Original Article

Formulation and Evaluation of Nisoldipine Loaded Solid Lipid Nanoparticles and Nanostructured Lipid Carriers: Application to Transdermal Delivery

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ABSTRACT

Background: Nisoldipine is an anti hypertensive drug. Nisoldipine exhibits poor oral bioavailability (5%) because of rapid metabolism in the gut and liver. To overcome hepatic first pass metabolism and to enhance bioavailability, lipid based drug delivery systems (Solid lipid nanoparticles (SLN) and nano structure lipid carriers (NLC)) can be exploited. **Objectives:** In this work, effort was made to prepare novel particulate carrier systems such as stable Solid Lipid Nanoparticles and Nanostructured Lipid Carriers for transdermal delivery of Nisoldipine (NSP). For this investigation, Carbopol 934 used as gel forming agent for hydrogel preparation. **Methods:** Aqueous dispersions of lipid nanoparticles made from Dynasan 114, 116, 118 were prepared by hot homogenization technique followed by ultrasonication and then optimized formulations of SLN and NLCs were incorporated into the freshly prepared hydrogels. Prepared lipid nanoparticles were characterized for particle size, zeta potential, entrapment efficiency, stability and *in-vitro* release profile. Also percutaneous permeation of SLN and NLCs were investigated in rat abdominal skin. **Results:** Analyzing the particles size by photon correlation spectroscopy (PCS) using Malven zeta sizer, which shows that the SLN and NLCs were in the range of 130-330 nm at room temperature. For all the tested formulations (SLN and NLCs), the entrapment efficiency was 72-97%. *In-vitro* drug release studies were performed for 24 hr. In these two cases the percentage drug release from gels enriched with SLN/NLC showed sustained release over period of 24 hr. In agreement with these results NSPNLC (Nisoldipine nanostructured lipid carriers) dispersion showed faster release. Formulation E4 showed faster release has less particle size and more zeta potential. **Conclusion:** Based on these, it was selected for further stability and *ex-vivo* studies. Both the SLN and NLC showed a sustained drug release over a period of 24 hr, but the sustained effect was more pronounced with the SLN and NLC gel formulations.

Key words: Nisoldipine, Solid lipid nanoparticles, Nanostructured lipid carriers, *In-vitro* release studies, *Ex-vivo* permeation studies, Lyophilization.

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INTRODUCTION

The development of a novel drug delivery system for a particular drug is difficult due to the differences of the drug solubility in the vehicle components and the vast range in cutaneous fluxes. In the majority of pharmaceutical formulations intended for transdermal/topical and dermatological therapy the drug molecules are dissolved in a liquid phase of oil in water (o/w) or water in oil (w/o) emulsion. However, due to the low viscosity of the inner phase of the systems it is difficult to achieve a prolonged or controlled release of a drug. Noninvasive drug delivery systems are gaining market share at the expense of oral and parenteral delivery. Technology advances are expanding the number of drug candidates that can be solubilized and delivered via transdermal gels. The trend towards increased home care and

drug therapy, self-administration will accelerate interest in transdermal gel formulations.[1]

In order to overcome the drawbacks associated with the colloidal systems, such as emulsions, liposomes and polymeric nanoparticles, Solid lipid nanoparticles were developed at the beginning of the nineties.[2] SLN having a mean particle size in submicron size between 50nm and 1000nm and are made up of materials which are biocompatible and biodegradable with capability of incorporating hydrophilic and liphophilic drugs.[3] A clear advantage of the use of lipid particles as drug carrier systems is that the matrix is composed of physiological components, i.e. excipients with generally recognized as safe (GRAS) status for oral and topical administration, which decreases the danger of acute and chronic toxicity. Preparation of SLN by exchanging the liquid lipid (oil) of the emulsions by a solid lipid,[4] which can bring many advantages in comparison to liquid core.[5] SLNs are low cost products. In fact, the excipients and production lines are relatively cheap.

The second generation of lipid nanoparticles are called nanostructure lipid carriers (NLC).[6,7] The difference between NLC and SLN is the fact that the concept of these former is performed by nano structuring the lipid matrix, in order to increase the drug loading and to prevent its leakage, giving more flexibility for modulation of drug loading and to prevent its leakage, giving more flexibility for modulation of drug release. For achieving of this approach by mixing solid lipid with liquid lipids in NLC, instead of highly purified lipids with relatively similar molecules in SLN. This mixture has to be solid at least at 40°C. The result is less ordered lipid matrix with many imperfections, which can accommodate a higher amount of drug.[7,8] Different substances have been entrapped into lipid nanoparticles, ranging from lipophilic and hydrophilic molecules, including labile compounds, such as peptides and proteins during the last 10 years.[9] The purpose of this project is to prepare and characterize solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) loaded with Nisoldipine, for transdermal drug delivery. The possibilities to use lipid nanoparticles for sustained or controlled release application have been discussed.

Nisoldipine is an anti hypertensive drug. Nisoldipine exhibits poor oral bioavailability (5%) because of rapid metabolism in the gut and liver. To overcome hepatic first pass metabolism and to enhance bioavailability, lipid based drug delivery systems (SLN and NLC) can be exploited. These systems incorporated into hydrogels for transdermal application increase the bioavailability. In the present study solid lipid nanoparticles and nano structured lipid carriers are employed to incorporate Nisoldipine to bypass the first pass metabolism, thereby to increase its bioavailability and to improve activity.

