Nanoemulsions as Versatile Formulations for Paclitaxel Delivery: Peroral and Dermal Delivery Studies in Rats

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Pathogenesis of psoriasis involves the keratinocytes in epidermis as well as the angiogenesis involving deeper skin layers. So, the drug delivery strategy should be customized to localize paclitaxel (PCL) inside both layers. In this investigation, in order to achieve penetration of PCL into deeper skin layers while minimizing the systemic escape, a nanoemulsion (NE) was formulated and evaluated its *in vivo* pharmacokinetic performance. Further, the same formulation was explored for peroral bioavailability enhancement of PCL. Upon dermal application, the drug was predominantly localized in deeper skin layers, with minimal systemic escape. When orally administered as NE, PCL was rapidly absorbed reaching a steady-state value of 3.5 μg/ml in 30 minutes, and steady-state levels persisted up to 18 hours. This has amounted to an absolute bioavailability of 70.62%. Inhibition of P-glycoprotein efflux by D-α-tocopheryl polyethyleneglycol 1,000 succinate and labrasol would have contributed to the enhanced peroral bioavailability of PCL. This investigation provides direct evidence on the localization of large molecular weight, lipophilic drug, PCL, in dermis. Further, the NE formulation has enhanced the peroral bioavailability significantly to more than 70%. The developed NE formulation was safe and effective for both peroral and dermal delivery of PCL.

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INTRODUCTION

Paclitaxel (PCL), a novel antineoplastic agent with unique molecular mechanism of action, is active clinically against advanced ovarian and breast cancer, and is under investigation for therapy of various cancers (Straubinger, 1995). Although it is therapeutically very effective, its poor biopharmaceutical properties severely limit its clinical usage. PCL, being a poorly soluble and poorly permeable drug, is classified under class IV of Biopharmaceutic Classification System (BCS) (Kasim *et al.*, 2003). It is currently administered by infusion of ethanolic solution containing Cremophor EL diluted in intravenous (i.v.) fluids. However, the presence of Cremophor EL is reported to be responsible for many of the adverse reactions of formulation (Gelderblom *et al.*, 2001). Therefore, to prepare Cremophor-free formulations for PCL administration, many alternative formulation approaches such as liposomes (Straubinger *et al.*, 1993; Dhanikula and Panchagnula, 2005a), mixed micelles (Alkan-Onyuksel *et al.*, 1994), and microemulsions (He *et al.*, 2003) were proposed. Apart from solubility, PCL, being a P-glycoprotein (P-gp) substrate (Varma and Panchagnula, 2005), oral bioavailability is very poor. Approaches to overcome poor oral bioavailability have received limited success so far (Sparreboom *et al.*, 1997; Kimura *et al.*, 2002; Woo *et al.*, 2003), prompting a clear need to develop PCL for administration by alternative routes using Cremophor-free formulations.

Previously, the regional pharmacokinetic advantage of PCL localized delivery was demonstrated (Dhanikula and Panchagnula, 1999, 2004; Dhanikula *et al.*, 2005b). Enhancing local levels of drug at therapeutic target, while keeping the systemic drug concentrations low will help in the treatment of diseases such as psoriasis and melanoma, against which PCL is pharmacologically effective (Amato *et al.*, 1998; Lee *et al.*, 1998; Kunstfeld *et al.*, 2003), but clinically limited owing to poor distribution into skin layers. Dermal drug delivery systems are designed to obtain a local effect in the desired skin layer upon their application on skin surface. Hence, they are intended to localize maximum drug concentration in skin layer of interest with a minimum net drug transport across skin (Behl *et al.*, 1993). Therefore, during pharmacokinetic evaluation of dermal delivery systems, the dosage from performance has to be viewed from two aspects: efficacy (dermal bioavailability) and systemic safety

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Abbreviations: AUC, area under the curve; i.v., intravenous; LD, lethal dose; LDV, laser Doppler velocimetry; LSOL, labrasol; MW, molecular weight; NE, nanoemulsion; PCL, paclitaxel; P-gp, P-glycoprotein; SC, stratum corneum; TEWL, transepidermal water loss; TPGS, D-α-tocopheryl polyethyleneglycol 1,000 succinate

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Table 1. Composition of PCL formulations tested *in vivo*

Component	Formulation I (blank)	Formulation II[1] (Taxol)	Formulation III[2] (nanoemulsion)
Labrasol+vitamin E-TPGS (3:1)	480 mg	—	1,920 mg
Labrafil M1944CS	20 mg	—	80 mg
Paclitaxel	—	20 mg	20 mg
^{14}C-paclitaxel	—	1 μCi	1 μCi
Cremophor EL	—	1,676.5 mg	—
Ethanol	—	1,244.7 mg	—
Routes of administration	Dermal	i.v. (10 mg/kg), Oral (10 mg/kg)	Dermal (8 mg/kg), oral (10 mg/kg)

i.v., intravenous; NE, nanoemulsion; PCL, paclitaxel; TPGS, D-α-tocopheryl polyethyleneglycol 1,000 succinate.
i.v. administration was performed to assess pharmacokinetic parameters and absolute bioavailability.
[1]Formulation II (Taxol) was prepared in the laboratory using the procedure and formula reported previously (Gao *et al.*, 2003).
[2]For oral administration, the NE formulation was compared with Taxol. Only NE is applied dermally as patch and compared with blank formulation patch and occlusion control for any potential irritancy.

