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Development of SLN and NLC Enriched Hydrogels for Transdermal Delivery of Nitrendipine: In Vitro and In Vivo Characteristics

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The purpose of this research was to investigate novel particulate carrier systems such as solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLC) for transdermal delivery of nitrendipine (NDP). For this investigation, four different gel-forming agents were selected for hydrogel preparation. Aqueous dispersions of lipid nanoparticles made from trimyristin (TM) were prepared by hot homogenization technique followed by sonication and then incorporated into the freshly prepared hydrogels. The particle size was analyzed by photon correlation spectroscopy (PCS) using Malvern zetasizer, which shows that for all the tested formulations, more than 50% of the particles were below 250 nm after 90 days of storage at room temperature. DSC analysis was performed to characterize the state of drug and lipid modification. Shape and surface morphology were determined by scanning electron microscope (SEM) and transmission electron microscope (TEM), which revealed fairly spherical shape of the formulations. The antihypertensive activity of the gels in comparison with that of oral NDP was studied using desoxy corticosterone acetate (DOCA)-induced hypertensive rats. It was observed that both carbopol SLN (A1) and carbopol NLC (B1) gels significantly controlled hypertension from the first hour ($p < .05$). The developed gels increased the efficacy of NDP for the therapy of hypertension. Both the SLN and NLC dispersions and the gels enriched with SLN and NLC possessed a sustained drug release over a period of 24 h, but the sustained effect was more pronounced with the SLN and the NLC gel formulations. Further, they were evaluated for zeta potential, entrapment efficiency, in vitro release, ex vivo permeation, and skin irritation studies.

Keywords solid lipid nanoparticles; nanostructured lipid carrier; hydrogel; transdermal delivery; nitrendipine; antihypertensive

INTRODUCTION

The purpose of topical and dermatological dosage forms is to conveniently deliver drug molecules across a localized area of the skin. To develop an ideal dosage form, one must take into account the flux of the drug across skin, the retention of the dosage form on the skin's surface, the reservoir capacity of the dosage form, and the patients' acceptability of the formulation. Drug delivery from colloidal systems such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) dispersed in a hydrogel appears to be unique when compared with the delivery from traditional topical and dermatological formulations. Lipid matrices with a brick wall structure where the drug is molecularly dispersed, such as SLN of matrix model or of drug-enriched core model, may exhibit sustained release properties. Alternatively, the presence of a liquid core where the drug molecules have more freedom to move may accelerate the release rate. Therefore, SLN and NLC based semisolid systems can provide optimum drug delivery profiles.

SLN and NLC are colloidal lipidic systems that have been proposed for several routes of administration, such as parenteral, oral, and topical route (Mehnert & Mäder, 2001; Müller et al., 1995). Both carrier types are based on solid lipids; however, they can be distinguished by their inner structure. SLN consist of pure solid lipids, and NLC contain a certain

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percentage of additional liquid lipids leading to imperfections in the crystal lattice. The main difference between SLN and NLC is the fact that the concept of the latter is performed by nanostructuring the lipid matrix, in order to increase the drug loading and to prevent its leakage, giving more flexibility for modulation of drug release. This approach is achieved by mixing solid lipids with liquid lipids in NLC, instead of mixing highly purified lipids with relatively similar molecules in SLN. This mixture has to be solid at least at 40°C. The result is a less ordered lipid matrix with many imperfections, which can accommodate a higher amount of drug (Müller, Radtke, & Wissing, 2002a; Müller, Radtke, & Wissing, 2002b; Müller, Radtke, & Wissing, 2004; Müller & Wissing, 2003).

Concerning topical administration, these systems possess occlusive properties because of film formation on the skin surface. They reduce the transepidermal water loss (de Vringer & de Ronde, 1995) and therefore enhance the penetration of drugs through the stratum corneum by increased hydration (Jenning et al., 1999). It has also been reported that the occlusion factor of SLN and NLC is related to their particle size, that is, it increases with the decrease of the mean particle diameter (Wissing, Lippacher, & Muller, 2001). Lipid aqueous dispersions with small nanoparticles and narrow size distributions are preferably obtained by emulsification of the molten matrix lipid in a hot aqueous phase with adequate emulsifying agents and subsequent crystallization of the dispersed lipid matrix (Bunjes, Koch, & Westesen, 2003). A clear advantage of the use of lipid particles as drug carrier systems is because the matrix is composed of physiological components, that is, excipients generally recognized as safe (GRAS) status for oral and topical administration, which decreases the danger of acute and chronic toxicity. Therefore, our systems were prepared by hot homogenization technique followed by sonication. Concerning liquid dispersions of lipid nanoparticles, they usually need to be incorporated in convenient dosage forms to obtain a topical application form having the desired semisolid consistency.

The main purpose is to investigate the latest developments of innovative solid lipid carriers, particularly SLN and NLC, for transdermal delivery of nitrendipine (NDP). In this study, NDP, a dihydropyridine calcium antagonist which has a very low solubility in vitro, has been used as a poorly water-soluble model drug for the study of the transdermal features of aqueous SLN and NLC dispersions. It is a potent peripheral vasodilator, which effectively reduces blood pressure (BP), when given at doses of 5–20 mg per day. After single, 20-mg oral dose of NDP, peak plasma concentrations (which vary widely from 10–50 μg/L) are achieved within 1–2 h. It was reported to be well absorbed following oral administration but undergoes extensive first-pass metabolism, leading to poor bioavailability of 10–20%. In addition to pharmacokinetic properties, NDP has low dose, low molecular weight (360.4), extensive first-pass effect, and lipophilic nature (octanol/water partition coefficient 2.88). Once the poor solubility of the drug in the carrier matrix results in a low affinity of the molecule for the carrier and, therefore, poor in vivo bioavailability, different lipid matrices have been chosen according to the solubility of the selected drugs in several lipids. All the above properties are enough indicators that NDP might be a good choice as a drug candidate for transdermal delivery. DSC analysis is used to investigate the crystalline structure of the SLN/NLC and the drug. Characterization of degree of lipid crystallization and lipid modification is helpful in understanding the drug incorporation and release pattern. In vitro drug release studies are important to understand the in vivo performance of the dosage form.

The aims of this study were to (a) develop SLN and NLC enriched hydrogels for transdermal delivery, (b) perform in vitro and in vivo permeation studies through rat skin, and (c) evaluate the efficacy of transdermal gels against hypertension-induced rats. The purpose was to provide the delivery of the drug at a controlled rate across intact skin to improve bioavailability and hypertension control for longer period from transdermal gels.

This article was also designed to assess the possibility to develop SLN and NLC based semisolid formulations using a well established hydrogel as transdermal vehicle. They were also evaluated for their particle size, zeta potential, entrapment efficiency, occlusive properties, and skin irritation studies.

MATERIALS AND METHODS

Materials

The following materials were used from the indicated sources without further purification procedures. NDP was a kind gift from U.S. Vitamins (Mumbai, India). Trimyristin (TM) (Dynasan 114) was generously supplied by Sasol (Witten, Germany); Captex 355 EP/NF (triglycerides of caprylic and capric acid) was donated by Abitec Corporation (Janesville, Winsconsin, USA); Soy phosphatidylcholine 99% (Epikuron 200) was donated by Degussa Texturant Systems (Deutschland, Hamburg). Tween 80 and dialysis membrane-70 were purchased from Hi-Media (Mumbai, India). For hydrogel preparation, Carbopol 934 (polyacrylate) was purchased from BF Goodrich (Cleveland, Ohio, USA), Xanthan gum was purchased from Sigma-Aldrich (Mumbai, India), hydroxy propyl cellulose (HPC) was purchased from Hi-Media (India). Chitosan (degree of deacetylation, 80.8%) was gifted by Central Institute of Fisheries Technology and India Sea Foods (Kochi, India). Centrisart filters (molecular weight cutoff 20,000) were purchased from Sartorius (Goettingen, Germany). The other chemicals were of analytical reagent grade.

