolerability. These results indicate that SLNs could be a promising carrier for topical delivery of IT. The effects of IT-SLNs as topical delivery of IT for acne therapy needs further research on human skin.

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**S A Ž E T A K**

**Povećana fotostabilnost, smanjena permeacija i irritacija izotretinoina iz kruto-tekućih nanočestica**

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Cilj rada bio je pripraviti nove kruto-tekuće nanočestice izotretinoina (IT-SLNs) i ispitati povećanje fotostabilnosti, smanjenje permeacije kroz kožu i smanjenje irritacije nakon uklapanja izotretinoina u nanočestice. IT-SLNs pripravljeni su metodom vrucé visoko-tlačne homogenizacije. Veličina čestica, zeta potencijal, morfološka i termotropska svojstva određena su transmisjskom elektronskom mikroskopijom (TEM), odnosno diferencialnom pretražnom kalorimetrijom (DSC). IT-SLNs imale su prosjecni promjer 74,05 ± 8,91 nm i visoku učinkovitost uklapanja (EE) od 80,6 ± 1,2 %. Rezultati pokazuju da uklapanje izotretinoina u SLNs značajno smanjuje njegovu fotodegradaciju. *In vitro* ispitivanje permeacije pokazuje da se IT-SLNs mogu nakupljati u različitim slojevima kože i spriječiti sistemsku apsorpciju izotretinoina kroz kožu. IT-SLNs također značajno povećavaju IT akumulaciju u površinskim slojevima ljudske kože (stratum corneum). IT-SLN bio je značajno manje irritabilan nego komercijalno dostupni IT-GEL pa je pogodan pripravak za topičku primjenu izotretinoina.

**Ključne riječi:** SLN, izotretinoin, fotostabilnost, irritacija kože, permeacija kroz kožu

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