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Research Article

Formulation and Evaluation of Lidocaine Lipid Nanosystems for Dermal Delivery

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Abstract. The objective of the present investigation was to formulate solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for improving the dermal delivery of a local anesthetic agent lidocaine (LID). SLN and NLC were characterized for particle size distribution, polydispersity index, entrapment efficiency, X-ray powder diffraction pattern (XRD), thermal behavior by differential scanning colorimeter (DSC) and surface morphology by transmission electron microscopy (TEM). LID-loaded SLN and NLC were formulated into hydrogels for topical application. The *in vitro* permeation profiles of LID SLN gel, LID NLC gel, and a marketed LID formulation (Xylocaine® gel) were evaluated by using guinea pig skin. The *in vivo* efficacy of LID SLN gel, LID NLC gel, and a marketed LID formulation (Xylocaine® gel) gel was evaluated on guinea pig using pinprick test. LID SLN showed a particle size of 78.1 nm with a polydispersity index of 0.556, whereas LID NLC showed a particle size of 72.8 nm with a polydispersity index of 0.463. The entrapment efficiency of LID in both SLN and NLC was 97% and 95.9%, respectively. The TEM studies revealed the almost spherical nature of LID SLN and NLC formulations. The XRD and DSC studies of LID SLN suggested amorphization of drug in the carrier system. The SLN formulation was stable with respect to particle size, polydispersity, and entrapment efficiency for 6 months at 40°C/75% relative humidity (RH). Negligible leakage was observed for the NLC formulation when stored for 1 month at 40°C/75% RH. *In vitro* permeation studies indicated that LID SLN gel and LID NLC gel significantly sustained the LID release compared to that of Xylocaine® gel. The *in vivo* efficacy results supported the results of the *in vitro* permeation studies wherein the LID SLN gel and LID NLC gel resulted in fivefold and sixfold increase in duration of anesthesia, respectively, compared to that of Xylocaine® gel.

KEY WORDS: lidocaine; local anesthetic effect; nanostructured lipid carriers (NLC); solid lipid nanoparticle (SLN).

INTRODUCTION

Drug delivery to or via the skin presents both unique opportunities and obstacles due to skin structure, physiology, and barrier properties. The skin, the largest organ of the body, may be viewed either as a natural protective barrier against penetration of toxic exogenous compounds, excessive loss of water and other essential compounds, or as a promising portal of entry of drugs for local and/or systemic action.

Lidocaine (LID) is an effective and reliable local anesthetic of rapid onset, intermediate action, and low systemic toxicity. The marketed preparation of LID includes topical ointment, gel, and solution (marketed under the trade name Xylocaine®) which contains LID hydrochloride (HCl) in various concentrations ranging from 1% to 5% *w*/*w*. ELMA®, another successful formulation, is a eutectic mixture of LID HCl and procaine HCl (1). These formulations are characterized by immediate release and short duration of action.

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In percutaneous or dermal applications, the drug should remain on the skin surface as its uncharged, lipophilic form for a substantial time, so that it penetrates the stratum corneum and desensitize the underlying pain receptors within the skin. New topical drug delivery systems such as particulate carriers are now being designed to enable a drug to reach the desired pharmacological site of action at a controlled rate and have a sustained duration of action. Targeting of topically applied drugs to the different skin layers by using particulate carriers has become a major center of research in dermatology (2). A number of carrier systems like microemulsion, liposomes, and nanoparticles have been investigated for dermal delivery of drugs. These systems may enhance drug permeation in skin, increase duration of local action, and prevent systemic absorption of drugs thereby reducing side effects associated with the drugs (3).

Local anesthetics incorporated into liposomes have shown greater effectiveness, making shorter application periods possible as well as reduction in side effects (4, 5). Furthermore, microemulsion-based LID delivery systems were formulated which had four times higher flux rate compared to conventional emulsion (6). Combination of iontophoresis and microemulsion has also been reported to

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result in better permeation and retention of LID in the skin layers (7). Anestaform™, a new technology from Onset Therapeutics, incorporates the Delevo™ Foam Technology for delivery of local anesthetic agent in which the active ingredients is incorporated in a foam vehicle that utilizes a semisolid emollient microemulsion system to deliver the active ingredient rapidly and efficiently (8).

Solid lipid nanoparticles (SLN) have emerged as an alternative to liposomes due to various advantages such as improved physical stability, low cost compared to phospholipids, and ease of scale-up and manufacturing (9–11). Moreover, their potential in epidermal targeting (12–15), follicular delivery (16), controlled drug delivery (9), and photostability improvement of active pharmaceutical ingredients (17, 18) has been very well established.

