DEVELOPMENT OF PROTRANSFERSOMAL SYSTEM FOR EFFECTIVE TRANSDERMAL DELIVERY OF NIFEDIPINE

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ABSTRACT

The purpose of the current study was to investigate the feasibility of new vesicular drug carrier system Protransfersome gel (PTG) as transdermal drug delivery system for Nifedipine. PTG formulations of Nifedipine were prepared by coacervation phase separation method and characterized for vesicle shape, size, entrapment efficiency, turbidity, and drug permeation across rat skin and were evaluated for their stability and bioavailability. For optimization of PTG, different formulations (PTG-1 to PTG-12) were prepared using the various quantity of drug, alcohol and varying the ratio of polymer and surfactant. The maximum entrapment efficiency (97.9 ± 0.35%) and optimum vesicles size (597 ± 4.0 nm) have been attained by optimizing the amount of surfactant (4 mg), alcohol (100 µl), and PC : S ratio (85 :15). The skin permeation studies were performed for 24 hrs on hairless abdominal skin of rat using the Franz diffusion cell. The flux value obtained from PTG (56.15 ± 2.14 μg/cm²/hr) is about 3-fold higher than that of the drug suspension (19.28 ± 2.14 μg/cm²/hr). PTG formulation showed good stability at 4 ± 1°C and after 3 months of storage, there was no change in liquid crystalline nature, drug content, and other characteristic parameters observed. The PTG formulation is non-irritant. Hence, the prepared formulation can be considered to be safe for topical application. In vivo pharmacokinetic study of PTG showed a significant increase in bioavailability of Nifedipine by 6.452 times with reference to an oral delivery of drug. From the CLSM study, it was confirmed that the penetration of the PTG entrapped dye was enhanced to the dermis from where it can go directly to the blood circulation.
KEYWORDS: Transdermal delivery, Protransfersome gel, skin permeation, stability, bioavailability enhancement.

INTRODUCTION
Hypertension is an increasingly important medical and public health issue\(^1\). Hypertension plays a major etiologic role in the development of cerebrovascular disease, ischemic heart disease, cardiac and renal failure\(^2\). Several classes of medications, collectively referred to as antihypertensive drugs, are currently available for treating hypertension\(^3\).

Nifedipine, a calcium channel blocker used in the treatment of hypertension and angina pectoris. Nifedipine (20–60 mg) once-daily, orally given in the treatment of hypertension\(^4\). Its solubility is poor in both lipophilic and hydrophilic media \(^5-6\). The treatment requires a constant release of the drug into systemic circulation. Since, its half life is 2-4 hrs requires frequent dosing of the drug\(^4-5\). Even though Nifedipine is rapidly and almost completely absorbed from GI tract but it undergoes extensive first pass metabolism (around 60%) resulting in a poor bioavailability (45%) after oral administration\(^7\). Hence, to improve its therapeutic efficacy, patient compliance and to reduce the frequency of dosing and side effects as well as to avoid its extensive first pass metabolism, transdermal drug delivery approach was considered to be better suitable for Nifedipine.

Delivery via the transdermal route is an interesting option in this respect because a transdermal route is convenient and safe\(^8\). The various vesicular systems, as a transdermal carrier, have been gaining attention because they not only act as depot for delivery of contents but also act as penetration enhancers. The various carrier systems like liposomes, ethosomes and transfersomes comprising of phospholipid that are reported to be harmless and non irritating to the skin but the poor stability is the major problem in the development of these vesicular systems at industrial and clinical levels\(^9\).