(percutaneous absorption owing to drug escape). The disposition of drug, once it crosses stratum corneum (SC), is a complex process. It can be made to cross SC and localize in the epidermis and dermis with formulation approach such as by using skin depot formers, or by using vesicles and nanoemulsions (NEs). Further, PCL with high molecular weight (MW) (853.9 Da) and lipophilicity (Log P 3.5) (Dhanikula and Panchagnula, 1999) would not be taken up efficiently by systemic circulation owing to poor rate of dissolution in the scanty intercellular fluid as well as lesser diffusion coefficient by virtue of high MW. This will lead to the formation of depot in the deeper skin layers, leading to high local drug concentrations coupled with minimum systemic escape.

NEs are thermodynamically stabilized dispersions of oil in water (Tenjarla, 1999). These novel formulations enhance drug delivery into and across skin primarily by increasing concentration gradient across skin (Kreilgaard, 2002a). NEs also perturb the skin barrier function by virtue of the presence of surfactants, which are well-known penetration enhancers (Kreilgaard, 2002a, b).

As pathogenesis of psoriasis involves the keratinocytes in the epidermis (Frye *et al.*, 2003) as well as the angiogenesis involving deeper skin layers (Kunstfeld *et al.*, 2003), the drug delivery strategy should be customized to localize PCL concentrations inside these two layers. However, PCL being a large molecule (853.3 Da), the deep skin penetration is very difficult. Previously, we demonstrated that skin bilayer alteration alone is not sufficient to enhance the transdermal permeation of PCL (Panchagnula *et al.*, 2005). In this investigation, in order to achieve penetration of PCL into deep skin layers while minimizing the systemic escape, NEs were formulated and evaluated for their *in vivo* drug delivery performance. Further, the same NEs were evaluated for oral bioavailability enhancement as well. All excipients used in the formulation of NEs are approved for peroral drug delivery (Table 1).

RESULTS AND DISCUSSION

Physical characteristics of formulation

Physical characteristics of formulation are summarized in Table 2. The formulation was infinitely dilutable without any phase separation. It has showed a nanoglobule size (21.58 nm), with minimal size distribution (poly dispersity index 0.13), and zeta potential approaching zero. The globule size and polydispersity increased slightly to 41.7 nm and 0.2037, respectively, upon infinite dilution, but still remained in nanosize range. No large volume fraction or liquid crystalline phases were observed, until infinite dilution of formulation.

Table 2. The screening summary of formulation[1]

Character	Remarks
Dilution potential	≈100%
Effect of dilution	Stable till infinity
Globule size (*z*-ave) (nm)	21.58
Polydispersity index	0.13
Zeta potential (mV)	−0.21±0.54
Globule size at infinite dilution (nm)	41.71
Polydispersity index at infinite dilution	0.21
Polarized light microscopy	Clear at original magnification ×630
pH	3.44

The values are expressed as mean±SEM.
The physical characterization was performed in triplicate.
[1]Composition of formulation is described in Table 1.

Pharmacokinetics of PCL in Sprague–Dawley rats

In order to assess the absolute bioavailability of PCL from different routes of administration using NE, initially the i.v. pharmacokinetic parameters were determined. PCL showed a two-compartment disposition upon i.v. administration (Figure 1). Plasma concentration of PCL had declined in a bi-exponential manner from peak concentration of 35 μg/ml to zero in 12 hours. Hence, the concentration profile was analyzed by two-compartment model using PCNONLIN. Typical of a lipophilic molecule, PCL has rapidly distributed (α half-life: 0.4152 hours) and then eliminated slowly

(β half-life: 3.0924 hours). The dose-independent pharmacokinetic parameters determined were in agreement with the values reported earlier (Gao *et al.*, 2003; He *et al.*, 2003) (Table 3). The area under the curve $(AUC)_{0-\infty}$ upon i.v. administration was used as reference to calculate the absolute bioavailability from oral and dermal administration of PCL. Non-linearity in pharmacokinetics of PCL was reported earlier in the range of 2–20 mg/kg (Gelderblom *et al.*, 2001). For that reason, formulations administered by different routes were compared at same dose of PCL (10 mg/kg).