Nanostructured lipid carriers (NLC) are the second generation of lipid nanoparticles wherein solid lipid of SLN is partially substituted by lipids which are liquid at room temperature (19). Owing to this modification, the drug is probably in the liquid lipid which in turn is surrounded by the solid lipid. This provides some degree of mobility to the drug which contributes to stability even when the solid lipid undergoes polymorphic changes. In addition, NLC may also provide for controlled drug release.

Incorporation of LID, a lipophilic agent, in lipid nanoparticulate system such as is anticipated to facilitate good loading in the carrier system and better permeability into dermal layer. An optimized lipid nanoparticulate formulation of LID is expected to have a fast onset and long duration of deep local anesthesia with reduced systemic absorption and side effects. Deep anesthesia in skin is useful to relieve pain in procedures involving the skin such as venipuncture, lumbar puncture, skin biopsy, and removal of hair in hirsutism.

This study presents the preparation, optimization, and characterization of SLN and NLC for dermal delivery of LID.

MATERIALS AND METHODS

Materials

Compritol 888 ATO (COM) and Precirol ATO 5 (PRE) were obtained as gift samples from Coloron Asia, Mumbai, India. Miglyol 810 (MIG 810) was a kind gift sample from S. Zaveri and Sons, Mumbai, India. LID was obtained as a gift sample from Exim Pharm International and Heer Pharma, Mumbai, India. Noveon AA1® (Polycarbophil) was obtained as a gift sample from Noveon, Mumbai, India. Tween 80, glycerol, sodium benzoate, and triethanolamine were purchased from S.D. Fines, Mumbai, India. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Preparation of LID SLN, NLC, and Nanoemulsion

The various process parameters for SLN, NLC, and nanoemulsion (NE) preparation are mentioned in Table I. LID SLN, NLC, and NE were prepared using the ultrasound dispersion method. Briefly, lipid mixtures of COM and PRE in different ratios (as indicated in Table I) were melted at temperatures of 85–90ºC. LID was added to the above molten lipids. Furthermore, Tween 80 solutions of 10 and 20 mM concentrations at temperatures of 85–90ºC were added to the lipid phase to form coarse emulsion. The coarse emulsion was sonicated using Sonifier® Model 250 (Branson Ultrasonics, USA) probe sonicator at 30 W for 1 to 4 min (as indicated in Table I). The resulting nanosuspension was cooled at 4°C (ice bath) to form SLN.

LID NLC was prepared in the same manner as LID SLN. The lipid mixture comprised of COM and MIG 810 at varying concentrations (Table II). Tween 80 solution at 20 mM concentration was used and the sonication time was 4 min.

LID NE was prepared in the same manner as that of LID SLN in which the solid lipid was replaced with MIG 810. Tween 80 was used at 20 mM concentration and the sonication time was 4 min.

The lipid load (the total amount of lipid present in the formulation) for SLN, NLC, and NE formulations in optimization and *in vitro* permeation study was 2.5% *w*/*w*. Furthermore, for *in vivo* efficacy test, SLN, NLC, and NE formulations containing 2% *w*/*w* of LID were prepared using 5% *w*/*w* lipid load and drug was added in the ratio of 1:2.5 with respect to the amount of lipid used, respectively.

Characterization of LID SLN and NLC

Determination of Percent Entrapment Efficiency

The SLN and NLC dispersions were diluted with distilled water and centrifuged using Minispin (Eppendoff, United States) at 13,200 rpm for 25 min. The lipid mass at the top was separated following which LID was extracted with methanol and absorbance was measured at 262 nm on a V-530 UV-visible spectrophotometer (Jasco, Japan) against similarly treated blank formulation. The percent entrapment efficiency (%EE) was calculated based on the following formula:

$$\%EE = (A/B) \times 100$$

where A is the amount of LID entrapped in the lipid and B is the initial amount of LID added in the formulation.

Particle Size Distribution

The average particle size and polydispersity index of the lipid particulate dispersions obtained via various experiments were determined in duplicate by the photon correlation spectroscopy N5 Submicron Particle Size Analyzer (Beckman Coulter, Wipro, India). Measurements were carried at an angle of 90° at 25°C. Dispersions were diluted with double-distilled water to ensure that the light-scattering intensity was within the instrument's sensitivity range. Double-distilled water was filtered through 0.45 μm membrane filters (Pall Life Sciences, Mumbai, India) prior to determination of particle size.