Methods

Preparation of Aqueous SLN and NLC Dispersions and Gels

NDP (0.2%, wt/vol), Dynasan 114 (5%, wt/vol), and phosphatidylcholine 95% (2%, wt/vol) were dissolved in 10 mL mixture of chloroform and methanol (1:1). Organic solvents were completely removed using a rotoevaporator (Laborota 4000, Heidolph, Germany). Drug-embedded lipid layer was melted by

heating at 58°C above melting point of the lipid. An aqueous phase was prepared by dissolving Tween 80 (1%, wt/vol) in double distilled water (sufficient to produce 10 mL of preparation) and heated to same temperature of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out (at 12,000 rpm and temperature 70°C) using a Diax 900 homogenizer (Heidolph, Germany) for 3 min. Coarse hot oil in water emulsion so obtained was ultrasonicated (12T-probe) using a Sonoplus ultrahomogenizer (Bandelin, Germany) for 20 min. Nitrendipne solid lipid nanoparticles (NDPSLN) were obtained by allowing hot nanoemulsion to cool to room temperature.

NLC were prepared in exactly the same manner as the SLN dispersions, only partially replacing 30% of the solid lipid matrix by Captex 355 EP/NF (caprylic/capric triglycerides).

Gels Enriched with Lipid Nanoparticles

Gels were prepared using four polymers: namely, Carbopol 934 (1%), Xanthan gum (1%), HPC (2%), and Chitosan (1%). For the preparation of hydrogel, the gel-forming polymer was dispersed in double distilled water containing glycerol (10%). Aqueous SLN and NLC dispersions (20%) and hydrogels were mixed in a high-speed stirrer (Remi, Mumbai, India) at approximately 100 rpm for 5 min to yield gels containing a final concentration of 5% lipid nanoparticles. The hydrogels composed of carbopol 934 and chitosan were adjusted to pH 6.5 and 4.0, respectively. The SLN and NLC dispersions were used as reference. The SLN and NLC loaded NDP hydrogels were stored at room temperature for 90 days.

EVALUATION OF SLN AND NLC DISPERSION AND GEL

Physicochemical Properties

The SLN and NLC dispersions were characterized for their physicochemical properties such as color, odor, and stability after centrifugation. Centrifugation was performed at 200 *g* for 30 min. The gels were evaluated for color, odor, and pH.

Measurement of Size and Zeta Potential

Size and zeta potential of NDPSLN and nitrendipne nanostructured lipid carrier (NDPNLC) were measured by photon correlation spectroscopy (PCS), with the help of Malvern zeta sizer. Samples were diluted appropriately with the aqueous phase of the formulation for the measurements, and the pH of diluted samples ranged from 6.8 to 7.4. Zeta potential measurements were done at 25°C, and the electric field strength was around 23.2 V/cm.

Assay and Entrapment Efficiency

The prepared NDPSLN/NDPNLC dispersions (0.2 mL) or gel (0.5 g) was diluted to 10 mL with chloroform/methanol (1:1). Final dilution was made with mobile phase, and NDP content was determined by HPLC.

The entrapment efficiency of the system was determined by measuring the concentration of free drug in the dispersion medium/aqueous phase of undiluted NDPSLN/NDPNLC dispersion. Ultracentrifugation was carried out using Centrisart, which consists of filter membrane (molecular weight cut-off, 20,000 Da) at the base of the sample recovery chamber. About 1 mL of undiluted sample of NDPSLN/NDPNLC dispersion was placed in the outer chamber, and the sample recovery chamber was placed on top of the sample. The unit was centrifuged at 12,000 rpm for 30 min. The SLN/NLC along with encapsulated drug remained in the outer chamber, and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of the NDP in the aqueous phase was estimated by HPLC.

Occlusion Test

The in vitro occlusion test was adapted from de Vringer (1992). Beakers (100 mL) were filled with 50 mL of water, covered with filter paper (cellulose acetate filter, 90 mm, cutoff size: 4–7 μm), and sealed. About 200 mg of sample was spread evenly with a spatula on the filter surface (18.8 cm^2), leading to an applied amount of 10.6 mg/cm^2. A visible film formation on top of the filter paper was observed during the experiment. The samples were stored at 32°C (skin temperature) and 50–55% relative humidity (RH) for 48 h. The samples were weighed after 6, 24, and 48 h, giving the water loss due to evaporation at each time (water flux through the filter paper). Beakers covered with filter paper but without applied sample served as reference values. Every experiment was performed in triplicate ($n = 3$). The occlusion factor F was calculated according to the following equation:

$$F = (A - B/A) \times 100,$$

where A is the water loss without sample (reference) and B is the water loss with sample.

An occlusion factor of zero means no occlusive effect compared with the reference, and 100 is the maximum occlusion factor.

HPLC Analysis of Nitrendipine

Mobile phase was prepared by mixing acetonitrile, freshly prepared double distilled water, and glacial acetic acid in the ratio of 60:40:0.1, by volume (Kobayashi et al., 1993). Mobile phase was degassed with the help of bath sonicator. The chromatographic system consisted of a Shimadzu LC-10AT solvent delivery pump equipped with a 20-μL loop and rheodyne sample injector. Wakosil II 5C18RS (SGE) (25cm × 4.6 mm i.d.) analytical column was used. Detector used was SPD-10A VP dual wavelength UV-Visible detector (Shimadzu, Japan), and the eluate was monitored at 235 nm. The sensitivity was set at 0.001 AUFS. Flow rate was kept at 1 mL/min. The data were recorded using Winchrome Software.

DSC Analysis

DSC analysis of NDP, TM, physical mixture (PM), and lyophilized NDP–TM was performed using Perkin-Elmer DSC-7 model. The instrument was calibrated with indium. All the samples (~5 mg) were heated in aluminum pans using dry nitrogen as the effluent gas. The analysis was performed with a heating range of 20–200°C and at a rate of 20°C/min.

Preparation of mixtures of SLN/NLC components for thermal analysis are as follows:

- NDP
- TM
- PM of NDP and TM [SLN].
- Lyophilized NDPSLN
- Captex 355
- PM of NDP, TM, and Captex 355 [NLC]
- Lyophilized NDPNLC

The ratios of NDP to TM were similar to that of weight ratios in SLN and NLC formulation. NDP, TM, PM, Captex 355, lyophilized NDPSLN, and lyophilized NDPNLC were subjected to same thermal cycles.

High-Resolution Transmission Electron Microscopy and Scanning Electron Microscopy

Bright-field transmission electron microscope (TEM) images were taken using a Jeol 3010, Japan, high-resolution transmission electron microscope (HRTEM) operated at 300 keV. Samples for TEM were prepared by dropping a dispersion of the nanoparticles on copper grid-supported Formvar films.

The sample for the scanning electron microscope (SEM) analysis was prepared by sprinkling the microspheres on one side of double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. The SEM analysis of the microspheres was carried out by using Jeol JSM 5300. The microspheres were viewed at an accelerating voltage of 15–20 kV.

In Vitro Drug Release

In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm and molecular weight cut-off between 12,000 and 14,000 was used. Membrane was soaked in double distilled water for 12 h before mounting in a Franz diffusion cell. One milliliter of SLN/NLC dispersion or 0.5 g of semisolid preparation was applied to the donor compartment, and the receptor compartment was filled with 1% SLS in phosphate buffer, pH 7.4 (12 mL). During the experiments, the solution in the receptor side was maintained at 37 ± 0.5°C and stirred at 800 rpm with teflon-coated magnetic stirring bars. At fixed time intervals, 100 µL of the sample was withdrawn from receiver compartment through side tube and analyzed by HPLC.