In order to overcome the stability problem liquid crystalline pro-ultraflexible lipid vesicles “Protransfersome” were proposed, that will be converted into ultraflexible lipid vesicles transfersomes also known as elastic liposomes, \textit{in situ} by absorbing water from the skin. Protransfersomes provide higher stability and better skin penetration ability than the traditional lipid vesicles, e.g. liposomes, niosomes etc. The proposed PTG is a liquid crystalline gel in which the drug is intercalated within phospholipids\(^10\).
MATERIAL AND METHODS

Materials
Nifedipine was obtained as a gift sample from Suchem Laboratories, Vatva, Ahmedabad, India. Soya lecithin and disodium hydrogen phosphate were purchased from Nice Chemicals Pvt. Ltd, Kochi (Kerala). Sodium deoxycholate was purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Sephadex-G-50 and Rhodamine 123 were purchased from Sigma Aldrich, USA. Isopropanol, methanol, potassium dihydrogen phosphate, sodium chloride were purchased from Rankem Pvt. Ltd, New Delhi. n-Octanol was purchased from Sd Fine Chem Ltd, Mumbai. n-Propanol was purchased from Qualigens Fine Chemicals, Mumbai. HPMC was purchased from Leo Chem, Bangalore. PEG-400 was purchased from Yarrow Chem Products, Mumbai. All other reagents used in the study were of analytical grade. Double distilled water was used for all experiments. Wistar albino rats were used for all the animal experiments.

Preparation of Formulations
Protransfersome gel was prepared by coacervation phase separation method with slight modifications. Accurately weighed 15 mg of sodium deoxycholate, 85 mg of soya lecithin and 1.0 mg of drug were taken in a clean and dry wide mouth glass vial of 5.0 ml capacity and 100 µl isopropanol was added to it. All the ingredients were then mixed by using a magnetic stirrer at a temperature of 60°C, the open end of the glass vial was covered with a lid to prevent the loss of solvent from it. The stirring was continued until all the ingredients were dissolved. Then the 100 µl of aqueous phase (Phosphate buffer pH 7.4) was added at the same temperature with continuous stirring, which lead to the formation of the less viscous liquid composition that further got converted into Protransfersome gel on overnight cooling in dark.

Vesicle size and size distribution analysis
The size and size distribution of the transfersome formed from the hydration of the PTG were determined using a digital microscope (BA-310, Motic, USA) at an angle of 90°. The Protransfersosomal formulation was hydrated with 10 ml of saline solution (0.9% NaCl) using manual shaking for 5 minutes. A drop of transfersome dispersion prepared from Protransfersome was spread on a glass slide and examined under the digital microscope (X40). The vesicles size of hydrated transfersome dispersions prepared from Protransfersome was also analyzed by zetasizer (Malvern Instruments, UK). Zetasizer yields
the mean vesicle size and the polydispersity index (PI) as a measure of the width of the particle size distribution.

**Determination of drug entrapment efficiency (%) and drug content (%)**

The drug entrapment efficiency was determined after separating the unentrapped drug by the minicolumn centrifugation method. Then the eluted vesicles were lysed using n-propanol and analyzed for drug content. Drug entrapment efficiency was expressed as percentage of total drug entrapped\(^{[12-13]}\). The percentage of drug entrapped and drug content was calculated by using the following equations.

\[
\text{Drug entrapment efficiency (\%) } = \frac{\text{Amount of drug entrapped}}{\text{Amount of drug used}} \times 100
\]

\[
\text{Drug content (\%) } = \frac{\text{Amount of drug entrapped}}{\text{Amount of PTG taken}} \times 100
\]

**Rate of hydration (Spontaneity)**\(^{[14]}\)

Spontaneity of Protransfersomal formulation is described as number of transfersomes formed after hydration of the PTG (without sonication). PTG were transferred to the bottom of a small stoppered tube and hydrated with 2 ml saline solution and then diluted to ten times with the same saline solution and kept aside without agitation. After 15 min, a drop of aqueous layer was withdrawn and placed on the Neubauer’s chamber. The number of transfersomes vesicles per cubic mm was counted by digital microscope (Motic). The transfersomes in 5 large squares were counted and calculated by using the following formula;

\[
\text{Total number of transfersome counted in 5 squares} \times \text{Dilution factor} \times 4000
\]

\[
\text{Total number of squares counted (5 squares)}
\]

**Degree of deformability**\(^{[15]}\)

Degree of deformability is an important and unique parameter of transfersomal formulations because it differentiates transfersomes from other vesicular carriers like liposomes that are unable to cross the stratum corneum intact. Transfersomes formed on hydration were passed through a pore of known size (50 nm) at a constant pressure for 5 minutes. Vesicle size and size distributions were noted after each pass by digital microscopy. The degree of deformability was calculated by using the following formula;

\[
E = J \times (r/r_p)^2
\]

where,
E = elasticity of vesicles membrane; J = amount of suspension extruded in 5 min.; \( r_v \) = vesicle size; \( r_p \) = pore diameter.