Transmission Electron Microscopy

Morphology of the LID SLN and LID NLC was studied by transmission electron microscopy (TEM). The particulate carriers were negatively stained using uranyl acetate. The stained grid was then air dried and examined immediately

Table I. Formulation Parameters and Characterization of LID SLN (n=3)

Formula no.	Lipid composition COM/PRE	Surfactant concentration (mM)	Sonication time (min)	Entrapment efficiency ± SD (%)	Mean particle size ± SD (nm)	Polydispersity index ± SD
BI	1:9	10	1	73.05±0.982	124.25±4.737	0.481±0.0197
BII	1:1	20	4	92.15±0.495	74.25±5.161	0.684±0.0919
BIII	9:1	10	1	78.52±0.134	247.85±2.333	0.685±0.0063
BIV	9:1	20	4	97.37±1.414	78.1±2.687	0.556±0.0862
BV	9:1	10	4	89.43±1.414	120.1±1.414	0.344±0.0516

under Jel-1010 Electron microscope (Jeol, Japan). The electron micrographs were obtained in the range of ×2,500 to ×120,000 magnifications.

Differential Scanning Colorimeter Studies of LID SLN

SLN without LID and LID-loaded SLN were freeze dried. The thermograms of individual components and of SLN were recorded on Chromatopac R6A (Shimadzu, Japan) thermal analyzer. An accurately weighed amount (5 mg) of individual components was transferred to aluminum pans and the samples were scanned from 30°C to 110°C at the heating rate of 5°C/min using an empty aluminum pan as reference.

X-ray Diffraction Pattern Studies of LID SLN

X-ray diffraction patterns (XRD) were recorded to evaluate the physical nature of the formulations. No-drug SLN and LID-loaded SLN were freeze dried so as to keep the formulation in powdered form. XRD patterns were recorded on a Philips Analytical X'Pert PRO Powder X-ray diffractometer at a scanning rate of 1°min^{-1} between 10° and 50° 2θ range.

Stability Studies of LID SLN and LID NLC

The optimized SLN and NLC dispersions were studied for stability. Samples were stored in amber-colored glass vials with rubber closures and sealed with aluminum caps. The LID SLN formulations were stored at 4±2°C, 25±2°C/60±5% relative humidity (RH) and 40±2°C/75±5% RH for a period of 6 months and aliquots were withdrawn at time intervals of 0, 15, 30, 60, 90, and 180 days. LID NLC formulation stored at 4±2°C, 25±2°C/60±5% RH and 40±2°C/75±5% RH for a period of 1 month and aliquots were withdrawn at time intervals of 0, 7, 14, 21, and 30 days.

The nanoparticulate dispersions were evaluated with respect to percent LID retained, particle size, and polydispersity index.

Formulation of Topical Gel of SLN, NLC, and NE

The SLN, NLC, and NE were prepared in two strengths of 1% and 2% LID for *in vitro* and *in vivo* efficacy test, respectively. Briefly, 0.3 g of accurately weighed Noveon AA1, 30% glycerol, and 0.1% sodium benzoate was added to SLN, NLC, and NE dispersion containing LID. The total amount of LID topical gel was 10 g. The mixture was kept aside for 1 h for swelling and was intermittently stirred with a glass rod. Later, triethanolamine was added dropwise to make the pH at 7–7.5. The topical gels containing LID were prepared 1 day before the start of the experiments.

In Vitro Skin Permeation Studies of LID SLN, NLC, and NE

In vitro permeation of LID from the lipid nanoparticulate-based gel and marketed formulation (Xylocaine® gel, AstraZeneca, India) was evaluated using hairless abdominal guinea pig skin samples excised from animals aged about 3–4 months. The guinea pigs (weight range, 300–400 g) were obtained from Haffkine Institute, Mumbai, India.

The skin samples were mounted on modified Franz diffusion cell with a surface area of 6 cm^2 and a receptor volume of 17 ml such that the dermal side of the skin was exposed to the receptor fluid and the stratum corneum remained in contact with the donor compartment. The receptor compartment consisted of phosphate buffer pH7.2. Accurately weighed gel formulations (Xylocaine®, SLN, NLC, or NE) corresponding to 5 mg of LID was applied on the donor compartment side of the skin. The donor compartment was covered with an aluminum foil to avoid evaporation. The temperature was maintained at 37±0.5°C. One milliliter of receptor medium was withdrawn at time intervals of 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 15, 18, and 24 h and

Table II. Composition and Characterization of LID NLC (n=3)

Formula no.	Compritol/total lipid (% *w/w*)	Miglyol 810/total lipid (% *w/w*)	Entrapment efficiency (%)	Mean particle size (nm)	Polydispersity index
N1	95	5	91.25±0.561	74.3±3.654	0.487±0.0121
N2	90	10	95.96±0.985	72.8±2.135	0.463±0.0421
N3	85	15	92.15±1.234	76.1±1.654	0.456±0.0712
N4	80	20	89.89±0.265	83.7±3.012	0.571±0.0257
N5	60	40	53.95±2.365	78.2±4.251	0.601±0.0719

was replaced by the same volume of fresh buffer to maintain the sink condition. The absorbance value of the solutions was read on a UV-visible spectrophotometer at 262 nm against that of aliquots withdrawn from skin permeation study of the blank formulation at corresponding time points and the LID content in the aliquot withdrawn was determined with reference to the standard plot.

