Development and Evaluation of Topical Formulation Containing Solid Lipid Nanoparticles of Vitamin A

Submitted: August 5, 2005; Accepted: June 14, 2006; Published: November 17, 2006

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ABSTRACT

The purpose of this research was to investigate novel particulate carrier system such as solid lipid nanoparticles (SLN) for topical application of vitamin A palmitate and to study its beneficial effects on skin. Topical gels enriched with SLN of vitamin A were prepared. The solid lipid nanoparticulate dispersion was prepared using high-pressure homogenization technique and was incorporated into polymeric gels of Carbopol, Pemulen, Lutrol, and Xanthan gum for convenient application. The nanoparticulate dispersion and its gels were evaluated for various parameters such as particle size, in vitro drug release, in vitro penetration, in vivo skin hydration, and skin irritation. The solid lipid nanoparticulate dispersion showed mean particle size of 350 nm. Differential scanning calorimetry studies revealed no drug-excipient incompatibility. In vitro release profile of vitamin A palmitate from nanoparticulate dispersion and its gel showed prolonged drug release up to 24 hours, which could be owing to embedment of drug in the solid lipid core. In vitro penetration studies showed almost 2 times higher drug concentration in the skin with lipid nanoparticle-enriched gel as compared with conventional gel, thus indicating better localization of the drug in the skin. In vivo skin hydration studies in albino rats revealed increase in the thickness of the stratum corneum with improved skin hydration. The developed formulation was nonirritant to the skin with no erythema or edema and had primary irritation index of 0.00. Thus it can be concluded that SLN represents a promising particulate carrier having controlled drug release, improved skin hydration, and potential to localize the drug in the skin with no skin irritation.

KEYWORDS: Solid lipid nanoparticles, topical, gel, vitamin A.

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INTRODUCTION

The efforts to improve drug effectiveness have led to developments in drug delivery technology. Targeted drug delivery implies selective and effective localization of pharmacologically active ingredient at preselected target in therapeutic concentration, while restricting its access to nontarget areas, thus maximizing the effectiveness of the drug. The carrier is one of the most important entities required for successful transportation of the drug. Colloidal drug delivery system is a rapidly developing area that has contributed significantly to the progress in the field of controlled and targeted drug delivery. Colloidal carriers are one of the approaches for the controlled delivery of drugs by the dermal route.

As a vehicle for controlled release of active substances and targeting to skin layers, nanodisperse systems such as liposomes, nanoemulsions, and lipid nanoparticles are gaining more and more importance. Solid lipid nanoparticles (SLN) are the new generation of nanoparticulate active substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use. Small lipid vesicles in the range of nanometers have the advantages, but avoid the disadvantages, of other colloidal carriers.[1] SLN were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. Compared with polymeric nanoparticles, SLN have lower toxicity because of the absence of solvents in the production process and also relatively low cost for the excipients. SLN represents a particulate system, which can be produced with an established technique of high pressure homogenization, allowing production on industrial scale. This method also protects the incorporated drug against chemical degradation as there is little or no access for water to enter the inner area core of the lipid particle.[2]

Solid lipid nanoparticles appear promising as a drug carrier system, and therefore were investigated for topical application of vitamin A. Stratum corneum is the main barrier in the percutaneous absorption of topically applied drugs. Small size and relatively narrow size distribution of SLN permit site-specific delivery to the skin.[3,4] SLN have high affinity to the stratum corneum, and therefore an enhanced bioavailability of the encapsulated material to the skin is achieved. SLN enhance the penetration and transport active substances particularly lipophilic agents and thus intensify the concentration of these agents in the skin.[5]

