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International Journal of Pharmaceutics 346 (2008) 124–132
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Pharmaceutical Nanotechnology

Nanostructured lipid carrier (NLC) based gel of celecoxib

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Received 13 March 2007; received in revised form 29 May 2007; accepted 29 May 2007
Available online 15 June 2007

Abstract

Nanostructured lipid carriers (NLC) based topical gel of celecoxib was formulated for the treatment of inflammation and allied conditions. NLC prepared by the microemulsion template technique were characterized by photon correlation spectroscopy for size and scanning electron micrograph (SEM) studies. Drug encapsulation efficiency was determined using Nanosep® centrifugal device. The nanoparticulate dispersion was suitably gelled and assessed for *in vitro* release and *in vitro* skin permeation using rat skin. Efficacy of the NLC gel was established using a pharmacodynamic study, i.e., aerosil-induced rat paw edema model. The skin permeation and rat paw edema pharmacodynamic studies were carried out in comparison with a micellar gel which had the same composition as that of the NLC gel except for the solid lipid and oil. The NLC based gel described in this study showed faster onset and elicited prolonged activity until 24 h.

Keywords: Celecoxib; Nanosturctured lipid carriers (NLC); Topical gels; Micellar; Anti-inflammatory; Prolonged action

1. Introduction

In the last decade, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been looked upon as promising carriers for presenting several attractive features for transdermal drug delivery. SLN are identical to an oil-in-water emulsion, except that the liquid lipid (oil) portion of the emulsion is replaced by a solid lipid having a mean photon correlation diameter (PCS) ranging between 80 and 1000 nm (Müller et al., 2000a). SLN are particles made from solid lipids or lipid blends and are produced by one of the following techniques, namely, high pressure homogenization (Müller and Lucks, 1996), microemulsion template technique (Gasco, 1993), solvent emulsification evaporation technique (Sjöström and Bergenståhl, 1992), solvent displacement technique (Hu et al., 2002; Schubert and Müller-Goymann, 2003), solvent emulsification diffusion method (Trotta et al., 2003; Quintanar-Guerrero et al., 2005), phase inversion (Heurtault et al., 2002) and a very recently introduced membrane contractor technique (Charcosset et al., 2005; Ahmed El-Harati et al., 2006). NLC, the new generation of lipid nanoparticles, overcome the limitations associated with the SLN, namely, limited drug loading, risk of gelation and drug leakage during storage caused by lipid polymorphism (Müller et al., 2000b). NLC consists of a mixture of spacially very different lipid molecules, i.e., solid lipid(s) is blended with liquid lipid(s) (oils) (Müller et al., 2004). The resulting matrix of the lipid particles shows a melting point depression compared to the original solid lipid; however, the matrix remains solid at body temperature (Müller et al., 2002a).

Both SLN and NLC possess numerous features that are advantageous for topical route of application (Müller et al., 2000c, 2002a,b; Mehnert and Mäder, 2001). SLN and NLC are colloidal carrier systems providing controlled release profiles for many substances (Müller et al., 1995; Zur Muhlen et al., 1998; Souto et al., 2004; Souto and Müller, 2005). These carriers are composed of physiological and biodegradable lipids exhibiting low systemic toxicity and low cytotoxicity (Müller et al., 1997). Most of the used lipids have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical preparations. The small size of the lipid particles ensures close contact to stratum corneum and can increase the amount of drug penetrating into mucosa or skin. Due to their solid lipid matrix, a controlled release from these carriers is possible. This becomes an important tool when it is necessary to supply the drug over prolonged period of time, to reduce systemic absorption, and when drug produces irritation in high concentrations. As a result of film formation after topical application, occlusive

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doi:10.1016/j.ijpharm.2007.05.060

properties have also been reported for SLN (Wissing and Müller, 2001, 2002a,b).

Celecoxib (CXB), a selective COX-2 inhibitor, has been approved for the treatment of rheumatoid arthritis, osteoarthritis, acute pain, familial adenomatous polyposis and primary dysmenorrhea (Krishnan et al., 2003; Marshall, 1999; Simon et al., 1998). An arthritic condition demands a controlled release drug delivery system for a prolonged period so that can satisfy the goals of the treatment like reduction of pain and inflammation, maintenance of functional ability, slowing of disease progression and prevention of adverse effects of drugs. Moreover, topical formulations of COX-2 inhibitors are being developed as a novel pharmacologic approach for the treatment of COX-2 mediated skin diseases like inflammation, pain and nociception, skin tumors, injury and wounds (Lee et al., 2003). The development of a novel delivery system for a particular drug for topical application is complex due to the wide diversity of the drug solubility in the vehicle components and the vast range in cutaneous fluxes. In the majority of the pharmaceutical formulations intended for topical and dermatological therapy, the drug molecules are totally dissolved in a liquid phase of oil-in-water (o/w) or water-in-oil (w/o) emulsions. However, due to the low viscosity of the inner phase of the afore-mentioned systems, it is difficult to achieve a prolonged or controlled release of a model drug (Siekmann and Westesen, 1998). The need of the hour is design of a topical drug delivery system of CXB that could not only increase the presence of the drug locally and for a prolonged period, but also reduce the risk of systemic toxicity as a result of the reduced dose. SLN and NLC have been shown to exhibit a controlled release behavior for various active ingredients such as ascorbyl palmitate (Üner et al., 2005), clotrimazole (Souto et al., 2004), ketoconazole (Souto et al., 2005), suncreens (Müller et al., 2002a,b) and other antifungal agents (Souto and Müller, 2006).

