*Drug Development and Industrial Pharmacy*, 32:911–918, 2006

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600814676

informa
healthcare

# Formulation and Evaluation of Nanostructured Lipid Carrier (NLC)–based Gel of Valdecoxib

**M. Joshi and V. Patravale**
Pharmaceutical Division, Mumbai University Institute of Chemical Technology (MUICT), N. P. Marg, Matunga, Mumbai-400019, India

**ABSTRACT** Nanostructured Lipid Carrier (NLC)–based topical gel of Valdecoxib was formulated with the aim of faster onset yet prolonged action for the treatment of inflammation and allied conditions. NLCs prepared by microemulsion template technique were characterized by photon correlation spectroscopy for size. Drug encapsulation efficiency was determined using Nanosep® centrifugal device. The nanoparticulate dispersion was suitably gelled and characterized with respect to drug content, pH, spreadibility, rheology, and *in-vitro* release. Safety of the NLC-based gel was assessed using primary skin irritation studies, and efficacy was confirmed using pharmacodynamic study, namely the Aerosil-induced Rat Paw edema model. The developed NLC-based gel showed faster onset and elicited prolonged activity up to 24 hours.

**KEYWORDS** Valdecoxib, Nanosturctured lipid carriers (NLC), Topical, Anti-inflammatory, Prolonged action

## INTRODUCTION

Solid lipid nanoparticles and nanostuctured lipid carriers have attracted much attention in the last decade in transdermal drug delivery. Solid lipid nanoparticles (SLNs) are identical to an oil-in-water emulsion wherein the liquid lipid (oil) of the emulsion has been replaced by a solid lipid that has a liquid-to-solid phase transition well above the body temperature (37°C) (Müller et al., 2000a). SLNs are particles made from solid lipids or lipid blends produced by one of the following techniques (Patravale et al., 2004), viz. High Pressure Homogenization, High Shear Homogenization, Microemulsion Templates, Solvent Injection Method employing water-insoluble solvent, and Solvent Emulsification Diffusion method using a partially water-miscible solvent (Müller et al., 1995). To overcome some of the limitations of SLNs, viz. limited drug loading and drug leakage during storage, nanostructured lipid carriers (NLC) have been developed (Müller, 2000b; Müller et al., 2000c). They consist of a solid lipid matrix with a high content of liquid lipid (Müller et al., 2004).

We would like to acknowledge Gattefosse France, Colorcon Asia, Noveon Inc., BASF India, and Cipla Ltd. for the gift sample of excipients and drugs, and the University Grants Commission for a fellowship.

Address correspondence to Dr. V Patravale, Reader in Pharmaceutics, Pharmaceutical Division, Mumbai University Institute of Chemical Technology (MUICT), N. P. Marg, Matunga, Mumbai-400019, India; Tel: +91-22-24145616; Fax: +91-22-24145614; E-mail: vbp_muict@yahoo.co.in

Both SLN and NLC possess a number of features advantageous for topical route of application (Müller et al., 2000a; Müller et al., 2002; Mehnert & Mäder, 2001). Solid lipid nanoparticles and nanostructured lipid carriers are colloidal carrier systems providing controlled release profiles for many substances. These carriers are composed of physiological and biodegradable lipids of low systemic toxicity and also 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, controlled release from these carriers is possible. This becomes an important tool when it is necessary to supply the drug over a prolonged period of time, when it is necessary to reduce systemic absorption, and when the drug is irritating in high concentrations. As a result of film formation after topical application, occlusive properties are also reported (Wissing & Müller, 2001; Wissing & Müller, 2002a; Wissing & Müller, 2002b).

Valdecoxib was used as a highly lipophilic model drug in the present study. Valdecoxib is a nonsteroidal anti-inflammatory drug that acts by inhibition of cycloxygenase II (COX-II) and is used in the treatment of inflammation and arthritis. The available marketed topical formulation of Valdecoxib contains 56% alcohol which may have drying effect on the skin after chronic usage. Therefore, the aim of the study was to develop a nonalcoholic delivery system for Valdecoxib with faster onset yet prolonged action for the treatment of inflammation and arthritis without causing a drying effect. The study presented herein reports for the first time the utilization of a microemulsion (ME) template method to generate NLCs. This method is preferred over the other reported methods due to its specific advantages, such as that it obviates the need for specialized equipment to produce nanocarriers (Patravale et al., 2004); it requires no energy to generate nanocarriers (Gasco, 1997); particle size is easily controlled by controlling the size of the emulsion droplet, and scale-up is easy (Patravale et al., 2004).

