Formulation of Nanocarrier Systems for Transdermal Drug Delivery.

Thesis
Presented By
Sara Mahmoud Hassan Soliman
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Under The Supervision of

Prof. Dr. Nabaweya Abd El-Aziz
Professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy-October 6 University

Prof. Dr. Omaima Naim El-Gazayerly
Professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy-Cairo University

Dr. Nevine Shawky Abd El-Malak
Associate Professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy-Cairo University

Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy-Cairo University
Lacidipine is a calcium channel blocker developed for oral administration. It is used in the treatment of hypertension and atherosclerosis. It also possesses an antioxidant effect. Lacidipine is a highly lipophilic drug of poor water solubility and undergoes extensive first-pass hepatic metabolism with a mean absolute bioavailability of about 10% (range 3-59%). It is completely metabolized in the liver by cytochrome P450 3A4 to pharmacologically inactive metabolites. This very low oral bioavailability restricts its use. So, lacidipine could be an excellent candidate for transdermal delivery.

Transdermal drug delivery systems (TDDS) are one of the most rapidly advancing areas of novel drug delivery, which are designed to deliver a therapeutically effective amount of drug across a patient’s skin. Transdermal drug delivery systems offer many advantages over oral dosage forms including: avoiding hepatic metabolism, gastric degradation, minimizing systemic side effects due to site specific drug delivery, the easier administration and more patient compliance in addition to the possibility of immediate withdrawal of the treatment if necessary. However, only a few drugs could be delivered effectively through transdermal route due to the inability of most drugs to penetrate the barrier nature of stratum corneum.

Therefore, in order to reach therapeutic drug concentrations in certain skin layers or in the blood circulation, the uppermost barrier, the stratum corneum (SC), has to be overcome. This process is affected by various factors, e.g., the physicochemical properties of the drug and the vehicle used for administration.

Lacidipine has poor wettability and aqueous solubility which limit its formulation as transdermal dosage form and makes this challenging. So, the aim of this study was formulation of nanocarrier systems with a high degree of drug release and permeation,
namely proniosomes and nanoemulsion, for transdermal delivery of lacidipine to avoid its extensive first pass metabolism.

The work in this thesis is divided into three chapters:

**Chapter I**: Preparation and Evaluation of Lacidipine Proniosomes for Transdermal Delivery.

**Chapter II**: Preparation and Evaluation of Lacidipine Nanoemulsions for Transdermal Delivery.

**Chapter III**: In Vivo Performance of the Optimized Lacidipine Formulations.

**Chapter I**

*Preparation and Evaluation of Lacidipine Proniosomes for Transdermal Delivery.*

Proniosomes are non-ionic based surfactant vesicles, which may be hydrated immediately before use to yield aqueous niosomes dispersions. Proniosomes can be converted into the niosomes in-situ by absorbing water from the skin. These proniosome-derived niosomes are as good as or even better than conventional niosomes. Proniosomes enhance the uptake of drugs through the skin, reduce the side effects and improve the bioavailability.

So, the aim of this chapter was to develop and characterize proniosomal gel bearing lacidipine for transdermal delivery so as to avoid its extensive first pass metabolism and improve its permeation through the skin, leading to improved bioavailability, and minimum side effects. Lacidipine loaded proniosomes were prepared, characterized and optimized using $2^3$ full factorial design to define the optimal conditions regarding the selected factors to produce proniosomes with minimum vesicle size, high entrapment efficiency and high release efficiency.

*Investigations and results obtained could be summarized as follows:*


1) The amount of cholesterol ($X_1$), the amount of soya lecithin ($X_2$), and the amount of non-ionic surfactant (Cremophor RH 40) ($X_3$) were selected as three independent variables. The vesicle size, the percentage of entrapment efficiency (%EE) and the percentage release efficiency (%RE) after 24 h through semi-permeable membrane (cellulose nitrate with pore size 0.45µm) were selected as dependent variables. All data were statistically subjected to analysis of variance (ANOVA) using Design-Expert® software.

2) Eight proniosomes systems containing 1% lacidipine were prepared by a coacervation phase separation method.

