INTRODUCTION
1.1 PAIN

1.1.1 Pain and Its Classification

Pain is a multidimensional phenomenon that may vary in intensity, location, time pattern, and quality. It is the most common symptom for which patients seek medical attention [Mark P; 2005]. Distinguishing between different types of pain is critical for proper treatment; it is classified by its duration into acute and chronic pain. Chronic pain may depend upon its source of production: nociceptive pain is transmitted by nociceptors from the site of injury or tissue damage (for example, inflamed joints in arthritis) while neuropathic pain is initiated or caused by a primary lesion or dysfunction in the nervous system (further subdivided into central and peripheral, involving the central and peripheral nervous systems, respectively); while the visceral pain involves the internal organs and mixed pain is of mixed origin [Fig 1.1] [Harstall C; 2003].

Fig 1.1: Various Types of Pain [Marc R; 2006]

Nociceptive pain is a basic and essential functional of the peripheral and central nervous system. It has a warning function and signalizes imminent or actual tissue damage. As such nociceptive pain preserves body integrity [Manfredi, M; 1981]. Neuropathic pain on the other hand is a pain arising as a direct consequence of a lesion or disease affecting the
somatosensory system [Treede RD; 2008]. Function is altered at different levels of the peripheral and central nervous system.

Neuropathic pain has been shown to impair patients’ overall health-related quality of life, including important aspects of physical and emotional functioning such as mobility and ability to work [O'Connor AB; 2009]. Neuropathic pain has been recently redefined by Neuropathic Pain Special Interest Group (NeuPSIG) of the International Association for the Study of Pain (IASP) as “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”. The current IASP definition is “pain initiated or caused by a primary lesion or dysfunction of the nervous system”. The new definition proposed by NeuPSIG replaces “dysfunction” with “disease” to distinguish neuropathic pain from pain such as that caused by neuroplastic changes in response to strong nociceptive stimulation. The term “nervous system” is replaced by the “somatosensory system” to distinguish neuropathic pain from pain caused by lesions in other parts of the nervous system, e.g., pain associated with muscular spasticity associated with lesions of central motor pathways [Maija H; 2011].

Damage to the somatosensory system represents a potential risk for the development of neuropathic pain, and such damage to the nervous system can be caused by a variety of disorders ranging from simple nerve cuts to complex genetic disorders compromising axonal transport. The sites of the disorders giving rise to neuropathic pain are likewise multiple and dispersed, extending from the bottoms of terminal nerve fibers to the highest centers in the cerebral cortex. Neuropathic pain constitutes a rather well-described symptom constellation despite diversities in causes and anatomy [Finnerup NB; 2010]. Neuropathic pain is as a complex set of abnormal physiologic processes incited by trauma, a noxious event or a disease state. Accordingly, it should not be considered as a syndrome in and of itself, but as a symptom of other neurologic dysfunctions [Nicholson BD; 2003].

Diabetic peripheral neuropathy (DPN) and Post herpetic neuralgia (PHN) are two important clinical neuropathic pain syndromes that have been extensively studied with regard to establishing treatment regimens for neuropathic pain. Painful diabetic
neuropathy is a distal, symmetrical, axonal-sensory neuropathy usually involving the feet and legs initially and later the hands. DPN and PHN are associated with spontaneous, episodic pain and evoked pain, including allodynia in response to touch, cold, or heated tactile stimuli, and light brush (dynamic allodynia). DPN has been defined as including “symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes [Boulton AJM; 1998 & 2005].” The causes of DPN are not clearly understood but are believed to include release of toxic metabolites, alterations in growth factors, a compromised microvasculature, and possible inflammatory events. Although DPN usually involves the extremities, it may also involve thoracic or cranial regions where it presents either symmetrically or asymmetrically, and may occur from ischemia or nerve entrapment in addition to the progression of the disease [Pappagallo M; 2005]. Herpes zoster or shingles is a viral infection whose pathology is characterized by acute inflammation. In a small minority of patients with the painful but self-limiting condition of herpes zoster, the pain persists after the healing of the acute lesions and a chronic pain state develops [Watson CP., 1998]. This pain is referred to as post herpetic neuralgia (PHN). The pain of PHN is unrelenting and is characterized by burning, aching or itching with superimposed lancinating pains. PHN is a very painful neuropathic condition that is still present between 1 and 6 months after the herpes zoster (shingles) has cleared, representing a transition from acute herpes zoster to PHN. It has been described as an intermittent pain or can present as a persistent, but fluctuating, pain. Patients with PHN describe it as burning, itching, and throbbing or a shooting pain, and dyesthesias may be present. Like DPN, PHN has been associated with neuro-inflammation and a loss of large and small sensory fibers. The etiology of neuropathic pain states encompasses many other conditions, including prolonged treatment with chemotherapeutic agents, infections such as HIV, and from idiopathic and genetic sources [Michael HO; 2005]. HIV infection and HIV medications are both associated with the development of neuropathy which usually manifests as distal, symmetrical, predominantly sensory, polyneuropathy [Bailey RO, 1988; Corblath D, 1988; Fuller GN, 1993]. Causative factors include nerve infiltration by HIV and the toxicity of antiretrovirals such as didanosine (ddI) or zalcitabine (ddC) [Fuller GN, 1991; Grafe MR, 1989; Griffin J, 1993; Jamie P, 1992; Rizzuto N, 1995; Simpson D, 1992]. Painful HIV-
associated neuropathy is also a toxicity of antiretroviral therapy and as such it limits patients’ ability to remain on antiviral regimens containing these life saving compounds. It has been documented that painful HIV-associated neuropathy can lead to patient refusal to take anti-retroviral therapy, with potentially life threatening consequences.