Hence, in the present investigation, the feasibility of NLC as a novel carrier system for topical application of CXB, with regard to the modulation of the release of CXB, was checked. The role played by the oily phase component of the NLC was also judged by comparing the *in vitro* release, permeation, and *in vitro* pharmacodynamic activity with a micellar gel. The micellar gel was prepared using the excipients as that in the NLC gel, except the solid lipid and the oil. NLC were prepared from microemulsion templates and the procedure is as described in our previous publication (Joshi and Patravale, 2006).

The developed topical gel could also have implications in chemoprevention of skin cancer as the topical application of COX-2 inhibitors is known to inhibit ultraviolet-B (UVB) mediated cutaneous inflammation. Various topical drug delivery systems have been suggested for the same (Subramanian et al., 2005; Yener et al., 2003).

2. Materials and methods

2.1. Materials

Celecoxib (CXB) was obtained as a gift from Cadila Pharmaceuticals Ltd., Ahmedabad, India; Emulsynt Glyceryl dilaurate (GDL) was a kind gift from ISP through Anshul Agencies, Mumbai, India; Capmul MCM (glyceryl mono-dicaprylate) from Abitech Corporation through Indchem International, Mumbai, India; Capryol 90 (propylene glycol monocaprylate, containing 90% monoesters), Gelucires (glycerol esters of saturated fatty esters), Apifil pastils (PEG-8 Beeswax), Transcutol (purified diethylene glycol monoethyl ether), Labrasol (caprylocaproyl macrogol-8 glycerides) from Gattefosse France through Colorcon Asia Pvt. Ltd., Mumbai, India; Cremophor RH 40 (PEG 35 Castor oil), Solutol HS 15 (poly-oxyethylene esters of 12-hydroxystearic acid) from BASF India Ltd., Mumbai, India; Glyceryl monostearate from Fine Organics Pvt. Ltd., Mumbai, India; Miglyol 812 (caprylic/capric triglyceride) and Softigen 767 (PEG-6 caprylic/capric glycerides) from Sasol GmBH through S. Zhaveri and Co., Mumbai, India. While PEG 400, Tween 20, Tween 80 were purchased from SD Fine Chemicals, Mumbai, India. The gelling agent Carbopol Ultrez 10 was obtained as a gift sample from Noveon, India. All the other chemicals were of the analytical grade.

2.2. Screening of components (solubility studies)

The solubility of CXB was determined in different solid lipids, oils, surfactants and solubilizers (Shen and Zhong, 2006). An excess of drug was added individually to oil, surfactant and solubilizer (5 ml each) in screw capped tubes. After 24 h, each sample was centrifuged and 0.5 ml of the clear supernatant layer was diluted suitably with methanol, and analyzed by high performance liquid chromatography (HPLC). One of the most important factors that determines the loading capacity of the drug in the lipid is the solubility of drug in melted lipid. However, equilibrium solubility studies can not be carried out in this case. Hence, we used a modified method (Joshi and Patravale, 2006) to identify the solid lipid having better solubilization potential for CXB. For studying the solubility in solid lipids, 100 mg of the CXB was taken in a test tube, the solid lipid was added in increments of 0.5 g, and the test tube was heated in a controlled temperature water bath kept at 80 °C. The amount of lipid required to solubilize the CXB

Table 1
Solubility of celecoxib in different oils, surfactants and solubilizers

	Solubility
Oils (mg/g)	
Miglyol 812	10.73 ± 2
Capmul MCM	53.02 ± 5
Capryol 90	84.23 ± 8
Surfactants (mg/g)	
Tween 20	307.38 ± 30
Tween 80	381.12 ± 40
Labrasol	397.91 ± 40
Cremophor RH 40	239.74 ± 20
Softigen 767	378.73 ± 30
Solutol HS 15	404.33 ± 40
Solubilizers (mg/ml)	
PEG 400	301.66 ± 30
Transcutol	554.16 ± 50

Table 2
Amount of solid lipid required to solubilize 100 mg of celecoxib

Solid lipid	Amount (g)
Glyceryl dilaurate	1.5
Gelucire 62/05	1
Gelucire 50/13	0.5
Gelucire 53/10	1.2
Gelucire 44/14	2.0
Apifil pastils	1.2
Glyceryl monosterate	1.0

in the molten state was estimated. The results are compiled in Tables 1 and 2.