3) The Physicochemical Properties of the prepared proniosomes systems were evaluated by:
   a) **Drug content**: The percentage of lacidipine in the prepared proniosomes systems was varied from 100.09±1.20% to 103.35±0.37%.
   b) **Physical Appearance**: All the prepared lacidipineproniosomes systems were homogeneous and yellowish clear viscous liquid. No sign of drug precipitation was observed.
   c) **Optical Microscopic Examination**: The dry thin film of the hydrated lacidipineproniosomal systems was examined for the formation of niosomes using optical microscope The micrographs showed presence of homogenous population of vesicles with spherical shape.
   d) **Zeta Potential (Surface Charge) Determination ($\zeta$)**: The zeta potential of the hydrated lacidipineproniosomal systems was measured by Laser Doppler velocimetry using Malvern Zetasizer. It was found that the niosomes formed from lacidipineproniosomes systems had negative zeta potential values which were in the range of $-18.85\pm0.65$ to $-39.75\pm0.85$ mV.
   e) **Vesicle Size Determination**: The mean vesicle sizes and span of the niosomes formed from lacidipineproniosomes systems were measured by using Malvern Zetasizer. All systems were found in nano size range of 162.43±0.77 nm to 547.30±2.10 nm with adequate values of polydispersity index of 0.352±0.038 to 0.676±0.036. It was found that the vesicles size significantly increased with
increasing the amount of cholesterol and decreased with increasing the amount of lecithin and Cremophor RH 40.

f) **Determination of Percentage Entrapment Efficiency (%EE) of Lacidipine Proniosomes:** The entrapment efficiency of the lacidipine in proniosomes systems was determined by filtration through Whatman filter paper (Grade No. 1, 11 µm). The results of percentage entrapment efficiency of lacidipine in niosomes were found ranging from 44.06±0.06% to 98.01±0.683%. The percentage entrapment efficiency significantly increased with increasing the amount of Cremophor RH 40 and decreased with increasing the amount of lecithin and cholesterol.

g) **In Vitro Release Studies of Lacidipine Proniosomes:** In vitro release of lacidipine from the prepared proniosomal systems through semi-permeable membrane (cellulose nitrate with pore size 0.45µm) was performed using a USP Dissolution Apparatus II with slight modification at a rotation of 100 rpm. Studies were carried out at 37±1ºC in 500 ml of 30% methanolic solution (v/v) for 24 hours. The results revealed that:

- The percent release efficiency of lacidipine from all proniosomes systems was higher compared to the control drug suspension.
- The percent release efficiency of lacidipine significantly decreased with increasing the amount of cholesterol and increased with increasing the amount of lecithin and Cremophor RH 40.
- It was found that all the proniosomes systems and plain drug suspension showed best fitting to Higuchi diffusion model.

4) **Optimization of Lacidipine Proniosomes:** Optimization was performed to find out the level of independent variables (X1, X2, and X3) by applying the point prediction method of the Design Expert software. The system **F4** containing cholesterol (10 mg), soya lecithin (80 mg), Cremophor RH 40 (270 mg), absolute ethanol (0.25 ml) and water (0.1 ml) was found to fulfill the maximum requisite of an optimum system based on its minimum mean vesicle size (162.43 nm), maximum EE (98.01%), maximum release efficiency (88.33%) values and maximum desirability (0.98). The
optimized system (F4) was then formulated as proniosomal gel using Carbopol 940 (1% w/w).

5) **Evaluation of Lacidipine Proniosomal Gel:**
   
a) **Visual inspection of the optimized lacidipine proniosomal gel formulation (GF4):**
   Visual inspection of the optimized lacidipine proniosomal gel formulation (GF4) showed yellow translucent homogeneous gel. No sign of drug precipitation and no phase separation were also observed.

b) **Transmission Electron Microscope (TEM):**
   The morphology of the hydrated proniosomal gel in distilled water was investigated using TEM. Photograph of TEM revealed that, the optimized proniosomal gel (GF4) appeared as spherical nano vesicles.

c) **pH Determination:**
   The pH of 10% w/w aqueous solution was measured by pH meter and found that, pH value of the optimized lacidipine proniosomal gel formulation (GF4) (4.74±0.01) was within the physiologic range of transdermal application (pH 4-7 unit).