Vitamin A, a fat-soluble vitamin, is involved in the formation and maintenance of healthy skin, hair, and mucous membranes. It has the ability to increase skin elasticity and decrease skin roughness and the peroxidation of skin lipids.[6] It works by exfoliating the surface layer of skin, thus speeding up cell turnover, making the skin look fresher, smoother, and younger. It increases skin moisture, cellular renewal and decreases skin wrinkling. Topical vitamin A acts as an antioxidant on the skin. It prevents tissue atrophy and the loss of collagen that is generally found with aging. Vitamin A helps to restore normal soft skin and reduces keratoses in sun-damaged skin. Incorporation of vitamin A in SLN would help to target the drug to the desired site of action (ie, skin). This study investigates the formulation and characterization of SLN of vitamin A. The lipid nanoparticles were incorporated in gels for convenient topical application and were evaluated for in vitro skin penetration and in vivo skin hydration.

Materials and Methods

Materials

Vitamin A palmitate was a gift of BASF Ltd (Mumbai, India). Compritol 888 ATO (Glyceryl behenate) was provided by Gattefossé (Gennevilliers, France). Carbopol 940 and Pemulen TR-1 were obtained from Noveon Inc (Brussels, Belgium). Xanthan gum and Lutrol were provided by Signet Chemicals Ltd (Mumbai, India) and BASF Ltd, respectively, as gift samples. Surfactants, sodium lauryl sulfate (SLS) and sorbitan monooleate were purchased from S. D. Fine Chemicals, Mumbai, India. All other chemicals and solvents were of analytical reagent grade.

Preparation of Solid Lipid Nanoparticles

The lipid phase (5%) was melted. Drug (25% of the lipid phase) was dispersed in the lipid melt to obtain clear solution. The dispersion medium (ie, distilled water with surfactant [SLS 4%]) was heated to the temperature of the lipid melt. The lipophilic surfactant sorbitan monooleate (5%) was dissolved in the lipid phase. The hot lipid phase was emulsified in the dispersion medium by high speed stirring using Ultra-turrax T 25 (IKA-Werke, Staufen, Germany). This dispersion was then subjected to high pressure homogenization using APV 2000 (Invensys, Copenhagen, Denmark) homogenizer at 1200 bars and 9 cycles. The obtained nanodispersion was allowed to cool to room temperature, forming lipid nanoparticles by recrystallization of the dispersed lipid.

Preparation of Gels

Gels Enriched With Nanoparticles

Gels were prepared using 4 polymers: Carbopol 940 (1%), Pemulen TR-1 (1%), Xanthan gum (1%), and Lutrol F 127 (15%). For the preparation of the gel, glycerol (10%), nanoparticulate dispersion (20%), and water were weighed in a beaker and stirred. Required quantity of gelling agent was dispersed in the aqueous phase under continuous stirring. Neutralization was performed using triethanolamine in the case of Carbopol 940 and Pemulen TR-1 to attain pH 7.0.

Conventional Gel

Conventional gel of vitamin A was prepared by incorporating the vitamin A palmitate in Carbopol gel at the same concentration as nanoparticulate-enriched gel. In brief, vitamin A palmitate was dispersed in part of water containing SLS. In remaining part of water glycerol and Carbopol 940 (1%) were dispersed. The part containing vitamin A palmitate was added to the other part and neutralized with triethanolamine to get the desired consistency.

Evaluation of Solid Lipid Nanoparticulate Dispersion and Gel

Physicochemical Properties

The nanoparticulate dispersions were characterized for physicochemical properties such as color, odor, and stability after centrifugation. Centrifugation was performed at 2000 rpm for 30 minutes. Gels were evaluated for color, odor, and pH.

Drug Content

Assay was performed as described in Indian Pharmacopoeia (IP) 1996 by Method B.[7] In brief, a weighed quantity of nanoparticulate dispersion or gel was refluxed with hydroquinone, potassium hydroxide solution, and ethanol. Vitamin A was extracted by shaking with ether. After evaporation of solvent, the residue obtained was dissolved in 2-propanol and the amount of the drug was determined using Jasco UV-visible (VIS) spectrophotometer at 324 nm (Jasco Inc, Easton, MD).

