Development of Topical Gel of Capsaicin Loaded Solid Lipid Nanoparticles (SLNs): \textit{in vitro} and \textit{in vivo} Evaluation

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\textbf{Abstract:} Purpose: The aim of this study was to evaluate and compare the \textit{in vitro} and \textit{in vivo} topical potential of solid lipid nanoparticles (SLNs) gel for capsaicin. \textit{Methods:} SLNs were prepared by high shear homogenization and ultrasonication using capsaicin. Then SLNs gel was formulated by hydration method using carbopol 940. \textit{Results:} The mean particle size, width of distribution, zeta potential and entrapment efficiency of SLNs were 100 nm, 0.374, -48.36 mV and 63.5 \pm 2.9 respectively. The scanning electron microscopy (SEM) image revealed that capsaicin in SLNs was in amorphous state. Capsaicin release was prolonged to 14 hr with encapsulation (94.3\pm3.8\%). Drug loaded SLNs showed transdermal flux of 30.43\pm2.5 \mu g/cm^2/h indicating synergistic effects of encapsulation of capsaicin in SLNs. Permeation of optimized formulation was found to be about 1.6 fold times higher than plain drug. Drug loaded SLNs showed 51\% remission in inflammation at the end of study (28th day). In the paw edema test, SLN-5 showed the best permeation and effectiveness. The results of the present study demonstrated SLN-5 gel formulation possesses great potential for enhanced skin accumulation, prolonging drug release and improving the site specificity of capsaicin. \textit{Conclusion:} SLNs containing capsaicin could be prepared successfully by using an ultrasonic technique, which will not only sustain the release of drug but also increase permeation of drug to dermal layer of skin. The \textit{in vitro} and \textit{in vivo} studies showed that SLN-5 formulation prepared without organic solvent could be a new, alternative dosage form for effective therapy.

\textbf{Key words:} Solid lipid nanoparticles (SLNs), capsaicin, topical gel

\textbf{INTRODUCTION}

Solid lipid nanoparticles (SLNs) have emerged as an alternative carrier system to traditional carriers, such as polymeric nanoparticles, emulsion, noisomes and liposomes, and they attract great attention as a novel colloidal drug carrier for topical use [1]. The advantages of the carrier include negligible skin irritation, controlled release and protection of active substances [2]. Because they are composed of non-irritative and non-toxic lipids, SLNs seem to be well suited for use on inflammed 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 transdermal water loss and favors the drug penetrating through the stratum corneum [3-4]. Besides the nonspecific occlusion effect, the enhanced drug penetration might be related with SLNs themselves, the highly specific surface area of nanometer sized SLNs facilitate the contact of encapsulated drug with stratum corneum [3]. 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 [5-6]. Other advantages of SLNs are reported to be as avoidance of organic solvents, drug stability, high drug payload, and incorporation of liophilic and hydrophilic drugs [1]. SLNs have been used to improve the skin/dermal uptake of several drugs such as triptolide, isotretinoin, podophyllotoxin and prednicarbate [7-11, 12] which supports that SLNs can be employed as the carrier for the topical delivery of capsaicin.

Capsaicin (8-methyl N-vanillyl-6 nonenamide), the active compound of hot peppers of the genus...
Capsicum, exhibits broad bioactivity [13] including antinociception, antihypertensive and lipid-lowering activities [14-16]. Capsaicin is also used topically to treat various diseases such as rheumatoid arthritis, osteoarthritis, diabetic neuropathy and post therapeutic neuralgia [17]. The present work is focused on the preparation, characterization, in vitro percutaneous permeation and skin targeting behaviors capsaicin-loaded SLNs. Topical capsaicin is well absorbed from the skin. Maximal cutaneous concentrations of capsaicin are rapidly achieved when capsaicin is applied topically. These concentrations are greater with isopropyl preparations compared with propylene glycol or mineral oil preparations. [18]. In mice, capsaicin is distributed widely to the brain, spinal cord and liver after intravenous administration. [19]. Capsaicin is a fat soluble, odorless, pungent tasting, off-white solid with a melting point of 62–65 ºC and a molecular weight of 305.4 kDa. As it is not water soluble, alcohols and other organic solvents are used to solubilize capsaicin in topical preparations and sprays [20]. The drug had a poor water solubility, short biological half life and high lipophilicity that made it an excellent candidate for SLNs encapsulation. Topical application circumvents the hepatic metabolism and thus is suitable to develop delivery systems to attain both systemic and local effects for capsaicin. The present investigation was aimed at formulating topical gel of capsaicin loaded SLNs and explore its potential for topical delivery.