The amount of drug retained in the skin was calculated by the formula:

$$\%\ \text{LID retained in the skin} = \text{Total amount of LID in the gel} - \left[\text{Amount of the drug permeated at the end of 24 h} + \text{Amount of LID } \textit{on} \text{ the skin at the end of 24 h}\right].$$

In Vivo Efficacy of the LID SLN and NLC Gel Formulations

The activity of the LID SLN, LID NLC, and LID NE was compared to a marketed LID gel (Xylocaine®) containing 2% LID HCl. The topical gel of LID SLN, LID NLC, and LID NE was prepared as described for *in vitro* studies, except that the content of LID was 2% *w*/*w*.

In vivo efficacy was evaluated on male guinea pigs (n=6). The protocol for the study was approved by the Institutional Ethics Committee of Bombay College of Pharmacy, Mumbai, India and the study was done in accordance to the CPCSEA protocol. *In vivo* efficacy was evaluated by employing the pinprick test for accessing the local anesthetic activity (5, 20). The guinea pigs respond to the pin prick with a shivering reflex. The lack of shiver can serve as an indicator of the local anesthetic effect.

The hairs on the back of the guinea pigs were shaven with a clipper and the remaining hairs were removed using a depilatory with a rest period of 24 h to recover from any skin injury that might have occurred during shaving of the skin.

An area of 6.25 cm^2 was marked on both the sides of the shaven back of the guinea pigs. A single dose each of accurately weighed gel formulations (Xylocaine®, SLN, NLC, or NE) corresponding to 10 mg of LID was applied on the 6.25-cm^2 area to the shaven back of the guinea pig. The treated area was covered with Parafilm® to provide occlusion for 30 min. A modified lancet was prepared by inserting a sterile lancet in a pen-like device that is used by diabetic patients for routing blood glucose determination and used. The advantage of this device over a needle is that it provides more uniform stimulus intensity and minimizes probable skin injury. Response was checked immediately after removal of the occluding tape and at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480, 510, and 540 min. A washout period of 48 h was given to the animals before the start of next experiment. The same guinea pig was used for testing of conventional marketed gel (reference), gels containing blank SLN/NLC/NE, and gels containing LID SLN/NLC/NE to avoid intersubject differences. Gels of "blank" SLN/NLC (no drug) served as vehicle control and test was also performed on untreated skin which served as negative control. All preparations were tested on all the six guinea pigs (n=6).

RESULTS

Preparation of LID SLN and LID NLC

The ultrasound dispersion method was found satisfactory for the preparation of LID SLN and NLC. The colloidal dispersions were found satisfactory with respect to visual observation and did not sediment in 15 days.

Characterization of LID SLN and NLC

%EE of LID SLN and NLC

Tables I and II give the process parameters and characterization of LID SLN and LID NLC, respectively.

The %EE of LID in SLN varied from 70% to a maximum of 97%. Formula BIV SLN prepared with lipid mixture as 9:1 of COM/PRE, surfactant concentration as 20 mM, and sonication time of 4 min had the maximum entrapment efficiency of 97%.

The optimized LID NLC formula N2 was found to have 90% *w*/*w* of COM and 10% *w*/*w* of MIG 810 as the lipid mixture. The surfactant concentration and sonication time was kept unchanged at 20 mM and 4 min, respectively. This formula was found to have 95.96% entrapment efficiency.

Mean Particle Size (in Nanometers) of LID SLN and NLC

The LID SLN formulations were found to have narrow size distribution with the particle size varying from 70 to 250 nm (Table I). Formula BII exhibited the least particle size of 70 nm followed by formula BIV which had the mean particle size of 78 nm. Formula BII was prepared using the same process parameters as that of formula BIV, except that the lipid ratio was 1:1 of COM/PRE. The mean particle size of LID NLC varied from 72 to 84 nm. The optimized LID NLC formula N2 had a particle size of 72.8 nm (Table II).

Polydispersity Index of LID SLN and NLC

The polydispersity index is a ratio that gives information about the homogeneity of the particle size distribution in a given system. Ideally, it should be <0.3 (21). A small ratio of polydispersity index indicates narrow size distribution of the system. LID SLN and NLC had the polydispersity index in the range of 0.3 to 0.8. This indicates that the LID SLN and NLC had a narrow size distribution. The polydispersity index of LID SLN formula BIV was found to be 0.556 and LID NLC formula N2 showed a polydispersity index of 0.463.