**Morphology using transmission electron microscopy**

The morphology of the optimized hydrated PTG formulation was also determined by high resolution transmission electron microscopy. A drop of transfersomal dispersion was applied to a carbon-coated 300-mesh copper grid and left to adhere on the carbon substrate for about 1 min. A drop of 2% aqueous solution of uranyl acetate was applied for 35 seconds and again the solution in excess was removed by the tip of filter paper. The sample was air-dried and observed under the transmission electron microscope at 90kV\[^{10}\].

**Ex vivo skin permeation studies\[^{4}\]**

*Ex vivo* permeation studies give us valuable information about the product behavior *in vivo*. The drug permeated dictates the amount of drug available for absorption. The goal of permeation study is to compile a kinetic profile that reflects the concentration of active ingredient changes in time as it diffuses through the skin. The skin permeation studies were carried out on the optimized PTG formulation and was compared with that of drug suspension. For each formulations drug release profiles were studied for 24 hrs in triplicate.

**Procedure**

The abdominal hair of albino rats (wistar strain), weighing 150 - 200 g, was shaved using a hand razor. Care was taken not to damage the skin surface. The rats were sacrificed by excess chloroform inhalation and immediately the entire skin from the abdominal area was excised. This section of skin was then cleaned of any subcutaneous fat and blood vessels, while maintaining the integrity of the viable epidermis and stratum comeum. To obtain stripped skin, the excised skin was positioned with the dermis facing outermost and the dermis was then repeatedly peeled away using cellophane tape. This operation was repeated 15 times using a fresh piece of tape each time. Then epidermis was prepared surgically by heat separation technique, which involved soaking the entire abdominal skin in water at 60°C for 45s, followed by careful removal of the epidermis. The epidermis was washed with water and the treated skin was used immediately after preparation, so as to preserve its integrity for as long as possible.

The skin was sandwiched between donor and receptor compartment of the Franz diffusion cell with an effective diffusion area of 2.26 cm\(^2\) and a cell volume of 25 ml. The stratum
corneum surface of the skin faced the donor compartment, whereas the dermis faced the receptor compartment and apparatus was assembled. The receptor compartment was filled with of phosphate buffer saline (PBS, pH 7.4) containing 40% (v/v) of polyethylene glycol (PEG) 400. PEG 400 was added to maintain the sink conditions. The diffusion cells were maintained at 37°C using a magnetic stirrer and the fluid in the receptor compartment was stirred continuously. The formulations were gently placed in the donor compartment. The samples were withdrawn periodically for 24 hours. The samples were then analysed spectrophotometrically at 350 nm for presence of the drug. Each preparation was studied three times and the result of each preparation is the average value of three experiments.

**Permeation data analysis**

The drug flux (permeation rate) of Nifedipine at steady state \((J_{ss}, \mu g/cm^2/h)\) through skin was calculated from the following equation;

\[
Transdermal flux (J_{ss}) = \frac{\text{Amount of drug permeated}}{\text{time} \times \text{area}}
\]

The lag time was obtained from the X - intercept of linear portion. The permeability coefficient \((P, \text{cm/hr})\) was calculated by dividing transdermal flux \((J_{ss})\) with the concentration of the drug in donor cell \((C_d)\) by using the following equation;

\[
P = \frac{J_{ss}}{C_d}
\]

where, \(C_d\) is the initial concentration of drug in donor compartment.