2.3. HPLC analysis of CXB

The method described by Dhabu and Akamanchi (2002) was used for the analysis. The HPLC system consisted of Jasco PU 2080 Plus Intelligent HPLC Pump, Jasco, Japan, equipped with Merck C18-10 4.6 i.d. × 250 mm, 10 μm particle size column and a Jasco UV 2075 Intelligent UV–vis Detector, Jasco, Japan, with a Rheodyne 7725 injector USA managed by Jasco Borwin Chromatography software version 1.05. The mobile phase (methanol:water in the ratio of 75:25) was run at a flow rate of 0.8 ml/min and the ultraviolet absorption was read at 251 nm.

2.4. Formulation of NLC and micellar dispersions

2.4.1. Formulation of microemulsion

Selection of following components of microemulsion system was based on CXB-solubilizing capacity of the excipient. The selected components were as follows:

- Lipid phase
  (1) Solid lipid: Glyceryl dilaurate.
  (2) Oil phase: Capmul MCM.
- Surfactant phase
  (3) Surfactant phase: Cremophor RH 40.
  (4) Solubilizer: Transcutol.
  (5) Aqueous phase: double distilled water.

The components selected for the formulation of microemulsion system were GRAS listed.

2.4.2. Pseudoternary phase diagram

The boundaries of the microemulsion domains were determined with the aid of pseudoternary phase diagrams with the above components as the constituents of microemulsion. The lipid phase consisted of a 1:1 mixture of Capmul MCM and Glyceryl dilaurate. The surfactant phase consisted of a mixture of Cremophor RH 40 and Transcutol (ratio 1:1.66), while the aqueous phase was double distilled water. These ratios were based on series of trial and error experiments for which the data is not shown. The lipid phase was heated to melt the solid lipid. The required quantities of surfactant phase and the lipid phase were heated to the same temperature and gently mixed to form

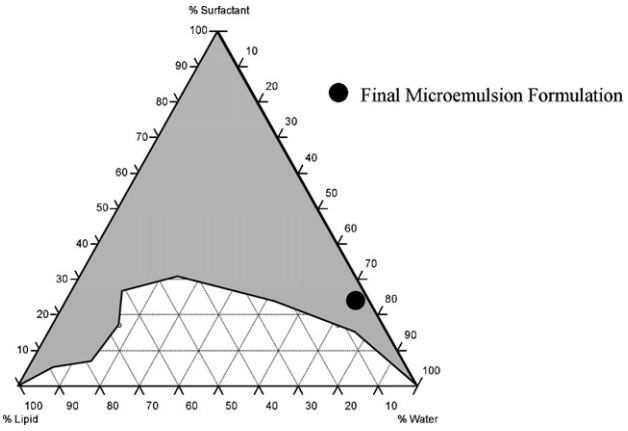


Fig. 1. Microemulsion phase diagram plotted with a lipid phase of 1:1 mixture of Capmul MCM and Glyceryl dilaurate and surfactant phase of mixture of 1:1.66 Cremophor RH 40 and Transcutol and double distilled water as aqueous phase.

a monophasic mixture that was slowly titrated with aliquots of distilled water and stirred at 60 °C for a sufficiently long time to attain equilibrium. After equilibrium was reached, the mixtures were checked visually for transparency and through crossed polarizers for optical isotropy. Only those systems which appeared black when visualized through the crossed polarizers were deemed to be within the microemulsion region. No attempts were made to completely identify the other regions of the phase diagrams. The pseudoternary phase diagram is as depicted in Fig. 1.

2.4.3. Characterization of the microemulsion

2.4.3.1. Freeze–thaw cycling. The microemulsion was subjected to freeze–thaw cycles (−4 to 40 °C) of 24 h for a period of 1 week and assessed for physical instabilities such as phase separation and precipitation.

2.4.3.2. Optical birefringence. The microemulsion was checked both visually and using crossed polarizers for optical isotropy to confirm absence of other phases.