Transmission Electron Microscopy of LID SLN and NLC

The TEM studies were carried out to get more insight about the morphology of the nanoparticulate systems. TEM photomicrographs of SLN formula BIV (Fig. 1) and NLC formula N2 (Fig. 2) reveals the almost spherical shape of LID

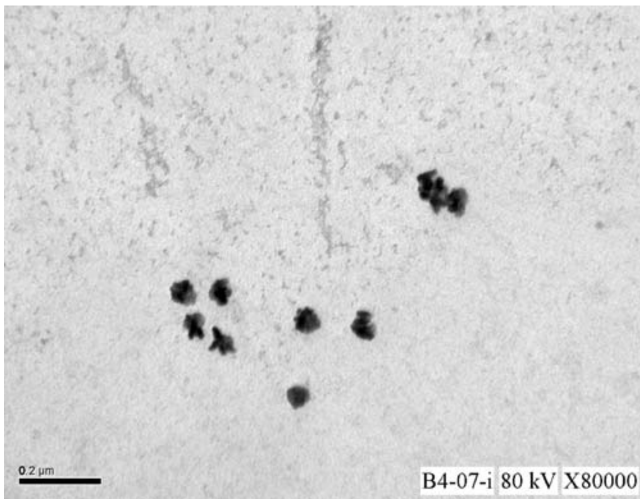

Fig. 1. TEM images of LID SLN

SLN and NLC with some irregularities at the periphery of the particulate.

The particle size as determined by the TEM was in line with that determined by the PCS.

DSC Studies of LID SLN

Figure 3 shows the differential scanning colorimeter (DSC) thermograms of the LID SLN formulation along with its individual components.

LID is a crystalline molecule with a sharp endotherm having onset temperature at 62.6°C, peak transition temperature at 70.4°C, and recovery temperature at 86.0°C.

The SLN without LID and LID SLN dispersions were freeze dried to protect the physical state of the lipids. Trehalose was used as the cryoprotectant in the ratio of 1:10. SLN without LID showed a broad endothermic peak at 69.5°C, whereas LID SLN exhibited a broad endothermic peak at 66.3°C. The height of the endotherm in the thermogram of LID SLN is less than that for the physical mixture (composed of LID, COM, PRE, Tween 80, and trehalose in the same ratio as that of LID SLN).

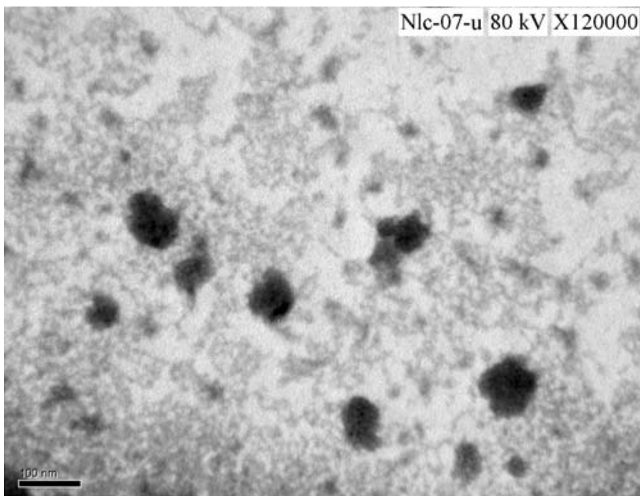

Fig. 2. TEM images of LID NLC

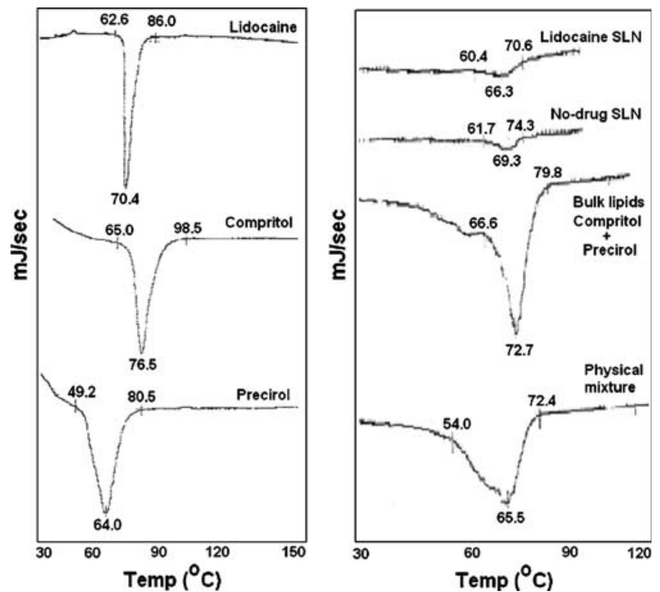

Fig. 3. DSC thermogram scans of LID SLN and its components

XRD Studies of LID SLN

Figure 4 shows the XRD patterns of LID, COM, PRE, COM/PRE (9:1) mixture, trehalose, physical mixture, freeze-dried SLN without LID, and freeze-dried LID SLN. X-ray scans of SLN confirmed that the individual components lost their crystalline nature when incorporated into SLN. LID powder was highly crystalline as evident from sharp peaks in the scan. XRD patterns of freeze-dried SLN did not display a sharp peak, indicating at least partial loss of crystalline nature. It is to be noted that a sharp peak seen at the 2θ value of around 25° in the X-ray scan of SLN without LID,