EXPERIMENTAL

Materials

Capsaicin was obtained ex-gratis from Ashian Herbex Ltd., Hyderabad. Glyceryl behenate, sodium collate, soyalecithin were bought from Sigma chemicals (USA). All other chemicals and solvents were of analytical grade.

Preparation of Solid Lipid Nanoparticles

SLNs were prepared by high shear homogenization and ultrasonication as in our previous studies. Capsaicin (80 mg) was added to Compritol 888 ATO (4 g) previously melted at 80 ºC. This hot lipid phase further was dispersed in a surfactant solution (1.5%, w/w), at 8000 rpm, 80 ºC for 1 min, using a high-speed stirrer (Ultra Turrax T8, Alliance Analytical Inc., California, USA). The surfactants used were poloxamer and sodium cholate. The obtained pre-emulsion was ultrasonified using a probe sonicator (Vibra cell, Sonics, USA). In order to prevent recrystallization during homogenization, production temperature was kept at least 5 ºC above the lipid melting point. The obtained nanoemulsion (O/W) was cooled down in an ice bath to form SLNs and finally diluted up to 200 ml with deionized water. Nanoparticle dispersions were stored at 4 ºC. Capsaicin was incorporated into SLNs, and niosomal formulations at saturating concentration to obtain equal thermodynamic activities. To determine the maximum amount of drug that could be added, increasing amounts of drug were added during preparation of SLNs formulation. Six formulations of SLNs (SLN-1 to SLN-6) were prepared using drug concentration in the range of 2-12%. Similarly 5 formulations of niosomes (Nio-1 to Nio-5) were prepared containing drug in the range of 2-10%. It was assumed that the presence of capsaicin crystals would indicate that the formulation was saturated with drug. Therefore, all drug loaded formulations were examined over a period of 14 days using phase contrast microscope (Leica, DMLB, Switzerland) for appearance of drug crystals. From these results SLN- 5 (drug 10%) and Nio-2 (8%) was optimized and used for further studies.

Preparation of Carbopol Gel

Carbopol 940 (1% w/w) was dispersed in small quantity of distilled water to prepare an aqueous dispersion which was allowed to hydrate for 4-5 hours. Propylene glycol (10% w/w), glycerine (30% w/w) and 0.025% w/w of drug were added subsequently to the aqueous dispersion followed by neutralization with 0.3% triethanolamine to adjust pH 6. The final weight of the gel was adjusted to 100 g. The entrapped air bubbles were removed using vacuum and left the gel overnight.

Preparation of Drug Loaded SLNs Gel

SLNs gel was prepared using the same formula. For this purpose equivalent amount of SLNs suspension containing 0.025 g drug was centrifuged and the pellets obtained were incorporated instead of the drug.
Preparation of Niosomal Gel
Niosomes were prepared by cast film method [21]. Phosphatidylcholine, span80, cholesterol used for niosomes preparation were dissolved in minimum quantity of chloroform in a round bottom flask. The organic solvent was removed by rotary evaporator under reduced pressure to form a thin film on the wall of flask. Final traces of solvent were removed under vacuum overnight. The deposited films were hydrated with water by rotation at 60rev/min for 1 h. To prepare smaller vesicles, dispersion was bath sonicated for 15 min. Niosomal gel was prepared as described in above section.

Physicochemical Characterization of SLNs
The prepared SLNs were evaluated for size, zeta potential, Scanning Electron Microscopy (SEM), Transmission electron microscopy (TEM), differential scanning calorimetry (DSC), entrapment efficiency (EE%) and stability studies as in our previous studies.

Measurement of Size and Zeta Potential of SLNs
The size and zeta potential of SLNs were measured by photon correlation spectroscopy using a Zetasizer 3000 HSA (Malvern, UK). Samples were diluted appropriately with the aqueous phase of the formulation for the measurements, and the pH of diluted samples ranged from 6.9 to 7.2. Zeta potential measurements were done at 25ºC and the field strength was 20 V/cm on a large bore measuring cell (4 mm). Samples were diluted with bi-distilled water.