MATERIALS

Nisoldipine obtained as a gift sample from Aurabindo Pharma, Hyderabad, India. Dynasan 114, 116 were generously supplied by Sasol, Witten, Germany. Dynasan 118 and Oleic acid were supplied from S.D. Fine-Chem Ltd, India. Carbopal 934p, Glycerol and Dialysis membrane-70 were purchased from Hi-media (Mumbai, India). Soya Lecithin (Lipoid E80) was obtained from Lipoid, Germany. Centrisart filters (molecular weight cutoff 20,000) were purchased from Sartorius (Goettingen, Germany). The other chemicals were of analytical reagent grade.

METHODS

Characterization by Differential Scanning Calorimetry (DSC)

DSC is one of the basic techniques used to investigate drug-excipients compatibility. It quantifies the enthalphic changes during endothermic or exothermic effects. The instrument was calibrated with indium (calibration standard, purity >99.99%) for melting point and heat of fusion. About ~5mg sample was taken for analysis into standard aluminum pans. An empty pan was used as reference. The heating rate was increased at the rate of 20°C/min with heating range of 20-200°C and the obtained thermo grams were observed for any type of interaction. DSC analysis of Nisoldipine (NSP), Dynasan-114, 116 and 118 and lyophilized Nisoldipine products were analyzed.

Preparation of Aqueous SLN and NLCs Dispersions and Gels of Nisoldipine

Nisoldipine (drug 10 mg), solid lipid (100-300mg) and soya lecithin (100mg) were dissolved in 10 mL mixture of chloroform and methanol (1:1). Organic solvents were completely removed using a Rota evaporator (Laborota 4000, Heidoph, Germany). A drug embedded lipid layer was melted by heating at 58°C above melting point of the lipid. Aqueous phase was prepared by dissolving polaxamer 407 (0.5 to 2% w/v) in double distilled water (sufficient to produce 10 mL of preparation) and heated to the same temperature of the oil phase. Hot aqueous phase was added to the oil phase and homogenization was carried out (at 12,000 rpm) using a Diax 900 homogenizer (Heidolph, Germany) for 10min. Coarse hot oil in water emulsion so obtained was ultrasonicated (12T- probe) using Vibracell probe

sonicator (Bandelin, Germany) for 20 min. Nisoldipine loaded solid lipid nanoparticles were obtained by allowing hot nano emulsion to cool to room temperature.[10] Nanostructured Lipid Carriers (NLC) was prepared in exactly the same manner as the SLN dispersions; only replacing 30% of the solid lipid matrix by oleic acid. Quantity of ingredients used for the preparation of SLNs and NLCs of Nisoldipine given in the Table 1.

Optimization of Formulation variables

For development of SLN/NLCs optimize the formulation variables like lipid content, concentration of surfactant and optimize the process variables like homogenization time, ultrasonication time was observed. For effect of lipid content three different amounts of lipid (100, 200 and 300) were selected for the preparation of SLN of Nisoldipine. The formulations were stored at room temperature and examined for physical stability. The formulations were also used for *in-vitro* release studies. In order to observe the effect of polaxamer 407 concentration on particle size and physical stability, different concentrations of polaxamer 407 (0.5%, 1.75% and 2%) were used in the formulation of SLN and NLCs of Nisoldipine. The homogenization time was varied from 5 min to 10 min while homogenization speed (12,000rpm) and ultra sonication time were kept constant. In another study, the homogenization time was kept constant at 10 min and the ultrasonication time was varied from 15 to 20 min.

Preparation of gels enriched with Lipid Nanoparticles

Gels were prepared using Carbopol 934p (2%) polymer. For the preparation of hydrogel, the gel forming polymer (was dispersed in double distilled water containing glycerol (10%). 20% of aqueous SLN and NLCs dispersions and hydrogels were mixed in a high-speed stirrer (Remi, Mumbai, India) at approximately 100 rpm for 5 min to yield hydrogels containing a final concentration of lipid nanoparticles about 5%.[11] The hydrogels composed of carbopol 934p were adjusted to pH 6.5 with triethanolamine. The dispersions of SLN and NLCs were used as reference.

Evaluation of SLN and NLCs dispersion and Gels

Physicochemical Properties

After centrifugation the dispersions of SLN and NLCs were characterized for their physicochemical properties such as color, odor and stability. The gels enriched with SLN and NLCs were evaluated for color, odor and pH. Measurement of Particle size, PDI and zeta potential of SLN.

The size, PDI and zetapotential of Nisoldipine SLNs and NLCs were measured using photon correlation spectroscopy (PCS), with the help of a Malvern Zetasizer (Nano ZS90). The prepared SLN and NLCs 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. Measurement of zeta potential was done at 25°C and the strength of electric field strength was around 23.2 V/cm.

Transmission Electron Microscopy

Transmission electron microscope (TEM) images were obtained by using a Jeol 3010, Japan, transmission electron microscope operated at 300keV. TEM samples were prepared by dropping dispersion of the particles on copper grid supported formvar films.

Determination of drug content and entrapment efficiency

About 0.2mL of the NSPSLN/NSPNLCs formulation was diluted to 10mL with chloroform and methanol mixture (1:1) and then further dilutions were made with the mixture of above solvents. The diluted samples were estimated by UV- visible spectrophotometer at λ_{max} 238nm for the amount of Nisoldipine present.