d) **Test for Spreadability:**
   By pressing 0.5 g of the optimized lacidipine proniosomal gel formulation (GF4) between two glass slides, and then the formed diameter was measured. Spreadability value revealed that GF4 was suitable for transdermal application.

e) **In Vitro Drug Permeation**
   was carried out through excised rabbit skin over a period of 24 hours in 500 ml of 30% methanolic solution (v/v) at 37±1°C using a USP Dissolution Apparatus II for the optimized lacidipine proniosomal gel formulation (GF4) and the control lacidipine emulgel. The results showed that the optimized proniosomal gel formulation (GF4) exhibited higher lacidipine permeation through excised rabbit skin compared to the control emulgel.

6) Storage of the optimized lacidipine proniosomal gel (GF4) at 40°C for 3 months showed no significant change in the physical appearance, pH and spreadability. Regarding the chemical stability of the stored formulation using HPLC stability indicating method, it was found that the optimized proniosomal gel (GF4) possessed lacidipine percentage within the range permitted by the United States Pharmacopoeia (90-110%) and in vitro drug permeation of the optimized proniosomal gel formulation (GF4) was not altered.
Taking into consideration the good efficiency in systemic delivery together with lack of irritancy and excellent safety profile, Cremophor RH 40 proniosomal gel could be considered as very promising candidates as absorption and penetration enhancer for delivering of the antihypertensive lacidipinetransdermally. GF4 will be candidate formulation for in vivo study in chapter III.

**Chapter II**

*Preparation and Evaluation of Lacidipine Nanoemulsions for Transdermal Delivery.*

Nanoemulsions are thermodynamically stable transparent or translucent dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant usually in combination with cosurfactant having a droplet size in the range of 10-100 nm.

Nanoemulsions have received great attention for various applications including, dermal and transdermal drug delivery due to higher storage stability, ease of preparation, lower preparation cost, thermodynamic stability, permeation enhancement activity of their components, and a high solubilizing capacity for various drugs over conventional topical formulation vehicles.

Therefore, the aim of the present work in this chapter was to develop a nanoemulsion gel using a $2^3$ full factorial design for transdermal delivery of the antihypertensive lacidipine so as to avoid extensive first pass metabolism and improve the permeation through the skin.

**Investigations and results obtained could be summarized as follows:**

1) Solubility of lacidipine in different oils was found to be as follows: Capryol™ 90 >Capmul® MCM EP >Labrafil® M 1944 CS >IPP; in surfactants: Cremophor RH40 >Labrasol® >Tween 60 and in cosurfactants: Transcutol™ HP >PEG 400 >PG.
2) Based on the solubility studies, Capryol™ 90 and IPP were chosen to represent the oily phases; Cremophor RH 40 was used as surfactant and Transcutol® HP was used as cosurfactant for constructing phase diagrams.

3) Two pseudo-ternary phase diagrams were constructed to determine the nanoemulsions region and to obtain the concentration range of components for the existing nanoemulsions zones.

4) A $2^3$ full factorial design was used to define the optimal conditions regarding the selected factors to produce nanoemulsion with high physical stability and high release efficiency. The drug oil solubility ($X_1$), the oil concentration ($X_2$), and the water concentration ($X_3$) were selected as three independent variables. The physical stability and the percentage release efficiency ($\%RE$) after 24 h through semi-permeable membrane (cellulose nitrate with pore size 0.45µm) were selected as dependent variables. All data were statistically subjected to analysis of variance (ANOVA) using Design-Expert® software.