1.1.2 Definition of Pain symptoms and signs in patients with neuropathic pain [Ralf B; 2010].

Some of commonly accustomed negative and positive sensory symptoms and its sign are in patients with neuropathic pain are as enlisted in Table 1.

**Table 1.1: Definitions of Important Neuropathic Pain Symptoms & Related Signs**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td><strong>Negative symptoms and signs</strong></td>
<td></td>
</tr>
<tr>
<td>Hypoesthesia</td>
<td>Reduced sensation to non-painful stimuli</td>
</tr>
<tr>
<td>Pall-hypoesthesia</td>
<td>Reduced sensation to vibration</td>
</tr>
<tr>
<td>Hypoalgiesia</td>
<td>Reduced sensation to painful stimuli</td>
</tr>
<tr>
<td>Thermal hypoesthesia</td>
<td>Reduced sensation to cold or warm stimuli</td>
</tr>
<tr>
<td><strong>Spontaneous sensations or pain</strong></td>
<td></td>
</tr>
<tr>
<td>Paraesthesia</td>
<td>Non-painful ongoing sensation (skin crawling sensation)</td>
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<tr>
<td>Paroxysmal pain</td>
<td>Shooting electrical attacks for seconds</td>
</tr>
<tr>
<td>Superficial pain</td>
<td>Painful ongoing sensation, often a burning sensation</td>
</tr>
<tr>
<td><strong>Evoked pain</strong></td>
<td></td>
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<tr>
<td>Mechanical dynamic allodynia</td>
<td>Pain from normally non-painful light moving stimuli on skin</td>
</tr>
<tr>
<td>Mechanical static hyperalgesia</td>
<td>Pain from normally non-painful gentle static pressure stimuli on skin</td>
</tr>
<tr>
<td>Mechanical punctate, pin-prick hyperalgesia</td>
<td>Pain from normally stinging but non-painful stimuli</td>
</tr>
<tr>
<td>Temporal summation</td>
<td>Increasing pain sensation (wind-up-like pain) from repetitive application of identical single noxious stimuli</td>
</tr>
<tr>
<td>Cold hyperalgesia</td>
<td>Pain from normally non-painful cold stimuli</td>
</tr>
<tr>
<td>Heat hyperalgesia</td>
<td>Pain from normally non-painful heat stimuli</td>
</tr>
<tr>
<td>Mechanical deep somatic hyperalgesia</td>
<td>Pain from normally non-painful pressure on deep somatic tissues</td>
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1.1.3 Pathophysiology of Neuropathic Pain

Basic and human research indicates that several mechanisms can lead to neuropathic pain. The condition of neuropathic pain cannot always be explained by a single etiology or specific lesion, but multiple mechanisms contribute, as well as a complex interaction between damaged and non-damaged neurons account for the pain signal [Baron R; 2006]. Furthermore, the condition is complicated by the various distinct cellular changes in the peripheral and central nervous system following a specific injury.