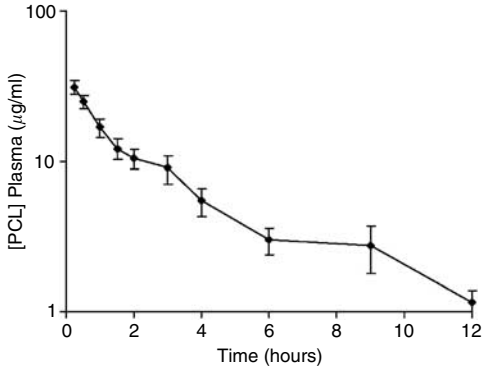

Figure 1. i.v. plasma concentration profile of PCL administered via femoral vein (10 mg/kg).** Taxol formulation was injected through femoral vein over a period of 3 minutes. The drug showed two-compartment disposition characterized by rapid distribution followed by slow elimination. The dose-independent parameters correlated well with literature reports (Gao *et al.*, 2003; He *et al.*, 2003). The $AUC_{24-\infty}$ was calculated from formula C_{24}/β, and $AUC_{0-\infty} = AUC_{0\text{-}24} + AUC_{24-\infty}$. **This profile is the mean ± SEM of 4–5 Sprague–Dawley rats.

Table 3. i.v. pharmacokinetic parameter of PCL administered as Taxol (10 mg/kg)

Parameter	Units	Magnitude
A	μg/ml	25.98
B	μg/ml	14.91
α	h^{-1}	1.6692
β	h^{-1}	0.2241
$AUC_{0\text{-}\infty}$	μg/ml h	85.84
K_{10}	h^{-1}	0.4981
K_{12}	h^{-1}	0.6441
K_{21}	h^{-1}	0.7511
V_d	ml	61.13
Cl_{total}	ml/h	30.44
AUMC	μg/ml h^2	306.22
MRT	h	3.73

AUC, area under the curve; AUMC, area under first moment plasma concentration; i.v., intravenous; MRT, mean residence time; PCL, paclitaxel. The AUC_{0-t} was calculated using linear trapezoidal rule and $AUC_{t-\infty}$ was estimated from C_t/K_{el}, then by adding these two components, $AUC_{0\text{-}\infty}$ was calculated. V_d – volume of distribution and Cl_{total} – total plasma clearance rate are dose-independent pharmacokinetic parameters and dependent on physiology and drug molecule. AUMC is the area under first moment plasma concentration–time profile, and MRT (mean residence time) is the ratio of AUMC and AUC.

Oral bioavailability enhancement of PCL using NE formulation

Upon oral administration of marketed Taxol formulation, owing to poor peroral absorption, plasma levels characteristically remained below 300 ng/ml (Figure 2), and the absolute bioavailability, calculated on the basis of $AUC_{0-\infty}$, was 10.62% (Figure 3). In contrast, PCL delivered from NE

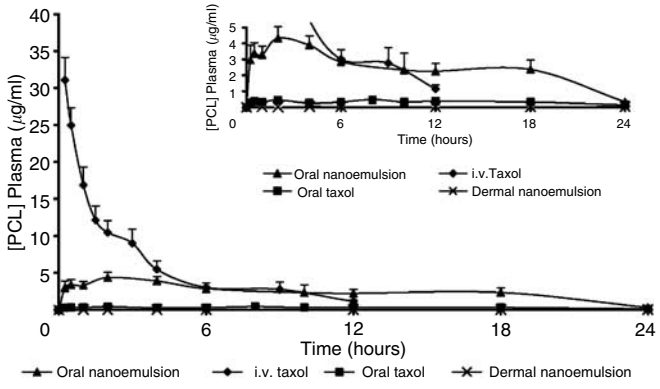

Figure 2. Plasma concentration profile of PCL when administered orally as NE and its comparison with i.v. and oral Taxol. The inset graph is the magnified bottom portion in order to show clearly the plasma drug concentrations obtained by non-i.v. routes. Oral NE and Taxol plasma concentrations are mean ± SEM of six rats, i.v. profile is the mean ± SEM of 4–5 rats, whereas the dermal application profile is expressed as mean ± SEM of four rats. NE formulation (coded as LMEV_24 in text) containing TPGS as co-surfactant was administered by various routes to calculate relative and absolute bioavailability. PCL formulation commercially available as Taxol, containing Cremophor EL and dehydrated ethanol was used as reference formulation. Oral absolute bioavailability of PCL was very low (10.62%) and improved seven times (70.25%), when administered as NE. PCL was administered at 10 mg/kg dose orally and intravenously. Dermally it was administered at 8 mg/kg dose. As it is a case of infinite dosing, and rate control is with skin, the amount of applied dose is not significant.