2.4.4. Formulation of NLC from microemulsion templates

NLC were prepared from warm microemulsion templates as described in our previous publication of NLC based gel of valdecoxib (Joshi and Patravale, 2006) which is based on the original method by Gasco (1993) for the preparation of SLN. This method was adapted because of its obvious advantages such as elimination of the need of both the specialized equipments (Patravale et al., 2004) energy required to generate nanocarriers (Gasco, 1997). The lipid phase consisted of molten lipid mixed with oil and CXB. The surfactant, solublizer and water comprised the aqueous phase. Both the phases were maintained at a temperature above the melting point of the lipid (60 °C). At this temperature, the two phases were mixed using a cyclomixer to form a microemulsion. This warm microemulsion was diluted in cold water (2–3 °C) under mechanical stirring *over head* stirrer (Remi, India) at 3000 rpm for 10 min to form the NLC dispersion such that the concentration of CXB in the final dispersion remained 1% (w/w).

Table 3
Compostion of 1% celecoxib micellar dispersion

Ingredient	Quantity (%)
Phase A	
Celecoxib	1
Gelucire 44/14	5
Phase B	
Cremophor RH 40	23
Transcutol	10
Water	32

2.4.5. Formulation of micellar dispersion

For the purpose of comparison and to understand the role of solid lipid and oils in NLC system, micellar dispersion having similar composition as that of the NLC gel was prepared omitting the solid lipid and oil. Gelucire 44/14 was used as the solubilizer. The final formula of 1% micellar dispersion is as depicted in Table 3. CXB was dissolved in gelucire by heating in a water bath maintained at 60 °C to form phase A. The phase B consisted of mixture of Cremphor RH 40, Transcutol and double distilled water. The two phases were mixed at same temperature using a cyclomixer to achieve micellar dispersion.

2.5. Characterization of the nanoparticulate dispersion

2.5.1. Determination of particle size and polydispersity index of the nanoparticulate dispersion

All measurements were performed in triplicates using a Beckman N4 plus submicron particle size analyzer (Wipro, India) at a temperature of 25 ± 2 °C and at 90° to the incident beam applying the principle of photon correlation spectroscopy (PCS). Dispersions were diluted with double distilled water to ensure that the light scattering intensity (between 6e+004 and 1e+006), was within the instrument's sensitivity range. Double distilled water was filtered through 0.45 μm membrane filters (Pall Life Sciences, Mumbai) prior to particle size determination.

2.5.2. Scanning electron micrographic studies

Images were recorded on a Hitachi S-576 Scanning electron micrograph (magnification: 50×; accelerating voltage: 20.0 kV). Analysis was performed at 25 ± 2 °C. The NLC dispersion was diluted appropriately and sonicated. Few drops of the dispersion was placed on the grid and allowed to dry. After the samples dried thoroughly, the image was captured (Fig. 2).

2.5.3. Determination of drug encapsulation efficiency

Determination of the amount of drug incorporated in NLC is of prime importance, since it influences the release characteristics. The amount of drug encapsulated per unit weight of the nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium. A known dilution of the NLC dispersion was prepared and 100 μl of it transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an ultrafilter (MWCO100KD, Pall Life Sciences). The Nanosep® was centrifuged at 15,000 × *g* (Eltek TC 4100 D Research Centrifuge) for 40 min. The filtrate was diluted appropriately and

Fig. 2. SEM of celecoxib NLC.

the amount of CXB in both the phases was estimated by HPLC analysis.

The entrapment efficiency was calculated by the following equation:

$$\text{Percentage entrapment efficiency} = \left[\frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}}\right] \times 100$$

where "$W_{\text{initial drug}}$" is the mass of the initial drug added and the "$W_{\text{free drug}}$" is the mass of the free drug detected in the filtrate of lower chamber of Nanosep® post-centrifugation of the aqueous dispersion.

2.6. Formulation of NLC based and micellar gels

Both the nanoparticulate dispersions was gelled using different gelling agents like carbopols, xanthan gum and carrageenan. Based on the compatibility with nanoparticulate dispersion, feel aesthetic appeal and ease of spreadability Carbopol (Ultrez 10) was selected as the gelling agent. Carbopol was dispersed using an overhead stirrer at the speed of 800 rpm (Remi, Mumbai, India) for 3 h. Different concentrations of Ultrez 10 ranging from 0.5 to 1% were used for gelling and the concentration giving the optimum viscosity was chosen for further studies. Carbopol (Ultrez 10) 0.6% was added to the nanoparticle dispersion under overhead stirring at 800 rpm. Stirring was continued till the carbopol got dispersed. The carbopol dispersion was neutralized using 0.05% (w/w) triethanolamine.