## MATERIALS

Valdecoxib was obtained as a gift from Cipla ltd., Mumbai, India. Glyceryl Dilaurate was a kind gift from ISP through Anshul Agencies, Mumbai, India. Gelucires (glycerol esters of saturated fatty esters), Caproyl 90 (propylene glycol monocaprylate, containing 90% monoesters), and Transcutol (purified diethylene glycol monoethyl ether) were obtained from Gattefosse France through Colorcon Asia pvt. ltd, Mumbai, India. Solutol HS 15 (macrogol 15 hydroxy stearate) and Cremophor RH 40 (PEG 35 castor oil) were obtained from BASF India ltd., Mumbai, India. The gelling agent Carbopol Ultrez 10 was obtained as a gift sample from Noveon, Mumbai, India. All other chemicals were of analytical grade.

## METHODS

### Screening of Components (Solubility Studies)

The solubility of Valdecoxib was determined in different solid lipids, oils, surfactants, and solubilizers. An excess of drug was added individually to oils, surfactants, and solubilizers (5 mL each) in screw-capped tubes. After 24 h, each sample was centrifuged, and 0.5 mL clear supernatant layer was diluted suitably and analyzed by HPLC. The results are depicted in Fig. 1. For the solubility studies in solid lipids, 100 mg of the drug was taken in a test tube. The solid lipid was added in increments of 0.5 gm and the test tube was heated in a controlled temperature water bath. The amount of lipid required to solubilize the drug in molten state was noted. The results are as shown in Fig. 2.

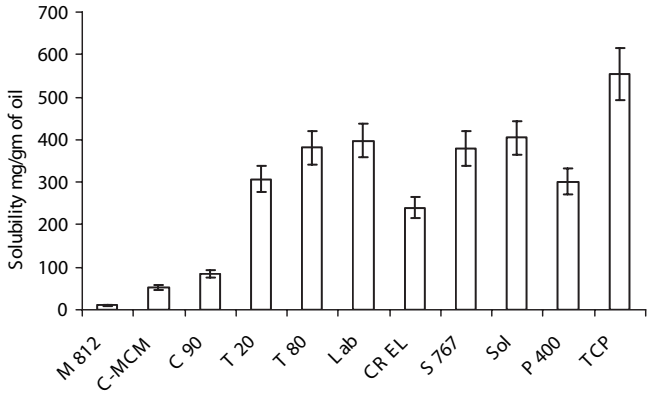


**FIGURE 1** **Solubility of Valdecoxib in Different Oils, Surfactants and Solubilizers, M 812 Miglyol 812, C-MCM Capmul MCM, C 90 Caproyl 90, T 20 Tween 20, T 80 Tween 80, Lab Labrasol, CR EL Cremophor EL, S 767 Softigen 767, Sol Solutol HS 15, P 400 PEG 400, TCP Transcutol P.**

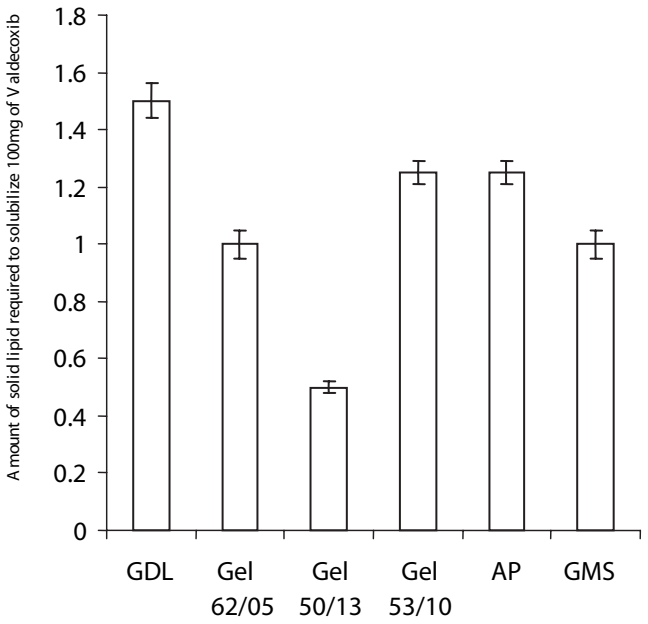


**FIGURE 2** **Solubility of Valdecoxib in Different Solid Lipids, GDL Glyceryl Dilaurate, Gel 62/05 Gelucire 62/05, Gel 50/13 Gelucire 50/13, Gel 53/10 gelucire 53/10, AP Apifil Pastills, GMS Glyceryl Monostearate.**