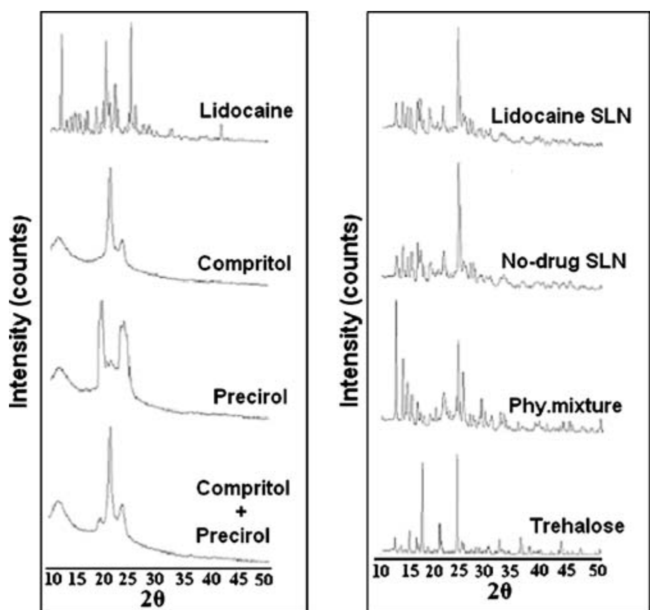

Fig. 4. XRD pattern of LID SLN and its components

LID SLN, physical mixture with LID, and trehalose which is one of the peaks characteristic of trehalose.

In Vitro Permeation Study of LID SLN, LID NLC, and LID NE

Table III lists the LID formulations that were studied for *in vitro* permeation studies and the results are shown in Fig. 5.

Marketed gel Xylocaine® showed complete permeation of LID at the end of 6–8 h. The LID NE exhibited a moderate onset of permeation and complete permeation of LID was observed at the end of 12 h. LID SLN provided biphasic permeation pattern wherein about 50% of drug was permeated in 6–8 h followed by a sustained release till 24 h. The LID SLN formula BIV showed uniform drug permeation profile. During the initial 1–2 h of the study, burst of LID permeation from SLN was observed. Furthermore, the system was able to achieve a slow permeation for 6–8 h wherein about 50% of the entrapped drug was permeated. The SLN system was able to sustain the LID release up to 24 h whereby complete permeation of LID was observed. The LID NLC showed a slow permeation with 25% of LID permeation at the end of 8–10 h and 47% of LID permeated at the end of 24 h and 50% of LID was retained in the skin after 24 h.

In Vivo Performance of LID SLN, LID NLC, and LID NE

The *in vivo* efficacy testing was performed for the following LID formulations: Xylocaine®, untreated skin (control), blank NE, LID NE, blank SLN, LID SLN, blank NLC, and LID NLC. The guinea pig response to the pinprick test for various formulations is shown in Fig. 6.

The guinea pigs with untreated skin responded by giving a shivering response on every prick. Treatment of the skin with blank SLN/NLC did not cause much change in the responses compared to those by untreated skin confirming that blank SLN/NLC themselves did not contribute to local anesthetic action.

The conventional marketed gel formulation (Xylocaine®) had a fast onset of action and the skin was totally anesthetized, but the total duration of anesthesia lasted for only 1 h. Deep local anesthesia is the duration in which the number of painful pricks felt is zero and total duration of anesthesia is duration in which the number of painful pricks is ≤4 (5, 20). The LID SLN formulation BIV had a moderate onset of action and the total time of deep local anesthesia lasted for 2 h, while the total duration of anesthesia was up to 6 h which is significantly longer than that of Xylocaine® (p value <0.05).

The LID NLC formulation N2 showed a moderate onset of action of 1 h and the total time of deep local anesthesia lasted for 2.5 h, while the total duration of anesthesia was up to 7 h which is significantly longer than that of Xylocaine® (p value <0.05).