2.7. In vitro release from the gel using USP paddle over disk method

The paddle and vessel assembly from USP type II apparatus, with the addition of a small stainless steel disk assembly designed for holding the gel at the bottom of the vessel, was used. The temperature was maintained at 32 ± 0.5 °C. Phosphate buffer solution (pH 7.4), 900 ml of was placed in the vessel and equilibrated at 32 ± 0.5 °C. One gram of the gel was

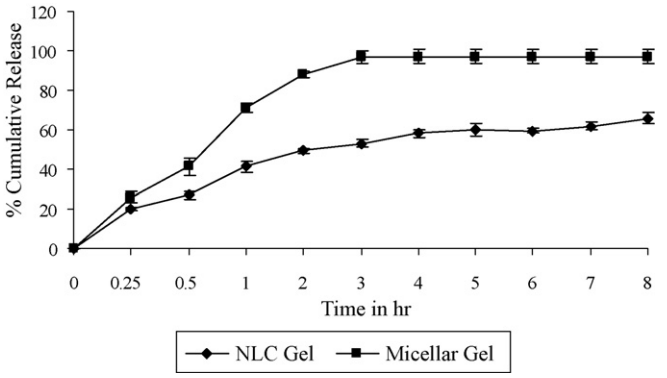


Fig. 3. *In vitro* release profile of CXB from NLC and micellar gels using USP paddle over disk method at the end of 8 h.

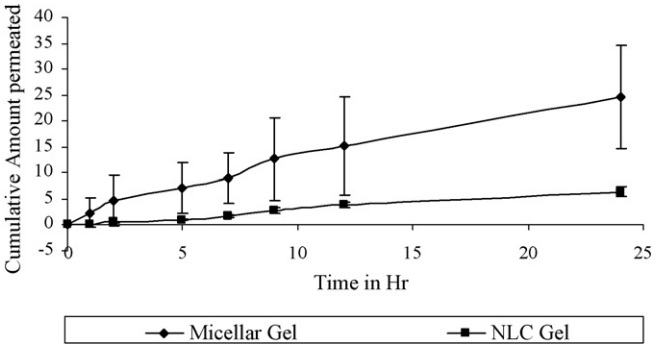


Fig. 4. *In vitro* permeation profile of CXB from NLC and micellar gels using excised rat skin at the end of 24 h employing a Franz diffusion assembly.

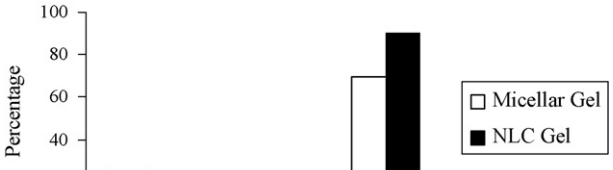
applied on the disk assembly, assuring that the release surface was as flat as possible. The disk assembly was gently inserted at the bottom of the dissolution vessel. The speed of rotation of the paddle was maintained 25 rpm. Aliquots were withdrawn at hourly intervals and analyzed spectrophotometrically at 254 nm. Baseline correction was done using a placebo gels which underwent similar treatment to determine *in vitro* release to nullify any possible absorption arising from the lipids or surfactants. A graph of percentage cumulative release against time (h) was plotted as depicted in Fig. 3. To describe the kinetics of the CXB release from the gel, mathematical models such as zero-order, first-order, Higuchi's model were used. The criterion for selecting the most appropriate model was based on a goodness-of-fit test.

2.8. *In vitro skin permeation studies*

For the purpose of this study, skin from the abdominal area of young male albino rat weighing 500 ± 30 g was used. The rat was sacrificed by decapitating in head. Subsequently, hair present in the abdominal area of the animal was carefully cut as short as possible using scissors, without damaging or scratching the skin surface. In the next stage, the skin was surgically removed, cleaned of muscle, fat, or vasculature, and kept at −4 °C for 24 h before experimentation. The skin specimens were thus prepared and individually placed in Franz-type diffusion cells having a surface area 3.14 cm^2, set at 32 °C using a thermostated water bath. Ten milliliter of phosphate buffer saline (PBS) (pH 7.4) was used as the receptor phase. Excised sections of rat skin were then carefully placed between the donor and receptor compartments of the diffusion cells and firmly adhered in place. Assurance was made that the skin was completely in contact with the receptor phase, eliminating any air bubbles. The stopper was placed on the withdrawal port for removing the receptor phase. Subsequently, 4.5 mg/cm^2 of the gels were uniformly placed in the donor phase, in contact with the excised section of rat skin. Receptor phase was stirred constantly throughout the experiment and the temperature maintained at 32 °C. At set intervals of 0, 1, 2, 5, 7, 9, 12 and 24 h, 2.5 ml of the receptor phase was removed and immediately replaced by the equal volume of PBS (pH 7.4) solution. All the experiments were performed in triplicate. The amount of CXB released into the receptor phase from the formulations was determined by HPLC. By determining the amount of CXB released at various time intervals, the cumulative% of CXB permeated versus time (h) graphs were plotted for both the gels (Fig. 4). Another graph of amount of celecoxib diffused per unit area (*Q*/*A*) versus time (h) was plotted. The linearity of this region was checked using statistical testing of linear regression. Having confirmed the linearity of this region, the slope of the line (flux) was calculated. The amount of formulation remaining on the skin was collected carefully and diluted suitably with the mobile phase and the amount of CXB that remained on the skin (i.e., in the donor compartment) was calculated by HPLC. To calculate the amount of CXB deposited within the skin, the skin was mincedin, transferred to a test tube and subjected to vortexing for 15 min in a mobile phase using a cyclomixer. The resulting solution was filtered through a 0.45 μm membrane, injected into HPLC and concentration was found out. The results of skin deposition studies are depicted in Fig. 5.