Particle Size Analysis

The particle size analysis of the selected formulation was performed using Malvern Mastersizer 2000 (Malvern Instruments, Worcestershire, UK) and the theory used was laser diffraction with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration.

Transmission electron microscopy was performed using JEOL 1010 (JEOL Ltd, Tokyo, Japan). One drop of nanoparticulate dispersion was placed on the grid, dried for 3 to 5 minutes, and drained on the filter paper. The grid was further dried by keeping it in the Petri plate; then it was loaded in the transmission electron microscope, and areas

were scanned for observation of nanoparticles. The picture was taken under the electron microscope and is shown in Figure 1.

Thermal Analysis

Differential Scanning Calorimetry (DSC) was performed by Mettler-Toledo DSC 821^e (Columbus, OH) instrument, and an empty standard aluminum pan was used as reference. DSC scans were recorded at heating rate of 10°C/min. Figure 2 shows the DSC thermogram of pure glyceryl behenate as bulk material and the thermogram of nanoparticulate dispersion containing SLN loaded with vitamin A palmitate.

In Vitro Drug Release

In vitro release studies were performed with Keshary Chien diffusion cells. Cellulose nitrate membrane filters (0.1-μm pore diameter, Whatman India Liason Office, Mumbai, India) were soaked with isopropyl myristate to simulate lipophilic properties of stratum corneum and mounted on diffusion cell. Phosphate-buffered saline (PBS) containing 3% polysorbate 80 was used as receptor fluid. One milliliter of liquid nanoparticulate dispersion or 0.5 g of the semisolid preparation was applied to the donor compartment. Samples were collected over a period of 24 hours and analyzed using Jasco UV-VIS spectrophotometer at 329 nm.

In Vitro Skin Penetration Studies

In vitro skin penetration studies were performed with human cadaver skin using Keshary Chien cells. Human cadaver skin from the abdominal region, after removing hair and subcutaneous fat tissue, was mounted on the Keshary Chien diffusion cell. PBS containing 3% Polysorbate 80 served as receptor fluid. A small quantity (0.5 g) of the gel was applied to the skin surface. At the end of 24 hours, the amount of drug in the receptor compartment, the drug remaining on the skin, and the drug concentration in the skin was determined by extraction into a suitable solvent followed by spectrophotometric analysis using Jasco UV-VIS spectrophotometer.

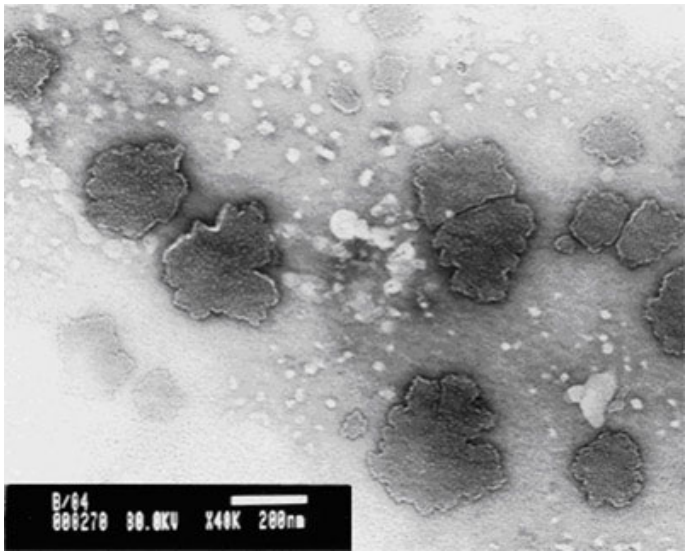

Figure 1. Transmission electron micrograph of solid lipid nanoparticulate dispersion.