**Fig 1.2: Schematic representation of the generation of pain**

Normal: Central terminals of c-afferents project into the dorsal horn and make contact with secondary pain-signaling neurons. Mechanoreceptive Aβ afferents project without synaptic transmission into the dorsal columns (not shown) and also contact secondary afferent dorsal horn neurons. (B) C-fiber sensitization: Spontaneous activity in peripheral nociceptors (peripheral sensitization, black stars) induces changes in the central sensory processing, leading to spinal-cord hyperexcitability (central sensitization, gray star) that causes input from mechanoreceptive Aβ (light touch) and Aδ fibers (punctuate stimuli) to be perceived as pain (allodynia). (C) C-fiber loss: C-nociceptor degeneration and novel synaptic contacts of Aβ fibers with “free” central nociceptive neurons, causing dynamic mechanical allodynia. (D) Central disinhibition: Selective damage of cold-sensitive Aδ fibers that leads to central disinhibition, resulting in cold hyperalgesia. Sympat, sympathetic nerve. [http://www.endotext.org/diabetes/diabetes31/diabetes31.htm].
A. **Peripheral changes following nerve damage:** Inflammatory and damaged cells in the peripheral nervous system following a lesion or a disease play an important role in the development of neuropathic pain. Cells release their intracellular content in consequence of an injury in the peripheral nervous system, which in turn sensitize nociceptors to further stimulation [Pasero C; 2004]. In addition, a lesion in the peripheral nervous system triggers changes in the number and location of ion channels, especially sodium channels on the damaged C-fibers as well as TREK-1 and TRPV1 channels causing heat hypersensitivity. These channels accumulate along the primary afferent fibers and in the dorsal root ganglion, resulting in a lowered threshold and an increased spontaneous firing, termed ectopic discharges [Costigan M; 2000]. Normally, adjacent afferent fibers have no contact and thereby no impact on the activity of each other. However, after nerve injury, chemical or electrical connections between injured and uninjured nerve fibers may form “cross talk” or ephaptic conduction. Through this connection, the properties of the uninjured afferent fibers are altered and non-painful stimuli may cause excitation of normally “silent” nociceptors [Bridges D; 2001].

B. **Central nervous system responses:** Next to the changes in the periphery, continued nociceptor input into the dorsal horn of the spinal cord increases the responsiveness to incoming stimuli and contributes to plasticity changes in the central nervous system. A major process in the central sensitization is manifested as increased excitability, initiated and maintained by the sensitized primary afferent fibers. These fibers sensitize the spinal cord by presynaptic release of tachykinins (substance P and neurokinin A) and neurotransmitters (glutamate, calcitonin generelated peptide and GABA). Glutamate acts on AMPA receptors, while the tachykinins bind to neurokinin receptors on the postsynaptic membrane. The binding of substance P to its receptors triggers the release of intracellular calcium, thereby increasing the neuronal excitability and facilitating up-regulation of another kind of ionotropic glutamate receptor; the NMDA receptor [Beydoun A; 2003]. Under normal circumstances, glutamate has no effect on NMDA receptors because the receptor channels are blocked by magnesium ions at resting membrane potentials. However, during central sensitization, the increased action potentials remove the magnesium ions, resulting in further influx of calcium ions into the cell. The increased intracellular calcium contributes to maintenance of the central
sensitization, due to its action as secondary messenger. This activates protein kinase C, leading to phosphorylation of the NMDA receptor that leaves the receptor in an open state, due to continuous removal of the magnesium ions [Woolf CJ; 1999]. The central sensitization may manifest in three ways: the threshold to noxious stimuli is lowered, the response to stimuli increased and the area available to receive stimuli enlarged which is evidenced as disinhibition in the spinal dorsal horn and descending facilitation from the brainstem and various plastic changes in the pain processing areas of the brainstem and the cerebral cortex. In conclusion, the pain transmission system involves a number of factors both peripherally and in the central nervous system. These include besides signal disinhibition in the spinal dorsal horn, descending facilitation from the brainstem, plastic changes in the pain processing areas of the brainstem and the cerebral cortex. The exact role of the various functional mechanisms is still not completely understood.

**Fig 1.3:** Cascade of events following peripheral and central nervous system lesion resulting in central sensitization. [AMPA-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; Glu-glutamate; mGlu-metabotropic glutamate; NMDA-N-methyl-D-aspartate].
1.1.4 Current State of Art: Pharmacologic Treatments

The treatment options available for the management of neuropathic pain are almost as diverse as the etiologies. Because of the diversity of the underlying initiating events, patient populations and manifestations of the different types of pain, there is no way to predict the possible outcome of a particular therapy. Although there are many pharmacologic and non pharmacologic therapies available, it has been estimated that sufficient pain relief is obtained in only about one-half of neuropathic pain patients. Because there is no way of predicting the response of an individual to a particular therapeutic intervention, there is no single ideal treatment option. Therapeutic strategies are based on “trial and error” [Dworkin RH; 2002].

Mechanistic Stratification of Drugs used to treat Neuropathic Pain [Debra BG; 2004]

- Drugs that modulate peripheral sensitization by inactivation of voltage-dependent sodium channels- Phenytoin, Tricyclic antidepressants, Lidocaine, Mexiletine, Carbamazepine
- Drugs that modulate central sensitization by interacting with high-threshold N type calcium channels-Lamotrigine, Carbamazepine, Gabapentin.
- Drugs that enhance the descending inhibitory pathways- Opioids, Selective norepinephrine reuptake inhibitors (SNRIs), Selective serotonin reuptake inhibitors (SSRIs), Tramadol, Tricyclic antidepressants.
- Drugs that modulate central sensitization by their effects on NMDA Receptors- Dextromethorphan, Ketamine, Methadone.