2.9. *Pharmacodynamic efficacy of the NLC gel determined by aerosil induced rat paw edema method and comparison with micellar gel*

The protocol for animal testing was approved by Institutional Animal Ethical Committee (IAEC) No. UICT/PH/IAEC/1204/15. Animal care and handling throughout the experimental procedure were performed in accordance to the CPCSEA guidelines. Male Wistar strain rats having body



	Avg Permeation	Avg Deposition	Avg in Donor
Micellar Gel	24.67	5.79	69.54
NLC Gel	6.12	3.58	90.3

Fig. 5. *In vitro* deposition and permeation of CXB from NLC and micellar gels using excised rat skin at the end of 24 h employing a Franz diffusion assembly.

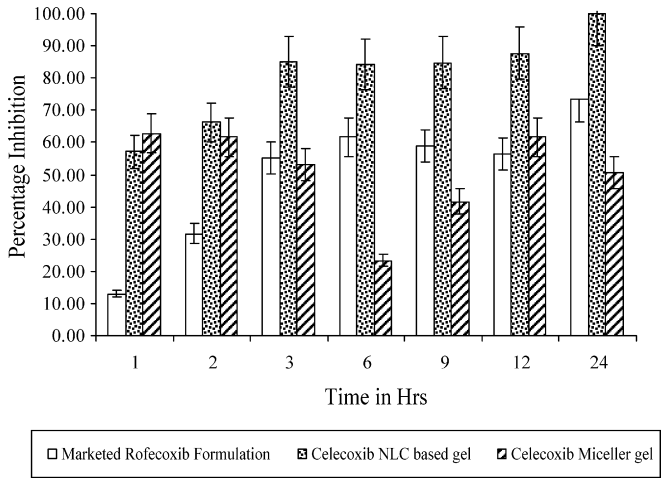


Fig. 6. *In vivo* comparison of the percentage inhibition produced by NLC based celecoxib gel, micellar gel and marketed gel in aerosil-induced rat paw edema method.

weight between 150 and 180 g were randomly divided into four groups of six rats each with each group receiving different topical treatment. 0.1 ml of 2.5% aerosil suspension (Vogel, 1997) prepared in distilled water was injected in the right hind foot of each rat under the planter aponeurosis. The rats of the standard group were treated with commercial marketed nanosized formulation of Rofecoxib. The first experimental group was treated with developed NLC based formulation of celecoxib, the second experimental group with micellar gel, while the control group was given no topical treatment. Briefly the right hind edematous paws of the rats were applied with 0.1 g each of the respective gels and it was allowed to get dry. Measurements of the foot volume were performed by the displacement technique using plethysmometer immediately before and after of injection of aerosil and subsequently every hour after the respective topical treatment. The edema rate and percentage inhibition of each group was calculated as follows:

$$\text{Edema rate}\,(E) = \frac{V_t - V_o}{V_o}$$

$$\text{Inhibition}\,(I)\,(\%) = \frac{E_c - E_t}{E_c} \times 100$$

where V_o is the mean paw volume before the aerosil injection, V_t the mean paw volume after the aerosil injection at time 't', E_c the edema rate of the control group and E_t is the edema rate of the treated group injection at time 't'. A graph of percentage inhibition obtained for different experimental groups which is normalized against control group versus time (h) is as depicted in Fig. 6.

2.9.1. Statistical analysis

Data was expressed as mean ± standard deviation and the edema rates of the three groups were statistically assessed by one-way analysis of variance (ANOVA). Differences in the inhibition rates between CXB treated groups and the control group were evaluated by Dunnett's t-test and a P value of <0.05 was considered as significant.

3. Results and discussion

3.1. Screening of components (solubility studies)

Among the selected oils that were screened (Table 1), maximum solubility of CXB was found in Capryol 90 followed by Capmul MCM. Among the surfactants (Table 1), Labrasol followed by Tween 80, Softigen 767 and Cremophor RH 40 showed reasonable solubilizing potential for CXB. Transcutol P and solutol HS 15 proved to be the best solublizers for CXB (Table 1). Thus, saturation solubility studies helped to further streamline the choice of excipients for microemulsion formation. Of the solid lipids screened, it was observed that Glyceryl dilaurate (GDL), Gelucire 62/05 (Gel 62/05), Gelucire 50/13 (Gel 50/13), Gelucire 53/10 (Gel 53/10), Apifil Pastills (AP), Glyceryl monostearate (GMS) were able to solubilize CXB to an appreciable extent.