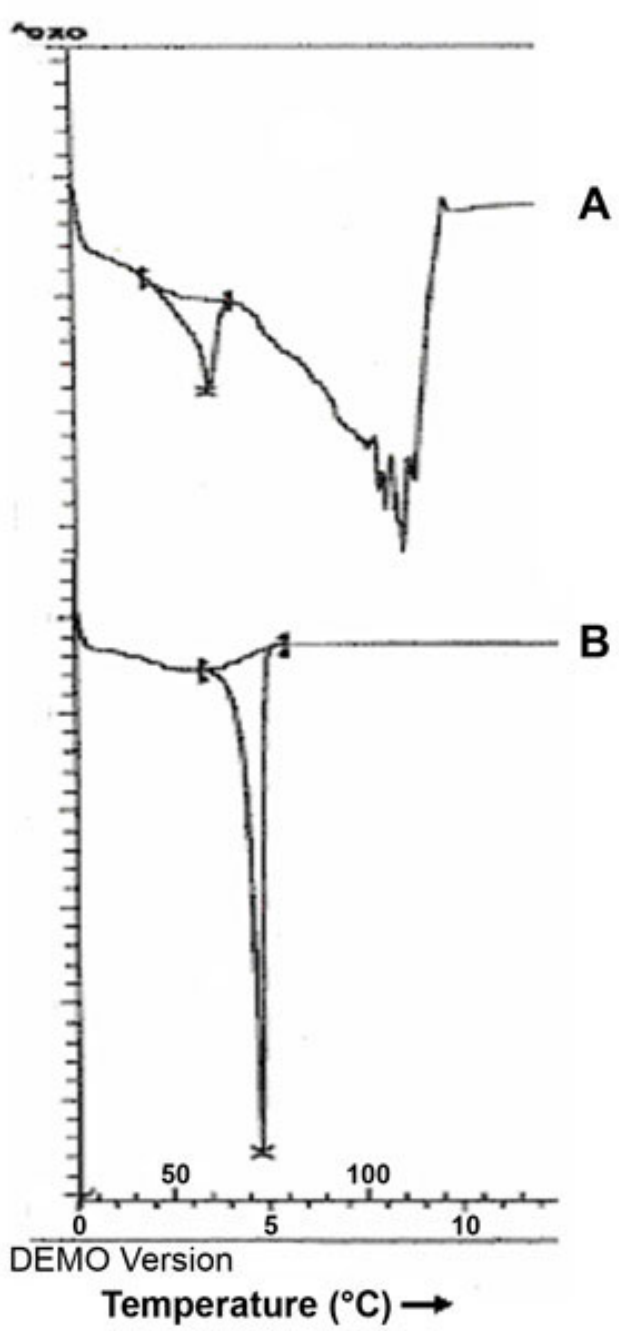

Figure 2. DSC thermogram of pure glyceryl behenate (A), and nanoparticulate dispersion containing solid lipid nanoparticles loaded with vitamin A palmitate (B).

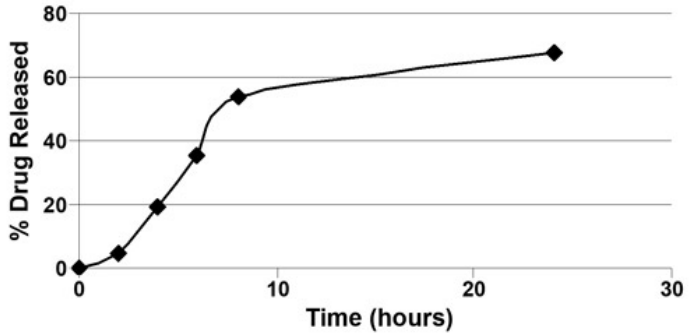

Figure 3. In vitro release profile of vitamin A palmitate incorporated in nanoparticulate dispersion.

In Vivo Skin Hydration Studies

The skin hydrating effect of the selected formulation was investigated in vivo and compared with the conventional gel. The topical formulations were applied to the shaved skin of female albino rats. After 24 hours, the animals were humanely killed and the skin was isolated, vertically sliced using microtome, and stained with hematoxylin and eosin. The slides were observed under optical microscope and thickness of stratum corneum was measured. The photomicrographs were taken using hund-Wetzlar Image analyzer (Helmut Hund Gmbh, Wetzlar, Germany).