Skin Membrane Preparation

The abdominal hair of Wister male rats, weighing 160 ± 25 g, was shaved using hand razors 24 h before treatment. After anesthetizing the rat with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was in contact with a saline solution for 1 h before starting the diffusion experiment. All surgical and experimental procedures were reviewed and approved by the animal and ethics review committee of Faculty of Pharmaceutical Sciences, Kakatiya University (Warangal, Andhra Pradesh, India).

Ex Vivo Permeation Studies

A system employing improved Franz diffusion cells with a diffusional area of 3.56 cm^2 was used for permeation studies. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor. Two milliliters of SLN/NLC dispersion/0.5 g of the gel of NDP was applied to the skin surface in the donor compartment, and the receptor compartment of the cell was filled with 12 mL of phosphate buffer (pH 7.4). During the experiments, the solution in the receptor side was maintained at 37 ± 0.5°C and stirred at 800 rpm with teflon-coated magnetic stirring bars. After application of the test formulation on the donor side, 100 µL aliquots were collected from the receptor side at designated time intervals (1, 2, 4, 8, 12, 18, and 24 h). Thereafter, an equivalent volume of receptor fluid was supplied to the receiver compartment immediately after each sample collection. At the end of 24 h, the amount of drug remaining on the skin and the drug concentration in the skin was determined by extraction into a suitable solvent followed by HPLC analysis.

In Vivo Studies

The animals used for in vivo experiments were adult male Wistar albino rats (230–250 g) procured from the central animal house of the University College of Pharmaceutical Sciences, Kakatiya University. The animals were kept under standard laboratory conditions, at 25 ± 1ºC and 55 ± 5% relative humidity with a 12 h light/dark cycle. The animals were housed in polypropylene cages, four per cage, with free access to a standard laboratory diet (Lipton Feed, Mumbai, India) and water ad libitum. Guidelines of the institutional animal ethics committee were followed for in vivo experiments.

Pharmacokinetic Evaluation of SLN and NLC Enriched Hydrogels on Animals

Wistar albino rats were used as the animal models for the bioavailability studies. The animals were selected after superficial examination of the skin surface for abnormalities. Only rats weighing between 230 and 250 g were selected for the study. About 10 cm^2 of skin was shaved on the dorsal side.

Before application of the gels, rats were kept under observation for 24 h for any untoward effects of shaving; they were fasted over this period. The rats were divided into three groups ($n = 6$). Group I was administered NDP orally (5 mg/kg), group II received SLN enriched hydrogel (A1), and group III received NLC enriched hydrogel (B1). The blood samples were withdrawn at different time intervals (1, 2, 3, 4, 8, 12, and 24 h). Plasma samples were separated by centrifugation (Centrifuge Micro 17 TR, Inchun, Korea) and stored in vials at −70°C until they were analyzed by HPLC.

Pharmacokinetic Analysis

Plasma concentration versus time data for NDP in individual rats was analyzed by using Kinetica (Version 1.1). The statistical significance of the differences between the formulations was analyzed by Student's *t*-test using Graph Pad InStat 3 software. A difference below the probability level of .05 was considered statistically significant. Pharmacokinetic parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-24}, and $AUC_{0-\infty}$ were estimated. The pharmacokinetic parameters were estimated for each rat individually, and the average of six values was calculated.

Efficacy of Transdermal Gels Against Hypertension in Rats

A BP measuring instrument (Stoelting, Wood Dale, IL, USA) with a noninvasive tail cuff and a digital BP display panel was used. The rats were trained to stay in the rat holder in a calm and nonaggressive state during BP measurement. After the rats' initial BPs were recorded, hypertension was induced by injecting 1% NaCl desoxy corticosterone acetate (DOCA) (20 mg/kg/week subcutaneously) (Aqil et al., 2004; Aqil et al., 2006; Katayose & Kataoka, 1997). Two weeks later, rats with a minimum mean BP of 150 mmHg were selected. The animals were divided into four groups ($n = 6$). Group I served as control, group II received NDP 5 mg/kg orally, group III received formulation A1, and group IV received formulation B1. BP was measured at different time intervals (1, 2, 4, 6, 12, and 24 h).

Skin Irritation Test

The hair on the dorsal side of Wistar albino rats was removed by clipping 1 day before this portion of the experiment (Namdeo & Jain, 2002). The rats were divided into four groups ($n = 6$). Group I served as the control, group II received transdermal gel A1, group III received transdermal gel B1, and group IV received a 0.8% vol/vol aqueous solution of formalin as a standard irritant (Mutalik & Udupa, 2004). A new gel, or new formalin solution, was applied daily for 7 days. Finally, the application sites were graded according to a visual scoring scale, always by the same investigator.

RESULTS AND DISCUSSION

Characterization of the Investigated Formulation

For the production of aqueous NDPSLN and NDPNLC dispersions containing 20% of lipid matrix, the formulations given in Table 1 were chosen.

For this investigation, four different hydrogel types were prepared using optimal stabilizer combination of water, gel-forming polymer, and glycerol as hydrating agent. The aqueous NDPSLN and NDPNLC dispersions were admixed to the freshly prepared hydrogels. The final composition of the investigated NDPSLN and NDPNLC containing hydrogels were shown in Table 2.

Physicochemical Properties

The NDPSLN and NDPNLC dispersions were light yellowish in color, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation. Gels loaded with NDPSLN and NDPNLC dispersions were light yellow in color and odorless with smooth appearance. The pH of the gels was in the range of 4.65 and 6.70.

Particle Size Analysis

The particle sizes of lipid nanoparticles before and after incorporation into four different hydrogels were shown in Figures 1 and 2. The polydispersity index (PI) is higher for Xanthan gum, approximately 0.3–0.4; and for chitosan hydrogel, the PI is approximately 0.4. After analysis, the formulations were stored at room temperature for 90 days. For all the tested formulations, more than 50% of the particles were below 250 nm after 90 days of storage at room temperature. The incorporation into hydrogels did not result in particle aggregation.

Zeta Potential (ζ)

The determination of zeta potential was performed in aqueous NDPSLN and NDPNLC dispersions stored at room temperature. The incorporation of NDP decreased the electrical

TABLE 1
Composition of the Investigated NDPSLN and NDPNLC Formulations (% wt/wt)

Formulations	Nitrendipine	Dynasan 114	Epikuron 200	Captex 355	Polysorbate 80
A (NDPSLN)	0.2	10	2	—	2.5
B (NDPNLC)	0.2	7	2	3	2.5

TABLE 2
Final Composition of the Investigated A (NDPSLN) and B (NDPNLC) Enriched Hydrogel Formulations (% wt/wt)

Formulations	Dynasan 114	Captex 355	Polysorbate 80	Gel-Forming Agent	Glycerol
A1	10	—	2.5	1% Carbopol 934	5
A2	10	—	2.5	1% Xanthan gum	5
A3	10	—	2.5	2% HPC	5
A4	10	—	2.5	1% Chitosan	5
B1	7	3	2.5	1% Carbopol 934	5
B2	7	3	2.5	1% Xanthan gum	5
B3	7	3	2.5	2% HPC	5
B4	7	3	2.5	1% Chitosan	5

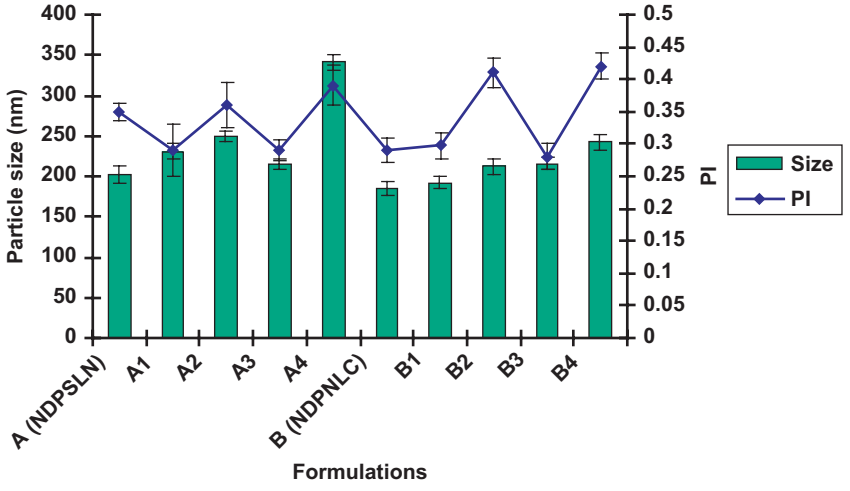

FIGURE 1. Particle size analysis of nitrendipine solid lipid nanoparticles (NDPSLN) and nitrendipne nanostructured lipid carrier (NDPNLC) formulations after day 1 of storage at room temperature.