Owing to the dispersible nature of gelucires, Glyceryl monostearate and Glyceryl dilaurate were preferred over gelucires to make NLC. The dispersible nature of gelucires could prevent formation of a solid structure in the ultimate dispersion, which is a prerequisite for fabrication of an NLC (Müller et al., 2002b). Therefore, gelucires were not considered for further studies.

3.2. Formulation and characterization of the NLC based gel

The NLC generated using the microemulsion templates emphasis was on utilization of biocompatible surfactants and solubilizers like Cremophor and Transcutol. The shaded area under Fig. 1 indicates the area under the microemulsion existence region. The microemulsion, represented as a dot in Fig. 1, prepared using optimized quantity of selected components could survive the accelerated conditions of freeze–thaw cycling. This indicated that the microemulsions were thermodynamically stable. This test (Prince, 1977) places stress on the microemulsion. At temperatures below freezing, the formation of ice crystals in an oil/water (O/W) type of microemulsion may cause oil particles to elongate and flatten. In addition, the lipophilic portion of the emulsifier molecule could lose its mobility while the hydrophilic portions are simultaneously "dehydrated" due to the freezing action of water. As the sample is thawed, water is released and travels rapidly through the microemulsion. If the system can "heal" itself before coalescence occurs, then the microemulsion survives the test. However, if the rate of redissolution of the ingredients is slow, instability may occur in case of microemulsion, which is not related to normal temperature processes.

In the optical birefringence studies the microemulsion was found to be isotopically clear.

The average particle size of the NLC dispersion was estimated to be 160 nm with a polydispersity index of 0.624 indicating wide particle size distribution. Polydispersity is measure of particle homogeneity and it varies from 0 to 1. Closer the value of polydispersity to zero, higher the homology between the particles. The average particle size of the micellar dispersion was estimated to be 120 nm with a polydispersity index of 0.823 indicating wide particle size distribution. There was a marginal

increase in the particle size after gelling of upto 185 ± 8 nm for the NLC dispersion and upto 138 ± 9 nm for the micellar dispersion. The nanoparticulate nature of the NLC dispersion particles was further confirmed by SEM studies (Fig. 2). Moreover, findings of the SEM study also suggested a spherical nature of NLC.

The drug encapsulation efficiency within the NLC was found to be 35%. The low encapsulation is mainly due to the partitioning of the CXB between oil phase and the aqueous phase. CXB has fairly good solubility in the surfactants and solubilizers used which tends to pull CXB out of the oil phase. No attempts were made to separate the encapsulated CXB from the free CXB.

Xanthan gum yielded fluidy gels and exhibited tackiness. Therefore carbopol was used as the gelling agent. At 0.6% (w/w) Carbopol of Ultrez 10, both the gels, namely, the NLC gel and micellar gel showed optimum viscosity.

3.3. In vitro drug release studies

Drug release studies from gel is not only an important step during the developmental stages of new formulations but also a routine quality control test for assuring uniformity of the finished product. In the *in vitro* release studies, the NLC gel (Fig. 3) showed burst release in the first 15 min followed by a steady release. The observed release pattern of the NLC gel could be due to the following sequence of events: the diffusion of unencapsulated CXB, solubilization by the surfactant micelle, diffusion of CXB from oil nanodroplets in first 2 h, diffusion from the solid lipid surface and, subsequently, diffusion from the core. As indicated in Fig. 3, the CXB encapsulated within the core of solid lipid is not released even at the end of 8 h and a net drug release of only 65% could be obtained. The micellar formulation showed 100% drug release within 3 h. Both burst release as well as sustained release are of interest for dermal application. Burst release is useful to improve the penetration of drug and for faster onset of action, while a sustained release supplies the drug over a prolonged period of time.

The drug release data from the NLC gel was fitted into different models. The value of r^2 was found to be highest for the Higuchi model ($r^2 = 0.98$). This indicates that the test product follows matrix diffusion based release kinetics.

3.4. In vitro skin permeation studies

The *in vitro* permeation of CXB through rat skin from NLC based gel and micellar gel was calculated in terms of mean cumulative amount diffused at each sampling time point during time period of 24 h (Fig. 4). The flux values after 24 h from the NLC based gel was found to be 0.3555 and 1.2753 mcg/cm^2 h from the micellar gel. Moreover, plot of the amount of CXB permeated (from both the formulations) as a function of time, showed a linear relationship ($r^2 = 0.99$), thus indicating that CXB permeation followed pseudo-first-order kinetics.

