Chapter-3
Research Envisaged &
Selection of Drug
Candidate
3.1 OBJECTIVE OF THE RESEARCH
The objective of the present research work was to explore the feasibility of Sonophoresis as a viable drug delivery system using a model drug (Losartan potassium), both in vitro as well as in vivo, in rats. Optimization of ultrasound parameters for sonophoretic delivery of the drug in rats was achieved based on these experiments. Histopathological studies were carried out, in order to study the influence of ultrasound on skin structure. Further an effect of ultrasound application on the permeation of LP from proniosomal gel was compared with drug dispersed in aquasonic jelly using male albino rats. Skin pharmacodynamic studies were carried out by monitoring the effect of STDDS on blood pressure of methyl prednisolone acetate induced hypertensive rats. Determination of various pharmacokinetic parameters from the serum drug-concentration profile of LP following sonophoretic delivery of drug were carried out in vivo in rats. Further, Dermatokinetic studies were carried out to reinforce the in vivo results.

3.2 JUSTIFICATION OF THE PRESENT WORK
For transdermal drug delivery applications, permeability has to be increased by agents that interact with stratum corneum or perturb it. The range of therapeutic agents as candidates for this route can be increased by the use of a number of enhancement methods of transdermal permeation chemical (He et al., 2004), biochemical (Fang and Leu, 2006) and physical (Joshi and Raje, 2002; Sivamni et al., 2005) enhancement. All the aforementioned sources are expected to interact with the otherwise inert, dry and dead corneocytes of stratum corneum and perturb its symmetry which has to be detrimental to its integrity. The increase in the flux may be attributed through one or more of the following mechanisms:
- increased diffusion co-efficient (chemical enhancers, electroporation)
- provision of additional driving forces (iontophoresis and electroporation)
Clinical acceptance of chemical absorption promoters was initially very encouraging, but an unfortunate feature of many potent enhancers is that they irritate, as they also interfere with viable cell membranes. Industrial scientists therefore often limit their investigations for a suitable enhancer to materials known to be benign on skin, e.g., GRAS (generally recognised as safe) substances (Barry, 2001). Moreover, toxicity concerns of these enhancers
become the major barrier for enhancer application in transdermal formulation (Wang et al., 2005).

Research is going on in the field of iontophoresis utilizing many drugs, including big molecules like insulin and other peptides. Iontophoresis is the delivery of drugs by transdermal route under the influence of electric current. Effect of competitive ions, drug retention after withdrawal of current, charge accumulation, permanent ionization and lipophilicity of the drug, are the major restrictions in practical acceptance of the technique. The skin irritation associated with iontophoresis has been addressed by several studies and it is an issue preventing wide application of the technology. Therefore, search for newer methods continued and Sonophoresis is one of them. Sonophopresis is capable of expanding the range of compounds that can be delivered transdermally (Lavon et al., 2005; Lavon et al., 2007; Nanda et al., 2006). In addition to the benefits of avoiding the hepatic first pass effect and higher patient compliance, it possesses some additional advantages. The unique and promising release of drugs by Sonophoresis renders it an attractive candidate as a physical enhancer to administer drugs throughout the skin as reported in Table 2.3 (Chapter Two - Literature Review). The highly lipophillic nature of skin restricts the permeation of hydrophilic, high molecular weight and charged compounds through the stratum corneum into the systemic circulation. However, many therapeutically active drug molecules are hydrophilic. This research was aimed to see the effect of ultrasound on systemic delivery of hydrophilic losartan potassium. In sonophoretic drug delivery system, the patient’s metabolic machinery is not unnecessarily being burdened in processing a large quantity of drug as would happen with a drug undergoing significant hepatic first pass metabolism, when given orally, e.g. Losartan Potassium. The drug delivered sonophoretically enters the systemic circulation and exerts its pharmacological responses. Hypertension is a health condition in which the blood pressure (BP) is persistently elevated (high BP). Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure, and is a leading cause of chronic renal failure. Even moderate elevation of arterial BP leads to shortened life expectancy. Nearly 1 billion people worldwide have high BP (defined as > 140/90 mmHg), and that number is...
expected to increase to 1.56 billion people by next two and half decades. The prevalence of hypertension is predicted to increase by 24% in developed countries and by 80% in developing regions (Kearney et al., 2005). From 1999 to 2004, 78% of adults with hypertension were aware of their disease, 68% were treated for their hypertension with medications, and less than two-thirds were controlled to BPs below 140/90 mmHg with medication (Rao et al., 2008). WHO 1999 reports that one out of every three deaths in India is because of heart diseases. Amongst several risk factors associated with this growing menace, hypertension or high BP has been established as a risk factor for heart diseases (Shams et al., 2010).