charge at the surface of both SLN and NLC; when comparing both systems, NDPNLC revealed lower ZP values than the respective NDPSLN system. These results could be taken as an indication that NDP is entrapped in the lipid matrix of SLN and NLC. NDPSLN and NDPNLC were negatively charged when incorporated into Carbopol 934, Xanthan gum, and HPC hydrogels. The opposite was observed when incorporated into chitosan hydrogels. These results are due to the cationic character of the bioadhesive polymer.

As far as Carbopol 934 hydrogels, the carboxylic groups have to be neutralized with sodium hydroxide to exhibit gel-forming properties. This neutralizing agent could enhance aggregation because of the action of sodium ions as electrolyte, which can reduce the ζ values of the particles (Müller, Lucks, Stampa, & Müller, 1990). As a consequence of this lower ζ value, aggregation may occur. This phenomenon is well known for lipid nanoemulsions (Freitas & Müller, 1999), as well as for SLN when incorporated into polyacrylate hydrogels (Freitas, 1998). Figures 3 and 4 compare the ζ values of the aqueous NDPSLN and NDPNLC dispersions before and after their incorporation into hydrogels measured on day 1 and after 90 days of storage at room temperature. Figures 3 and 4 reveal that during storage time, the ζ value of the surface lipid nanoparticles remains practically unchanged (e.g., B2, A1, and B1) or slightly decreased (e.g., A2 and A3). In comparison with NDPSLN formulations with same liquid content, NDPNLC formulations show lower ζ values. NDPSLN and NDPNLC aqueous dispersions can be incorporated into hydrogels consisting of more or less uncharged polymers without significant changes in the particle size characteristics and ζ values (Jenning, Schäfer-Korting, & Gohla, 2000). In case of gel-forming polymers with very polar groups like chitosan, possible interactions between negative surface charge of the lipid nanoparticles and polar groups of this polymer must be taken into account.

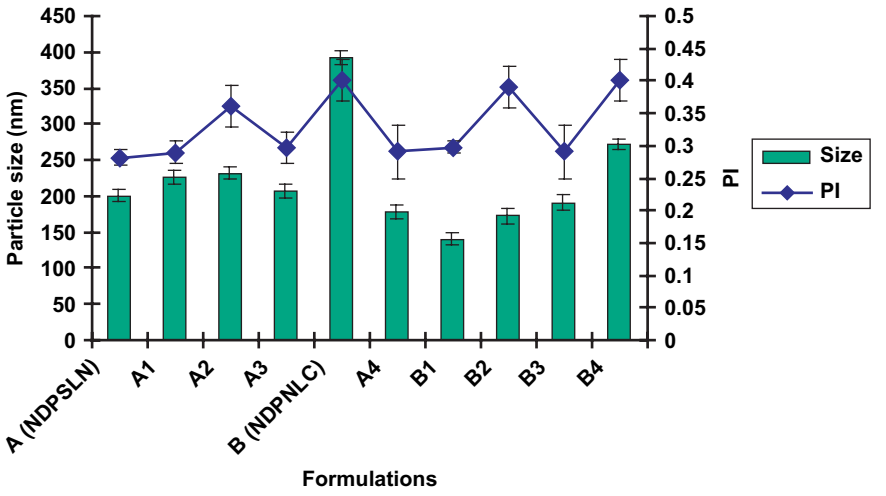

FIGURE 2. Particle size analysis of nitrendipine solid lipid nanoparticles (NDPSLN) and nitrendipne nanostructured lipid carrier (NDPNLC) formulations after day 90 of storage at room temperature.

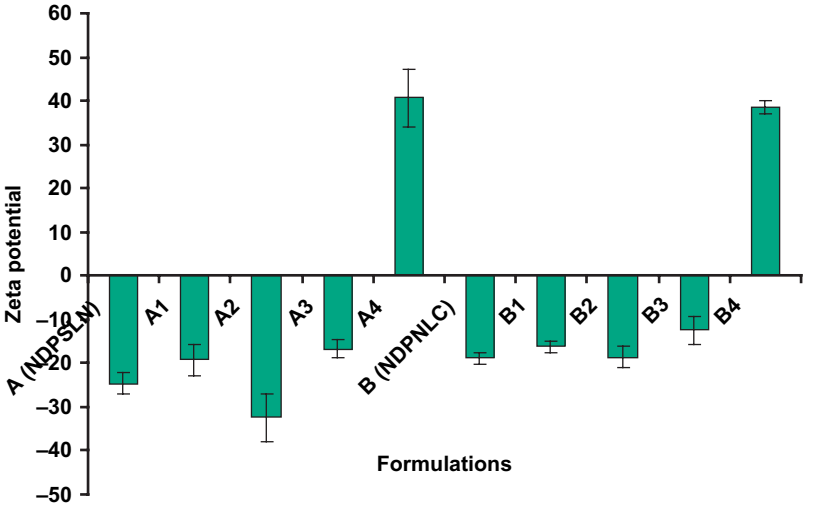

FIGURE 3. Comparison between the zeta potential (ZP) values of the aqueous nitrendipine solid lipid nanoparticles (NDPSLN) and nitrendipine nanostructured lipid carrier (NDPNLC) dispersions before and after their incorporation into hydrogels after day 1 of storage at room temperature.

Assay and Entrapment Efficiency

A high amount of NDP could be incorporated in the SLN and NLC dispersions. As high as 30% of the drug with respect to the lipid could be incorporated, such high incorporation is possible because of the lipophilic character of NDP. Assay results showed that concentration of NDP in the total system ranged from 0.92 to 1.01 mg/mL for different formulations.

The percentage of incorporated drug in the lipid matrix (entrapment efficiency) was evaluated over a period of 90 days. Entrapment efficiencies of all the formulations were lowered by 2.8–4.5% after 90 days of storage at room temperature. Incorporation of NDP led to high entrapment efficiency, probably because of their lipophilic character. NDPNLC is responsible for higher entrapment efficiency in comparison with NDPSLN formulation. This result is due to the binary mixture of liquid and solid lipids, resulting in only a very weak crystallization (Jenning, Mader, & Gohla, 2000; Jenning, Thünemann, & Gohla, 2000). For all the tested formulations, the entrapment efficiency was higher than 75%.