Primary Skin Irritation Studies

Primary skin irritation studies of the selected formulation were performed using albino rabbits in accordance with the guidelines of the Consumer Product Safety Commission.[8] Formalin was taken as positive control and plain gel was used as negative control in the study. The study was approved by the Institutional Ethics Committee (IAEC C. U. Shah College of Pharmacy, Mumbai, India).

Results and Discussion

Preparation of Solid Lipid Nanoparticle Dispersion

Solid lipid nanoparticle dispersion of vitamin A palmitate were successfully prepared by melt homogenization method. Homogenization at pressures lower than 1000 bar did not result in achievement of all the particles in the submicron range. Homogenization pressure of 1200 bar with 9 cycles resulted in colloidal dispersion.

Physicochemical Properties

The nanoparticulate dispersion was light-yellowish in color, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation (2000 rpm for 30 minutes). Gels loaded with nanoparticulate dispersion were light yellow in color, odorless, with smooth appearance. The pH of the gels was in the range of 4.75 and 6.60.

Drug Content

A high amount of drug could be incorporated in the nanoparticulate dispersion. As high as 25% of vitamin A palmitate with respect to the lipid could be incorporated as compared with the 5% reported.[9] Such high incorporations were possible because vitamin A palmitate is fat soluble. With 20% nanoparticulate dispersion being incorporated in the gel, the final concentration of vitamin A palmitate in the gels was 0.25%. Drug assay showed 97% to 100% contents of labeled amount.

Particle Size Analysis

The particle size analysis of the nanoparticulate dispersion by laser diffraction using Malvern Mastersizer showed a mean particle size of 350 nm. Transmission electron micrograph (TEM) of SLN dispersion of vitamin A illustrates the spherical shape of nanoparticles entrapping the drug, vitamin A palmitate (Figure 1). It also clearly shows the homogeneous monolayer coating of surfactant at the periphery of the nanoparticles surrounding the lipid core. The TEM studies showed particle size ranging approximately from 100 to 500 nm, which was in confirmation with particle size analysis obtained with laser diffraction.

Thermal Analysis

DSC is a highly useful means of detecting drug-excipient incompatibility in the formulation.[10] Glyceryl behenate alone and in formulation was studied using DSC. For the bulk material of glyceryl behenate, the melting process took place with maximum peak at 71.97°C. DSC thermogram of SLN dispersion showed an endotherm at 63.73°C, which can be attributed to melting of glyceryl behenate in SLN (Figure 2).

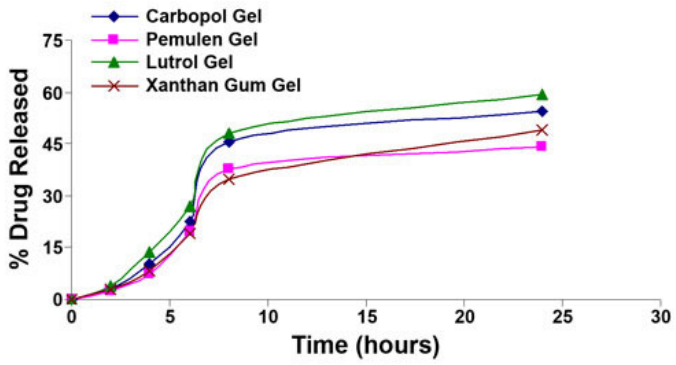

Figure 4. In vitro release of vitamin A palmitate from gels enriched with nanoparticulate dispersion.