Losartan potassium (LP) is one such drug that is used frequently for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents. The drug is generally given for a longer duration of time. This causes daily dosing schedule and patient inconvenience. Its absolute bioavailability is approximately 33% after oral dosing. The low bioavailability is primarily because of incomplete absorption and partly because of presystemic metabolism. Hence, it appears clearly that the bioavailability of LP can be increased by bypassing the hepatic first pass metabolism. The dose and consequently, many of its side effects, can be reduced in this manner.

Transdermal drug delivery has been recognized over the years to by pass the hepatic first pass metabolism and offer a drug systemically (Subedi et al., 2010). Hence, losartan potassium was selected as a model drug for the present research work. In this study, we hypothesized that transdermal drug delivery with Sonophopresis would yield greater bioavailability of low molecular weight and poorly absorbed and hydrophilic drug like losartan potassium.

Therefore, the aim of our present work was to develop a Sonophoretic transdermal drug delivery system for Losartan Potassium and to evaluate it on male albino rats.

### 3.3 PLAN OF WORK

1. Selection of drug and its physicochemical evaluation.
2. FTIR studies to ascertain that the drug is stable when it is subjected to ultrasound energy.
3. *In vitro* studies

- Effect of ultrasound on flux rate of drug across an animal skin
- Study of ultrasound variables like *intensity, duration of exposure, mode of application* on drug permeation.

4. Histopathological observations, in order to ascertain the effects of ultrasound on a biological membrane like skin.

5. *In vivo* studies:

- Pharmacological evaluation
- Pharmacokinetic and Dermatokinetic studies

6. Interpretation of results, conclusion and future scope of work.

### 3.4 SELECTION OF DRUG CANDIDATE FOR TRANSDERMAL DELIVERY

One important goal for the pharmaceutical industry is the identification of molecules with potential for becoming approved drugs (*Magnusson et al., 2004*). The drug development process selects molecules having optimal pharmacological activity in biological assay of choice (*Guy and Hadgraft, 1992*). Although, it may appear to be a simple task to select lead for pharmaceutical product development based on therapeutic rationale and compound safety and efficacy, the practicalities of this procedure are actually more complex. For the most part, therapeutic efficacy is dependent on the ability of a compound to cross biological barriers, travel to the target site and interact with receptors. However, it is often more appropriate in dermatological therapy to select compounds based on their inability to breach relevant biological barriers. Transport across SC is largely a passive process and physicochemical properties of a permeant are an important determinant of its ability to penetrate and diffuse across the membrane (*Roberts et al., 2002*).

The ideal properties of a molecule that would penetrate stratum corneum well are:

- Low molecular mass, preferably less than 500 Da.
- Adequate solubility in oil and water so that membrane concentration gradient may be high.
- Low melting point, correlating with good solubility as predicted by ideal solubility theory (*Pathan and Setty, 2009*).
3.4.1 Description of Losartan Potassium (LP)

![Chemical Structure of LP](image)

**Figure. 3.4.1.1 Chemical Structure of LP**

**Chemical Name:** (2-butyl-4 chloro-1 (2-(1H tetrazol-5yl) (1, 1' biphenyl- 4 yl) methyl)-1-H-imdazole- 5-methanol) (IP, 2007)

**Proprietary names:** Cozaar; Lortaanz;Losaprex; Neo-Lotan; Oscaar. It is also an ingredient of Hyzaar and Losazid.

**Indian Brand Names:** Adpace tab, Arbitace tab, Biosartan R 2.5, Loram, Losagem-H, Sartace tab.

**Marketed forms of LP:** Only oral dosage forms are available. 25, 50 and 100 mg tablets, 2.5 mg Capsules and Oral Suspension are available, ranging from 1.5 mg/ml to 10 mg/ml.

**Sponsor Company of drug:** It is currently marketed by Merck and Co. under the trade name Cozaar.

**Molecular Weight:** 461.01Da

**Formula:** C_{22}H_{22}ClKN_{6}O

**Packaging and storage:** Preserve in well-closed containers. Store at controlled room temperature.

**Dissociation Constant:** pK_{a}5 to 6.

**Partition Coefficient:** Log P(octanol/water), 4.01 (for Losartan).

**Melting Point:** 183.5-184.5 C

**Half life:** 1.5-2 hrs

**Oral Bioavailability:** Around 33%

**Volume of distribution:** 34 L; active metabolite:12 L.
**Plasma clearance:** losartan, 600 ml/min; active metabolite, 50 ml/min.

**Protein binding:** $>98\%$, mainly to albumin (both losartan and active metabolite).

**Physical Properties and Description**

LP is a white to off-white crystalline powder. It is freely soluble in water, soluble in alcohols, and slightly soluble in common organic solvents, such as acetonitrile and methyl ethyl ketone (Merck, 2000). LP is considered class III in the biopharmaceutics classification system, because it has high solubility and low permeability.