Highest value of flux was obtained for micellar gel, which can be attributed to the presence of surfactants in the system. The flux value for the NLC gel was found to be lowest, and it can be attributed to the slow permeation of CXB from the gel owing to encapsulation of the CXB in the lipid core.

The deposition potential of the gels was assessed at the end of 24 h after application. CXB 'percentage permeated', 'percentage deposited' and 'percentage remained on the skin' were calculated. The results are as depicted in Fig. 5.

Highest permeation was obtained for micellar gel, which can be attributed to its high flux value. Micellar gel showed higher deposition potential for the CXB as compared to the NLC gel. The deposition potential of the micellar gel was found to be approximately two-fold more than the NLC gel. But the average values of CXB present in the donor compartment of the NLC gel indicates that approximately 90% of the drug is still in the donor compartment at the end of 24 h, suggesting that the permeation and deposition values might present an altogether different picture at a time period beyond 24 h. However, studies need to be carried out beyond 24 h to validate this hypothesis. These observations confirmed the earlier findings about nanoparticles being deposited in the skin, thus acting as a depot to give sustained release. Although, we did not attempt to establish the mechanism of CXB permeation through skin from these gels, we believe that CXB gets transported while being entrapped with in the lipid matrix as observed with most of the topical nanoparticle formulations (Lombardi Borgia et al., 2005). While getting transported across the skin, NLC probably expel CXB from lipid matrix as a consequence of polymorphic transitions occurring in the solid lipid. This phenomenon has been hypothesized in the literature (Mei et al., 2003; Jain et al., 2005). SLN have been shown to improve the dermal localization of several topical therapeutic agents (Maia et al., 2002; Liu et al., 2007). This was one of the reasons to employ NLC approach for topical delivery of CXB as its dermal localization is highly desirable for enhancing the localized treatment of arthritis and thus reducing its systemic toxicity problems. Further studies would be focused on human cadaver skin and would be carried out for longer duration in order to get proper insight into potential of NLC based gels in CXB delivery.

3.5. Pharmacodynamic efficacy of the gel determined by aerosil induced rat paw edema method

In the *in vivo* pharmacodynamic efficacy test using aerosil as the phlogistic agent (Fig. 6), the described NLC formulation showed activity upto 24 h, reaching peak activity of 100%, thus rendering prolonged activity of the NLC gel. Commercial formulation also exhibited higher activity even at the end of 24 h, which could be attributed to its nanosized nature. But there seems to be statistically significant ($P < 0.05$) difference in the activity of micellar gel and NLC gel compared to commercial formulation at the end of 24 h. However, micellar formulation that gradually showed increasing activity from 1 to 3 h showed diminished activity (less than 20%) at the end of 6 h. The activity of the micellar gel again showed dramatic increase after 9 h. The initial rapid onset of action of the NLC gel was confirmed by more than 50% inhibition at the end of 1 h and 60% inhibition at the end of 2 h, which was comparable to the percentage inhibition shown by the commercial gel. This could be correlated

to the *in vitro* release studies. The sustained activity of the NLC based gel even at the end of 24 h could be because of the CXB encapsulated within the solid matrix, while the rapid onset is because of the CXB encapsulated in the oil and surfactant phase which forms the outer phase of the dispersion.

The results of the *t*-test and the ANOVA confirmed that difference in the mean values of edema rate and percentage inhibition of the NLC based gels and marketed formulation is because of their respective efficacy and not because of sampling error at $P < 0.001$.

It is noteworthy that inspite of the flux being four-fold lower, the NLC Nanogel exhibited marked increase in efficacy. This could be attributed to the form in which drug is presented, i.e., the Nano form. It is also postulated that NLC could elicit an immune response favoring an anti-inflammatory effect. However, this requires confirmation.

4. Conclusion

The NLC based gel containing celecoxib wherein the drug is dissolved in a mixture of solid lipid and liquid oil was formulated using a simple microemulsion template technique. NLC gel exhibited faster onset yet effecting a prolonged action as evident from a comparative study with micellar gel with respect to *in vitro* release, *in vitro* permeation and phamacodynamic studies.

The developed technology platform is proposed to be used for many other potential actives required to elicit prolonged action by topical route of delivery. It is further suggested that by changing the ratio of solid lipid and liquid lipid; release, permeation and hence pharmacodynamic activity can be modulated.

Acknowledgements

We would like to acknowledge ISP, Anshul Agencies, Gattefosse France, Colorcon Asia, Abitech Corporation, Indchem International, Noveon Inc., BASF India and Cadila Ltd. for the gift samples of excipients and drug. MDJ is deeply thankful to the University Grants Commission for Fellowship.

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