Transmission Electron Microscopy (TEM)
TEM (JEM-100CXII, Japan) is a method of probing the microstructure of rather delicate systems such as micelles, liquid crystalline phases, vesicles, emulsions and also nanoparticles [13]. Without surfactants the lyophilized SLNs were dispersed directly into tristilled water. Then copper grid coated with carbon film was put into the above solution several times. After being stained by 2% phosphotungstic acid (PTA) solution and dried under room temperature, the sample was ready for the TEM investigation.

Scanning Electron Microscopy (SEM)
The morphology of the lyophilized empty and capsaicin loaded SLNs were determined using SEM (Model LEO 1455VP, LEO, Cambridge, England). The lyophilized SLNs were evenly distributed onto a conductive tab on a stud and then sputter coated with gold in a cathodic evaporator.

Stability Studies
The prepared formulations were tested for stability on storing them in amber colored glass vials at 4 °C and 25 °C for 30 days. On 7th, 14th and 30th days, they were evaluated for vesicle size, zeta potential, EE% and shape.

Entrapment Efficiency (EE %)
2ml of nanosuspension solution of certain concentration was prepared and added to Sephadex G-25 column. Then methanol aqueous solution was passed through the column. The collected washing out liquid was measured by the UV-detection (Unico, UV-2102PC, USA) at 310 nm. The amount of drug that was not incorporated into the SLNs could be obtained by the UV-detection absorption percent. EE% could be achieved by the following equation.

\[
EE\% = \frac{W_{initial\text{drug}} - W_{free\text{drug}}}{W_{initial\text{drug}}} \times 100\% \tag{1}
\]

Differential Scanning Colourimetry (DSC)
Thermal behavior of capsaicin loaded SLNs was analyzed using DSC-7 (Perkin-Elmer, USA). Approximately 10mg of sample 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 [22-23].

HPLC Analysis of Capsaicin
The drug content of capsaicin was analyzed by a HPLC system consisting of a Hitachi L-7100 HPLC pump, a Hitachi L-7200 sample processor and a Hitachi L-7480 fluorescence detector. A 25 cm long, 4 mm inner diameter C18 column (LichroCart 250-
4, Merck) was used. The mobile phase for capsaicin was 55% pH 4 citrate-phosphate buffer and 45% acetonitrile at a flow rate of 1.0 ml/min. The column effluent was passed through the fluorescence detector set at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. The detection limit of capsaicin was 20ng/ml.

**In-Vitro Drug Release Studies using Cellophane Membrane**

The in vitro release of capsaicin from different capsaicin formulations was studied using locally fabricated Keshry Chein diffusion cell through the cellophane membrane (molecular weight cut off 12000 to 14000). Pure capsaicin was dissolved in 1:1 (v/v) ethanol-pH 7.4 citrate-phosphate buffers at a concentration of 20 mg/ml and used as control. The prepared formulation SLN-5 gel, SLN-5(suspension), NIO-2 gel, plain capsaicin gel, Zostrix® (marketed formulation) cream with 0.075% capsaicin (the same concentration of capsaicin as 20 mg/ml pure drug solution) was transferred to a dialysis bag (size cut off = 2.5 nm) immediately. The dialysis bag was placed in a 50 ml-beaker containing 1:1 (v/v) ethanol-pH 7.4 citrate-phosphate buffer. The outer phase was stirred continuously. At predetermined time intervals sample was withdrawn and replenished with same amount of receptor fluid. The drug content in outer phase was analyzed by using HPLC as described in above section.

**In Vitro Skin Permeation Study**

The in vitro permeation experiments were determined by using Franz diffusion cell. The dorsal skin of wistar rat (8–9 weeks old) was mounted on the receptor compartment with the SC-side facing upwards into the donor compartment and the dermal side facing downwards into the receptor. A 10 ml of 1:1 (v/v) ethanol-pH 7.4 citrate-phosphate buffer was used as the receptor medium. The donor compartment of the cell was filled with 2ml of drug solution, SLN-5(suspension), 1g SLN-5 Gel, NIO-2 Gel, Zostrix® (marketed formulation) with 0.075% capsaicin and plain capsaicin gel of formulation vehicle containing the test drug. The top of donor was covered with paraffin paper. The available diffusion area of cell was 0.785 cm². The receptor phase of cell was sustained at 37°C and stirred by a magnetic stirrer at 600 rpm. At appropriate intervals, 200 µl aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. All studies were performed in triplicate. The sample was analyzed by HPLC method described earlier [24].