Enhancement ratio \((Er)\) was calculated by dividing the transdermal flux \((J_{ss})\) of the respective formulation by the \(J_{ss}\) of the plain drug formulation;

\[
Er = \frac{J_{ss \ of \ formulation}}{J_{ss \ of \ plain \ drug}}
\]

**STABILITY STUDIES**

Stability studies were conducted for 3 months to determine the effect of storage condition on the optimized PTG containing drug and blank formulations. After determining the initial morphological characteristics, vesicles size and percentage entrapment of the drug in the PTG formulation, the optimized and blank formulations were divided into 2 sets and were stored at temperature \(4°C \pm 1°C\) in refrigerator (GL-185, LG, India) and at \(25 \pm 2°C, 60 \pm 5\%\) RH (as
per ICH guidelines) in programmable environmental test chamber (CHM 10S, REMI, India) in screw capped amber colored glass bottles. Effect of storage temperature on morphological characteristics, vesicles size and residual drug content (%) were evaluated for 3 months. After 10, 20, 30, 60 and 90 days, they were viewed under digital microscope for change in consistency, liquid crystalline structure, and appearance of drug crystals. Transfersomes formed from PTG formulation were also characterized for vesicles size and percentage drug content. Stability studies of each formulation were carried out in triplicate. Results of storage stability were evaluated employing one way ANOVA and Dunnett post test by using the software PRISM (Graph Pad) 5.0[16-18].

SKIN IRRITATION STUDY

The skin irritation was done as a test of product safety. The animal albino rats were used for this study. The backs of the animals were clipped free of fur with an electric clipper at least 4 hr before application of the sample. The rats were divided into three groups (n=3) as follows; Group 1: Control group  
Group 2: HPMC Gel entrapped drug (Standard)  
Group 3: Protransfersome Gel (Nifedipine)

Then samples were applied to previously shaved skin and spread uniformly with a glass road. At the end of the test period the residual material was wiped off with gauze wetted with warm soapy water and the treated area were evaluated for erythema/edema at 24, 48, 72 hrs post-treatment[19]. In an attempt to make a subjective test more quantitative scores have been assigned to measure the degree of redness and puffiness as reported in Code of Federal regulations 1980[20].

After observing the irritation scores, the primary irritation index was calculated. For each animal, the primary irritation scores were added together for the test substance for both erythema and edema at each time specified and divided by the total number of observations. The scores for each animal was added and divided by the total number of animals to give the primary irritation index.

Pharmacokinetic evaluation

In vivo study of optimized Protransfersomal formulation was performed on Wistar male rats (250±20 g). This study was approved by the ethical committee CPCSEA (Committee for the
Purpose of Control and Supervision of experiments on animal, Ministry of Culture, Government of India).

The animals were weighed immediately on procurement and were kept in a rat cage in a well-maintained faculty animal house. The rats were maintained under hygienic condition and supplied with a standard laboratory diet and water ad libitum and monitored on a regular basis. The experimental rats were then divided into three groups, each carrying six animals. Rats were kept on fasting 12 h before drug administration and until 24 h post dosing. Water ad libitum was given throughout the study. The Nifedipine dose selected was 3 mg/kg \(^{[21]}\).

The first group treated as control and second group received oral dose of drug suspension containing 3 mg Nifedipine using a rubber canula under non-anaesthetic condition. The third group received transdermal dose of PTG formulation. The formulations were applied to the previously shaved rat skin with the entire release surface in intimate contact with the stratum corneum. The microporous adhesive tape was rolled over to keep the formulation secured at the site of application. The skin was carefully wiped with 70% ethanol prior to application of vesicular formulation. The rats were anesthetized using ether, and blood samples (0.5 ml) were withdrawn from the retro orbital plexus of eye at 0.5, 1, 2, 3, 4, 5, 6, 12, 24 and 48 hrs in microcentrifuge tubes. The blood sample volume withdrawn was immediately replaced with an equal volume of physiological saline. The serum was separated by placing the tubes in a centrifuge 15 minutes at 3000 rpm and then serum was stored at −21°C until drug analysis was carried out using high performance liquid chromatography (HPLC).