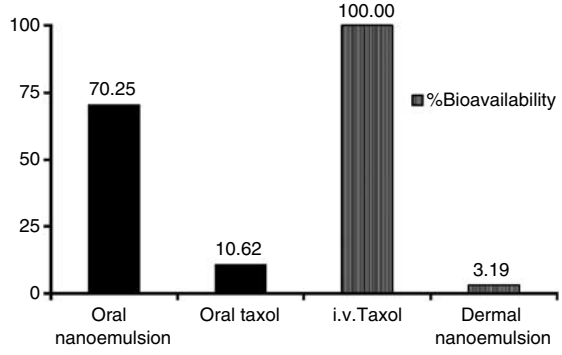

Figure 3. The absolute bioavailability of PCL administered from various formulation via different routes of administration. The numbers indicate the absolute bioavailability of formulations. The absolute bioavailability was calculated from the ratio of $AUC_{0-\infty}$ of each formulation with that obtained upon i.v. administration of same dose of PCL.

was rapidly absorbed reaching a steady-state value of 3.5 μg/ml in 30 minutes, and steady-state levels persisted up to 18 hours. This has amounted to an absolute bioavailability of 70.62% (calculated on the basis of $AUC_{0-\infty}$). Such a high extent of oral bioavaliabilty is remarkable in light of the fact that, although many reports on formulations with improved oral bioavailability of PCL were published, none had shown above 30% absolute bioavailability (Kimura *et al.*, 2002; Gao *et al.*, 2003; Woo *et al.*, 2003). Another interesting observation is the persistence of plasma drug concentrations from 0.5 to 18 hours at 3 μg/ml (Figure 2, inset), suggesting that drug is absorbed through out the gastrointestinal track. Significant amount of drug was absorbed from distal gastrointestinal track as well (ileum and colon), where gastrointestinal transit time lasts from 4 to 24 hours. Owing to the fact that P-gp expression is high in this area of gastrointestinal track, especially ileum, its absorption could not be enabled without the inhibition of P-gp by D-α-tocopheryl polyethyleneglycol 1,000 succinate (TPGS) and labrasol (LSOL).

P-gp is a membrane transporter that actively pumps xenobiotics out of cells. Given the very broad specificity of P-gp, its activity in the intestine can reduce the oral bioavailability of a wide range of drugs. PCL oral bioavailability has been reported to be mainly limited by the P-gp-mediated efflux (Sparreboom *et al.*, 1997; Dintaman and Silverman, 1999; Kimura *et al.*, 2002; Varma *et al.*, 2003; Woo *et al.*, 2003). Many surfactants and excipients have been shown to inhibit P-gp, and thus potentially enhance drug absorption. Excipients including LSOL and TPGS were reported to inhibit P-gp, thus enhancing the permeation of digoxin, a known P-gp substrate, using everted gut sac model (Cornaire *et al.*, 2004). TPGS increased the absorption flux of amprenavir (Yu *et al.*, 1999), and has been characterized as an inhibitor of P-gp-mediated drug transport in the human intestinal Caco-2 cell monolayers and other cell lines (Dintaman and Silverman, 1999; Bogman *et al.*, 2003). It has been shown to enhance the bioavailability of cyclosporine in human volunteers (Chang *et al.*, 2005) and of colchicine in rats (Bittner *et al.*, 2005). Recently, improved intestinal absorption of macromolecular hydrophilic drug vancomycin hydrochloride was reported using formulations containing LSOL and TPGS (Prasad *et al.*, 2003).

Further, excipients used in all formulations were approved for oral use. LSOL, chemically containing caprylocaproyl macrogol-8 glycerides, is official in European Pharmacopoeia, and approved for oral use. Its lethal dose $(LD)_{50}$ is reported to be as high as 22 g/kg in rats. TPGS is an oral dietary supplement of vitamin E, and is reported to be non-toxic with an acute LD_{50} of more than 7 g/kg in rats (Wu and Hopkins, 1999). The oil, M1944, chemically known as oleoyl macrogol-6 glycerides, also is orally approved excipient, and is listed in European Pharmacopoeia.

Penetration and permeation of PCL upon dermal application of NE formulation

In order to facilitate application of correct dose of formulation, and control area of application, the NE was incorporated into a prototype patch and applied on dorsal skin of rat using a medical grade adhesive. Before application of the patch, it is necessary to ensure that patch does not control the release of drug from formulation. Therefore, drug release was evaluated from the patch using US Pharmacopeia 6 type dissolution method. PCL release from dermal patch of 4 cm^2 area showed complete release in 6 hours, of which 75% occurred within 3 hours (data not shown). As a result, patch was not expected to be rate controlling, and permeation was controlled by skin itself.

Upon dermal application, the drug was predominantly localized in different layers of skin, with minimal systemic escape. By i.v. route, peak plasma concentrations reached up to 35 μg/ml, and concentrations in different layers of skin were in the range of 1 μg/4 cm^2 (Figure 4). On the contrary, upon dermal administration, even up to 48 hours drug concentrations were below 100 ng/ml, as a result systemic bioavailability reduced to mere 3.19% (Figure 3). Such differential distribution of drug represents an excellent degree of passive targeting to the tissue of interest, that is, the epidermis and dermis, and satisfies the objective of minimizing systemic escape, thereby effectively reducing body burden, simultaneously maximizing local concentrations. Distribution of drug into different skin layers and its variation with time is depicted in Figure 4. Local concentration of PCL in SC was around 0.5 μg till 4 hours. However, in effective dose and live dermis, levels reached up to 4 and 10 μg, respectively, by 4 hours. PCL is redistributed into different layers and the equilibrium levels persisted at 3 and 7 μg, respectively, in the epidermis and live dermis till 48 hours. Concentration gradient for maintaining this equilibrium is supplied by a high concentration of drug in SC. For the purpose of dosing, based on *ex vivo* lag time for steady state (5 hours) and the time to reach peak levels of drug in the