**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 above section. The excessive SLN-5 gel, NIO-2, Zostrix® dispersion or cream was removed and the skin samples with a penetration area of 3.8cm² were rinsed with alcohol and water to eliminate the capsaicin remained on the surface and then gently dried with a cotton wool [25]. Heat-separated membrane separation was carried out based on the reported method [26]. The skin samples were immersed in distilled water at (60±1) °C for 2 min and then the epidermis was removed from the dermis using a dull scalpel blade [27-28]. The epidermis sample or dermis cut into small pieces was soaked in 5ml 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 60min 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 [29-30].

**Lyophilization**

The SLNs were lyophilized using a programmable freeze-dryer (Shin PVTFD10R, Shinil Lab, Korea). Cryoprotectant was added to the SLN dispersion before freezing. Trehalose, mannitol, sucrose and fructose were screened at the level of 5% and 10% w/v for their cryoprotectant efficiency. Slow freezing was carried out on the shelves in the freeze dryer (shelf temperature “40 °C). The samples were lyophilized for 24 h from 40 °C to 25 °C at an increasing rate of 5 °C/h. Lyophilized products were reconstituted by sonication (2 min, 500W, Power Sonic 510, Korea).
In Vivo Studies in Rats

Arthritis Induction in Rats
Arthritis was induced in the right knee joint of each rat. On two occasions, 1 week apart, rats were injected subcutaneously with an emulsion of equal volumes of methylated bovine serum albumin (m-BSA) and Freund's Complete Adjuvant (FCA). Dose of this emulsion was 50 µl per rat per immunization cycle. Fourteen days after the second immunization, the rats were given two intra-articular injection of m-BSA (0.5 mg in 50 µl saline) into the right knee. One day after the induction dose, the rats developed inflammation in the right knee which peaked after 3 days. The development of arthritis was assessed by histopathological results [31].

Treatment of Antigen-adjuvant Induced Arthritis
The inflammation was assumed to be 100% on seventh day. Thus seven days after the intra-articular dose of m-BSA; the rats were divided into three groups (n=6). One group of rats was rubbed with plain capsaicin gel. Second group of rats was rubbed with capsaicin loaded SLNs gel onto the right knee (inflamed) and gel without capsaicin was rubbed onto the left knee joint. The last group was kept as positive control in which only gel without capsaicin was rubbed onto the right knee.

Measurement of Joint Knee Diameter
The development of arthritis was monitored at regular intervals by measuring the knee joint diameter, with the knee flexed to the same extent, using digital micrometer. The percentage average inflammation was measured by using formula.

\[
\text{% Average Inflammation} = \frac{\text{Average joint dia. of right knee} - \text{Average joint dia. of left}}{\text{Avg joint dia. of right knee day7} - \text{Avg joint dia.of left knee day7}}
\]

Histopathology Studies
4 weeks after the m-BSA challenge, representative rats from first two groups were killed. The knee joints were removed into and fixed in formalin buffer (15%) one week before histological processing. The joints were decalcified by dipping in 7% nitric acid for 5 days and were then embedded in paraffin wax (high m.p. 58°C), then sectioned at 5 µm and stained with haematoxylin/saline. The sections were evaluated under light microscopy for proliferation of fibroblasts, cartilage damage, and presence of inflammatory infiltrate and hyperplasia of synovial membrane.

Anti-inflammatory Effect Test
The formulations of capsaicin were evaluated for their anti-inflammatory activity on a carrageenan-induced rat paw edema model [32-33]. Inflammation was produced in the rats (Male, Wistar, weighing 200-250 g) using 100 µL of 1% carrageenan (wt/vol) in saline. This was injected into the plantar surface of the rats' left hind paw. To evaluate the topical anti-inflammatory activity of the formulations SLN-5 gel, plain capsaicin gel, NIO-2 gel, Zostrix® gel, groups of animals (n = 3) with carrageenan-induced paw edema were examined. Thirty minutes later, 100 µg of SLN-5 gel, plain capsaicin gel, NIO-2 gel, Zostrix® was applied topically on the edematous paw. A fifth group of rats was used as a control (gel without capsaicin). The increase in paw thickness was measured with the help of digital micrometer before (time 0) and 1, 2, 3, 4, 5, and 6 hours after carrageenan administration. The percentage of paw thickness increase from time 0 was calculated.