Entrapment efficiency of the system was determined by measuring the concentration of free drug (unentrapped) in aqueous medium as reported previously by Venkateshwarlu and Manjunath, 2004.[12] The aqueous medium was separated by ultracentrifugation using centrisart tubes (Sartorius, USA) which consist of filter membrane (M.Wt.cut off 20,000 Da) at the base of the sample recovery chamber. About 1mL of the undiluted sample of NSPSLN/NSPNLCs formulation was placed in the outer chamber and sample recovery chamber placed on top of the sample and centrifuged at 12,000 rpm for 15 min. The SLN/NLCs along with the encapsulated drug remain in the outer chamber and aqueous phase moves into the sample recovery chamber through the filter membrane. The amount of the NSP in the aqueous phase was estimated by UV-spectrophotometer at λ_{max} 238nm. The entrapment efficiency of Nisoldipine SLN/NLC was calculated using the formula:

$$Entrapment\ efficiency = \frac{Amount\ of\ Nisoldipine\ in\ lipid\ phase}{Assay\ value} \times 100$$

In vitro drug release studies of SLN and NLC and gels enriched with SLN and NLC

In-vitro release studies were performed using modified franz diffusion cells.[13] Dialysis membrane having pore size 2.4 nm and molecular weight cut off between 12,000-14,000 was used for the release studies. Dialysis membrane was soaked overnight in double distilled

water for 12h prior mounting in a modified franz diffusion cell. 3mL of SLN/NLCs dispersion/ 0.5g of lipid nanoparticles incorporated hydrogel were placed in the donor compartment and the phosphate buffer pH 7.4 with 0.75% sodium lauryl sulphate (SLS) was filled into receptor compartment. During the process, the receptor side solution was maintained at 37±0.5°C and stirred at 800 rpm with Teflon coated magnetic stirring beads. At fixed time intervals, 1mL of sample was withdrawn from receptor compartment through side tube analyzed by UV- visible spectrophotometer at λ_{max} 238nm.

Data obtained from *in-vitro* release studies were fitted to various kinetic equations to find out the mechanism of Nisoldipine release from NSPSLN, NSPNLC, NSPSLN gel and NSPNLC gel. The kinetic models used were zero order equation, first order equation, higuchi release and korsemayer peppas equation.

Ex vivo permeation studies

The *ex-vivo* permeation studies for optimized formulations was performed in sodium dihydrogen phosphate buffer (7.4) with 0.75% SLS using Franz's diffusion cell with diffusional area of 3.14 cm^2. In these studies, rats abdominal skin is used for the permeation.[14] The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor. 3mL of SLN/NLC dispersion of Nisoldipine and gels enriched with SLN/NLC sample was placed on the skin surface in the donor compartment and 26 mL of media was placed in the receptor compartment. During the experiments, the solution in receptor side was maintained at 37±0.5°C and stirred at 800 rpm with Teflon coated magnetic beads. After application of the test formulation on the donor side 1 mL of samples were collected from a receptor compartment at designated time intervals (1, 2, 4, 8, 12, 20 and 24hr). Thereafter, an equivalent volume of receptor fluid was supplied to the receiver compartment immediately after each sample collection. The amount of NSP in receptor fluids were analyzed by UV-Visible spectrophotometer at λ_{max} 238nm.[15]

Nisoldipine fluxes through the skin were calculated by plotting the cumulative amount of drug permeated through skin against time and determining the slope of the linear portion of the curve. At steady state, drug fluxes (µg/cm^2/hr) were calculated by dividing slope of the linear portion of the curve by the area of the skin surface through which diffusion took place. The target flux is calculated using the following formula.[16]

$$J_{target} = C_{SS} CL_T BW/A$$

Where, A is the effective diffusional area (3.14 cm^2), BW the standard human body weight of 60 kg, C_{SS} the Nisoldipine concentration at the therapeutic level and CL_T the total clearance.

Physical stability studies

Nisoldipine loaded solid lipid nanoparticles were stored at room temperature and refrigerated temperature for 60 days and average size, zeta potential and poly dispersity index were determined. The number of samples estimated was in triplicate (*n*=3).

Lyophilisation of NLCs preparation

Lyophilisation was done to enhance the stability of the NLCs preparation. To the NLCs preparation 10% mannitol was used as a cryoprotectant. The samples were frozen overnight and those frozen samples were subjected to lyophilisation at -56°C for a period of 24 hr.

RESULTS AND DISCUSSION

Characterization of the investigated formulation

NSPSLN were prepared by hot homogenization followed by ultrasonication method using three different solid lipids like Dynasan 114, 116 and 118. For optimizing the process variables, optimal particle size and PDI were observed when the homogenization was carried out for a period of 10 min and ultrasonication time was carried out for a period of 20 min. Further increase in homogenization time and ultrasonication time had no considerable effect on particle size. Similarly, for selecting formulation variables, optimized lipid concentration was 200mg increasing the lipid concentration beyond 300mg resulted higher particle size and release was slowed down. The optimized concentration of Soya lecithin and surfactant (poloxamer 407) was found to be100mg and 1.75% w/v respectively, the final composition of NSPSLN and NSPNLCs were shown in Table 1.

The optimized SLN formulations with three different lipids (Dynasan 114, 116, 118) are F4, S4 and E4. The best of these three formulations i.e., E4 is used to prepare the NLCs. NLCs were prepared in exactly the same manner as the SLN dispersions, only partially replacing 30% of the solid lipid matrix by liquid lipid (oleic acid), which was discussed earlier.