LP is a potent, highly specific angiotensin II type 1 ($\text{AT}_1$) receptor antagonist with anti-hypertensive activity (Chopra et al., 2006). It is first member of a new class of non-peptide angiotensin II receptor antagonist (Mufano, et al., 1992). It develops a gradual and long-lasting effect as antihypertensive, becoming a new alternative to this frequent chronic disease treatment (Lastra et al., 2003). The decrease in the blood pressure is produced by competitive antagonist action of $\text{AT}_1$ receptor and release of aldosterone and adrenaline from adrenal glands, renal action promoting salt and water reabsorption. The multiple action of LP may also provide the rational use of drug in the treatment of congestive heart failure (Vijayan et al., 2010). LP may also delay progression of diabetes nephropathy and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes, hypertension and proteinuria (>900 mg/24 hours) (Rossi S, 2006). The LIFE study demonstrated that LP was significantly superior to atenolol in the primary prevention of adverse cardiovascular events (myocardial infarction or stroke), with a significant reduction in cardiovascular morbidity and mortality for a comparable reduction in blood pressure (Dahlof et al., 2002). The drug is listed in Merck Index, Martindale’s Extra Pharmacopoeia, United States Pharmacopoeia and Indian Pharmacopoeia, 2007.

**3.4.1.1 Pharmacokinetics**

Losartan potassium is less absorbed from the gastro intestinal tract and the bioavailability is only 33% due to first pass metabolism in liver (Cytochrome 450 enzymes) (Vijayan et al., 2010). Peak plasma levels observed within 1 hr, and the $C_{\text{max}}$ and area under the concentration vs. time curve to infinity were proportional to the dose, $P < 0.05$. The elimination half-life, 108 to 153
min, was longer than that observed after a single i.v. dose, 41 min. and may reflect both continuous absorption and enterohepatic recirculation because the major route of excretion was via the bile. Losartan potassium was not distributed extensively to tissues; apparent volume of distribution at steady-state of 0.30 litres/kg and was highly but not extensively bound to plasma proteins (Christ et al., 1994).

3.4.1.2 Dose Requirement and Combination Therapy
In patients with mild to moderate hypertension, losartan potassium 50 to 100 mg once daily as monotherapy lowers blood pressure to a similar degree to enalapril, atenolol and felodipine extended release tablets. Losartan potassium combined with hydrochlorothiazide reduces blood pressure further than either drug given separately. About one-third of patients with severe hypertension have responded to the combination product. Losartan potassium appears to be effective in elderly patients. Losartan potassium is very well tolerated (Goa and Wagstaff, 1996).

3.4.1.3 Pharmacological Category and Dose
Angiotensin II receptor blocker required 50 to 100 mg daily.

3.4.1.4 Therapeutic Uses
Losartan potassium is prescribed alone or in combination with other medications to help control hypertension, or high blood pressure. Although losartan potassium treats high blood pressure, it cannot cure the condition. Losartan potassium works to block the action of the substance angiotensin II, which causes blood vessels to tighten, in order to relax blood vessels and lower blood pressure (Rang and Dale, 2007). Losartan potassium may also be used in patients with left ventricular hypertrophy (LVH) and high blood pressure to decrease the risk of stroke, as well as in patients with diabetic nephropathy, a condition resulting from diabetes that involves improper functioning of the kidneys. Losartan Potassium does not block the degradation of vasoactive substances such as bradykinin, enkephalins etc. and may not cause side effects such as cough related to ACE inhibitor–induced bradykinin accumulation (Lang et al., 1997).
### 3.4.1.5 Mechanism of Action

As a selective and competitive, nonpeptide angiotensin II receptor antagonist, losartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II; losartan interacts reversibly at the AT1 and AT2 receptors of many tissues and has slow dissociation kinetics; its affinity for the AT1 receptor is 1000 times greater than the AT2 receptor. Angiotensin II receptor antagonists may induce a more complete inhibition of the renin-angiotensin system than ACE inhibitors; they do not affect the response to bradykinin, and are less likely to be associated with non renin-angiotensin effects (e.g., cough and angioedema). Losartan increases urinary flow rate and in addition to being natriuretic and kaliuretic, increases excretion of chloride, magnesium, uric acid, calcium, and phosphate (*Merck Index*).

#### 3.4.1.6 LP Side Effects

- Impaired renal function
- Difficulty in swallowing
- Dose related orthostatic hypotension
- Hyperkalaemia (*Sweetman, 2005*)

#### 3.4.1.7 Drug Interactions

LP may increase levels of blood potassium which can lead to serious heart problems (arrhythmias). Therefore, concomitant use of other substances such as potassium-sparing diuretics (spironolactone, triamterene, and amiloride), potassium supplements, or salt substitutes containing potassium may lead to dangerous increases in serum potassium.