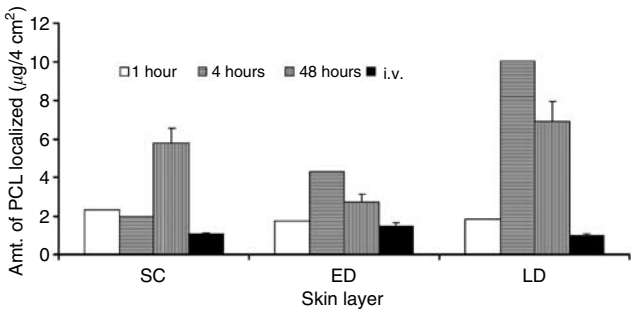

Figure 4. Distribution of PCL. Distribution of PCL into different layers of skin upon dermal administration as patch against systemic administration by i.v. route, showing regional pharmacokinetic advantage of localized delivery when compared to systemic delivery. Forty-eight hours data represent the mean ± SEM of four replicates, whereas at 1 hour and 4 hours, one animal each was killed to obtain tissue distribution of drug. In case of i.v. dosing, each bar is the mean ± SEM of four replicates. Upon i.v. administration, drug distribution is uniform in all skin layers at 1 μg/4 cm^2, whereas peak systemic concentration is 35 μg/ml. On the contrary, upon dermal application, maximum local concentration in live dermis layer reached to 10 μg, whereas systemic concentrations were below 100 ng/ml. This proves the regional pharmacokinetic advantage of these NEs when compared to systemic administration for the therapy of diseases involving the skin.

epidermis and live dermis, application of formulation for 4 hours would be sufficient.

Further, mass balancing indicated (Table 4) that upon i.v. administration, 11.7% of drug is distributed into skin tissues, 14.9% remained in plasma compartment, and remaining drug is distributed to other tissues. Whereas upon dermal administration, total formulation bioavailability was observed to be 6.2%, of which dermal bioavailability was 5.95% and systemic bioavailability was mere 0.22%. This clearly proves that drug is confined to skin layers without any systemic escape.

When a drug molecule crosses SC and enters into the live epidermis and dermis, its fate can follow one of the following paths based on its inherent properties:

- Taken up by blood vessels to distribute into systemic circulation.
- Get metabolized by enzymes.
- Localize there owing to poor aqueous solubility and diffusion coefficient.
- Directly penetrate into deeper tissues such as adipose tissue as well as muscle.

Among these pathways, first three are generally accepted, but there is controversy regarding the actual depth and quantity of drugs delivered to the local subcutaneous structures after topical administration. Some initial reports suggested that distribution of topically applied drug substances to underlying tissues occur mainly through systemic blood supply and direct penetration, if present at all, is minimal (Dawson *et al.*, 1988; Radermacher *et al.*, 1991). However, contrary to the accepted concept, the blood supply to the dermis is not capable of resorbing certain chemicals proportionately to their penetration through the epidermis. Consequently, local deep penetration of a series of lipophilic pesticides and steroids was demonstrated in mice and rats (Marty *et al.*, 1989), and salicylate salt localization was demonstrated in pigs (Baldwin *et al.*, 1984). Singh and Roberts (1996) have proposed structure–deep tissue penetration relationships for drugs with diverse physicochemical properties. Apparently, the concentration of any solute in a given tissue was dependent on concentration in preceding tissue, implying that direct redistribution is essentially concentration gradient driven. Further, the deep tissue penetration of drugs when applied from aqueous solution was greater for smaller solutes with adequate lipophilicity. Higaki *et al.* (2002) proposed a six-compartment pharmacokinetic model to explain the redistribution of drugs into deeper tissues when applied topically. From among the pharmacokinetic parameters analyzed, the clearance from viable skin to the muscle was found to correlate directly to the unbound drug fraction in viable skin, implying that the larger amount of unbound drug in viable skin significantly contributes to the direct penetration into muscle, rather than into systemic circulation.