Stability of LID SLN and LID NLC Formulations

LID SLN. The LID SLN formulations did not show visual physical instability up to a period of 6 months. The SLN formulations were stable with respect to particle size and polydispersity index. The LID SLN formulations were stable at 5°C (except formula BI) and no significant drug leakage (p value <0.05) was observed. SLN formula BII was stable at all the temperature conditions and showed insignificant drug leakage (p value <0.05) compared to the initial amount of 92% and was able to retain 90% of LID for 6 months at 40°C and 75% RH. The other SLN formulations showed varying amounts of drug leakage at 25°C and 40°C. SLN formulation BIV was stable with respect to particle size at all the temperature conditions and showed insignificant (p value <0.05) change in particle size compared to the initial size of 76.23 nm with that of 75.43 nm at the end of 6 months at 40ºC and 75% RH.

LID NLC. The LID NLC formula was stable with respect to visual physical stability, particle size, polydispersity index, and drug leakage up to 1 month. Insignificant (p value <0.05) amount of LID leaked during the storage period of 1 month and ~95% of LID was retained by the NLC at 40°C.

DISCUSSION

Use of COM and PRE for the preparation of SLN and NLC has been reported in the literature. It was thought worthwhile to use mixtures of the lipids to evaluate the influence of composition on entrapment efficiency. It was found that increasing the ratio of COM in the SLN formulation increases the entrapment efficiency. Furthermore, under the set of experimental conditions, the liquid lipid amount plays a crucial role with respect to entrapment efficiency of LID NLC. Increasing the liquid lipid beyond 10% decreased the entrapment efficiency. Also, decreasing the liquid lipid below 10% resulted in the decrease in entrapment efficiency. One probable reason for this could be that the increased lipid amount was not accommodated by the solid lipid and hence expelled out. Further decreasing the

Table III. Percent Drug Entrapment, Particle Size, and Polydispersity Index of Different Formulations Which were Studied for *In Vitro* and *In Vivo* Efficacy Testing of Prepared LID NLC, SLN, and NE

Formula	Drug entrapment (%)	Mean particle size (nm)	Polydispersity index
NLC	95.96	72.1	0.4315
SLN	97.37	76.6	0.556
NE	Not determined	65.2	0.4012

NLC nanostructured lipid carrier, *SLN* solid lipid nanoparticle, *NE* nanoemulsion

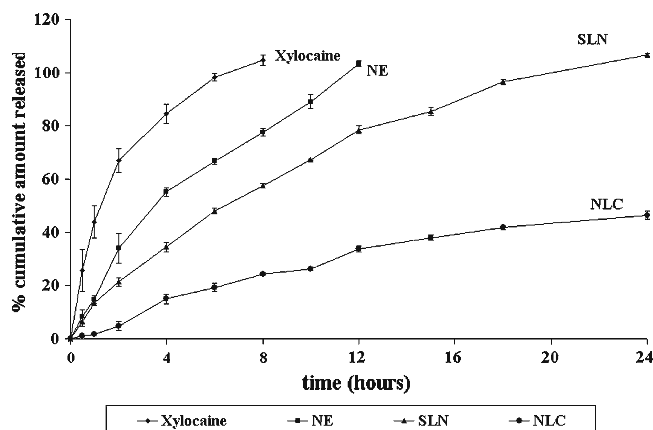

Fig. 5. *In vitro* permeation of different LID formulations across guinea pig skin ($n=3$)

liquid amount beyond 10% was not sufficient enough to hold LID.

It has been reported that decreasing the particle size results in an occlusive effect on the skin layer which leads to long duration of action of the drug. The present work revealed that within experimental parameters, as the amount of COM in SLN formulation increases, the particle size also increases. One probable reason for this could be the increase in viscosity of molten lipid phase which results in increase in the particle size of SLN preparation.

TEM photomicrographs of LID SLN formula BIV and NLC N2 formulations reveals the almost spherical shape of LID SLN and NLC with some irregularities at the edges of particulate carriers. The irregularities could be due to the method of preparation. The probe sonicator emits high-frequency waves which might cause irregularities on the particle surface compared to the solvent diffusion and solvent injection methods which are reported to result in smoother and spherical surface.

The physical state of lipids affects the permeability, leakage rates, and overall stability of the SLN (22–24). DSC studies give an insight of the phase transition in SLN that are responsible for the change in physical state of the system. The phase transition occurring in triglycerides (α, β', and β) is well documented in the literature. The height of the endotherm in the thermal scan of LID SLN is less than that of the physical mixture which was formulated with the same composition and ratio as that of LID SLN. This suggests loss of crystallinity of the lipids and LID after their incorporation into SLN. These results were further confirmed by the XRD studies where the XRD patterns of freeze-dried SLN did not display a sharp peak, indicating at least partial loss of crystalline nature.