## HPLC Analysis of Valdecoxib

The HPLC system consisted of Jasco PU 2080 Plus Intelligent HPLC Pump, Jasco, Japan, equipped with HiQsil C18-10 4.6 I. D. × 250mm, 10μm 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 (Acetonitrile: Water: Acetic Acid: Triethanolamine in the ratio of 55:45:0.1:0.03) was run at a flow rate of 1mL/min and ultraviolet absorption was read at 240nm.

## Formulation of Microemulsion

Selection of a microemulsion system was based on the drug-solubilizing capacity of the excipient. The selected components were

1. Solid Lipid: Glyceryl Dilaurate
2. Oil phase: Caproyl 90
3. Surfactant phase: Cremophor RH 40
4. Solubilizer: Transcutol and Solutol HS 15
5. Aqueous phase: double-distilled water

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

## 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 1:1 mixture of Caproyl 90 and Glyceryl Dilaurate. The surfactant phase consisted of a mixture of Cremophor RH 40, Solutol HS 15, and Transcutol (ratio 1:2:0.17) while the aqueous phase was double-distilled water. 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 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 both visually for transparency and through crosspolarizers 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 pseduoternary phase diagram is shown in Fig. 3.

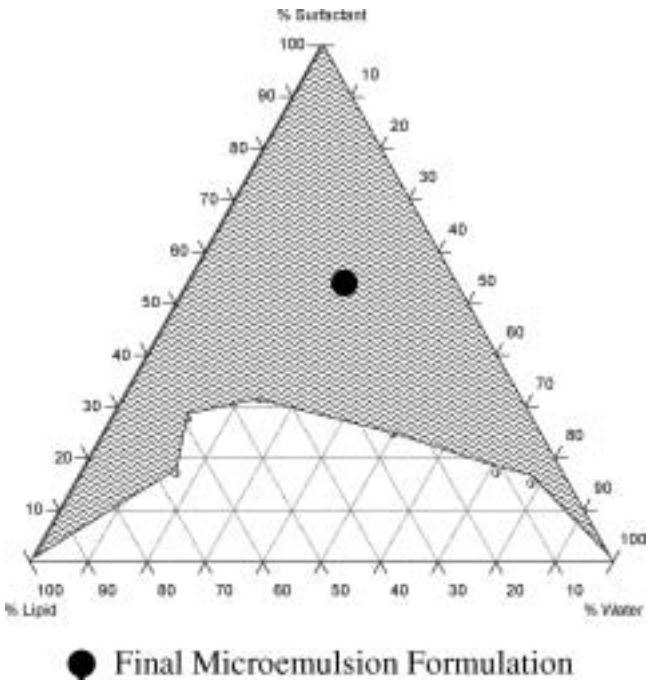


**FIGURE 3** **Microemulsion Phase Diagram.**

## Characterization of the Microemulsion

### *Freeze-Thaw Cycling*

Microemulsion was subjected to freeze-thaw cycles (–4 to 40°C) of 24 h for a period of one week and assessed for physical instabilities such as phase separation and precipitation.

### *Optical Birefringence*

The microemulsion was checked both visually and using cross-polarizers for optical isotropy to confirm absence of other phases.

## Formulation of NLCs From ME Templates

NLCs were prepared from warm microemulsion templates as per the method described by Gasco for the preparation of SLNs (Gasco, 1993 and Gasco 1997). The oil phase consisted of melted lipid mixed with the oil. Drug was dissolved in this mixture at 60°C. The aqueous phase consisted of surfactant, solublizer, and water. Temperatures for both phases were maintained above the melting point of the lipid (60°C). The oil phase was added to the aqueous phase, and both phases were mixed using a cyclomixer at this temperature to form a microemulsion. This warm microemulsion was diluted in cold water (2–3°C) under mechanical stirring to form NLC dispersion such that the concentration of Valdecoxib in the final dispersion remains 1%w/w.