For this investigation, hydrogel was prepared using optimal stabilizer combination of water, gel forming agent (Carbopol 934) and hydrating agent is glycerol. The selected aqueous dispersions of NSPSLN and NSPNLCs were admixed to the freshly prepared hydrogels.

Table 1: Formulation table for preparation of solid lipid nanoparticles and nano structured lipid carriers.

Formulation ingredients	Formulation code															
	F1	F2	F3	F4	F5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	NLC
Nisoldipine(mg)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Dynasan-114(mg)	100	200	300	200	200											
Dynasan-116(mg)						100	200	300	200	200						
Dynasan-118(mg)											100	200	300	200	200	170
Soyalecithin 95%(mg)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Poloxamer 407 (%)	1.5	1.5	1.5	1.75	2	1.5	1.5	1.5	1.75	2	1.5	1.5	1.5	1.75	2	1.75
Oleicacid (mL)																0.067
Double distilled water (mL)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Chloroform : Methanol (1:1)(mL)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Physicochemical properties

The aqueous dispersion of NSPSLN and NSPNLCs were light yellowish in color, odorless and fluid in nature. Even after centrifugation dispersion was stable and didn't show sedimentation. Hydrogel incorporated with aqueous dispersions of NSPSLN and NSPNLCs were light yellow in color and odorless with smooth appearance. The gels pH was at 6.5.

Particle size analysis

After preparation, the aqueous dispersions were stored at room temperature and their particle size and polydispersity index was measured using Photon correlation spectroscopy. After particle size analysis of the all formulations made with three solid lipids, results are summarized in Figure 1 and Table 2. The particle sizes of all formulations ranged from 160.23 nm to 330.25 nm, PDI from 0.20 to 0.44. It could also be observed that the particle size increased with the increase of lipid concentration and also PDI value decreased with the increase of lipid concentration. Based on the above results, selecting the optimizing formulation variables like lipid and surfactant concentrations were 200mg and 1.75% respectively. Optimizing process variables like homogenization time and ultrasonication time were 10min and 20min. Table 3 shows the photon correlation spectroscopy (PCS) mean particle size and polydispersity index after 1, 30 and 60 days of storage at room and refrigerator temperatures. It could also observe that increment of particle size and PDI value are higher when formulations are stored at room temperature compared to stored in refrigerator.

Zeta Potential (ζ)

Zetapotential measurement allows prediction about stability during the storage of colloidal dispersions. In general, aggregation of particles is less likely to occur for charge particles. For lipid nanoparticles the surface having the charge is negative. The zetapotential determination of aqueous of SLN and NLCs were performed during storage at room temperature and refrigerator conditions. The zeta potential and standard deviation values of all formulations are summarized in Figure 2 and Table 2. Agreement of these results with theory says that increased zetapotential values means that increased stability by electro repulsion. Increased zetapotential values were observed when increment in the concentration of lipid and surfactant. The zetapotential values of optimized E4 and E4 NLCs measured on 1st day, after 30days and after 60days of storage at room and refrigerator conditions, reveals that a slight decrease in the zetapotential of the lipid nanoparticles

during storage time at both temperature. In comparison to E4 (NSP SLN with Dynasan118) for with the same lipid content E4 NLCs formulation shows lower zeta-potential (Table 3).

Drug content and Entrapment efficiency

Drug content results showed that concentration of Nisoldipine in the total system ranged from 9mg to 9.6mg for different SLN/NLCs formulations. The percentage of drug which is incorporated in the lipid matrix (entrapment efficiency) was evaluated on day 1 for all formulations. Entrapment efficiency of all formulations ranged from 72 to 97% on day 1. The results are shown in Figure 3 and Table 2. Incorporation of Nisoldipine led to high entrapment efficiency, probably because of their lipophilic character. The sample with lower lipid concentration shows lower entrapment efficiency but in case of surfactant, higher concentra-

Table 2: Particle size, PDI and zetapotential of Nisoldipine in SLN and NLCs formulations (mean±SD).

Formulation code	Size (nm)±SD	PDI ±SD	Zeta potential (mV)±SD	Total drug content (mg)	Entrapment effficiency (%)
F1	160.23±5.96	0.44±0.028	15.9±1.87	9.09±0.05	72.47±0.21
F2	172.52±7.52	0.37±0.035	18.7±1.78	9.13±0.09	75.82±0.11
F3	230.95±3.45	0.36±0.013	20.2±1.01	9.37±0.02	82.71±0.31
F4	167.80±5.87	0.25±0.016	21.9±1.87	9.52±0.01	89.23±0.10
F5	192.23±2.89	0.39±0.012	21.7±1.78	9.16±0.07	86.08±0.24
S1	200.42±3.12	0.35±0.018	18.2±1.34	9.02±0.04	84.35±0.42
S2	260.52±4.25	0.31±0.021	23.4±1.31	9.23±0.01	87.58±0.21
S3	318.28±6.28	0.27±0.017	20.1±1.72	9.47±0.02	89.47±0.31
S4	245.30±4.92	0.21±0.031	25.9±1.56	9.54±0.07	91.38±0.21
S5	271.76±8.52	0.28±0.084	23.7±1.45	9.40±0.03	89.26±0.25
E1	220.23±5.26	0.26±0.028	22.1±1.58	9.03±0.05	86.36±0.32
E2	290.52±2.52	0.21±0.035	25.3±1.25	9.27±0.03	90.28±0.41
E3	330.25±3.45	0.27±0.013	23.2±1.01	9.39±0.08	91.75±0.25
E4	260.73±4.47	0.20±0.097	29.1±2.16	9.60±0.02	93.56±0.30
E5	300.29±5.79	0.23±0.015	26.3±2.06	9.23±0.01	90.06±0.09
NLC	140.79±12.9	0.29±0.013	27.9±1.97	9.66±0.07	97.07±0.07

Table 3: Particle size, PDI and zetapotential of Nisoldipine in SLN and NLCs formulations calculated after 1, 30 and 60 days of storage at room and refrigerated temperature (mean±SD).