The *in vitro* permeation studies were performed using formulations containing 1% *w*/*w* of LID. These formulations were found to be almost similar with respect to drug loading, particle size, and the polydispersity index (data not shown). *In vitro* permeation studies of marketed LID gel formulation Xylocaine® showed a fast onset and short duration of action which could be attributed to the fact that the gel contained LID as HCl salt which is water-soluble and permeated easily from the aqueous gel base. The LID NE exhibited a moderate onset of action. This can be accounted for the good solubilization capacity of the oil which retained the drug in it and lead to an initial slow release. The LID NE which lacks solid lipid matrix failed to retard the permeation of LID and complete permeation of LID was observed at the end of 12 h.

It has been reported that as the lipid content of the SLN dispersion increases, the formulation turns into gel with particulate aggregation and provides increase in skin permeation (22–24). Also, though surfactants help in preserving the lipid polymorphism, they may also act as permeation enhancers (9, 23). The LID SLN formula BIV showed uniform permeation profile. The burst in LID permeation from SLN in the initial 1–2 h could be due to SLN-free LID in the dispersion and LID adsorbed onto the surface of SLN. Furthermore, the system was able to achieve a slow permeation for 6–8 h wherein about 50% of the entrapped drug permeated. This can be attributed to the matrix of solid lipids in SLN compared to the mobile oily phase of MIG 810 in NE which facilitated faster and complete drug permeation in 10–12 h. Conversion of the α and β' states of COM to stable β ones could also have contributed to the drug expulsion which was available for permeation (9, 22, 23). The LID NLC system showed <50% LID release at the end of 24 h. The liquid lipid in the NLC provided a kind of crystalline imperfection in the lipid carrier system, which could have lead to retardation of lipid polymorphism (19) and hence provided better retention of LID in the system. Partitioning within and from lipids could have further retarded the LID release.

The *in vivo* efficacy studies were performed by preparing 2% *w*/*w* of LID. The LID SLN, NLC, and NE formulations had a lipid load of 5% *w*/*w* and the drug was added in the ratio of 1:2.5. The *in vivo* efficacy of the prepared LID lipid nanoparticulate systems was evaluated on guinea pigs using the pinprick test. The conventional marketed gel formulation had a fast onset of action and the skin was totally anesthetized. This indicated that the ionized form of LID penetrated much more rapidly than the free base but was rapidly absorbed and cleared off by the cutaneous capillaries (5). The LID SLN and NLC formulations had a moderate onset of action and the total time of deep local anesthesia lasted for 2–2.5 h, while the total duration of anesthesia was up to 6–7 h. The LID SLN/NLC hydrogel was comprised of LID unentrapped as well as the LID entrapped in SLN/NLC.

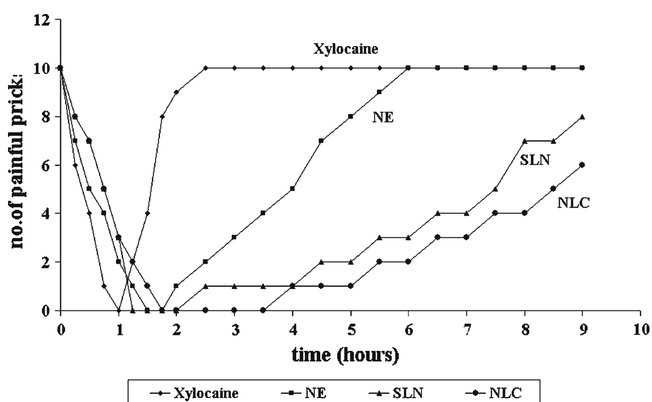

Fig. 6. *In vivo* efficacy of different LID formulations in guinea pig using pinprick model ($n=6$)

Initially, the SLN/NLC on the skin surface would release the drug slowly which along with the free drug in the hydrogel matrix would permeate the stratum corneum. In addition, the SLN/NLC along with the drug would penetrate the upper part of stratum corneum and get mixed with the skin lipids thereby acting as a depot from which the drug was released slowly.

The LID SLN/NLC dispersion were stored in amber-colored glass vial as light induced gelation tendency and hence particulate aggregation is reported in these systems (22, 25). The surfactant concentration plays a crucial role in modifying and preserving the β′–β modification (22). Also, due to the polymorphic transitions occurring in lipids, as time passes, the recrystallization index increases and the carrier is converted into more of crystalline nature resulting in expulsion of the drug from the carrier. LID SLN formulae BII and BIV were formulated using 20 mM Tween 80 which could have stabilized the lipid carrier system from LID leakage. The LID NLC formula was stable with respect to visual physical stability, particle size, polydispersity index, and drug leakage up to 1 month.

CONCLUSION

LID can be incorporated into SLN and NLC by the ultrasound dispersion method to give a stable system. LID SLN and NLC formulated as gel provided slow *in vitro* permeation of LID and, when applied topically, provided long duration of deep local anesthesia in guinea pigs.

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