5) Eight nanoemulsion systems containing 1% lacidipine were prepared and evaluated by:

a) Visual Inspection: All the prepared nanoemulsion systems were fluid, clear, homogeneous and transparent.

b) Drug content: The percentage of lacidipine in the prepared nanoemulsion systems was varied from 97.76±0.52% to 102.66±0.40%.

c) pH Determination: The pH of 10% w/w aqueous solution was measured by pH meter. The pH values of lacidipinenanoemulsion systems were found to be in the physiologic accepted range (pH 4-7 unit). Therefore, lacidipinenanoemulsion systems are safe for transdermal application.

d) Droplet Size Analysis: The mean droplet sizes and span of the prepared lacidipinenanoemulsion systems were measured by using Malvern Zetasizer. All the systems had droplets in the nano size range (13.76±0.13 to 27.87±1.87 nm). The polydispersity index (pdi) values of the prepared lacidipinenanoemulsionsystems are very low (0.06±0.012 - 0.39±0.037).

e) Assessment of Physical Stability: Examination of lacidipinenanoemulsion systems after centrifugation at 3,500 rpm for 30 min, heating-cooling cycle and
freeze-thaw cycle showed clear homogeneous nanoemulsions and no phase separation was observed except systems N2 and N4 which showed slight precipitate of drug. It was found that, the physical stability of lacidipinenanoeulsion systems significantly increased with increasing the drug oil solubility and decreasing the water concentration.

f) In Vitro Release Studies: The in vitro release of lacidipine from different nanoeulsion systems and the plain drug suspension was carried out through cellulose nitrate membrane over a period of 24 hours in 30% methanolic solution (v/v) at 37 ± 0.5°C. The results revealed that:

- The percent release efficiency of lacidipine from all nanoeulsion systems was higher than the plain drug suspension.
- The percent release efficiency of lacidipine significantly decreased with increasing the drug oil solubility and increased with increasing the oil concentration.
- Release kinetic studies showed that all nanoeulsion systems and plain drug suspension showed best fitting to Higuchi diffusion model.

6) Optimization of LacidipineNanoemulsion: Optimization was performed to find out the level of independent variables (X1, X2, and X3) by applying the point prediction method of the Design Expert software. The system N3 containing IPP (20 %), Cremophor RH 40/ Transcutol™ HP (3:1) (70 %) and distilled water (10 %) was found to fulfill the maximum requisite of an optimum system. The optimized system (N3) was then formulated as nanoeulsion gel using Carbopol 940 (1% w/w).

7) Evaluation of LacidipineNanoemulsion Gel:

a) Visual inspection of the optimized lacidipinenanoeulsion gel formulation (GN3) showed yellow transparent homogeneous gel. No sign of drug precipitation and no phase separation were also observed.

b) Test for Spreadability: By pressing 0.5 g of the optimized lacidipinenanoeulsion gel formulation (GN3) between two glass slides, and then the formed diameter was measured. Spreadability value revealed that GN3 was suitable for transdermal application.
c) **In Vitro Drug Permeation** was carried out through excised rabbit skin over a period of 24 hours in 30% methanolic solution (v/v) at 37±1°C for the optimized lacidipinenanoemulsion gel formulation (GN3) and the lacidipineemulgel. The results showed that the optimizednanoemulsion gel formulation (GN3) exhibited higher lacidipine permeation through excised rabbit skin compared to the control emulgel.

d) **Transmission Electron Microscope (TEM):** The morphology of the hydrated nanoemulsion gel in distilled water was investigated using TEM. Photograph of TEM revealed that, the droplets of the optimized nanoemulsion gel formulation were spherical in shape, discrete with size in nanometer range (< 100 nm).

8) Storage of the optimizedlacidipinenanoemulsion gel (GN3) at 40°C for 3 months showed no change in the physical appearance or color and no phase separation. Regarding the chemical stability of the stored formulation using HPLC stability indicating method, it was found that the optimizednanoemulsion gel possessed lacidipine percentage within the range permitted by the United States Pharmacopoeia (90-110%).

From these results, it can be concluded that the developed nanoemulsion gel is a good carrier for transdermal delivery of antihypertensive drug lacidipine. GN3 will be candidate formulation for in vivo study in chapter III.

**Chapter III**

*In Vivo Performance of the Optimized Lacidipine Formulations.*

Bioavailability is one of the most important aspects of pharmaceutical sciences. So, assessment of bioavailability of drugs is very important to detect extent of success of new formulations. Therefore, the aim of work in this chapter was to investigate the bioavailability of transdermal delivery of lacidipine from the optimized proniosomal gel (GF4) and from the optimized nanoemulsion gel (GN3) formulations in rabbits in comparison to the commercially available lacidipine product (Lacipil® 4 mg tablets). Skin
irritancy test and histopathological study were performed to confirm the safety of the optimized transdermal formulations.