Formulation Code	Day	At room temperature			At refrigerated temperature		
		Size(nm)	PDI	Zeta potential(mV)	Size(nm)	PDI	Zeta potential(mV)
E4	1	260.73±4.47	0.20±0.09	-29.1±2.16	260.73±4.47	0.20±0.09	-29.1±2.16
	30	285.56±8.58	0.28±0.14	-25.3±2.06	272.23±2.89	0.31±0.01	-26.1±2.04
	60	320.9±11.49	0.47±0.06	-22.4±2.08	291.01±8.54	0.34±0.03	-24.6±2.12
E4 NLC	1	140.79±12.9	0.29±0.01	-27.9±1.97	140.79±12.9	0.29±0.01	-27.9±1.97
	30	152.64±9.34	0.37±0.03	-23.3±1.67	147.09±3.14	0.32±0.01	-25.4±1.26
	60	165.28±7.36	0.44±0.05	-20.8±1.80	152.29±4.32	0.38±0.04	-22.1±1.32

tion shows lower entrapment efficiency, for all SLN formulations. Transition of dispersed lipid from meta stable form to stable form might occur slowly on storage because of small particle size and the presence of surfactants may lead to drug expulsion of from SLN formulations.[17-19] E4 NSP NLCs are responsible for higher entrapment efficiency in comparison to all SLN formulations. This result due to the binary mixture of liquid and solid lipids, resulting in only a very weak crystallization.[20,21] For all the formulations, the entrapment efficiency was higher than 80%.

Drug-Excipients compatibility studies (DSC Analysis)

A differential Scanning Calorimetry (METTLER) was used to study the thermal analysis of drug-excipients compatibility (Figure 4). Pure drug (Nisoldipine), freeze dried SLN preparations of three lipids Dynasan 114, 116 and 118 products were scanned in the temperature range of 50-250°C. Analysis performed under a Nitrogen purge. Nisoldipine showed a sharp endothermic peak at 157.34°C. Lyophilized product of Nisoldipine loaded Dynasan 114,116 and 118 SLN showed sharp endothermic peaks at 162.36°C, 163.46°C and 163.45°C respectively but intensity of the peak was decreased. This characteristic peak intensity was not observed in nisoldipine loaded SLN. The absence of detectable crystalline domains of nisoldipine in drug-loaded SLNs clearly indicates that nisoldipine encapsulated in SLNs is in the amorphous or disordered-crystalline phase or in the solid-state solubilized form in the polymer matrix. The endothermic peaks of Dynasan-114,116 and 118 were found approximately at ~ 61.51°C,~ 68.72°C and ~74.55°C respectively due to glass transition temperature (Tg) of Polymer. Thus the observation indicated that there was no interaction between Nisoldipine and three lipids.

Transmission Electron Microscopy

The image of TEM of the NSPSLN and NSPNLC was shown in Figure 5A and B, respectively. The result shows that the particles diameters vary from about 150 to 300 nm. There is no significant difference in the diameter between SLN and NLCs.

Drug release from SLN and NLC by dialysis method

From above results formulations containing Dynasan-114, 116 and 118 (F4, S4 and E4) were selected for *in-vitro* drug release studies. Results showed (Figure 6 and 7) maximum drug release of 65.54%, 71.16% and 75.96% respectively in pH 7.4 phosphate buffer with 0.75% SLS. The drug release pattern form formulation of SLNs F4, S4 and E4 showed biphasic release behavior consisting of initial burst release of 20% to 30% within one hour, followed by sustained drug release about 60 to 75% at the end of 24th hr. Presence of adsorbed drug on the surface of solid lipid nanoparticles is the reason for initial burst drug release and increased diffusional path length and hindering effect of surrounding solid lipid shell are the reasons for further sustained drug release. The SLNs with Dynasan 114, 116 and 118 incorporated into carbopol 934p gels (F4 gel, S4 gel and E4 gel) showed release of 29.94%, 24.68% and 37.93% respectively. E4 formulation showed highest percentage drug release among all the prepared SLNs, for that E4 formulation selected for further studies. NLC formulation (Dynasan 118) and gel loaded with NLCs of Dynasan 118 showed maximum release of 82.09% and 40.76% respectively. Further, all SLNs released drug relatively slowly when compared to that of NLCs. For preparation of NLCs 30% of solid lipid is replaced with liquid lipid (Oleic acid) due to this reason NLCs showing higher drug release compared to SLNs.

Release Kinetics

To know the release kinetics drug release data can be fit into different drug release kinetics results shown in Table 4. Release of drug from all the formulations followed zero order and higuchi, the best fit with the highest correlation coefficients were shown in zero order followed by higuchi plot. The mechanism of release is by diffusion as indicated by R^2 value of higuchi and n value of korsemeyer peppas equation. The percentage drug release was proportional to square root of time (Higuchi model) indicating that drug release from SLN and NLC and gels enriched with SLN/NLC is diffusion controlled. More over the plots of log percentage released vs. log time showed a high level linearity as given in Table 4, which in turn another conformation that the release is diffusion controlled.