The antihypertensive effect of losartan may be reduced by nonsteroidal anti-inflammatory drugs (indomethacin, ibuprofen, aspirin, and naproxen) (*Tripathi, 2001*).

### 3.4.2 ANALYTICAL METHODS

#### 3.4.2.1 Analytical Methods for Determination of LP

Several methods have been reported for the analysis of losartan potassium and its degradation products. In biological fluids, the active principle has been determined by high performance liquid chromatography (HPLC) (*Furtek et al., 1992; Kristoffersen et al., 2007; Lee et al., 1996; Yeung et al., 2000*). For applications in pharmaceutical products, there are methods that make use of HPLC (*Ansari et al., 2004; Lusina et al., 2005*), high...
performance thin layer chromatography (HPTLC) (Sathe and Bari et al., 2007), capillary electrophoresis (CE), capillary electrochromatography (CEC) (Quaglia et al., 2002) and spectrophotometry (Prabhakar and Giridhar, 2002).

The drug substance monograph on losartan potassium provided by the United States Pharmacopoeia recommends an HPLC method on a C-18 (4.0mm×25mm) column with a 0.1% solution of phosphoric acid in water and acetonitrile (60:40, v/v) as the mobile phase, flow rate at 1ml min⁻¹, column temperature at 35 °C, methanol as diluents and detection at 254 nm (Bonfilio et al., 2009).

Wenkhede et al., (2010) developed two UV- spectrophotometric and one reverse phase high performance liquid chromatography for the simultaneous estimation of LP in tablet dosage form.

Lastra et al., (2010) developed and validated an analytical UV derivative spectrophotometric method to quantify LP used as a single active principle in pharmaceutical forms. Simple, rapid and accurate, spectrophotometric method for the determination of losartan potassium by using bromothymol blue as a chromogen and phosphate buffer solution (pH 3-4) as a diluting agent was developed by Latteshjial et al., (2010).

A reversed-phase high-performance thin-layer chromatography method has been developed for the determination of losartan and its low level dimeric degradates (E and F). The method has been validated and shown to be sensitive, efficient, and reliable, and can be used as an excellent alternative to the HPLC stability testing of losartan potassium in COZAAR® tablets (McCarthy et al., 1998).

3.4.2.2 Analytical Techniques for Simultaneous Estimation of LP with other Drugs

A simple and accurate method for the Simultaneous estimation of losartan potassium (LP) and hydrochlorothiazide (HZ) has been developed by Gandhimathi et al., 2001. The method employs simultaneous equations to estimate these drugs in methanol, losartan potassium and hydrochlorothiazide showed maximum absorbance at 236 and 270 nm respectively. Losartan potassium and hydrochlorothiazide obeyed Beer Lambert's law in the concentration range from 2-20μg/ml and 1-50μg/ml.
respectively. The results of analysis have been validated statistically and by recovery studies.

Accurate and economical procedures for simultaneous estimation of amiloride and losartan potassium in two component tablet formulation have been developed by Gandhimathi et al., 2002. The first method employs solving a set of simultaneous equation. Amiloride has a absorbance maximum at 286 nm and Losartan Potassium has an absorbance maximum at 242 nm in methanol. The second method involved recording of second derivative spectra's of two drugs and measuring the absorbances at zero crossing points. Two sampling point selected were 348 nm and 296.5 nm for amiloride and losartan potassium for simultaneous estimation of two drugs. Both the drugs obeyed Beer's law in the concentration ranges employed for these methods.

Various formulations of losartan potassium as reported in literature are shown in Table. 3.4.2.2.1.

Table. 3.4.2.2.1 Formulations of LP as reported in the literature.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Delivery systems</th>
<th>Reported formulations</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Oral</td>
<td>Tablets</td>
<td>Behera et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Controlled Delivery</td>
<td>Microspheres</td>
<td>Rout and Nayak, 2009</td>
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<tr>
<td></td>
<td>Immediate Release</td>
<td>Matrix tablets</td>
<td>Raju et al., 2010</td>
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<td></td>
<td></td>
<td>Fast Dissolving Tablets</td>
<td>Kakade et al., 2010</td>
</tr>
<tr>
<td>2</td>
<td>Transdermal delivery</td>
<td>Transdermal patch</td>
<td>Vijayan et al., 2010</td>
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<td></td>
<td></td>
<td>Proniosomes</td>
<td>Thakur et al., 2009</td>
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Hence, LP was found to be a suitable drug candidate for transdermal drug delivery based on its favorable physicochemical and pharmacokinetic properties and was therefore selected as a model drug for present studies. To enhance drug delivery rate of a hydrophilic drug, sonophoretic transdermal drug delivery approach was used.