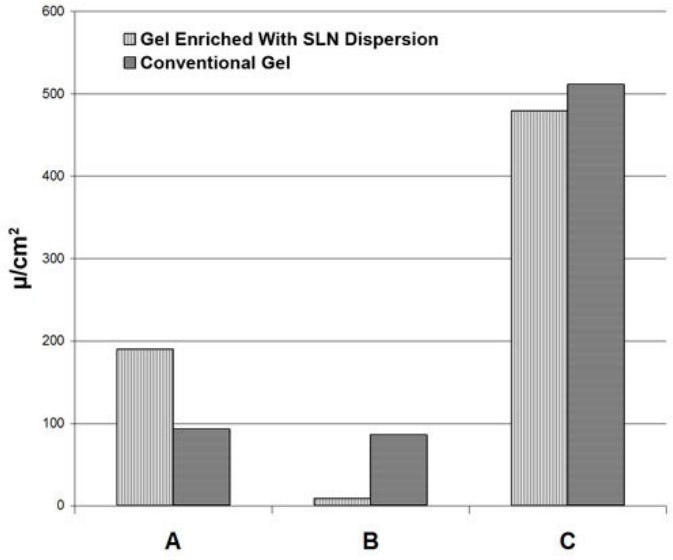

Figure 5. Comparison of the drug levels from in vitro skin penetration studies: (A) Penetrated in the skin, (B) Receptor compartment, and (C) Remained on the skin.

The peak of glyceryl behenate in the formulation shows a slight shift to the lower temperature side. This could be due to a reduction in particle size and an increase in surface area leading to a decrease in melting enthalpy as compared with the heat flow through larger crystals, which require more time. The higher melting enthalpy value suggests higher-ordered lattice arrangement. For the less-ordered crystal or amorphous state, the melting of the substance requires less energy than the perfect crystalline substance, which needs to overcome lattice force. The transformation of a sharp to a broad DSC peak due to the melting of glyceryl behenate and a decrease in the melting point is associated with numerous lattice defects and the formation of amorphous regions in which the drug is located. These results are in confirmation with the DSC studies reported earlier with glyceryl behenate SLN.[11] Hou et al have also made similar observations in DSC studies of mifepristone SLN prepared using glycerol monostearate when compared with the bulk matrix material.[12]

In Vitro Drug Release

Figure 3 shows the in vitro release profile of vitamin A palmitate from nanoparticulate dispersion. In the initial 2 hours, the drug release was less than 10% probably because of the slow diffusion of drug from the lipid. After 2 hours, the drug release rate increased with time until 8 hours following, which the rate declined. The prolonged drug release could be attributed to embedment of drug in the solid lipid matrix.

Comparing the drug release from nanoparticulate dispersion and nanoparticles in gels (Figure 4), the release of vitamin A palmitate was slower from the gel formulation: 54.38% at the end of 24 hours in Carbopol gel as compared with nanoparticulate dispersion, 67.52% at the end of 24 hours. Incorporation of nanoparticulate dispersion into gels further decreased the drug release. This result was probably due to the release-retarding effect of the polymeric matrix of gelling agents.

The drug release was found to be slowest in case of gel prepared using Pemulen. Xanthum gum gel showed faster release than Pemulen gel; however, because of its sticky texture, it was not investigated further. The drug release from gels prepared using Carbopol and Lutrol was similar, but the Carbopol gel was better in appearance and texture and was therefore selected for further evaluation.