To summarize, the dermal delivery of a high MW and lipophilic drug, owing to a combination of two adverse physicochemical properties, could not be enhanced using conventional penetration enhancers as well as vehicles (Panchagnula *et al.*, 2004a, 2005). Therefore, a combination strategy involving penetration enhancement *per se* using lipid bilayer alteration, along with enhanced concentration gradient and improved partitioning into skin, was proposed. The NEs have provided a custom-made opportunity to apply all these combinations in one formulation. PCL has indeed penetrated into the deeper skin layers up to subcutaneous tissue. PCL has penetrated within 4 hours, and maintained its high local concentration in respective skin layers up to 48 hours, with minimal redistribution. The reason behind the successful localization of drug in deep skin layers is owing to a combination of physicochemical properties of molecule as well as the selective calibration of penetration–permeation balance by the formulation. PCL penetration was enhanced using improved solubilization in vehicle, where PCL is expected to be at maximum thermodynamic activity, thus enabling a high concentration gradient across the skin along with improved partitioning and lipid bilayer perturbation using a combination of surfactant penetration enhancers. Surfactants are known skin penetration enhancers. They either alone or in the form of microemulsions or niosomes were reported to enhance skin permeation of drugs by enhancing skin permeability and solubility (Kreilgaard, 2002b; Nokhodchi *et al.*, 2003). Vaddi *et al.* (2001) had attributed the skin penetration enhancement of haloperidol

Table 4. Mass balancing of PCL upon dermal and systemic administration

Treatment	Dose applied (μg)	SC (μg)	ED (μg)	LD (μg)	Systemic (μg)
i.v. Taxol	1700	61.91 (3.64%)	82.92 (4.88%)	54.91 (3.23%)	235.3 (13.8%)
Dermal nanoemulsion	275	1.97 (0.72%)	4.35 (1.58%)	10.04 (3.64%)	0.61 (0.22%)

ED, epidermis; i.v., intravenous; LD, live dermis layers; PCL, paclitaxel; SC, stratum corneum.
All values are given in μg. Values in parenthesis indicate the percentage of administered drug localized in each compartment. In case of i.v. administration, the remaining 74% drug is either eliminated or distributed into other tissues, whereas the unaccounted 94% drug upon dermal application is either remaining in patch or eliminated from body. In case of i.v. administration, drug is distributed all over body, hence, for mass balancing purpose, surface area of rat was taken as 233 cm^2. However, in case of nanoemulsions, as is drug distributed in a local area of 4 cm^2, calculations were performed for this area of distribution. The amount of drug in systemic circulation was computed from $C_{ss,ave}{}^{*}V_d$ determined from i.v. dosing.
Upon i.v. administration, 11.7% of drug is distributed into skin tissues, 13.8% remained in plasma compartment, and remaining drug is distributed to other tissues or eliminated from body. Whereas upon dermal administration, total formulation bioavailability was observed to be 6.2%, of which dermal bioavailability was 5.95% and systemic bioavailability was mere 0.22%. This clearly indicates the skin localization of PCL with minimal systemic escape until 48 h post-administration.

by cetrimide, to an increased thermodynamic gradient accomplished by the extraction of skin lipids. Gupta *et al.* (2005) had attributed the skin permeation enhancement using microemulsions to an interaction between microemulsion components and the SC. In a comprehensive review on the microemulsions role on skin permeation enhancement, Kreilgaard (2002b) opined that the enhancement of drug solubility plays the main role in skin permeation enhancement of drugs from microemulsions. Once PCL has crossed SC, owing to its inherent lipophilicity and high MW, it could have moved slowly up to deeper subcutaneous layers, with the aid of NE components. However, owing to lipophilic nature of the drug, it is not efficiently taken up by systemic circulation during the transit.

Even though formulation constituents were all approved for dermal use at much higher levels than those utilized in the formulations, it is essential to evaluate irritancy potential when they are applied together in the form of formulation. In addition, it also helps to rule out the probability of cumulative toxicity. The visual scoring system adopted routinely for irritancy tests is invariably associated with errors in subjective evaluation. Therefore, in current investigation, irritancy potential of formulation was assessed by using biophysical tools transepidermal water loss (TEWL) and laser Doppler velocimetry (LDV). Cutaneous irritancy is accompanied by erythema and severe alteration of skin barrier function. Erythema and inflammation are characterized by increased blood flow, which is quantified by LDV. No statistically significant difference was observed between before and after treatment with formulation ($P > 0.05$), occlusion control, and blank formulation (Figure 5a). Similarly, difference in TEWL before and after treatment is statistically insignificant ($P > 0.05$) (Figure 5b), indicating that the skin barrier function is not altered irreversibly upon formulation application, and formulations are safe and non-irritant when applied for 48 hours *in vivo*.