Occlusion Test

Small particles possess an adhesive effect. The increased solubility of the active compound in the adhesion increases with decreasing particle size. Intensive in vitro studies were performed to quantify the occlusivity of SLN/NLC in the form of the so-called occlusion factor Lipid nanoparticles show

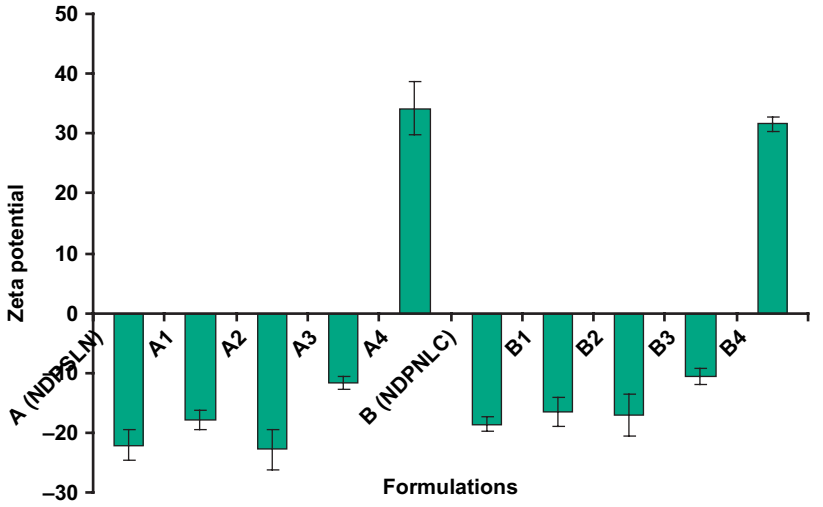

FIGURE 4. Comparison between the zeta potential (ZP) values of the aqueous nitrendipine solid lipid nanoparticles (NDPSLN) and nitrendipine nanostructured lipid carrier (NDPNLC) dispersions before and after their incorporation into hydrogels after day 90 of storage at room temperature.

adhesive properties, they tend to adhere to cells and surfaces, and because of the film formation of the nanoparticles on the skin, SLN possess occlusive properties (de Vringer, 1992; Jenning et al., 1999; Muller & Dingler, 1998; Wissing et al., 2001). The occlusion factor is dependent upon the sample volume, particle size, and crystallinity; therefore, samples of 200 mg were applied to the membrane of Franz cells (Wissing et al., 2001). The results of the occlusion test are shown in Figure 5. The occlusion factor depends strongly on the lipid concentration and the particle size of SLN/NLC. The results obtained from our study have shown a lower *F*, that is, lower occlusivity can be detected for NDPNLC than for NDPSLN dispersions. This result is due to the solid state of the lipid matrix of SLN, which disables the evaporation of water.

DSC Analysis

Figure 6A and B shows DSC curves of NDP, TM, PM, and lyophilized NDP–TM of both SLN and NLC, respectively. The thermograms of the lyophilized NDP–TM did not show the melting peak for the NDP around 160.6°C. This shows that NDP was not in crystalline state but it is in amorphous state. Endothermic peak of glucose used as cryoprotectant was observed at 148.5°C in NDP–TM curve. Similar results were reported by Cavalli, Aquilano, Carrlotti, and Gasco (1995) and Cavalli et al. (1997), stating that rapid quenching of the microemulsion does not allow the drug to crystallize. DSC analysis of camptothecin SLN prepared by high-pressure homogenization showed that camptothecin was in amorphous state (Yang & Zhu,

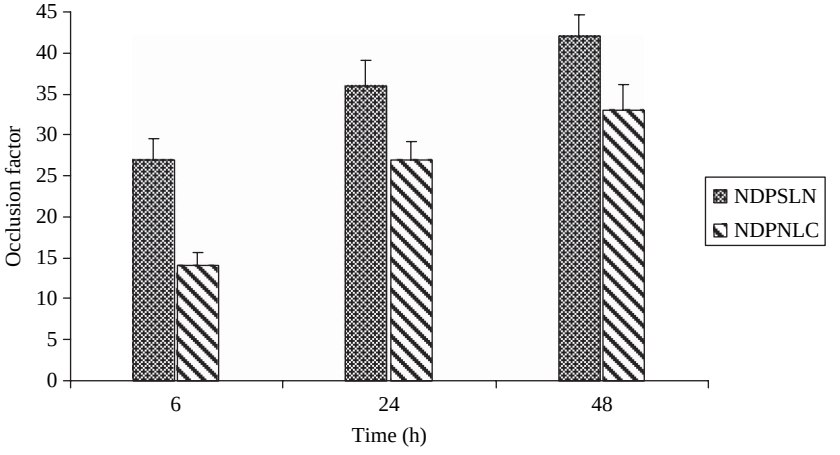

FIGURE 5. Occlusion factors of the investigated nitrendipine solid lipid nanoparticles (NDPSLN) and nitrendipine nanostructured lipid carrier (NDPNLC) formulation.

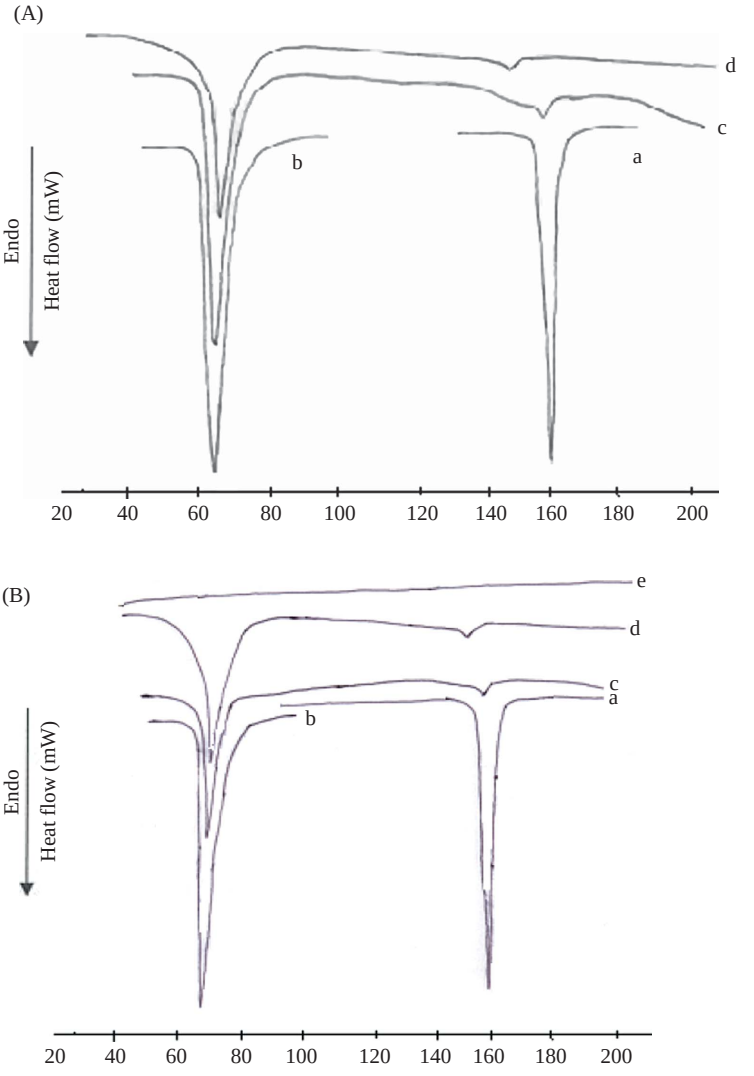

FIGURE 6. (A) DSC thermograms of nitrendipine (NDP) (a), trimyristin (TM) (b), physical mixture (PM) of NDP and TM (c), and lyophilized NDP solid lipid nanoparticles (SLN) (d). (B) DSC thermograms of nitrendipine (NDP) (a), trimyristin (TM) (b), physical mixture (PM) of NDP and TM (c), lyophilized NDP nanostructured lipid carrier (NLC) (d), and captex 355 (e).

2002). In our method, lipids and NDP were dissolved in a mixture of solvents, and subsequently, solvents were evaporated. This allowed homogeneous dispersion of drug in the lipid. Furthermore, methods of preparation (homogenization followed by ultrasonication) and the presence of surfactants do not allow the drug to crystallize. Thermodynamic stability of lipid nanoparticles depends on their existing lipid modification. Polymorphic transitions after crystallization of triglyceride nanoparticles are slower for longer-chain triglycerides than for shorter-chain triglycerides (Bunjes, Westesen, & Koch, 1996), whereas these transitions are faster for small size of crystallites (Westesen, Bunjes, & Koch, 1997). The type of surfactant and storage time affects the crystallinity of SLN/NLC and, consequently, degradation velocity (Olbrich, Kayser, & Muller, 2002).