**HPLC assay**

The nifedipine concentrations in the rat plasma samples were determined by the reported HPLC method with some modifications\(^{[22]}\). Samples of 0.05 ml were diluted with water to 1.0 ml and vortex-mixed after the addition of 10 ml of 1.0 M sodium hydroxide and 0.5ml of t-butylmethyl ether-isoctane (75:25, v/v); the organic layer was then separated by centrifugation. After removal of the upper organic layer, the extraction was repeated three more times, and the pooled extract was evaporated to dryness. To the resulting residue added the mobile phase and aliquots of 20 µl were injected onto the HPLC column. The concentration of unknown serum samples was calculated from standard calibration curve, which was drawn by adding a known amount of the drug to 0.05 ml of blank rat plasma and diluting it with water to 1.0 ml as described above. The mobile phase consisted of methanol :
water (85 : 15) and it was delivered at a flow rate of 1 ml/min. Detection was performed at the wavelength of 350 nm.

**Pharmacokinetic and statistical analysis**

Standard pharmacokinetic parameters obtained from each of the individual rat plasma concentration-time profiles of Nifedipine were calculated by noncompartmental methods using the computer program WinNonlin. The values of $C_{\text{max}}$ and $T_{\text{max}}$ were read directly from the arithmetic plot of time versus serum concentration of Nifedipine. The AUC was calculated by using the trapezoidal method. The half-life ($t_{1/2}$) was determined by linear regression of the log-linear portion of the plasma concentration-time profile. The apparent plasma clearance (CL) was calculated by dividing the dose by the AUC. The relative oral bioavailability of the different formulations has been evaluated by the equation:

\[
\text{Relative bioavailability} = \frac{\text{AUC Sample}}{\text{AUC Standard}}
\]

The pharmacokinetic data between oral and transdermal formulations were compared for statistical significance employing one-way ANOVA and Dunnett post test followed by using the software PRISM (Graph Pad) 5.0.

**CONFOCAL SCANNING LASER MICROSCOPY (CSLM) STUDY**

The abdominal hair of albino rats (Wister strain), weighing 150 - 200 g, was shaved using a hand razor. Care was taken not to damage the skin surface. For each formulation one animal was used. First, the Protransfersome gel loaded with the Rhodamine 123 and HPMC gel containing dye were formulated. Animals were then treated topically with fluorescent label formulations for 6 hrs and after 6 hrs rats were then sacrificed, treated skin was disected and fixed and evaluated for Rhodamine 123 penetration for formulations under the CLSM (Olympus FV-1000). The excitation wavelength was 570 nm and a 590 nm long pass filter was used for emission, the penetration of Rhodamine 123 labeled formulation in rat skin$^{[23]}$.

**RESULT AND DISCUSSION**

Protransfersomal formulation of Nifedipine was prepared by using the coacervation phase separation method. The method is based on the simple idea that the mixture of surfactant, alcohol and aqueous phase can be used to form the concentrated Protransfersome gel, which can be converted into stable vesicular transfersomal dispersion by dilution with excess aqueous phase.
Different additives were employed in the Protransfersome gel formulation. Lecithin was used as the vesicle forming agent, surfactant for providing flexibility, alcohol as the solvent and aqueous phase (buffering agent) as the hydrating medium. The biosurfactant, sodium deoxycholate was used because of its biocompatibility in comparing to other surfactants.

For optimization of PTG, different formulations (PTG-1 to PTG-12) were prepared using the various quantity of drug, alcohol and varying the ratio of polymer and surfactant. Formulation with maximum entrapment efficiency and optimum vesicles size considered as optimized formulation.

The maximum entrapment efficiency (97.9 ± 0.35%) and optimum vesicles size (597 ± 4.0 nm) have been attained by optimizing the amount of surfactant (4 mg), alcohol (100 µl), and PC : S ratio (85 :15). This optimized formulation was further investigated for characterization and permeation study.