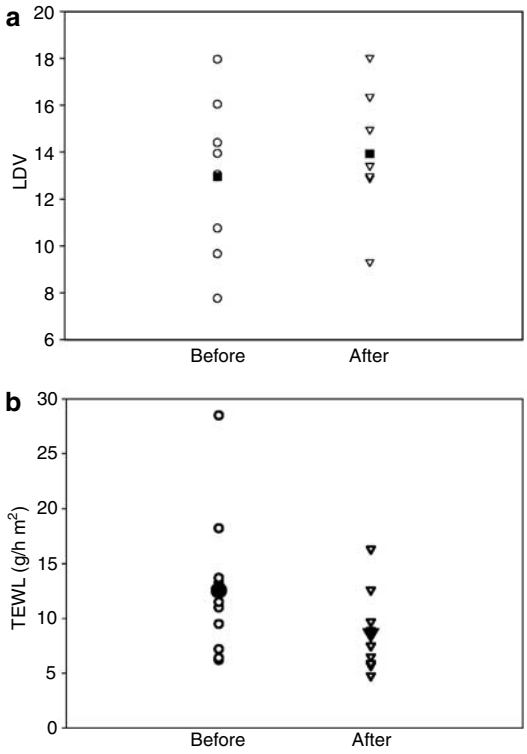

Figure 5. The irritancy evaluation of NE formulation using biophysical tools. (**a**) Skin local blood flow evaluation using LDV. (**b**) Skin barrier evaluation using TEWL. Using both tools, no significant difference was observed between naïve rats and NE-treated rats ($P > 0.05$), indicating that formulation is non-irritant and not altering barrier irreversibly *in vivo*.

Conclusion

Owing to high MW, lipophilicity, and P-gp efflux, PCL is difficult to deliver by oral and dermal routes. The combination of high lipophilicity and MW makes it an ideal candidate to localize in skin layers upon dermal application. In this investigation, pharmacokinetic proof is provided for the localization of PCL in deep skin layers using novel NE formulations. Further, using same NE formulation, peroral bioavailability also was enhanced to above 70%, which is a considerable enhancement over the previously reported approaches. The dual achievement of selective localization and systemic administration by changing route of administration of same formulation can be favorably utilized for the treatment of localized and systemically disseminated forms of cancers and psoriasis.

MATERIALS AND METHODS

Animal ethics compliance and radioactive material handling and disposal

The protocols for all the *ex vivo* and *in vivo* experiments performed on Sprague–Dawley rats were approved by the Institutional Animal Ethics Committee (IAEC, NIPER). The radioactive material was handled in the premises approved for the purpose in accordance with the statutory requirements of the Atomic Energy Regulatory Board (AERB, GOI) and the biological waste was disposed according to the IAEC, NIPER and AERB, GOI protocols.

Formulation of NEs

In case of formulation III, the drug (^{14}C-PCL and PCL) was dispersed in the internal oil phase (Labrafil, Gattefosse, France) by gentle mixing. TPGS was dissolved in LSOL by warming to above 60°C. After cooling to room temperature, this surfactant mixture was added to drug dispersion in oil. This forms the clear homogeneous concentrate NE spontaneously. In case of blank NE (formulation I), the oily phase was directly added to surfactant mixture. Formulation II (Taxol) was prepared in the laboratory using the procedure and formula reported previously (Gao *et al.*, 2003).

Physical characterization of NEs

The developed NEs were characterized with respect to pH, globule size distribution, zeta potential, and optical microscopy under polarized light after diluting the concentrated NE (about 500 μl) to 5 ml, 250 ml, and then to infinite dilution (more than 5 l). Globule size distribution and zetapotential were measured using Zetasizer-Nano ZS (Malvern, UK). The globule size distribution was measured by dynamic light scattering, whereas zeta potential was measured by phase analysis light scattering using mixed mode measurement (M3-PALS technique). Polarized light microscopy was performed under cross-polarizers.

Globule size distribution and zeta potential measurement. Globule size distribution and zeta potential were measured using Zetasizer-Nano ZS fitted with 633 nm red laser. Both measurements were performed simultaneously at various dilutions of same concentrated NE sample, using folded capillary cell, at 25°C, after sufficient equilibration. For the purpose of measurement, the dispersant was taken as water, and the refractive index of globules were taken from reported values of oil (1.470). The measurement was performed in triplicate and the parameters, *z*-ave size, polydispersity index, were used as measures for the calculation of mean size and size distribution, respectively. Before the measurement of zeta potential of each sample, its pH was measured with a pH meter using glass electrode (Thermo, Shield of Los Altos, CA).

Optical polarized light microscopy. As zetasizer can measure globule size less than 10 μm accurately, but in the presence of large volume fractions of oil, it will give erroneous results. Moreover, it cannot detect the presence of liquid crystalline phases. Hence, the NEs were observed at various dilutions under polarized light microscope. The samples were scanned at original magnification $\times 630$ under cross-polarizers to observe any specific patterns characteristic of liquid crystalline phases.

Evaluation of pharmacokinetic parameters of PCL in rats

Formulations of PCL administered *in vivo*, by various routes is given in Table 1. In order to assess pharmacokinetic parameters of PCL in Sprague–Dawley rats, commercial formulation, prepared in the laboratory using a procedure described earlier (Gao *et al.*, 2003), was administered at same dose as that used for oral administration.

The animals were anesthetized using urethane (intraperitoneal, 1.6 g/kg), and the femoral vein was exposed at right thigh muscle. A siliconized venous cannula was inserted, and after ligation using the silk thread, formulation II (Table 1) containing 6.8 mg/g of PCL was administered (10 mg/kg) slowly over 5 minutes, and then cannula was withdrawn after ligating both ends of vein using silk thread. The incision was sutured, after applying an anti-bacterial dusting powder. A total of 200 μl of blood was withdrawn from retro-orbital plexus at predetermined time points for 6 hours into heparinized microcentrifuge tubes (50 IU/ml of blood) and plasma was separated upon centrifugation at 2,000 *g* for 5 minutes. After each sampling, 500 μl of isotonic dextrose–normal saline solution was administered intraperitoneally to provide nourishment and to compensate for the depletion of fluid volume. The concentration of drug was estimated by liquid scintillation counting.