Ex vivo permeation studies

The *ex vivo* skin permeation of Nisoldipine loaded SLN formulations (F4, S4 and E4), NLC (E4 NLC), gels enriched with F4, S4, E4, NLC and drug solution were showed in Figure 8 and 9. NSPNLC exhibited the greatest (68.10%) percentage release of drug permeation in 24 hr. the amount of drug permeated per square centimeter of the effective diffusional area through the rat abdominal skin when plotted against time, the permeation profiles of drug seem to follow Higuchi's equation (R^2 =0.978 to 0.991) and zero order kinetics as it is evidenced by correlation coefficients (0.791 to 0.977). The flux for every formulation was shown in Figure

Table 4: Regression coefficient (R^2) values of Nisoldipine loaded SLN and NLC formulations through dialysis membrane.

Formulations	Zero order	First order	Higuchi	Korsmeyer- peppas	*n* value
F4	0.983	0.943	0.988	0.956	0.38
S4	0.943	0.874	0.96	0.978	0.345
E4	0.936	0.889	0.963	0.961	0.328
F4 gel	0.874	0.708	0.834	0.946	0.437
S4 gel	0.902	0.741	0.846	0.934	0.365
E4 gel	0.942	0.828	0.931	0.98	0.511
NLC	0.912	0.856	0.945	0.963	0.323
NLC gel	0.944	0.893	0.969	0.978	0.291

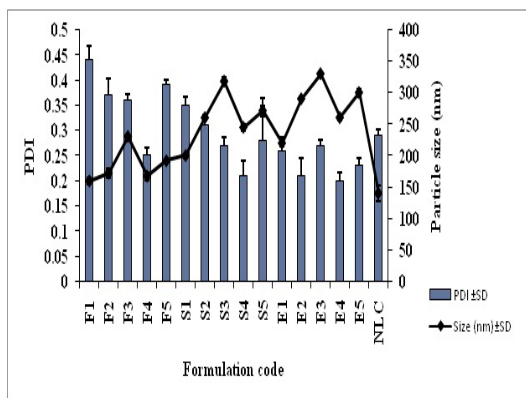

Figure 1: Particles size and PDI of all SLN and NLCs formulations.

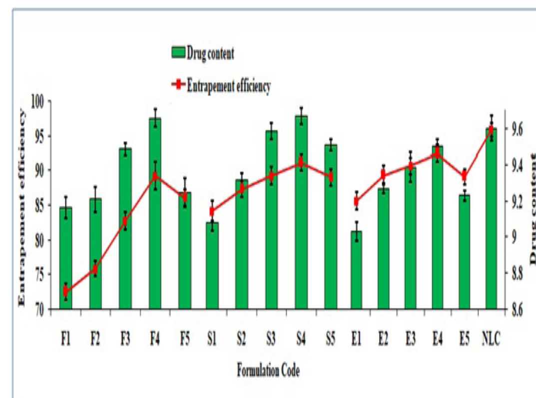

Figure 3: Drug content and entrapment efficiency of all formulations.

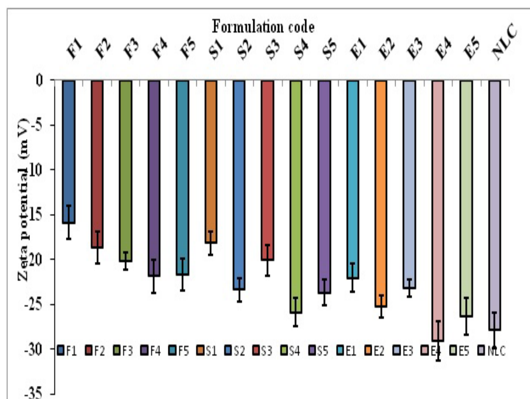

Figure 2: Zeta potential values of all SLN and NLCs formulations.

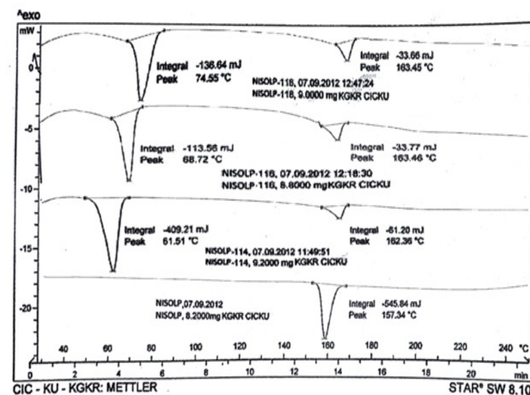

Figure 4: DSC studies of Nisoldipine and various formulations.