Skin Irritation Evaluation
Irritation evoked by experimental formulations on rat skin was microscopically judged after the end of experiments of in vivo percutaneous absorption. The site of application of each formula on the skin was excised and fixed in 10% neutral carbonated-buffered formalin for at least 24 h before routine processing. Each section was rinsed with running water, dehydrated using a graded series of ethanol solution and embedded in paraffin wax, and then frozen at –20°C prior to sectioning. The tissues were cut into small sections (6 µm) and stained with hematoxylin and eosin for histological evaluation. Sections were examined by light microscopy [34].

Statistical Analysis
Results were reported as arithmetic mean values ± standard deviation (x ± S.D.). Statistically significant differences were determined using Student’s t-test with P < 0.05 as a minimal level of significance.
RESULTS AND DISCUSSION

Formulation Optimization

A sufficient high-energy input was necessary to break down the droplets into the nanometer range \([35]\). Besides the production parameters, lipid matrix surfactant blend, viscosity of lipid and aqueous phase influenced the outcome of the procedure \([36]\). In the formulation, glycerol behenate was used as a lipid matrix as well as surfactant. It was suggested that this surface active partial glyceride facilitated emulsification and formed more rigid surfactant films and therefore improved the long-term stability of SLNs. For optimization of drug amount the formulations were prepared using varying concentrations of drug (2-12 w/w %) and these formulations were evaluated for particle size, zeta potential, morphological characterization and EE%. Many different drugs had already been incorporated in SLNs. According to professor M. Muller the prerequisite to obtain a sufficient loading capacity was a sufficiently high solubility of the drug in the lipid melt \([37]\). Relative higher drug EE% was one of the major advantages of SLNs. The maximum concentration of capsaicin that could be incorporated into the SLN-5 and NIO-2 formulations was found to be 10.0 mg and 8.0 mg with EE% of 63.5 ± 2.9% and 54.5± 4.8% respectively; on further increasing the amount of drug, drug crystals precipitated probably due to the saturation of the formulation. It should be stressed that these saturation concentrations are the total concentrations of capsaicin that could be incorporated in the formulation and not the entrapment value \([38-39]\). After one-month refrigerated storage the value only showed little decrease. Therefore, it was revealed that SLNs produced by this modified production method could achieve higher drug incorporation for lipophilic drugs like capsaicin. Drug loading was found to be 5.12%. The mean particle size and width of distribution were 100 nm and 0.374 respectively measured by LD (Mastersizer 2000) \([40]\), shown in (Figure 1), thus the drug loading SLN-5 showed a narrow distribution width and considerable small particle size. An outstanding feature of nanoparticles was the increase in saturation solubility and consequently an increase in the dissolution velocity of the compounds. According to the Kelvin and Ostwald-Freundlich \([41]\) equation, for small particles especially in the nanometer range, the saturation solubility could be increased significantly. Both the increase of saturation solubility and the enlargement of surface area contributed to the improvement of dissolution velocity by the Noyes-whitney equation \([42]\). Drugs in the form of nanoparticles (nanocrystals) had been reported to possess a full range of positive effects and the data on increase in bioavailability was quite impressive \([43]\). One aim of this experiment was to improve the bioavailability of the poorly soluble lipophilic drug by transforming it into nanoparticles. From the above discussions, it should be presumed that if the nanometer range particles could be obtained, the increase in bioavailability will become available. The zeta potential of drug loaded SLN-5 was -48.36 mV (Figure 2) Zeta potential can make a prediction about the stability of colloid dispersions. A high zeta potential (> |30| mV) can provide an electric repulsion to avoid the aggregation of particles \([44]\). The incorporation of capsaicin into SLNs found to have no influence on the zeta potentials.