Skin irritation is a common problem encountered with dermal and transdermal drug delivery limiting its wide acceptance among patients in spite of its obvious benefits. For this reason and since proniosomes and nanoemulsions are typically containing surfactants, skin irritancy test was performed on male Albino rats (150-180 g) to confirm the safety of the optimized lacidipineproniosomal gel formulation (GF4) and nanoemulsion gel formulation (GN3). Histopathological studies were also carried out to confirm previous irritation results.

The results obtained showed that, GF4 and GN3 transdermal formulations are safe and non-irritant to rat skin with no histopathological alteration observed in both epidermal and dermal layers of rat skin.

The LC-MS/MS method used for analysis was suitable for the determination of lacidipine in rabbit plasma and showing good linearity, acceptable precision and accuracy over the concentration range used (0.05 to 15 n g/ml), with mean % extraction recovery of 93.98±12.35. The chromatograms from the analysis of the rabbit's plasma showed that the retention times of lacidipine and hydrochlorothiazide were 1.62 and 0.50 min respectively.

Six male New Zealand rabbits weighing 3.0±0.3 Kg were used for this study. The bioavailability of lacidipine from the two optimized transdermal formulations (GF4 and GN3) was compared with the commercially oral available Lacipil® 4 mg tablet (GlaxoSmithKline). The rabbits were randomly divided into 3 groups, each containing two rabbits. A single-dose, three-phases with one week washout period randomized cross-over design was followed.

The results obtained showed that, the mean value of \( C_{p_{\text{max}}} \) and \( \text{AUC}(0-\infty) \) of the two optimized transdermal formulations (GF4 and GN3) were significantly (\( p<0.05 \))
higher than that of the market product (Lacipil® tablets). The relative bioavailability was 222.07% and 184.57% for GF4 and GN3 respectively with respect to the market product.

**AIM OF THE WORK.**

Lacidipine is a calcium channel blocker developed for oral administration. Lacidipine is used in the treatment of hypertension and atherosclerosis. It also possesses an antioxidant effect. Lacidipine is a highly lipophilic drug of poor water solubility and undergoes extensive first-pass hepatic metabolism with a mean absolute bioavailability of about 10% (range 3–59%). It is completely metabolized in the liver by cytochrome P450 3A4 to pharmacologically inactive metabolites. However the very low oral bioavailability restricts its use. So, this together made the drug an excellent candidate for transdermal delivery. Yet, very poor aqueous solubility of lacidipine in water and the excellent barrier function of the skin limit its formulation as transdermal dosage form and make this challenging.

For transdermal therapy, the key point of dosage design is to solubilize the drug in the vehicle and improve its release and permeability via skin without causing irritation or damage.

The aim of the present study was formulation of nanocarrier systems with a high degree of skin permeation, namely proniosomes and nanoemulsion for transdermal delivery of lacidipine.

Therefore, the work in this thesis is divided into three chapters:

**Chapter I**: Preparation and Evaluation of LacidipineProniosomes for Tranermal Delivery.

**Chapter II**: Preparation and Evaluation of LacidipineNanoemulsion for Tranermal Delivery.
Chapter III: In Vivo Performance of the Optimized Lacidipine Formulations.

**CONCLUSION.**

GF4 and GN3 transdermal formulations could provide an effective treatment for the management of chronic hypertension, where they succeeded in enhancing the drug bioavailability by proniosomes and nanoemulsion.

The proniosomes and nanoemulsion is a good nanocarriers for transdermal delivery of lacidipine and could provide an effective treatment for the management of chronic hypertension.

**INTRODUCTION.**

Lacidipine is a highly potent new calcium antagonist of the dihydropyridine class with long-lasting antihypertensive effects developed for oral administration (Micheli et al., 1990). In addition to calcium channel-modulated vasodilation, lacidipine displays antioxidant activity greater than that of other dihydropyridine calcium antagonists (McCormack and Wagstaff, 2003). Lacidipine (2-6 mg orally once daily) has antihypertensive efficacy similar to that of other long-acting dihydropyridine calcium antagonists, thiazide diuretics, atenolol (a β-blocker) and enalapril (an ACE inhibitor).