10. E4 SLN and E4 NLCs showed higher flux 0.019 mg/cm^2/hr and 0.018 mg/cm^2/hr. the results of drug release from NSPSLN, NSPNLC and gels enriched with SLN/NLC through the rat abdominal skin confirmed that Nisoldipine was released and permeated through the human skin. Finally percentage of drug permeated through rat kin was correlated against percentage of drug release by using *in-vitro* release test for optimized formulations, Figure 11 and 12 shows relationship between the percentage of Nisoldipine permeated in *ex-vivo* and percentage of Nisoldipine in *in-vitro*. The straight line and high correlation coefficient 0.979 and 0.993 of E4 NLCs and E4 NLCs gel respectively proved the good correlation between *ex-vivo* permeation and *in-vitro* drug release studies. Hence by the complete differences in the test conditions of *ex-vivo* and *in-vitro* release

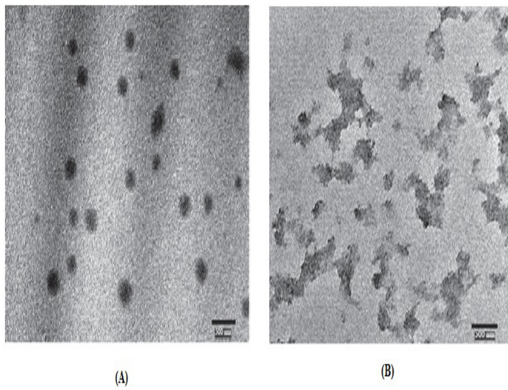

Figure 5: (A) Transmission electron microscope (TEM) image of nisoldipine solid lipid nanoparticles (NSPSLN). (B) TEM image of nisoldipine nanostructured lipid carrier (NSPNLC).

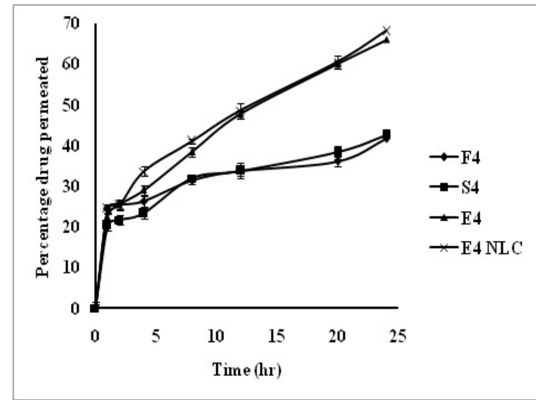

Figure 8: *Ex vivo* permeation studies of SLN/NLCs formulations.

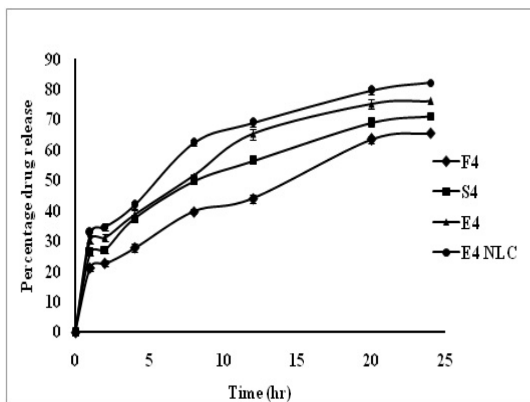

Figure 6: *In vitro* drug release studies of SLN/ NLCs formulations.

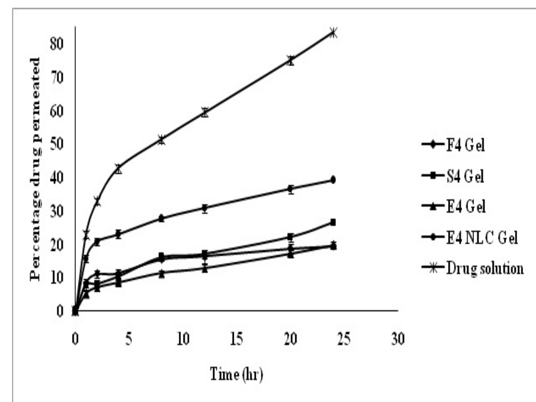

Figure 9: *Ex vivo* permeation studies of gel enriched with SLN/NLCs formulations and drug solution.

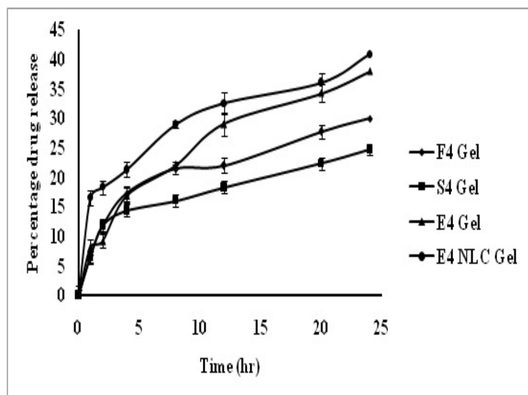

Figure 7: *In vitro* drug release studies of gels enriched with SLN/NLCs.

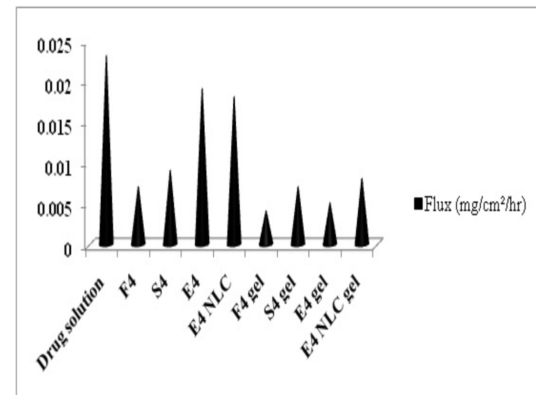

Figure 10: Calculation of flux of various SLN, NLCs formulation and drug solution.

studies, the high correlation and coincidence of *in-vitro* and *ex-vivo* release profile, it can be concluded that such a transdermal gel systems could be a useful carrier in transdermal drug delivery systems.

