Chapter-8
Summary
and
Conclusions
Transdermal delivery of cardiovascular agents offers numerous advantages. Among the cardiovascular agents, Losartan Potassium is widely used first-line drug in patients with increased cardiovascular risk. The drug possess distinct physicochemical properties (MW 461 Da, solubility (~1mg/mL), pKa 4.9, log P) and is formulated in sustained release tablets and spray dried microspheres as injectables. However, the drug is poorly bioavailable, show high incidence of adverse effects on oral absorption due to the effect of meals on its absorption profile, leading to poor patient compliance. Hence the development of transdermal drug delivery of this drug, which could maintain proper blood level for a prolonged period of time without adverse effects is in demand. Although the transdermal route represents a promising method for drug delivery, the hindrance created by the uppermost layer of skin, the stratum corneum (SC), limits the access of drug into the systemic circulation. The transport of Losartan Potassium across the formidable SC signified low intrinsic permeability to meet the therapeutic requirement. Approaches using chemical skin permeation enhancers were not attempted in the current investigation as these agents generally cause skin irritation. Similarly, the iontophoretic technique may not be of apposite as this technique is more prominent for hydrophilic drugs. On the other hand, Sonophoresis is a promising technique which can be used for enhancing the delivery of both hydrophilic and hydrophobic drugs. This research project was aimed to develop a Sonophoretic Transdermal Drug Delivery System (STDDS) for systemic delivery of model drug Losartan Potassium and evaluate it.

FTIR spectroscopy was used to confirm the non-degradative effects of ultrasound on the drug structure under experimental conditions.

Optimization of a formulation or process means finding the best possible composition or operating conditions. Traditionally, drug release is studied by changing one variable at a time approach. The method is time consuming and requires a lot of imaginative efforts. Moreover, a large number of experimental animals have to be sacrificed as skin is required everytime to determine the permeation rate. Also, it may be difficult to evolve an ideal drug release using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of
pharmaceutical variables by using established statistical tools such as factorial design.

Factorial design is a well-known important statistical optimization tool to study the effect of several factors influencing responses by varying them simultaneously and carrying out limited number of experiments. To optimize ultrasound variables for effective sonophoretic drug delivery using minimum number of animals, factorial design technique was followed here.

In the present study, albino male hairy rat was selected as the in vitro animal model for experiments, and in vivo studies were extended on the same animal model in spite of its small size. The in vitro studies for evaluation of LP permeation across the rat skin were done using modified Franz-diffusion cell. The donor compartment of the cell was modified to accommodate the transducer of ultrasound generator. The effect of various ultrasound parameters such as ultrasound intensity, mode of ultrasound application (pulsed and continuous) and duration of application were investigated to assess their influence on the transdermal permeation of LP. Total nine treatment groups were taken for experiments including control based on $2^3$ full factorial design plan. The three factors taken were intensity ($X_1$), duration of ultrasound application ($X_2$) and mode of ultrasound application ($X_3$) (continuous, C and pulsed, P) and they were varied at two levels, low level (-) and high level (+).

Hence, in vitro experiments were designed for the purpose of providing a framework for predicting sonophoretic drug delivery. It was observed from the results of permeation studies, out of the two intensities (0.5 and 2.5 W/cm$^2$) used, 2.5 W/cm$^2$ was found better. When duration of application of ultrasound (10 and 30 min.) was compared, application time 30 min. resulted in more flux. Finally, modes were compared at different intensities and duration of exposure. It was found that the results obtained in both the modes were comparable at given intensities and duration of exposure. However, the mode affected permeation significantly as is evident from the results of cumulative release data and flux at higher intensity and duration of application. Since, the motive of the study was to select an ideal set of parameters for further studies like pharmacodynamic studies and pharmacokinetic studies, hence, continuous mode at 2.5 W/cm$^2$ intensity and 30 min. duration was selected.
for further studies. This was also substantiated by supportive studies like histopathological studies and factorial design results.

Various mechanisms have been suggested to explain the enhancement of drug permeability via Sonophoresis, in literature. These mechanisms include: temperature increase, induction of convective flow and cavitation as well as radiation pressure (Tyle & Agrawala, 1989). However, Simonin has ruled out the involvement of radiation pressure after using mathematical calculations (Simonin, 1995).

The literature supports the observation that increasing temperature leads to an increment in skin permeability (Potts, 1989). Not only are there references reporting the phenomenon, there are also mechanistic studies which clearly relate enhanced percutaneous transport to specific biophysical events, including phase transitions within the intercellular lipid domains of the stratum corneum (Gay et al., 1994).

Other factors have previously been reviewed (Simonin, 1995). Cavitation, which is the oscillation generation, and subsequent violent collapse of gaseous micro-bubbles within the coupling medium and/or within the skin also help in explaining the enhancement of percutaneous absorption. It is hypothesized that cavitation occurs near the surface of the stratum corneum and hence temporarily disorganizes intercellular phospholipid bilayers and/or corneocytes by creating a transient aqueous channel in the stratum corneum, thus making transdermal absorption easier (Ueda et al., 1995; Mitragotri et al., 1996). Moreover, it has also been reported that sonication has a greater effect on the skin permeation of hydrophilic drugs which usually have low permeability (Boucaud et al., 2001).