Lacidipine is described by the following chemical name: Diethyl 4-\{(2-((tert-butoxycarbonyl)vinyl)phenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate and has the following structural formula:
The molecular formula of lacidipine is C_{26}H_{33}NO_6 and its molecular weight is equal to 455.5. The melting point range of lacidipine is 174-175ºC. Lacidipine is a white to pale yellow crystalline powder. It is practically insoluble in water, sparingly soluble in dehydrated alcohol, freely soluble in acetone and in dichloromethane (Lee and Bryson, 1994).

**Pharmacodynamic Properties of Lacidipine.**

Lacidipine is a once-daily, orally-administered lipophilic 1,4dihydropyridine calcium antagonist with an intrinsically slow onset of activity and long duration of action. The lipophilic nature of the drug results in the accumulation of lacidipine in membrane lipid bilayers from which it is slowly and continuously released (McCormack and Wagstaff, 2003).

Lacidipine blocks voltage-dependent L-type calcium channels, producing vasodilation, and thereby reduces total peripheral vascular resistance, resulting in a reduction in blood pressure (BP). In addition, lacidipine has antioxidant activity that is considered to play a role in reducing endothelial dysfunction induced by oxidative stress (McCormack and Wagstaff, 2003).

The onset of the antihypertensive effect occurs 0.5-1.0 hours post-dose, and is maintained throughout a 24 hours period during multiple dosing. Lacidipine
administration once daily in the morning reduces BP over 24 hours as demonstrated by ambulatory BP monitoring, although night-time BP is reduced to a lesser extent than day-time BP. Trough to peak plasma concentration ratios of 65% or more are commonly observed, indicating that once daily administration is appropriate (McCormack and Wagstaff, 2003).

The slow onset of activity means that lacidipine causes little or no reflex tachycardia or sympathetic activation during long-term administration. The high vascular selectivity of lacidipine results in little or no cardiodepression. Lacidipine also improves left ventricular function and, in animal models, exhibits vasoprotective properties in both the cardiovascular and cerebrovascular circulation (McCormack and Wagstaff, 2003).

There is pharmacodynamic evidence from in vitro and animal experiments to suggest that lacidipine may have antiatherosclerotic activity that is at least partly independent of BP lowering activity (McCormack and Wagstaff, 2003).

Adverse Effects of Lacidipine.

The adverse effects of lacidipine are similar to that of other dihydropyridine calcium antagonists; although, in some studies, the incidence of pedal oedema was lower with lacidipine than with other dihydropyridine calcium antagonists. The most common adverse effects are generally attributable to the drug’s vasodilatory actions and consist of headache, flushing, pedal oedema, dizziness and palpitations. Overall, approximately 32% of patients reported adverse events in uncontrolled therapeutic trials. Adverse events tend to occur in the first 6 months of therapy and diminish during long-term use (Lee and Bryson, 1994).

Pharmacokinetic Properties of Lacidipine.

After single-dose oral administration of lacidipine 2-6 mg to healthy volunteers, mean peak plasma concentrations (C_max) of 1.2-6.9 μg/l occurred at a median time of 1.0-1.8 hours post-dose, with high inter-individual variability. Administration of lacidipine 2-
6mg once daily for 8 days produced mean steady-state $C_{\text{max}}$ values of 1.2-5.2 μg/l. Plasma concentrations are increased in the elderly and in patients with hepatic impairment.

Lacidipine is rapidly but poorly absorbed from the gastrointestinal tract following oral administration, undergoes extensive first-pass hepatic metabolism and has a mean absolute bioavailability of approximately 10% (range 3-59%). Lacidipine is >90% bound to plasma proteins, mainly albumin. During multiple-dose administration, the terminal elimination half-life of lacidipine 2-6 mg/day was 13.2-18.7 hours. Lacidipine is completely metabolized in the liver by cytochrome P450 3A4 (CYP3A4) to pharmacologically inactive metabolites that are mainly eliminated by the biliary route and excreted in the faeces. Despite being metabolised by CYP3A4, pharmacokinetic interactions with grapefruit juice, simvastatin and digoxin are not considered clinically significant (Lee and Bryson, 1994). So, the very low oral bioavailability of lacidipine restricts its use and makes it an excellent candidate for transdermal delivery.