The PTG was viewed under digital microscope and observed birefringent streaks lamellar structures in liquid crystalline form [Photomicrograph 1 (a)]. Hydration of this gel formed spherical vesicular structure [Photomicrograph 1 (b)]. The transformation of lamellar liquid crystalline PTG to transfersomes can be ascribed to different degree of hydration of surfactant and phospholipid molecules. Initially, due to the presence of limited solvent, the PTG formed was a mixture of lamellar liquid crystals resembling palisades and vesiculating lamellas linked together. Further addition of water resulted in swelling of the lipid bilayer due to interaction of water with polar groups of surfactants and above a limiting concentration of solvent, the bilayers formed spherical structures randomly giving rise to vesicular structures.

The shape of the optimized PTG formulation was also confirmed through TEM study and are shown in Photomicrograph 2. Most of the vesicles are well identified, spherical and discreet having large internal aqueous space.

The skin permeation studies studies were performed for 24 hrs on hairless abdominal skin of rat using the Franz diffusion cell. In the initial 2.0 hrs of the release study, 18.16 ± 1.22% of PTG formulation and 04.28 ± 1.17 of drug suspension was released. It was observed that the 48.35 ± 2.98% and 60.27 ± 3.31% of the PTG formulation but 14.29 ± 2.16% and 20.49 ± 2.22% of drug suspension was released after 6.0 and 12.0 hrs, respectively. After 24.0 hrs 76.15 ± 2.9% of PTG formulation, but only 26.16 ± 2.23 of drug suspension was released.
The obtained results from the *ex vivo* drug release study were tabulated in Table 1 and Figure 1. From *ex vivo* drug release study, it was concluded that the PTG formulation having higher penetration ability as compared to drug suspension, so the higher amount of drug was permeated after 24 hrs from PTG formulation.

The skin permeation profile of PTG formulations and drug suspension at the same drug concentration has been shown in Table 2. The flux value obtained from PTG (56.15 ± 2.14 μg/cm²/hr) is about 3-fold higher than that of the drug suspension (19.28 ± 2.14 μg/cm²/hr). The very low skin permeability of drug suspension is due to extreme hydrophobicity and low solubility of Nifedipine in water. Better transdermal flux and no lag phase with PTG gel was perhaps a result of the combination of one or more of following mechanisms: (1) increased solubility of Nifedipine, (2) high association of drugs with vesicle bilayers, (3) increased partitioning of vesicles into the stratum corneum, (4) penetration enhancement effect of the short chain alkanols, and (5) elasticity of vesicle membrane.

The drug release from PTG formulations was subjected to different model dependent kinetics such as zero order, first order, Higuchi model and Korsmeyer and Peppas model release kinetics. The results of regression coefficient (R²) from the different drug release kinetic models were tabulated in Table 3.

The release profile exhibiting maximum R² value was found to obey that particular kinetics and R² value of Higuchi model is found to be greater as compared to other models for PTG formulations. Hence, the release kinetics of the PTG formulation has followed Higuchi model drug release mechanism. Higuchi model also called as diffusion release because it follows diffusion release mechanism having controlled and sustained release through diffusion mechanism.

**STABILITY STUDY**

The results obtained that the PTG have shown the minimum drug lost at 4°C ± 1°C, and fairly high retention of drug inside the vesicles was observed. At this low temperature condition % remaining drug content was good over a period of 3 months. While, storage at higher temperature 25±2°C leads to less % remaining drug content over a period of 3 months. The higher amount of drug leakage at elevated temperature may be due to the degradation of lipids constituting bilayers resulting in defects in membrane packing and loss of overall
rigidity that makes them leaky. With the increase in temperature, there is also increase in the fluidity of lipid bilayers, due to phase transition phenomenon.

The statistical analysis was carried out employing analysis of variance (ANOVA) and Dunnett post test by using the software PRISM (Graph Pad) 5.0. Through ANOVA and Dunnett post test it was concluded that no significant change in vesicles size and drug remaining (%) of PTG were found when these were stored at 4 ± 1°C compared to 25 ± 2°C. Hence, PTG formulation developed for transdermal delivery of Nifedipine having sufficient stability at 4 ± 1°C. So, it can be inferred from above discussion that the PTG formulation should be stored at lower temperature to minimize the drug lose.