HRTEM and SEM

The TEM image of the NDPSLN and NDPNLC was shown in Figure 7A and B, respectively, which shows that the particle diameters vary from about 150 to 300 nm. Both SLN and NLC were investigated, no significant difference in the diameter was obtained. The SLN dispersion has a completely different electron microscopic appearance, NLC show quite heterogeneous structures.

An image of a carbopol hydrogel loaded with the drug at 1,000 × magnification shows a rough surface with enhanced

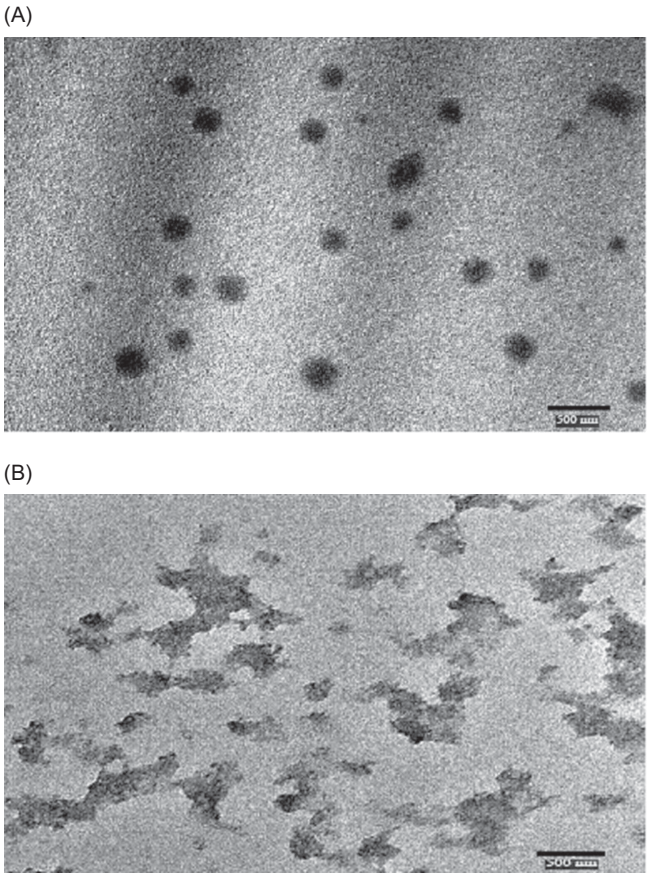

FIGURE 7. (A) Transmission electron microscope (TEM) image of nitrendipine solid lipid nanoparticles (NDPSLN). (B) TEM image of nitrendipne nanostructured lipid carrier (NDPNLC).

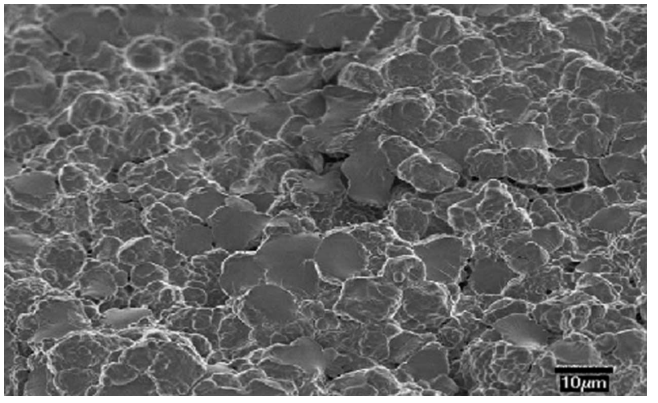

FIGURE 8. Scanning electron microphotograph of carbopol 934 hydrogel loaded with drug.

grain boundaries (Figure 8). The SEM studies were performed to determine the structure of the lipid nanoparticles incorporated in carbopol hydrogels and directly observe the particles entrapped inside the hydrogel matrix. Drug release studies and SEM pictures showed that drug-filled nanoparticles were successfully entrapped in the carbopol hydrogel matrices.

In Vitro Drug Release

The cumulative percentage release of NDP from NDPSLN (A) and NDPNLC (B) dispersions was investigated for a period of 24 h; each sample was analyzed in triplicate. Figure 9 shows the in vitro release profile of NDPSLN (A) and NDPNLC (B) dispersions. Concerning NDPSLN (A), in the initial 2 h, the drug release was less than 20% probably due to the slow diffusion of drug from the lipid. After 2 h, the drug release rate increased with time. NDPSLN (A) could prolong or retard the drug release because the drug molecules are entrapped in the solid lipid matrix. Soluble NDP is partitioned into aqueous phase from which it is dialyzed into the dialysis medium. NDP dissolved in lipid diffuses to the surface and undergoes partitioning between lipid and aqueous phase.

Concerning NDPNLC, the liquid lipid-enriched shell possessed soft and considerable higher solubility for lipophilic drugs character (Mühlen, Mühlen, Niehus, & Mehnert, 1996), in which the drug was easily loaded to higher amount and the drug could be easily released as well by the drug diffusion or the matrix erosion manners. Furthermore, the incorporation of liquid lipid into solid lipid matrix caused the NDPNLC become more imperfect and allowed loaded drugs easier to release, thus increasing the drug release rate when liquid lipid was included in NLC matrix. For the above reasons, it achieved the results of sustained release and increased drug release rate compared with NDPSLN.

Comparing the drug release from NDPSLN and NDPNLC dispersions and NDPSLN and NDPNLC in gels (Figures 10 and 11), the release of NDP was slower from gel formulation. The percentage drug release at the end of 24 h in NDPSLN (A1) and NDPNLC (B1) enriched carbopol gels was found to be 54.57 and 64.31%, respectively, whereas the percentage

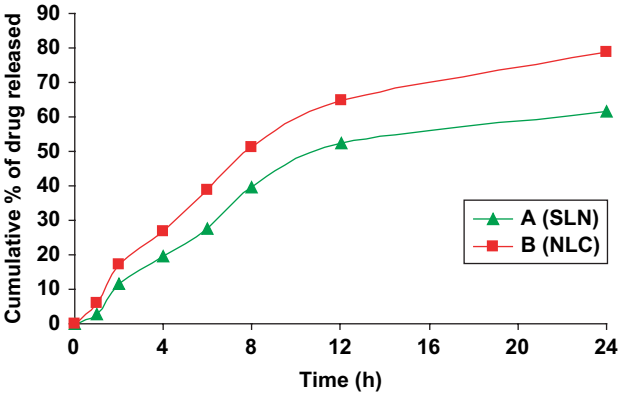

FIGURE 9. In vitro release profile of nitrendipine (NDP) in solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLC) dispersions, $M \pm SD$ ($n = 3$).

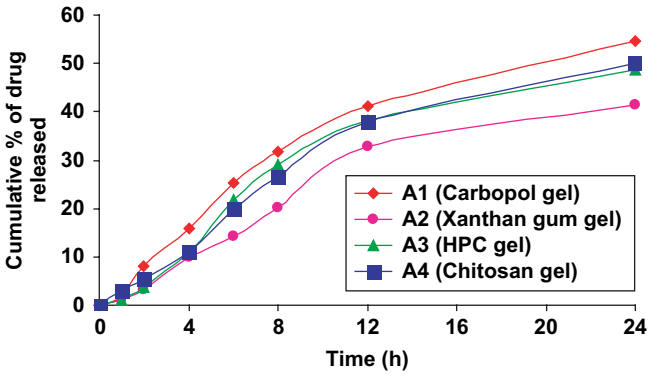

FIGURE 10. In vitro release of nitrendipine (NDP) from gels enriched with solid lipid nanoparticles (SLN) dispersion, $M \pm SD$ ($n = 3$).

drug release at the end of 24 h in NDPSLN (A) and NDPNLC (B) dispersions was 61.63 and 78.85%, respectively. Incorporation of SLN and NLC dispersion into gels decreased the drug release; this may be due to the release retarding effect of the polymeric matrix of the gelling agent.