**Patient** with hypertension must take antihypertensive drug on a long term basis. Although such drugs cannot give a radial cure, they can prevent heart failure and acute stroke induced by hypertension. The medical practitioner often facing a major problem is that the patients on oral antihypertensive do not follow proper drug regimen. Furthermore, patient with high blood pressure are treated with number of drugs at a time. In addition, oral antihypertensive therapy has many disadvantages such as (Yadav et al., 2012):

1. It undergo extensive first-pass metabolism.
2. Shows unpredictable or low bioavailability.
3. Dose dumping.
4. Dose inflexibility and increase in the cost of product.

So, formulating transdermal patches of antihypertensive drugs provide greater patient compliance (Yadav et al., 2012).
Transdermal Drug Delivery Systems (TDDS) are one of the most rapidly advancing areas of novel drug delivery, which are designed to deliver a therapeutically effective amount of drug across a patient’s skin (Chein, 1987). The first commercially available TDDS patch of scopolamine was approved by the U.S Food and Drug Administration in December 1979 for treatment of motion sickness.

Advantages of Transdermal Drug Delivery System (Yadav et al., 2012).

- It avoids GIT side effect, inactivation of drug by GIT enzymes, interaction of drug with food and first-pass metabolism of drugs in GIT.
- It provides controlled and sustained release of the medicament.
- It improves the bioavailability of drug.
- It provides uniform drug plasma concentration.
- It improves the patient’s compliance.
- It can be administered to non-responsive, unconscious and nauseating patient.
- It provides easy termination of drug in case of toxicity by removal of the formulation from the skin.

Disadvantages of Transdermal Drug Delivery Systems (Yadav et al., 2012).

- Transdermal drug delivery system is unsuitable for a drug that causes irritation at the site of application.
- It is suitable for potent drugs only.
- It is limited for the drugs which are imposed by skin permeability.

Despite the great potential of transdermal drug delivery systems, only a few drugs are available commercially as transdermal systems. The main reason is the barrier function of human skin that is considered to be the most impermeable epithelium to exogenous substances (Kreilgaard, 2002).

Human skin is an important target site for the application of drugs. The skin itself has three main layers: the epidermis, which is the outermost layer of the skin, covering the dermis that is the active part of the skin, holding the hair muscles, blood supply,
sebaceous glands, and nerve receptors. There is a fat layer underneath the dermis. The skin is a very heterogeneous membrane and has a variety of cell types, but the layer that controls the penetration of drugs is called the stratum corneum and, despite its thickness of only 15-20 μm, it provides a very effective barrier to penetration (Kogan and Garti, 2006).

The permeation of the drug through the skin has several routes: transcellular, intercellular, and appendageal (through eccrine (sweat) glands or hair follicles). Since the appendages occupy a very low surface area, this means of permeation is less significant under normal conditions (Kogan and Garti, 2006).

The intercellular pathway plays a major role in percutaneous uptake of drugs. It is well known that a complex mixture of essentially neutral lipids, which are arranged as a bilayer with their hydrophobic chains facing each other, forms a lipophilic bimolecular leaflet. Most of the lipophilic drugs pass through this region, and it is called a lipid pathway. The polar head group of lipids faces an aqueous region, forming a polar route that hydrophilic drugs generally prefer (Kogan and Garti, 2006).

Transdermal drug permeability is influenced mainly by three factors: the mobility of the drug in the vehicle, the release of the drug from the vehicle, and drug permeation through skin (Peltola et al., 2003). Therefore, the researchers are challenged to come up with formulations that increase the permeability of the drug without irreversibly changing the skin barrier function. In order to reach therapeutic drug concentrations in certain skin layers or in the blood circulation, the uppermost barrier, the stratum corneum (SC), has to be overcome. This process is affected by various factors, e.g., the physicochemical properties of the drug and the vehicle used for administration.