SKIN IRRITATION STUDY
The results showed that negligible irritation was shown either by the test formulations (PTG) or the standard formulation (HPMC). The PTG formulation is non-irritant. Hence, the prepared formulation can be considered to be safe for topical application. The photomicrograph of skin irritation study are shown in Photomicrograph 3.

IN VIVO STUDY
Pharmacokinetic studies were carried out on rats to judge the efficacy and bioavailability of the developed formulation against the oral dosage form. The data so obtained were subjected to Pharmacokinetic analysis. The mean $T_{\text{max}}$ of Nifedipine was 3 hr for oral treatment and 5 hr for transdermal treatment. A significant difference in $T_{\text{max}}$ value was observed between oral and transdermal treatments ($P>0.05$). A shift in the $T_{\text{max}}$ value toward higher side from transdermal treatment indicated the controlled release behavior of the formulation. The mean $C_{\text{max}}$ of Nifedipine was 0.998 ± 0.157 μg/ml for oral treatment and 1.892 ± 0.469 μg/ml for transdermal treatment. In the case of oral treatment the peak and valley pattern was quiet evident with the fluctuation in the plasma concentration whereas in transdermal treatment steady-state plasma concentration level was maintained (Figure 2). The mean AUC values after oral treatment was 8.436 ± 0.33 hr*μg/ml and after transdermal treatment was 54.435 ± 4.26 hr*μg/ml. There was a significant difference between the AUC values for transdermal and oral treatments ($P>0.05$). The difference in the AUC values clearly reflects that comparatively lesser amount of drug was available by oral administration to rat body because of high first pass metabolism of Nifedipine. Transdermal formulation in the present study was found to enhance the bioavailability of Nifedipine by 6.452 times with reference to an oral delivery of drug (Table 4). The increased bioavailability might be due to the elimination of
hepatic first pass metabolism in transdermal delivery. The plasma clearance was found to be 355.61 ± 13.23 and 55.11 ± 4.01 ml/hr for oral and transdermal drug delivery, respectively and the \( t_{1/2} \) was found to be 6.56 and 59.89 hr for oral and transdermal drug delivery, respectively. The high plasma clearance and low \( t_{1/2} \) for oral drug delivery due to the extensive elimination of drug from the body but in the case of transdermal drug delivery the plasma clearance was low and \( t_{1/2} \) was high, this indicated that PTG formulation enhanced the self life and delayed the clearance of drug from the body. Thus, the transdermal formulation PTG was found to provide prolonged steady-state concentration of Nifedipine with minimal fluctuations and improved bioavailability.

**CONFOCAL SCANNING LASER MICROSCOPY (CSLM) STUDY**

Rhodamine 123 is a hydrophilic fluorescence marker and does not normally get into the deeper layer of the skin. The depth of fluorescent penetrations into the skin was determined using the CLSM. The skin samples were fixed and confocal examination was done. When it applied in the form of HPMC formulation, it showed the penetration only 90µm depth as shown in **Photomicrograph 4**. However, this dye was transported extensively and reached the deeper layer, when applied in the form of PTG formulation. The PTG formulation containing dye showed the penetration upto 250 µm depth (**Photomicrograph 5**). These results proved that penetration of the PTG entrapped dye was enhanced to the dermis from where it can go directly to the blood circulation. The presence of fluorescence marker in the deeper layer of skin shows better skin penetration ability of the PTG formulation.
Photomicrograph 1: PTG formulation (a) and transfersome (b) (Magnification 40X)

Photomicrograph 2: TEM photomicrograph of transfersome

(b) On application of Protansfersome gel
Photomicrograph 3: Skin irritation study of control (a), PTG (b) and HPMC (c) treated animal for 24, 48 and 72 hrs.