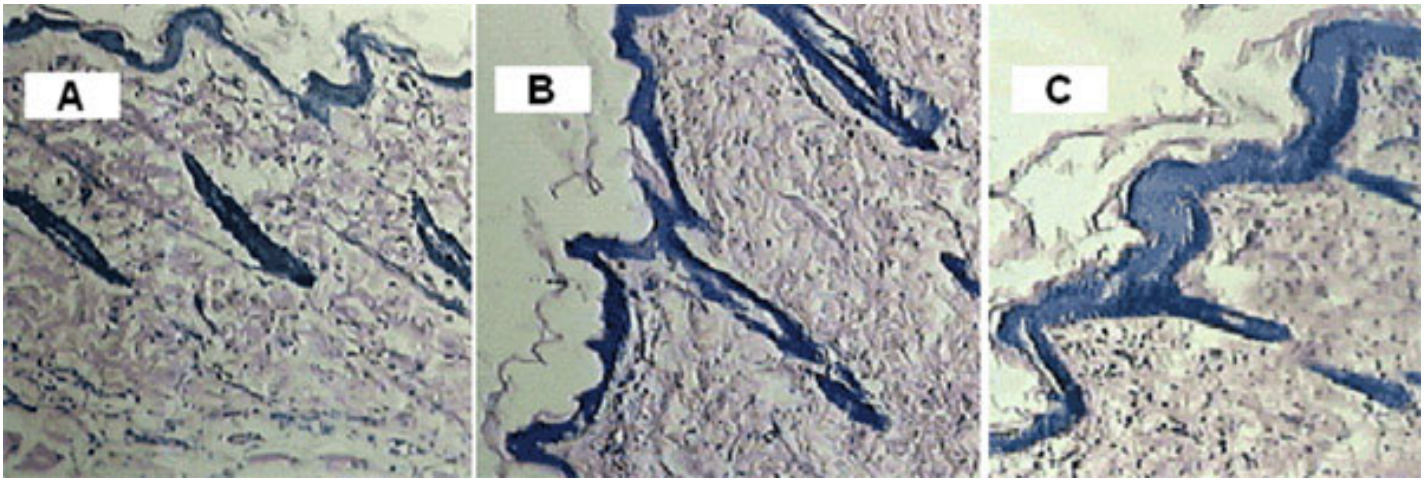

Figure 6. Photomicrographs of (A) untreated rat skin, (B) rat skin treated with conventional gel, and (C) rat skin treated with gel enriched with solid lipid nanoparticles. Magnification: 10×.

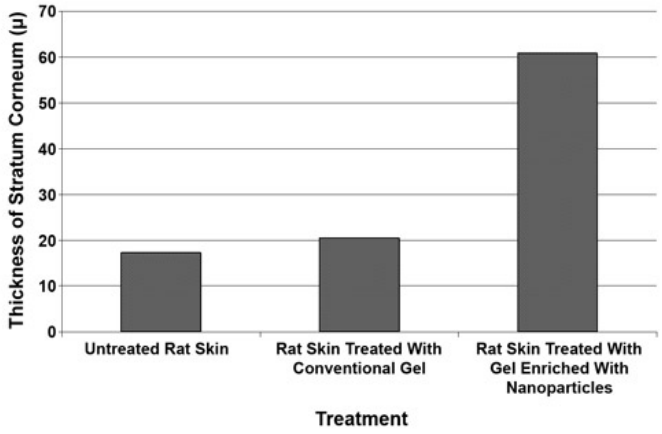

Figure 7. Effect of various formulations on the thickness of stratum corneum of rat skin.

In Vitro Penetration Studies

Carbopol gel enriched with SLN demonstrated almost 2 times higher drug levels in the skin after 24-hour treatment as compared with the conventional vitamin A gel (Figure 5). In this gel, the drug concentrated more in the skin with only 1.2% of the total drug being detected in the receptor compartment. In contrast, in the case of conventional gel almost 10 times more drug was detected in the receptor compartment; a much higher amount of drug remained unabsorbed (around 72%) in the case of conventional gel as compared with gel enriched with SLN, where around 67% drug remained unabsorbed on the skin. Also a much higher concentration per unit area of vitamin A palmitate was attained in the skin at 24 hours as compared with previous reports.[4] This finding could be owing to higher drug content in the developed formulation.

In dermatological treatment, improving the efficacy demands high drug levels in the skin. With nanoparticle dispersion, a greater quantity of drug remained localized in the skin, with lesser amounts penetrating into the receptor compartment as compared with conventional gel. Thus, drug-localizing effect in the skin seems possible with novel colloidal particulate drug carriers such as SLN. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin.