The percentage drug release was found to be slowest in case of NDPSLN (A2) and NDPNLC (B2) dispersions incorporated in Xanthan gum gel, that is, 41.45 and 47.72%, respectively. It has got a sticky texture; therefore, it is not investigated further. The percentage drug release from NDPSLN (A3) and NDPNLC (B3) dispersions incorporated into HPC gels was found to be 48.62 and 53.42%, respectively. The percentage drug release from SLN and NLC dispersions incorporated into chitosan gels was found to be 50.16 and 56.81%, respectively.

The carbopol gel was better in appearance and texture and was therefore selected for further evaluation. Carbopol 934 gels and the lipid used for the preparation of lipid nanoparticles (TM) have excellent skin properties. The first because of its thermal stability and optimum rheological properties and the second because the lipid composition of the epidermis is mainly based on triacylglycerols (25%) (Osborne & Amann, 1990).

Ex Vivo Permeation Studies

The goal of a permeation study is to compile a kinetic profile that reflects how the concentration of an active ingredient changes in time as it diffuses through the skin.

The in vitro skin permeation of NDPSLN and NLC was investigated through rat skin as shown in Figure 12. NDPNLC (B1) (area of 3.56 cm^2) exhibited the greatest (765.51 ± 53.21 $\mu g/cm^2$) cumulative amount of drug permeation in 24 h. The release kinetics was established by determining the diffusional release exponent from the plot of log of cumulative drug permeated versus log time. This plot yielded a straight line from which diffusional release exponent (n) was calculated and found to be between 0.89 and 0.96 for both SLN/NLC dispersions and SLN/NLC carbopol gel formulations, which showed that the release of drug from these formulations followed a non-Fickian pattern (Langer & Peppas, 1981; Saravanan, Bhaskar, Maharajan, & Sadasivan Pillai, 2004).

From the percent cumulative drug permeated versus time plot, the slope values were determined as the skin permeation rate. The cumulative amount of drug permeated at the end of 24 h was found to be 793.16 ± 41.82 and 898.14 ± 56.16 $\mu g/cm^2$ with skin permeation rate constants of 3.3585 and 3.7718 percent/cm^2/h for NDPSLN (A) and NDPNLC (B) dispersions, respectively. For the conventional gel, the cumulative amount of drug permeated at the end of 24 h was found to be 474.145 ± 24.1 $\mu g/cm^2$ with skin permeation rate constant of 2.0762 percent/cm^2/h. The cumulative amount of drug permeated at the end of 24 h was found to be 656.12 ± 44.82 and 765.51 ± 53.21 $\mu g/cm^2$, with skin permeation rate constants of 2.7923 and 3.2125 percent/cm^2/h for NDPSLN (A1) and NDPNLC (B1) enriched carbopol gels, respectively. SLN and NLC

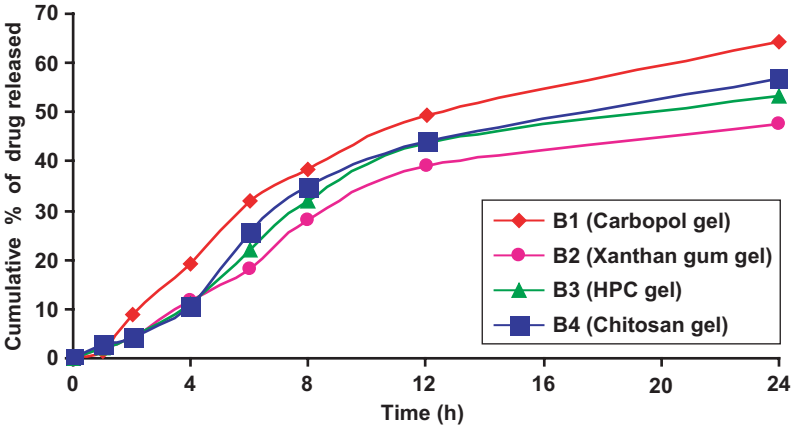

FIGURE 11. In vitro release of nitrendipine (NDP) from gels enriched with nanostructured lipid carrier (NLC) dispersions $M \pm SD$ ($n = 3$).

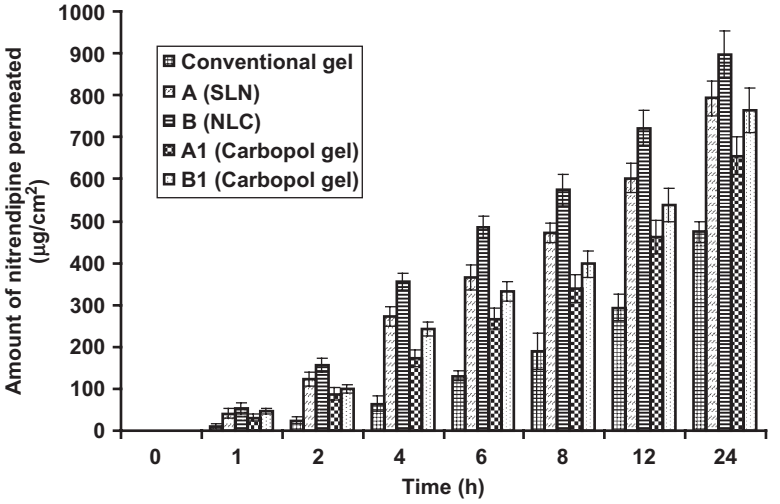

FIGURE 12. Ex vivo permeation studies of nitrendipine (NDP) from carbopol gels enriched with solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLC) dispersions through rat abdominal skin, $M \pm SD$ ($n = 3$).

enriched carbopol gels released drug slowly when compared with SLN and NLC dispersions, accounted for by the time drug takes to diffuse through the gel. The slower release of drug from SLN and NLC carbopol gels maintained the drug concentration for longer period.

The SLN/NLC dispersions and SLN/NLC gel formulations possessed a sustained drug release over a period of 24 h, but the sustained effect was more pronounced with SLN and NLC enriched gel formulations. The results of drug permeation from all the formulations through the rat abdominal skin confirmed that NDP was released and permeated through the rat skin and hence could possibly permeate through the human skin.

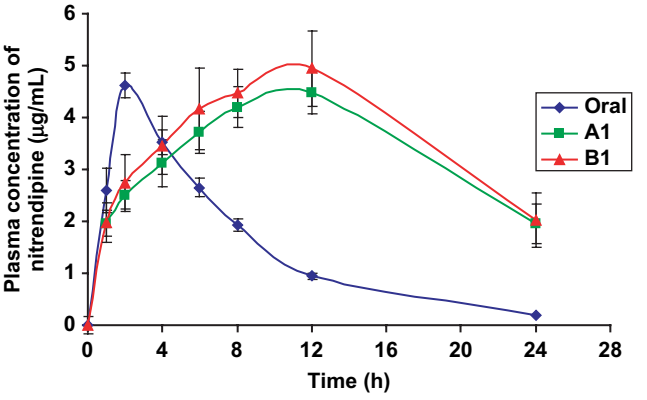

FIGURE 13. Plasma concentrations of nitrendipine (NDP) after oral and transdermal administration of NDP ($n = 6$).