Figure 1: Average Particle Size and Particle Size Distribution of SLNs Loading Model Drug

Figure 2: Zeta Potential of the Ultrafiltrated Dispersions of SLNs
Stability Studies

Vesicular formulations were stored at 4 °C and at 25 °C for 30 days. The stability testing data indicated that vesicular formulations stored at 4 °C were more stable than those stored at 25 °C. The zeta potential of different vesicular formulations stored at 25 °C were very unstable whereas the zeta potential values of formulations stored at 4 °C were more stable therefore the storage temperature of 4 °C is better than the room temperature in order to maintain favorable zeta potentials.

TEM and SEM Investigation

The TEM image (Figures 3a) shows the shape of the nanoparticles entrapping with the model drug. It was evident that the particles investigated revealed round and homogeneous shading, the particle size ranging approximately from 100 to 110 nm. SEM was used to examine the submicron size and morphology of the SLNs. The SEM image revealed that the SLNs were spherical in shape and homogeneously distributed around 100–200nm in diameter, and that the incorporation of capsaicin did not seem to cause morphological changes.

DSC Analysis

DSC was a tool to investigate the melting and recrystallization behavior of crystalline materials like SLNs (Figure 4) shows an overview of the melting process of bulk matrix, lyophilized drug-free placebo and drug loading powder. The heat flow is less than 1 mW. Thermogram of the lyophilized drug loaded SLNs did not show the melting peak of crystalline capsaicin, which indicates that capsaicin in SLNs, was in amorphous state. For this study, capsaicin and lipid were first dissolved in ethyl acetate. Subsequently, ethyl acetate was evaporated. Therefore capsaicin was dispersed in the lipid homogeneously. There are some similar results revealing that drug in SLN were in amorphous state [45-46]. The bulk glyceryl behenate melted at 82.28°C with almost the same melting point at 83°C.

In-vitro Drug Release Studies

The release rate from capsaicin loaded SLNs is an important parameter since a sustained release is necessary in order to decrease the dose dependent side effects of capsaicin and improve its therapeutic index. The drug entrapment and release rates were evaluated by dialysis method using spectrophotometric estimation [47]. Capsaicin loaded SLNs were stable in 1:1 (v/v) ethanol-pH 7.4 citrate–phosphate buffer (used as the receptor medium) and a slow release of drug from the complex was found. (Figure 5) shows the comparative % drug release from different drug loaded SLN-5 gel, SLN-5 suspension, and NIO-2gel formulations in comparison with plain capsaicin
gel, Cream Zostrix® and drug solution. Significant prolongation of capsaicin release across the dialysis membrane was achieved with the capsaicin loaded SLNs in comparison with plain drug. The cumulative amount of drug released in 1 hr from the SLN-5 gel formulation was 6.5 ± 0.3% compared with 8.89±1.2%, 14.5±0.8%, 78.75±2.8%, 45.78±0.8% and 95.8±4.1%, from SLN-5(suspension), NIO-2gel, plain capsaicin gel, Cream Zostrix® the control drug solution respectively. Capsaicin release was prolonged to 14 hr with encapsulation (94.3±3.8%). Drug release from the capsaicin was steady and slow and decreased as a function of time.

In Vitro Skin Permeation Studies

Skin permeation studies were carried out using abdomen rat skin. (Figure 6) shows % amount of capsaicin permeated across rat skin as a function of time from different formulations SLN-5gel, SLN-5 suspension and NIO-2gel, compared with marketed formulation (Zostrix®) and drug solution. The in vitro skin permeation of drug is mainly assessed by its flux, $J_{ss}$ ($\mu g/cm^2/h$). This was calculated from the slope of the linear steady portion of % cumulative amount released versus time (h) plot as shown in (figure 6). Another important parameter is diffusion lag time (LT) for the drug to reach the receptor compartment. Diffusional lag time is important parameter, which is necessary for calculating diffusivity, partition coefficient and permeability coefficient. All these skin permeation parameters for capsaicin have been calculated and reported in (Table 1). The value of transdermal flux for different formulations was observed between 22.52±0.6 to 50.20±2.3 $\mu g/cm^2/h$. This is about 1.2 to 2.5 times higher than that obtained from drug solution (18.15±0.6) and two-fold higher than Ceram (marketed formulation) and liposomal formulation. SLN-5 showed significant increase in transdermal flux to 30.43±2.5 $\mu g/cm^2/h$ indicating synergistic effects of encapsulation of capsaicin in SLNs.