In Vivo Skin Hydration Studies

Figure 6A shows the photomicrograph of untreated rat skin. The upper layer in the photomicrographs represents the top layer of the skin (ie, stratum corneum). As shown in the photomicrographs, application of conventional gel showed only slight change in the thickness of the stratum corneum (Figure 6B), while application of the gel enriched with SLN showed a significant increase in the thickness of the stratum corneum, almost 3-fold as compared with the conventional gel and 3.5-fold compared with the untreated rat skin (Figure 6C and Figure 7). This increase in thickness could be caused by increased water content in the stratum corneum on application of nanoparticle-enriched gel. With conventional gel, there was only marginal increase in the thickness of stratum corneum. Because of the small particle size of SLN, it probably covers the surface of the skin, which reduces transepidermal water loss (TEWL) and evaporation of water from the skin, thus increasing the moisture content of the skin leading to increased thickness of the stratum corneum. This improved skin hydration was probably responsible for the increased drug penetration into the skin. This action has been demonstrated by increased drug levels in the skin as observed in in vitro penetration studies.

Thus, an enhancement in skin hydration and improved skin penetration can be achieved by adding SLN to topical gels, without having the stickiness or greasiness of occlusive vehicles. SLN therefore represent a highly effective carrier for cosmetic and topical preparations, where improved skin hydration and drug penetration is desired.

Primary Skin Irritation Studies

The scores for erythema and edema were totaled for intact and abraded skin for all rabbits at 24 and 72 hours. The primary irritation index (PII) was calculated based on the sum of the scored reactions divided by 24 (2 scoring intervals multiplied by 2 test parameters multiplied by 6 rabbits). The developed formulation showed no erythema or edema

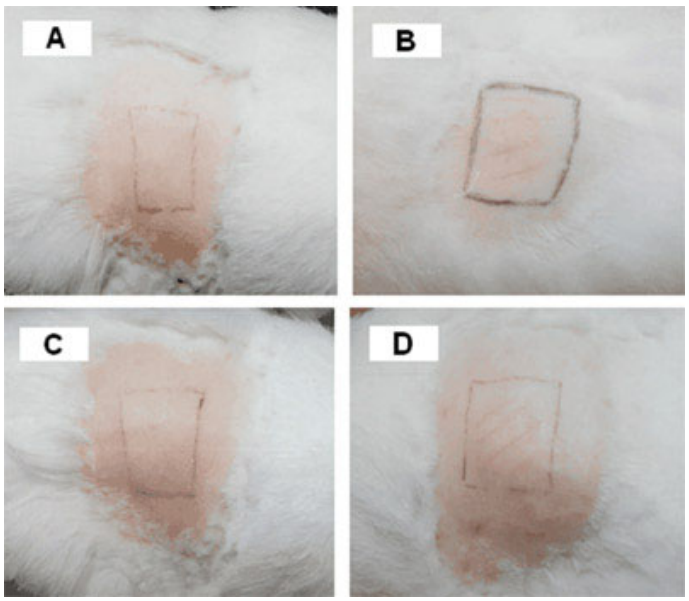

Figure 8. Photographs of rabbit skin treated with developed gel: (A) intact and (B) abraded after 24 hours; (C) intact and (D) abraded after 72 hours.

on the intact and abraded rabbit skin (Figure 8). The Primary irritation index of the formulation was calculated to be 0.00. Thus the formulation can be classified as a non-irritant to the rabbit skin.

CONCLUSION

In conclusion, SLN represent a highly effective, nonirritant carrier for cosmetic and topical preparations, where improved skin hydration and drug penetration is desired. Improved skin penetration can be due to enhanced contact of the active agent and skin resulting from the large particle surface area and film formation. SLN also have the potential to localize the drug at the site and could be useful for site-specific delivery of drugs to the skin.

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