Pharmacokinetic Evaluation of SLN and NLC Enriched Hydrogels on Animals

Figure 13 shows the blood plasma levels of NDP after transdermal and oral administration. The mean pharmacokinetic parameters recorded in Table 3 were calculated from the blood plasma concentrations of the drug. The maximum drug concentration, C_{max}, after oral administration was 4.63 ± 0.42 µg/mL, and T_{max} was 2 h. For the A1 formulation, C_{max} and T_{max} were 4.65 ± 0.35 µg/mL and 12 h and for the B1 formulation C_{max} and T_{max} were 5.11 ± 0.58 µg/mL and 12 h. All the pharmacokinetic parameters obtained with NDP transdermal gels were significantly different from those obtained with oral NDP administration. The results indicated that the elimination half-life of NDP was prolonged from oral administration (4.88 ± 0.70 h) to transdermal gel formulations (A1, 13.55 ± 4.05 and B1, 15.03 ± 8.32 h) in rats, which means that the drug remains in the body for a longer period and its action is more sustained. The transdermal gels also have a lower elimination rate constant, which further supports sustained action of drug from the gels. The significantly high AUC values observed with transdermal gels also indicate increased bioavailability of the drug from gels compared with oral administration.

Efficacy of SLN and NLC Enriched Transdermal Hydrogels Against Hypertension in Rats

The results in Table 4 indicate that the administration of DOCA produced significant hypertension in rats. The oral administration of NDP significantly ($p < .05$) controlled the hypertension initially, with the maximum effect observed at 2 h, but after 2 h, the BP started rising gradually until it was the same as the initial value at 24 h. By contrast, the administration of NDP through transdermal gels A1 and B1 resulted in a gradual decrease of BP, with the maximum effect from both the

TABLE 3
Mean Pharmacokinetic Parameters of Nitrendipine After Oral and Transdermal Administration

Parameters	Oral	A1	B1
C_{max} (μg/mL)	4.63 ± 0.42	4.65 ± 0.35	5.11 ± 0.58
T_{max} (h)	2	12	12
$t_{1/2}$ (h)	4.88 ± 0.70	13.55 ± 4.05	15.03 ± 8.32
$AUC_{0-\infty}$ (mg h/mL)	34.79 ± 1.65	78.43 ± 6.43*	84.22 ± 3.66*
AUC_{0-24} (mg h/mL)	36.17 ± 1.84	120.16 ± 25.06*	134.88 ± 39.57*

All values are expressed as $M \pm SE$ ($n = 6$), A1 and B1 are SLN and NLC enriched hydrogels, respectively, C_{max} indicates maximum concentration, T_{max} indicates time of maximum concentration, $t_{1/2}$ elimination half-life, AUC area under the plasma concentration—time curve.

*$p < .05$; significant compared with control.

gels observed at 12 h ($p < .05$). Although the gels produced a peak effect at 12 h, they decreased the BP significantly ($p < .05$) at the first hour and the effect continued for 24 h. This clearly indicates that the transdermal gels release the drug gradually over a period, which results in prolonged control of hypertension for 24 h. Oral NDP acted quickly and drastically, but then its effect dropped off, the gels did not decrease the BP greatly in the initial phase when compared with the oral form, as indicated by the significant ($p < .05$) difference between the oral-treated and the gel-treated groups at 2 h, but the effect of oral NDP started declining after 6 h because of its short half-life. Since the administration of NDP through gels resulted in sustained and continued drug release for 24 h, the gels were able to control the hypertension throughout the period. Clearly, the prepared transdermal gel formulations (A1 and B1) are capable of surmounting the shortcomings of oral administration of NDP, such as low bioavailability, short half-life, and high first-pass metabolism.

Skin Irritation Test

The skin irritation test of the transdermal formulations A1 and B1 showed a skin irritation score (erythema and edema) of less than 2 (Table 5). According to Draize, Woodward, and Calvery (1944), compounds producing scores of 2 or less are considered negative (no skin irritation). Hence, the developed transdermal gel formulations are free of skin irritation.

CONCLUSION

In this study, the utility of SLN and NLC hydrogels as carriers for transdermal delivery of NDP was exploited. The results suggest that these SLN and NLC hydrogels can serve as efficient promoters for NDP to permeate into skin. Both NDPSLN and NDPNLC represent a highly effective, nonirritant carrier for topical preparations, where improved drug penetration is desired. Improved skin penetration can be due to enhanced contact of the active agent and skin resulting from the large particle surface area and film formation. After 90 days of storage at different temperatures, the mean diameters of SLN and NLC remain practically the same (<1 μm), which emphasizes the physical stability of these lipid particles. The size distribution of SLN and NLC before and after incorporation into hydrogels revealed a unimodel profile. NDPNLC show higher entrapment efficiency due to their liquid parts. NDPNLC also show a faster release profile in comparison with NDPSLN. Both the SLN and NLC dispersions and gels enriched with SLN and NLC possessed a sustained drug release over a period of 24 h, but the sustained effect was more pronounced with the SLN and NLC gel formulations.

To sum up, advantages of the new formulations based on hydrogels for skin are their suitable viscosity, transparency, and high chemical stability of drug in the formulation, probably due to the special gel microstructure. Moreover, a lower drug release can be achieved. An additional advantage is the possibility of combining the drug-loaded SLN/NLC with the gel hydrophilic matrices, which results in an excellent adhering and constant releasing formulation. More polar materials such as carbopol hydrogel matrices adhere excellently on skin. This may be partly due to the humidity of skin and due to the presence of hair follicles and sweat glands, which contain aqueous channels.

In conclusion, the developed systems are promising alternative drug carriers for transdermal pharmaceutics. Controlled release purposes have been accomplished by incorporating NDP into the solid matrix of Dynasan114-based lipid nanoparticles.

Since the administration of NDP through gels resulted in sustained and continued drug release for 24 h, the gels were able to control the hypertension throughout the period. Clearly, the prepared transdermal gel formulations (A1 and B1) are capable of surmounting the shortcomings of oral administration of NDP, such as low bioavailability, short half-life, and high first-pass metabolism.

The NDP transdermal gels developed in this study have great utility and are a viable option for effective and controlled

TABLE 4
Antihypertensive Effect of NDPSLN and NLC Enriched Hydrogels in Comparison to Oral Route

		Mean Blood Pressure (mm Hg)						
Group	Treatment	Initial	1 h	2 h	4 h	6 h	12 h	24 h
I	Control	179.81 ± 7.92	176.52 ± 6.98	176.91 ± 7.01	178.31 ± 6.62	179.92 ± 6.99	178.92 ± 6.65	178.71 ± 7.21
II	Oral	178.8 ± 6.91	127.68 ± 6.91*	92.69 ± 5.92*	101.54 ± 5.71*	109.24 ± 6.62*	117.81 ± 5.01*	174.53 ± 6.92
III	A1	175.65 ± 7.17	154.71 ± 5.94*,**	135.52 ± 5.71*,**	114.98 ± 4.98*	110.71 ± 5.72*	105.43 ± 4.98*	114.81 ± 4.45*,**
IV	B1	182.72 ± 7.02	156.71 ± 6.01*,**	137.27 ± 5.82*,**	118.76 ± 6.21*	115.82 ± 4.89*	110.72 ± 4.69*	118.32 ± 4.63*,**

All values are expressed as $M \pm SEM$ ($n = 6$), A1 and B1 are NDPSLN and NDPNLC Enriched Hydrogels respectively.
*$p < .05$, significant compared with control.
**$p < .05$, significant compared with oral.

TABLE 5
Skin Irritation Scores Following SLN and NLC Enriched Transdermal Gel Administration

	Control		A1		B1		Formalin	
Rat No.	Erythema[a]	Edema[b]	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	1	2	0	2	2
2	0	0	1	0	0	0	3	1
3	0	0	1	0	1	1	3	2
4	0	0	2	1	1	0	2	3
5	0	0	1	0	2	1	3	3
6	0	0	2	0	2	2	3	2
Average	0	0	1.17 ± 0.3073*	0.33 ± 0.2108*	1.33 ± 0.3333*	0.67 ± 0.3333*	2.67 ± 0.2108	2.16 ± 0.3073

*$p < .05$, significant compared with formalin.
[a]Erythema scale: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, scar formation.
[b]Edema scale: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, severe.

management of hypertension. However, pharmacodynamic and pharmacokinetic evaluation of these systems in human volunteers is necessary to confirm these findings.

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