**Table 1**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>$J_{ss}$ ($\mu g/cm^2/h$)</th>
<th>LT $^b$ (h)</th>
<th>$D^c$ ($cm^2/h$)</th>
<th>$P^d$ ($cm/h$)</th>
<th>$R^e$</th>
<th>$D^f$ ($\mu g$)</th>
<th>$ER^g$</th>
<th>$ER^h$</th>
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<tr>
<td>SLN-5(gel)</td>
<td>30.43±1.2</td>
<td>2.1</td>
<td>9.891</td>
<td>15.22</td>
<td>0.978</td>
<td>307.56±1.5</td>
<td>12.4</td>
<td>1.59</td>
</tr>
<tr>
<td>SLN-5 (suspension)</td>
<td>28.19±2.1</td>
<td>1.71</td>
<td>.3891</td>
<td>14.09</td>
<td>0.972</td>
<td>198.37±2.7</td>
<td>4.34</td>
<td>1.47</td>
</tr>
<tr>
<td>NIO-2</td>
<td>26.15±0.6</td>
<td>.82</td>
<td>.3773</td>
<td>9.57</td>
<td>0.951</td>
<td>45.74±1.1</td>
<td>2.23</td>
<td>1.21</td>
</tr>
<tr>
<td>Cream(Zostrix®)</td>
<td>24.52±2.3</td>
<td>2.26</td>
<td>.2952</td>
<td>11.76</td>
<td>0.953</td>
<td>158.92±2.8</td>
<td>3.47</td>
<td>1.23</td>
</tr>
<tr>
<td>Plain capsaicin gel</td>
<td>20.15±0.6</td>
<td>.82</td>
<td>.2673</td>
<td>9.57</td>
<td>0.951</td>
<td>45.74±1.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Drug Solution</td>
<td>18.15±0.6</td>
<td>1.82</td>
<td>.3673</td>
<td>9.57</td>
<td>0.951</td>
<td>45.74±1.1</td>
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</table>

$J_{ss}$ = Transdermal flux; LT = Lag time; $D^c$ = Diffusion coefficient; $P^d$ = Permeability coefficient; $R^e$ = Correlation coefficient; $D^f$ = Amt. of drug deposited in skin; ER$^g$ = Enhancement ratio of drug deposition in skin.

ER$^h$ = Enhancement ratio of drug permeation in skin
SLN-5= Nanosuspension of solid lipid nanoparticles
Skin Uptake Behaviors

There was no significant difference in cumulative amounts of capsaicin in epidermis between the cream (Zostrix®) marketed formulation, NIO-2gel and SLN-5gel as shown in Figure 7(a) (p > 0.05), while the SLN-5 gel significantly increased the cumulative uptake of capsaicin in dermis as shown in Figure 7(b) (p < 0.05). The amount of capsaicin penetrated into dermis from SLN-5gel at 12 h was nearly 2 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 increase amount of drug in the dermis is also related to the occlusion properties. Following the water evaporation from the SLNs suspension 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-coenocytes gaps [48-49].

In Vivo Studies in Antigen Adjuvant Induced Arthritis Rat

Effect on Joint Diameter

Three days after intra-articular administration of m-BSA antigen, the first sign of inflammation in the form of joint swelling became apparent. Another significant feature was reduced mobility in right limb due to the onset of arthritis. Seven days later after antigen induction, the disease reached maximum severity (assumed to be 100%). The treatment was started on eighth day (well establishes) in all the groups. One group was treated with plain capsaicin, another with SLN-5 and last with gel containing no capsaicin. The joint diameters were measured using digital micrometer. A significant reduction in joint inflammation in rats treated with SLN-5 as compared to treated with plain gel and gel without drug was observed. The percentage inflammation rate in all the groups from day 7 (time of m-BSA challenge) to day 28 is shown in (Figure 8). The treatment with plain capsaicin gel did not show much reduction in joint inflammation within the study period of seven days, whereas the treatment with drug loaded SLNs showed 25% remission in inflammation rate after the 15 days and 51% at the end of study.