Photomicrograph 4: CLSM image of HPMC - dye gel
Photomicrograph 5: CLSM image of PTG – dye gel

Figure 1: *Ex vivo* release profile of PTG gel and drug suspension

Figure 2. Plasma drug concentration vs time graph of PTG and drug suspension
Table 1: Comparison of *ex vivo* skin permeation studies of formulations

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Cumulative % drug release</th>
<th>Drug suspension</th>
<th>Protransfersome gel</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.38 ± 0.09</td>
<td>04.23± 1.16</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>02.91± 0.8</td>
<td>11.29± 2.21</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>04.28 ± 1.17</td>
<td>18.16 ± 1.22</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>05.87 ± 1.19</td>
<td>26.07 ± 1.27</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>07.41 ± 1.23</td>
<td>33.18± 2.31</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>09.38 ± 2.20</td>
<td>40.78 ± 2.78</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>14.29 ± 2.16</td>
<td>48.35± 2.98</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>20.49 ± 2.22</td>
<td>60.27 ± 3.31</td>
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<tr>
<td>24</td>
<td>26.16 ± 2.23</td>
<td>76.15 ± 2.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Permeation parameters of drug suspension and PTG formulation

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Transdermal flux (Jss) (µg/cm²/hr)</th>
<th>Permeation coefficient (cm²/hr)</th>
<th>Enhancement ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug suspension</td>
<td>19.28 ± 1.65</td>
<td>0.0048 ± 0.0004</td>
<td>2.91 ± 0.13</td>
</tr>
<tr>
<td>Protransfersome gel</td>
<td>56.15 ± 2.14</td>
<td>0.0140 ± 0.0005</td>
<td></td>
</tr>
</tbody>
</table>

All the values are given as mean ± SD, n = 3

Table 3: $R^2$ values obtained on applying release kinetic

<table>
<thead>
<tr>
<th>Kinetic models</th>
<th>$R^2$ value of PTG formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>0.823</td>
</tr>
<tr>
<td>First order</td>
<td>0.947</td>
</tr>
<tr>
<td>Higuchi</td>
<td>0.955</td>
</tr>
<tr>
<td>Peppas corrn</td>
<td>0.939</td>
</tr>
</tbody>
</table>

Table 4. Pharmacokinetic Parameters of Nifedipine

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$t_{1/2}$ (hr)</th>
<th>$T_{max}$ (hr)</th>
<th>$C_{max}$ (µg/ml)</th>
<th>$AUC$ (hr*µg/ml)</th>
<th>Plasma clearance (ml/hr)</th>
<th>Relative bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug suspension</td>
<td>6.56</td>
<td>3</td>
<td>0.998 ± 0.157</td>
<td>8.436 ± 0.33</td>
<td>355.61 ± 13.23</td>
<td>6.452</td>
</tr>
<tr>
<td>PTG formulation</td>
<td>59.89</td>
<td>5</td>
<td>1.892 ± 0.469</td>
<td>54.435 ± 4.26</td>
<td>55.11 ± 4.01</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; n = 6

CONCLUSIONS

The results of the present investigation showed that the problems associated with the oral bioavailability of Nifedipine could be overcome by incorporating it into a new transdermal proultraflexible drug carrier, Protransfersome gel. Using coacervation phase separation method, Nifedipine has been successfully incorporated in Protransfersomal formulations.
which can be potentially useful for delivery of this drug. PTG formulation was also found to be quite stable at 4 ± 1°C over a three-month period. From *ex vivo* drug release study, it was concluded that the PTG formulation having higher penetration ability as compared to drug suspension, so the higher amount of drug was permeated after 24 hrs from PTG formulation. It follows diffusion release mechanism having controlled and sustained release through diffusion mechanism. The skin irritation study was concluded that the PTG formulation is non-irritant. Hence, the prepared formulation can be considered to be safe for topical application. From CLSM study, it was concluded that PTG formulation having sufficient skin penetration capability.

It can be concluded from the result obtained that the PTG formulation developed for transdermal delivery of Nifedipine possessed better skin permeation potential, better stability, and higher entrapment efficiency, easy to scale-up and ability as a self penetration enhancer. An 6.452-fold increase in peak plasma concentration of Nifedipine and its maintenance over 48 hours after topical application of optimized PTG formulation as compared to plain drug solution suggested that PTG formulation provided a better mode of systemic delivery of Nifedipine.

**REFERENCES**