## Formulation of NLC-Based Gel

The nanoparticulate dispersion obtained after diluting the warm microemulsion templates was gelled using different gelling agents like Carbopols, xanthan gum, and carrageenan. Based on compatibility with the nanoparticulate dispersion, ease of preparation, and aesthetic appeal; Carbopol (Ultrez 10) was selected as the gelling agent. Carbopol was dispersed using an overhead stirrer at the speed of 600rpm for 3hrs. Different concentrations of Ultrez 10 were used for gelling (0.5–1%) and the one giving optimum viscosity was chosen for further studies. Carbopol (Ultrez 10) 0.6% was added to the nanoparticle dispersion under overhead stirring at 800rpm. Stirring was continued until carbopol was dispersed. The carbopol dispersion was neutralized using 50% w/w Triethanolamine.

## Characterization of Nanoparticulate Dispersion

### *Determination of Particle Size and Polydispersity Index*

All measurements were performed in triplicate on a Beckman N4 plus submicron particle size analyzer at a temperature of 25°C ± 2°C and at 90°C to the incident beam applying the principal of photon correlation spectroscopy (PCS) on samples diluted to achieve an intensity between $5 \times 10^4$cps to $1 \times 10^6$ cps using particle-free distilled water.

### *Determination of Drug Encapsulation Efficiency*

A known dilution of the NLC dispersion was prepared, and 100µl of it was transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an ultrafilter (MWCO100KD, Pall Lifesciences, Mumbai, India). The Nanosep® was centrifuged at 15,000 rpm for 40 mins. The supernatant and the filtrate were diluted appropriately and the amount of drug in both the phases was determined using HPLC analysis.

The entrapment efficiency was calculated by the following equation:

$$\text{Entrapment Efficiency (\%)} = \left( \frac{W_{\text{inital drug}} - W_{\text{free drug}}}{W_{\text{inital drug}}} \right) \times 100$$

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

## Characterization of the Gel

### *Determination of Drug Content, Spreadability, and pH*

For determination of drug content, about one gram of the gel was weighed in a 100mL volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed on a Shimadzu UV-1650 PC UV-VIS Spectrophotometer managed by Shimadzu UV

probe version 2.10 at a λ-max of 240nm. The spreadability of the gel was determined using the following technique: 0.5 g gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate. The increase in the diameter due to spreading of the gels was noted. The pH of the 10%w/w gel was determined using Equip-tronic Digital pH meter Model EQ_610, standardized using pH 4.0 and 7.0 standard buffers before use. The results of characterization of gel are summarized in Table 1.

### *Rheological Studies on the Gel*

Brookefield Synchro-Lectric Viscometer (Model RVT) with helipath stand was used for rheological studies. The sample (30g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using T-C spindle at 0.5, 1, 2.5, and 5 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted. The measurements were carried in duplicate at ambient temperature. Direct multiplication of the dial readings with factors given in the Brookefield viscometer catalogue gave the viscosity in centipoises. The consistency index and flow index were calculated from the Powerlaw equation:

$$\tau = K\, r^{n}$$

Where: "τ" is shear stress; "r" is shear rate; "K" is consistency index; "n" is flow index.

Taking log of both sides,

$$\text{Log}\ \tau = \log K + n \log r$$

$$\text{Shear stress (dynes/cm}^2) = \text{Viscosity (cps)} \times \text{Rate of shear (sec}^{-1})$$

**TABLE 1** **Characterization of NLC based Valdecoxib Gel**

| Parameter | Value |
|---|---|
| Drug Content | 99.45 ± 2% |
| pH | 6.5 |
| Spreadiability | 6cm/sec |
| Viscosity at 5 rpm | $85 \times 10^5$cps |
| Flow Index 'n' | 0.386 |
| Consistency Index 'K' | $29.7 \times 10^5$ dynes/cm$^2$ |

Thus, from the plot of log of shear stress v/s log of shear rate, the slope of the plot representing flow index and antilog of the y-intercept indicating consistency index was calculated.

## *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°C ± 0.5°C. Phosphate buffer pH 7.4 (900mL) solution was placed in the vessel and equilibrated at 32°C ± 0.5°C. One gram of the gel was 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 kept 25rpm. Aliquots were withdrawn every hour and analyzed spectrophotometrically. A graph of % cumulative release against Time in hours was plotted as depicted in Fig. 4. To describe the kinetics of the drug release from the gel, mathematical models such as zero-order, first-order, and Higuchi's were used. The criterion for selecting the most appropriate model was based on a goodness-of-fit test.