Physical stability studies

Nisoldipine loaded SLN were stored at room and refrigerated temperature for 60 days and average size, zeta potential and poly dispersity index (PDI) were determined. Stability studies were conducted for optimized formulation (E4) and NLC which showed better size, PDI, zeta potential, entrapment efficiency and release characteristics. Some changes were noted in size, PDI and zeta potential values, which indicated the susceptibility for stability problems during storage at room temperature and 4°C. Hence, lyophilization technique

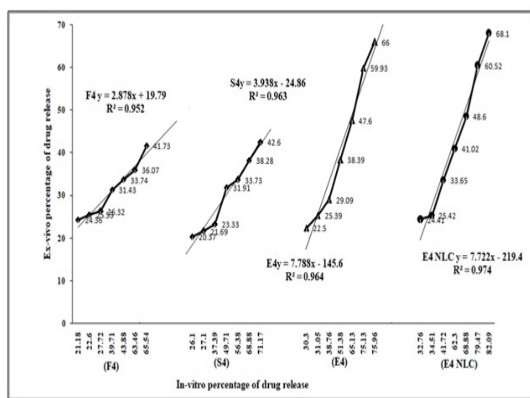

Figure 11: ***In vitro*** **and** ***ex vivo*** **correlation between the cumulative % of drug released** ***in vitro*** **and % of drug permeated** ***ex vivo*** **of optimized SLN and NLC.**

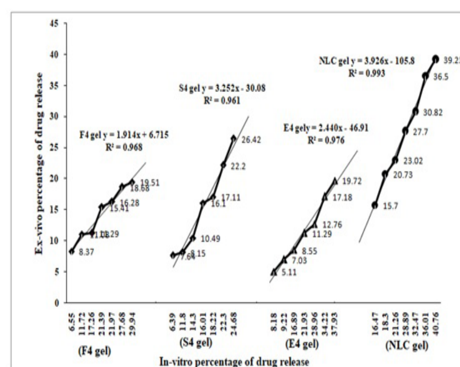

Figure 12: ***In vitro*** **and** ***ex vivo*** **correlation between cumulative % of drug released in vitro and % of drug permeated** ***ex vivo*** **of optimized SLN Gels and NLC Gels.**

Table 5: Characterization of optimized formulation E4 before and after lyophilization.

Parameters	Before lyophilization	After lyophilization
Size (nm)	260.73	623.3
PDI	0.2	0.39
Zeta potential (mV)	29.1	31.2

was used to overcome this problem. The optimized formulation (E4) was subjected to lyophilization. Mannitol (10%) was used as cryoprotectant. Characterization of optimized formulation E4 before and after lyophilization results were tabulated in Table 5. Due to lyophilization the size of NLCs increased due to adhesion of smaller NLCs particles and PDI was also found to be increased.

CONCLUSION

In this work, efforts were made to prepare stable solid lipid nanoparticles and nanostructured lipid carriers for characterization of *in-vitro* and *ex-vivo* release of Nisoldipine from SLNs, NLCs and gels with SLN/NLC. Stability studies were conducted for finally optimized formulation (E4) at room temperature and 4°C for 2 months; some changes were noticed in size, PDI and zeta potential values, which indicated the susceptibility for stability problems during storage at room temperature and 4°C. Hence, lyophilization technique was used to overcome this problem. NSPNLC dispersion showed faster release in comparison NSPSLN dispersion and gels enriched with SLN/NLCs. *In vitro* release of NSPNLC, NSPSLN dispersions and gels enriched with SLN/NLC followed Higuchi and zero order kinetics. Both SLN and NLC showed a sustained drug release over period of 24 hr but the sustained effect was more pronounced with the SLN and NLC gel formulations. In conclusion, the developed formulations are promising alternative drug carriers for transdermal drug delivery. Pharmacokinetic and pharmacodynamics evaluation of these systems in animals and humans is necessary to confirm these findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SLN: Solid Lipid Nanoparticles; **NLCs:** Nanostructured Lipid Carriers; **NSP:** Nisoldipine; **NSPSLN:** Nisoldipine solid lipid nanoparticles; **NSPNLCs:** Nisoldipine nanostructured lipid carriers; **PDI:** Poly Dispersity Index.

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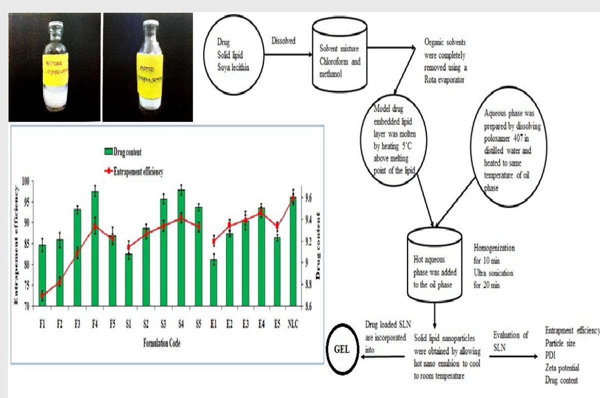
PICTORIAL ABSTRACT



SUMMARY

- Nisoldipine loaded SLN and NLCs were prepared by using different solid lipids and liquid lipids for application to transdermal drug delivery.
- Both SLN and NLC showed a sustained drug release over period of 24 hr but the sustained effect was more pronounced with the SLN and NLC gel formulations.

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