Various potential mechanisms to enhance drug penetration through the skin include directly affecting the skin and modifying the formulation so the partition, diffusion, or solubility is altered (Williams and Barry, 2004).
Nanocarriers such as proniosomes and nanoemulsion are one of the most important techniques for enhancement of transdermal permeation of drugs and have received increasing attention during the last years (Neubert, 2011 & Schwarz et al., 2012).

The aim of this study was formulation of nanocarrier systems with a high degree of drug release and permeation, namely proniosomes and nanoemulsion, for transdermal delivery of lacidipine.

SUMMARY

"Formulation of Nanocarrier Systems for Transdermal Drug Delivery."

The aim of this study was formulation of nanocarrier systems with a high degree of drug release and permeation, namely proniosomes and nanoemulsion, for transdermal delivery of lacidipine to avoid its extensive first pass metabolism.

Chapter I: Preparation and Evaluation of Lacidipine Proniosomes for Transdermal Delivery.

The aim of the present work was to develop proniosomal gel using cremophor RH 40 as non-ionic surfactant containing the antihypertensive drug lacidipine for transdermal delivery. Proniosomes containing 1% lacidipine were prepared, characterized and optimized using a $2^3$ full factorial design to define the optimum conditions to produce proniosomes with high entrapment efficiency, minimal vesicle size and high percentage release efficiency. The amount of cholesterol ($X_1$), the amount of soya lecithin ($X_2$), and the amount of cremophor RH 40 ($X_3$) were selected as three independent variables. The optimized system (F4) composed of cholesterol (10 mg), soya lecithin (80 mg), cremophor RH 40 (270 mg), absolute ethanol (0.25 ml) and water (0.1 ml) was then converted to proniosomal gel using carbopol 940 (1% w/w). In vitro permeation through excised rabbit skin study revealed higher flux (6.48±0.45) for lacidipine from the optimized proniosomal gel when compared to the corresponding emulgel (3.04±0.13)
The physical and chemical stability of the optimized formulation was retained after storage at 40 ± 0.2°C for three months.

Chapter II: Preparation and Evaluation of Lacidipine Nanoemulsions for Transdermal Delivery.

The aim of the present work was to develop a nanoemulsion gel for transdermal delivery of lacidipine. Pseudo-ternary phase diagrams were constructed using capryol™ 90 or isopropyl palmitate as oils, cremophor RH 40/ transcutol™ HP in the ratio of 3:1 as surfactant and cosurfactant respectively. Nanoemulsions containing 1% lacidipine were prepared, characterized and optimized using a $2^3$ full factorial design. The three independent variables selected were drug oil solubility ($X_1$), the concentration of oil ($X_2$), and the concentration of distilled water ($X_3$). The optimized system (N3) which composed of 20% isopropyl palmitate (w/w), 70% (w/w) cremophor RH 40/transcutol™ HP in the ratio of 3:1 and 10% (w/w) distilled water was then formulated as nanoemulsion gel using carbopol 940 (1% w/w). In vitro permeation through excised rabbit skin study revealed that the permeation of lacidipine from the optimized nanoemulsion gel was significantly higher than that from the control emulgel. The physical and chemical stability of the optimized formulation was retained after storage at 40 ± 0.2°C for three months.

Chapter III: In Vivo Performance of the Optimized Lacidipine Formulations.

The aim of work in this chapter was to investigate the bioavailability of transdermal delivery of lacidipine from the optimized proniosomal gel (GF4) and from the optimized nanoemulsion gel (GN3) formulations in rabbits in comparison to the commercially available lacidipine product (Lacipil® 4 mg tablets). Skin irritancy and histopathological investigation of rat skin revealed that, GF4 and GN3 transdermal formulations are safe and non-irritant to rat skin with no histopathological alteration observed in both epidermal and dermal layers of rat skin. The mean value of $C_{p_{max}}$ and $AUC_{(0-\infty)}$ of the two optimized transdermal formulations (GF4 and GN3) were significantly (p<0.05) higher than that of the market product (Lacipil® tablets). The
relative bioavailability was 222.07% and 184.57% for GF4 and GN3 respectively with respect to the market product.