## Skin Irritation Studies

The developed formulations were tested for primary skin irritation using Draize patch test (Verneer,

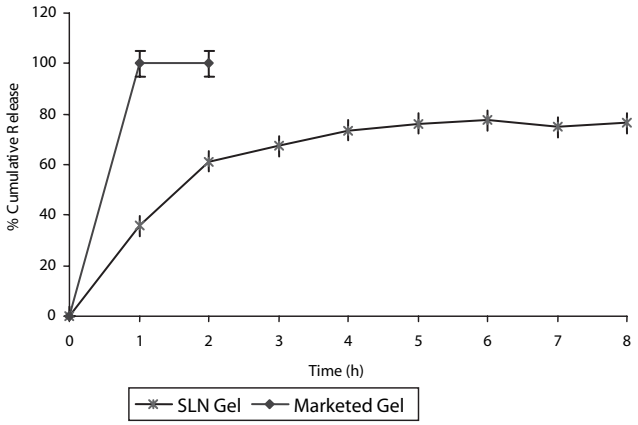


**FIGURE 4** **In-vitro Release From the Gel Using USP Paddle Over Disk Method.**

**TABLE 2** **Primary Irritation Index Values on the Skin at the End of 24, 48 and 72 h**

| | Irritation Score | | |
|---|---|---|---|
| | Time of Application | | |
| Formulation | 24hr | 48hr | 72hr |
| Developed NLC based Valdecoxib Gel | 0 | 0 | 0 |
| Marketed Valdecoxib Gel | 0 | 1 | 1 |

1991; Bronaugh & Maibach, 1982) in rabbits. The protocol for these animal studies was approved by the Institutional Animal Ethical Committee (IAEC) no. UICT/PH/IAEC/D405/5. For this study, three white New Zealand rabbits weighing 2.5–3 kg were used. The back and sides of the rabbits were clipped free of hair 24 h prior to the application of formulations. The following formulations were applied to the skin of the rabbits after 24 h:

- NLC based Valdecoxib gel
- Marketed Valdecoxib gel
- Placebo gel

0.5 g of each gel was applied on the hair-free skin of rabbits by uniform spreading within the area of 4 $cm^2$. The skin was observed for any visible change such as erythema (redness) or edema (swelling). Evaluation was done by using the scale given by Draize. The results of primary skin irritation test are depicted in Table 2.

## Pharmacodynamic Efficacy of the Gel Determined by Aerosil-Induced Rat Paw Edema Method

The protocol for these animal studies was approved by Institutional Animal Ethical Committee (IAEC) no. UICT/PH/IAEC/1204/15. Male wistar strain rats weighing 150–180g were randomly divided into three groups of six rats, each group receiving a different topical treatment. 0.1mL 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 the marketed formulation of Valdecoxib. The experimental group was treated with a developed NLC-based formulation of Valdecoxib while the control group was given no topical treatment.

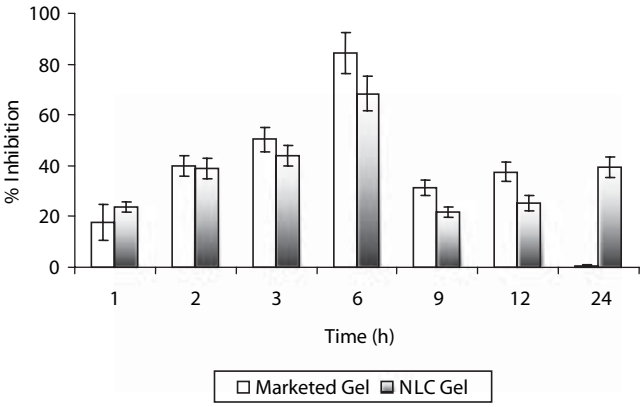


**FIGURE 5** **Comparison of the Percentage Inhibition Produced by NLC Based Valdecoxib Gel and Marketed Gel in Aerosil-Induced Rat Paw Edema Method.**

Measurements of the foot volume were performed by the displacement technique using plethysmometer immediately before and after the injection of Aerosil at fixed time intervals. The edema rate and percentage inhibition of each group was calculated as follows.

$$\text{Edema Rate (E)} = V_t - V_o / V_o$$

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

Where $V_o$ is the mean paw volume before Aerosil injection; $V_t$ is the mean paw volume after Aerosil injection. $E_c$ is the edema rate of the control group, and $E_t$ is the edema rate of the treated group. A graph of %inhibition Vs time in hours is shown in Fig. 5.