Our results are consistent with a cavitation-based mechanism since cavitation activity and duration increase with increasing acoustic pressure (e.g. intensity) and application time, respectively.

The appendageal routes were also reported in affecting permeation during Sonophoresis. Therefore, wistar rat skin was used as the model membrane to perform in vitro sonophoretic study since it has a greater number of hair follicles compared to hairless mouse (Bronaugh et al., 1982). Since drug flux was enhanced
significantly, it is suggested that the appendageal pathways are more susceptible to ultrasonic enhancement than the transcellular pathway. Group of researchers (Osamura, 1982; Fang et al., 1999) also demonstrated that clobetasol 17-propionate penetrates skin more readily through follicles than epidermis.

Future work can be planned in order to better understand the detailed mechanism of sonophoretic drug delivery enhancement.

Subsequent to this, a comparative study of losartan potassium permeation from proniosomal gel with drug dispersed in aquasonic jelly (coupling medium) was carried out on rat skin. Proniosomes were prepared using cholesterol and Span 40 by coacervation phase separation technique. The formation of proniosomes was confirmed by optical microscopy. Permeation studies from proniosomes and drug dispersed in aquasonic jelly were carried out using same ultrasound parameters. It was observed that aquasonic jelly gave better permeation results in comparison to proniosomes and hence was selected as vehicle and coupling media for further studies.

Though it is evident from factorial design results that all the three ultrasound variables have positive impact on losartan potassium delivery across the skin, the impact of these variables were in the following order; duration of ultrasound maximum, followed by intensity and then mode of application. When results of interaction between the chosen parameters were compared, the most significant interaction seen was between intensity and duration of ultrasound application.

Based on the results and observations of in vitro studies, research work was extended for further supportive experiments. As ultrasound is a form of mechanical energy, it was expected that it will interact with the biological membranes. Hence, effects of ultrasound on different layers of skin, showing cellular changes, were verified by histopathological studies. Results showed that intraepidermal cleft, collagen fibre degradation and cell damage were found equally in both modes of ultrasound application i.e. continuous and pulsed mode. However, the difference in the degree of subepidermal oedema, atrophy and liquefication of epidermis can be said to responsible for difference in enhancement at 2.5 W/cm² intensity applied for 30 min. at different modes.
Pharmacodynamic studies were designed with an aim to study the efficacy of sonophoretic transdermal drug delivery against hypertension. The study was carried out using Tail Cuff Method and methyl prednisolone acetate was used to induce hypertension in rats. The sonophoretic transdermal drug delivery system using losartan potassium was found to decrease the BP significantly (paired t-test, P < 0.001) in the proximity of the normal value. This indicated that drug was permeated into systemic circulation in rats. However, post treatment of BP values in control (I) and treatment group (III) were comparable (P<0.05). Hence, sonophoretic transdermal delivery system of LP was successful in reverting the rat BP to normal values. The above results suggested that the STDDS holds promise for the management of hypertension that needs to be validated by pharmacokinetic studies.

To quantify the concentration of drug reaching the systemic circulation, in-vivo experiments comprising of pharmacokinetic studies were carried out and analysis was done using HPLC. Sonophoretic delivery is expected to enhance the delivery of a drug through various skin layers into the systemic circulation. Thus, for a drug like LP, which has a significant hepatic first pass metabolism, the sonophoretic delivery should by-pass the hepatic first-pass metabolism, thereby resulting in a major therapeutic advantage. Hence, pharmacokinetic studies were carried out on rats to judge the efficacy of sonophoretic transdermal system against the oral dosage form. Mean AUC<sub>0-t</sub> value after oral treatment was 1023.0±118 ng.h/ml and after sonophoretic transdermal treatment was 1257.1±141 ng.h/ml. There was a significant difference between the AUC<sub>0-t</sub> values for sonophoretic and oral treatments (P< 0.05). According to results, the difference in the AUC<sub>0-t</sub> values clearly reflects that comparatively lesser amount of drug was available by oral administration to rat body because of high first pass metabolism of losartan potassium. Sonophoretic transdermal system in present study was found to enhance the bioavailability of losartan potassium by 1.22 times with reference to an oral delivery of drug. It is concluded from the results obtained that sonophoretic drug delivery results in an increase in systemic drug concentration.

Finally, dermatokinetic studies were carried out to establish the concentration profile of Losartan Potassium within the stratum corneum. Tape stripping technique was used for this study. HPLC method was used for quantifying the amount of drug on
tapes. The results substantiated the earlier results of pharmacokinetic studies. This new approach has the potential of giving a clear picture of skin kinetics. A detailed and well planned dermatokinetic study in future can be of great help in correlating various sonophoretic effects.

Based on the results of various studies planned and conducted as a part of the present work, it is concluded that sonophoretic transdermal drug delivery system is a potentially useful and safe technique for improving the penetration of BCS class III drug like Losartan Potassium for transdermal delivery. It is once again established that Sonophoresis is a good drug delivery system for drugs to be given for systemic action.

**FUTURE SCOPE**

An effort was made to develop and evaluate a sonophoretic delivery system for Losartan Potassium. Though the essential studies like *in vitro, in vivo, histopathological, pharmacodynamic and pharmacokinetic* (including dermatokinetic) studies were carried out on rats but there is still room for establishing its utility in the clinical set up. Clinical trials of sonophoretic delivery of Losartan Potassium are recommended for future studies.