Histopathology Results

Histological view of sagittal sections of the knee joint samples taken from normal rats, rats treated with SLN-5 gel and rats treated with plain capsaicin gel for both the limbs shown in (Figure 9). The knee joint section of normal rats (without induction of
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Arthritis) shows normal surface of joint cartilage and adequate joint space, whereas in case of rats with induced arthritis treated with plain SLNs gel without capsicain, erosion of cartilage, papillary infoldings of synovial membrane and irregular outer margin of cortex of the bone and also inflammatory infiltrate was seen with proliferation of fibroblast around the cortex of the bone. The knee joint sections of rats treated with drug loaded SLNs gel showed minimal papillary foldings, adequate joint space and shows possible recovery of joint cartilage as the surface of bone from both the sides. However the results obtained from the studies shows that certain damage could not be rectified as the treatment was started at the stage of well established arthritis. The results are in consonantly with early findings [49].

Anti-inflammatory Activity

It has been reported that carrageenan-induced edema can be divided into 2 phases. The first phase occurs throughout the first hour after carrageenan injection. It 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 to 5 hours after carrageenan injection. In this phase, the macrophages in carrageen and insulted dermal tissue release interleukin-1 to induce accumulation of polymorphic nuclear cells into the inflammatory area. This then releases the lysosomal enzymes and active oxygen to destroy connective tissues and induce paw swelling. In this study, the progress of the paw edema test was compatible with that found in the literature. Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness throughout the first hour after intraplantar injection of carrageenan and reached a peak of inflammation after 4 hours (Figure). The results of the paw edema test were evaluated using repeated-measures ANOVA, and the interaction was found to be significant between Factor 1 and Factor 2. This means that the paw edema differences among formulations for each hour were not similar. The difference in the increase of paw thickness between hours was significant. Because of interaction between factor 1 and factor 2, formulations were compared for each hour using 1-way ANOVA. Homogeneity of variance, analyzed using the Levene test, was observed at all hours. The Duncan test, used as post hoc analysis, found that when all formulations were compared with the control, a significant difference was found. The differences in paw thickness increase among formulations against time are shown in (Figure 9). In vitro and in vivo studies were compared. According to in vitro studies, SLN-5 had better penetration than NIO-2 gel, cream (Zostrix®), and plain capsicain gel. The result of better permeation of SLN-5 was also supported by in vivo studies.

Skin Irritation Evaluation by Pathologic Biopsy

To evaluate the influence of ingredients on the skin irritation, the skin was pathologically investigated after application of the capsicain gels. Figure 10 represents microscopic photographs of rat skin at 10 h after with or without treatment with SLN-5 gel and the commercially available product, respectively. After 10 hr of formulation application, the treatment side was observed and skin scores awarded by visual observation. The total irritation scores (TSI) of skin are listed in Table 2. The results revealed that the damage degree of skin (TSI) tended to increase slightly with the increases in penetration rate (PR). There was no significant difference (P±0.05) in TIS between the SLN-5, the penetration of which was highest, and the commercial product, indicating that the damage degree of SLN-5 was acceptable.  

![Figure 9: Percentage Increase of Paw Thickness after Sub Plantar Injection of carrageenan. Values are Means of 3 Determinations ± SD.](image)
## Table 2

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Plain drug solution</th>
<th>SLN-5(Gel)</th>
<th>Cream(Zostrix®)</th>
<th>NIO-2(Gel)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythema</td>
<td>Edema</td>
<td>Erythema</td>
<td>Edema</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
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<tr>
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<tr>
<td>6</td>
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<td>0</td>
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<tr>
<td>Ave.</td>
<td>0.83</td>
<td>0.33</td>
<td>0.50</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Scores are as defined 0 – no erythema, 1 – very slight erythema, 2 – well defined erythema, 3 – moderate to severe erythema. Similarly defined as edema.

## CONCLUSION

Capsaicin loaded SLNs can be prepared successfully by using modified high shear homogenization and ultrasound techniques as proved in previous studies. The surface structure of the formulation was spherical and rough. The encapsulation efficiencies were over 60% and the mean size was in the range of 100-150nm. The release rate of SLN-5 was much slower than that of NIO-2, cream (Zostrix®). The release pattern of the formulation was found to be of the non- fickian. Skin irritation studies indicated insignificant damage by the prepared formulation as compared to commercial formulation. According to both *in vitro* and *in vivo* studies, SLN-5 showed the best permeation and effectiveness. Our studies provided evidence that SLNs developed were valuable as topical delivery carrier to enhance the penetration of lipophillic drug capsaicin.

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## REFERENCES