## Statistical Analysis

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

# RESULTS AND DISCUSSION

## Screening of Components (Solubility Studies)

Among the oils screened (Fig. 1), maximum solubility of Valdecoxib was found in Caproyl 90 while among solid lipids (Fig. 2), the highest solubility of

Valdecoxib was found in Glyceryl Monostearate, Glyceryl Dilaurate, Gelucire 50/13 (Stearoyl macrogol-32 glycerides) and Gelucire 53/10 (PEG 32 glyceryl stearate). Owing to the solid nature of Glyceryl Monostearate and Glyceryl Dilaurate, they were preferable to gelucires for the fabrication of nanostructured lipid carriers. Among the surfactants (Fig. 1), Labrasol followed by Tween 80 showed good solubilizing potential for Valdecoxib, while Transcutol P proved to be the best solublizer (Fig. 1) for Valdecoxib.

The point highlighted in the phase diagram depicts the final formula of the microemulsion. This point was chosen as it could solubilize 100mg of drug with good stability.

## Formulation and Characterization of the NLC-Based Gel

The microemulsions prepared (Fig. 3) using the optimized quantity of selected components could survive the freeze-thaw cycling. This indicated the microemulsions to be thermodynamically stable. In the optical birefringence studies the microemulsion was found to be isotropically clear. The average particle size of nanoparticulate dispersion was found to be 157nm with a polydispersity index of 0.582 indicating homogeneity in particle size distribution. Polydispersity is a measure of particle homogeneity, and it varies from 0 to 1. There was a marginal increase (170nm ± 10nm) in the particle size after gelling of the NLC dispersion. The drug encapsulation efficiency in the nanoparticles was found to be 51%. The gel showed optimum viscosity at the concentration of 0.6% of Carbopol Ultrez 10. The drug content of the NLC-based gel was found to be 99.45 ± 2% and pH was found to be 6.5, which are within acceptable limits. Spreadability is an important property of topical formulation from a patient compliance point of view. Application of the formulation to inflamed skin is more comfortable if the base spreads easily, exhibiting maximum "slip" and "drag." The diameter was found to be 6 cm. The large diameter indicates better spreadability. In general, the gels that possess a high consistency index are less spreadable. The release of the drug from the formulation is governed by its components as well as by the consistency of the formulation. Viscosity of the NLC gel was found to be $85 \times 10^5$cps at 5rpm. Consistency index is a measure of consistency and is equivalent to apparent viscosity at a shear rate of 1 $Sec^{-1}$. The consistency index of the formulation was found to be $29.7 \times 10^5$. The flow index *n* is a measure of the deviation of a system from Newtonian behaviour ($n = 1$). A value of $n < 1$ indicates pseudoplastic flow or shear thinning; $n > 1$ indicates dilatant or shear thickening flow. The gel showed a flow index of 0.386, indicating pseudoplastic flow behaviour. Flow index confers an idea of the flowability of the formulation from the container. Generally, the thicker the base, the lower the flow index. The results of characterization of NLC gel are summarized in Table 1.

## In Vitro Drug Release Studies

The study of drug release from gel is an important step in the development stages of new formulations, as well as a routine quality-control test for assuring uniformity of the finished product. In the in vitro release studies the NLC gel (Fig. 4) showed burst release in the first two hours followed by a steady release. This could be due to diffusion of unencapsulated drug in first two hours followed by diffusion from the NLC surface and thereafter from the core. As can be seen in Fig. 4, the drug encapsulated within the core of NLC is not completely released even after at the end of 8 hours, and only 80% release can be obtained. Burst release as well as sustained release both are of interest for dermal application. Burst release can be useful to improve the penetration of drug. Sustained release supplied the drug over a prolonged period of time. The marketed formulation showed 100% release within 1 hr.

The release data from NLC gel was fitted to the 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.

## Primary Skin Irritation Studies

In the skin irritation studies (Table 2), the developed formulation showed no skin irritation on intact rabbit skin compared with the marketed formulation which showed slight irritation at the end of 48 hrs. The irritation in the marketed formulation may be due to the high content of alcohol in the formulation. In no case was edema seen over a period of 3 days. The irritation score (primary skin irritation index) studied

to assess the effect of cumulative application of the test formulations on skin irritation was 0 for the developed formulation and 1 for the marketed formulation at the end of 48 h and 72 h. This indicates better skin acceptability of the developed formulation for topical application over the marketed formulation.