Oral bioavailability assessment of formulation

Oral bioavailability assessment was performed in parallel groups of six rats each. To one group, formulation I, containing 6.8 mg/g PCL, was administered, and another group was administered with NE (formulation III, containing 10 mg/g of PCL) (compositions given in Table 1) by oral gavage (10 mg/kg). Blood was collected at predetermined time points for 24 hours, then processed and analyzed as described in previous section.

Dermal penetration and permeation of PCL from NE formulation

Preparation of prototype patch. In order to facilitate application of accurate dose and to control area of application, the NE was applied after incorporating a weighed quantity of formulation into a prototype patch of 4 cm^2 area. The patch was prepared using a procedure described previously (Panchagnula and Khandavilli, 2004b). Briefly, heat-sealable backing layer and microporous polyethylene membrane were sealed on three sides, and after incorporating a weighed quantity of formulation, the fourth side was sealed. All patches were prepared 12 hours before application onto animals. Before *in vivo* application, drug release from patch was studied using rotating disk (USP 6) dissolution apparatus. Dissolution was performed at 50 r.p.m., using 250 ml of ethanol:phosphate-buffered saline (1:3) as dissolution medium, maintained at 32°C in a closed vessel. Samples were withdrawn intermittently (1 ml with replacement) till 6 hours and released PCL was analyzed HPLC.

In vivo application of patch. After ensuring that drug release is not controlled by patch using dissolution testing, the patches were applied under occluded conditions on dorsal surface of animals and the penetration and permeation of PCL was estimated for 48 hours.

Rats were anesthetized with urethane (1.6 g/kg, intraperitoneal), and dorsal skin was shaven. Dermal administration was performed in three groups, keeping blank and occlusion control for the purpose of irritancy evaluation. Patches were affixed to the shaven dorsal skin using medical grade adhesive tape. To one group, blank formulation patch (formulation I, Table 1) was applied, and to another only the patch was applied for assessing occlusion effect on skin. To another parallel group, NE formulation patch (formulation II) was affixed. Blood was collected into heparinized tubes from retro-orbital plexus, which were processed similar to those samples obtained upon oral and i.v. administration.

After experimentation, the skin exposed to formulation was excised using scalpel after killing animal by cervical dislocation while it is under anesthesia. Subcutaneous tissue attached to the exposed skin was removed completely by scrapping with scalpel. The excised fraction consists both the dermis and subcutaneous fatty tissue. Upper tissue containing SC, live epidermis, and a part of dermis was treated for 4 hours with 0.5% trypsin solution in phosphate buffer (pH 8.0) absorbed on a filter paper. SC was then carefully peeled off from the underlying epidermis and dermis layers. Skin, by this process was, separated into three layers, SC, live epidermis, and dermis together (denoted as epidermis in Discussion), and subcutaneous tissue containing a part of dermis (denoted as LD). Separated layers were subsequently dissolved in tissue solubilizer and the amount of drug localized in each layer was quantified using liquid scintillation counting.

Measurement of TEWL and LDV. In order to assess the irritancy potential of NEs, two biophysical tools were employed. TEWL and LDV were measured before and after the application of the patch in all the three groups, that is, NE treatment, blank application, and occlusion control.

TEWL measurement was performed by placing the collared probe on the surface of dorsal skin of anesthetized rat. All measurements were performed in a single ventilated room having controlled temperature between 28 and 30°C, and relative humidity of 38–40%.

Before measuring the local blood flow of skin using LDV, the anesthetized animals were acclimatized to the room temperature maintained at 25°C for at least 1 hour. LDV measurement was performed by applying the probe at 5 mm distance over skin. This is

necessary to avoid any changes in local blood flow owing to the pressure exerted by probe if directly contacted. The continuity between skin and probe was maintained using normal saline drop in between. Each measurement was performed for a minimum of 60 seconds.

Data processing and statistics

Pharmacokinetic parameters upon i.v. administration were calculated by fitting plasma concentration profile to two-compartment model using PCNONLIN (version 4.0). AUC_{0-t} was calculated using linear trapezoidal rule. $AUC_{t-\infty}$ was estimated from the formula C_t/K_{el}. Then, $AUC_{0-\infty}$ was obtained by summation of these two components. Absolute bioavailability was calculated by taking the ratio of $AUC_{0-\infty}$ obtained from dermal and oral routes with that obtained upon i.v. administration. TEWL and LDV were compared before and after treatment, and with that of occlusion and blank controls, using *t*-test at 95% confidence interval. Skin affinity values for different layers were reported per 4 cm^2, the area of application of patch.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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