## 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. 5), the developed NLC formulation showed activity up to 24 h, reaching peak at 6 h, confirming prolonged activity of the gel. The marketed formulation exhibited higher activity for the first 12 h, which could be attributed to the presence of a high amount of alcohol, a known skin-penetration enhancer. However, the marketed formulation showed diminished activity (less than 5%) at the end of 24 h. The faster onset of action of the developed NLC gel was confirmed by more than 20% inhibition at the end of 1 h and 40% inhibition at the end of 2 h which was comparable to the percentage inhibition shown by marketed gel. This could be correlated to in-vitro release studies. The sustained activity of the NLC-based gel even at the end of 24 h could be explained by the drug's encapsulation within the solid matrix, while the faster onset is explained by the drug's encapsulation in the oil and surfactant phase, which forms the outer phase of the dispersion.

The results of the t test and ANOVA confirmed that differences in the mean values of the edema rate and the percentage inhibition of the NLC-based gels and marketed formulation is because of their respective efficacy and not because of sampling error at $P < 0.05$.

## CONCLUSION

The NLC-based gel containing Valdecoxib dissolved in a mixture of solid lipid and liquid oil in nanoparticulate form helped us to attain the objective of faster onset yet prolonged action as evident from in vitro release and phamacodynamic studies. The gel was also found to be safe and did not cause drying or irritation of the skin as revealed by the Draize patch test.

## REFERENCES


Bronaugh, R. L., & Maibach, H. L. (1982) In: *Safety and Efficacy of Topical Drugs and Cosmetics*. Kligman, A. M. and Leyden, J. J., Ed.; Grine and Stration Inc. New York., 51–59.

Gasco, M. R. (1993) Method of Producing solid lipid microspheres having narrow size distribution, US Patent No 5250236.

Gasco, M. R. (1997). Solid lipid nanospheres from warm microemulsions, *Pharmaceutical Technology Europe*, *9*, 52–58.

Mehnert, W., & Mäder, K. (2001). Solid lipid nanoparticles—production, characterization and applications. *Advanced Drug Delivery Reviews*, *47,* 165–196.

Müller, R. H., Mehnert, W., Lucks, J. S., Schwarz, C., zur Mühlen, A., Weyhers, H., Freitas, C., & Rühl, D. (1995). Solid lipid nanoparticles (SLN)—an alternative colloidal carrier system for controlled drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *41,* 62–69.

Müller, R. H., Rühl, D., Runge, S., Schulze-Forster, K., & Mehnert, W. (1997). Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. *Pharmaceutical Research*, *14,* 458–462.

Müller, R. H., Mäder, K., & Gohla, S. (2000a). Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics*, *50,* 161–177.

Müller, R.H. (2000b), Solid-liquid (semi-solid) lipid particles and method of producing highly concentrated lipid particle dispersions. German Patent Application 199,45,203.2.

Müller, R. H., Mäder, K., Lippacher, A., & Jenning, V. (2000c). Festflüssige (halbfeste) Lipidpartikel und Verfahren zur Herstellung hochkonzentrierter Lipidpartikel-dispersionen. PCT/EP00/04565.

Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews*, *54,* 131–155.

Müller, R. H., Wissing, S. A., & Kayser, O. (2004). Solid lipid nanoparticles for parenteral drug delivery, *Advanced Drug Delivery Reviews*, *56,* 1257– 1272

Patravale, V. B., Date, A. A., & Kulkarni, R. M., (2004). Nanosuspensions: a promising drug delivery strategy. *Journal of Pharmacy and Pharmacology*, *56,* 827–840.

Verneer, B. J. (1991). Skin irritation and sensitization. *Journal of Controlled Release*, *15,* 261–265.

Vogel, G. H. (1997) In: *Drug Discovery and Evaluation: Pharmacological Assays*: Vogel, W. H. Ed.; Springer, New York, 407.

Wissing, S. A., & Müller, R. H. (2001). A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. *International Journal of Cosmetic Science*, *23,* 233–243.

Wissing, S. A., & Müller, R. H. (2002a). The influence of the crystallinity of lipid nanoparticles on their occlusive properties. *International Journal of Pharmaceutics*, *242,* 377–379.

Wissing, S. A., & Müller, R. H. (2002b). Solid lipid Nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. *Journal of Controlled Release*, *81,* 225–233.