![Fig 1.4: Different mechanisms of pain and possible treatments](image)
C fibers are modulated by sympathetic input with spontaneous firing of different neurotransmitters to the dorsal root ganglia, spinal cord and cerebral cortex. Sympathetic blockers (e.g. clonidine) and depletion of axonal substance P used by C fibers as their neurotransmitter (e.g. by capsaicin) may improve pain. In contrast Aδ fibers utilize Na+ channels for their conduction and agents that inhibit Na+ exchange such as antiepileptic drugs, tricyclic antidepressants, and insulin may ameliorate this form of pain. Anticonvulsants (carbamazepine, gabapentin, pregabalin, topiramate) potentiate activity of g-aminobutyric acid and inhibit Na+ and Ca2+ channels, N-methyl-D-aspartate receptors, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors. Dextromethorphan blocks N-methyl-D-aspartate receptors in the spinal cord. Tricyclic antidepressants, selective serotonin reuptake inhibitors (e.g. fluoxetine), and serotonin and norepinephrine reuptake inhibitors inhibit serotonin and norepinephrine reuptake, enhancing their effect in endogenous pain-inhibitory systems in the brain. Tramadol is a central opioid analgesic. α2 antag, α 2 antagonists; 5HT, 5-hydroxytryptamine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; DRG, dorsal root ganglia; GABA, g-aminobutyric acid; NMDA, N-methyl-D-aspartate; SNRIs, serotonin and norepinephrine reuptake inhibitors; SP, substance P; SSRIs, selective serotonin reuptake inhibitors; TCA, tricyclic antidepressants [endotext.org].

Current pain management relies heavily on agents with analgesic properties. Non-narcotic analgesics (acetaminophen and aspirin), narcotic analgesics (opioids), non-steroidal anti-inflammatory drugs (NSAIDs), and thermal agents continue to be the mainstays of pain management [Irena M, 2010]. More recently, other medicines have been added, such as antidepressants, anticonvulsants, topical anesthetics and selective cyclo-oxygenase 2 (COX 2) inhibitors. Since 2004, concern over the cardiovascular safety of the COX 2 class drug has led to a continuous decline in their market share in favour of NSAIDs and opioids. Although current analgesic drugs help many, only one forth of patients with pain achieves adequate relief [Irena M, 2010]. Today, the administration of sodium-channel blockers with topical, regional, epidural, or intrathecal technique is used not only for the control of surgical pain but also for the management of chronic pain conditions [Amir R, 2006].
1.1.5 Current treatment of pain with sodium channel Blockers

The clinical diagnosis and treatment of pain is a difficult challenge because of the variety of mechanisms that underlie the condition, and the fact that different patient groups show diverse responses to the same therapy. Despite the wide-spread use of sodium channel blockers in the treatment of pain, their mode of action and sodium channel specificity have not been fully elucidated [Anindya B; 2009]. Some sodium channel blockers affect calcium-signaling, GPCRs and modulate neutrophil immune responses [Amir R; 2006]. The three main categories of drugs currently prescribed for the treatment of neuropathic pain are anticonvulsants, tricyclic antidepressants and local anesthetics, all of which appear to exert their therapeutic effects by modulating voltage-gated sodium channels [Amir R; 2006].

![Sodium channel inhibitors used in treatment of neuropathic pain.](image)

Sodium channel blockers inhibit the ectopic activity of sodium channels in both injured peripheral and demyelinated neurons, as well as block the over activity of sodium channels mediated by modifications such as phosphorylation and the up regulation of specific isoforms such as the tetradoxotxin resistant sodium channel [Oscar A; 2007]. These abnormalities contribute to prolonged nociceptive depolarization and result in a supralinear increase in neurotransmitter release. Overactive sodium channels remain in a persistently open conformation, which is preferentially bound by sodium channel blockers. Hence, agents that reduce neurotransmitter release from nociceptors generating ectopic pulses, such as topical local anesthetics and anticonvulsants, may relieve neuropathic pain. Topically applied local anesthetics may relieve neuropathic pain by...
Local anesthetics comprise the third major class of voltage gated sodium blockers demonstrating consistent efficacy against neuropathic pain. One report suggests that local anesthetics such as lidocaine, toccainide, and flecainide are more effective against peripheral neuropathic pain than central neuropathy; lidocaine patch has been approved as a topical treatment for post herpetic neuralgia. Its mode of action is through attenuation of both peripheral nociceptor sensitization and CNS hyperexcitability by sodium channel blockade. Lidocaine has been postulated to target sodium channels by stabilizing the open state, although lidocaine could also modify pain hypersensitivity through sodium channel-independent routes [Oscar A; 2007].

1.1.6 Topical Analgesics
Topical analgesics for neuropathic pain are an attractive treatment option because they deliver medication locally and are associated with minimal side effects and drug-drug interactions [Debra B; 2004]. Occasionally, local adverse effects (e.g., redness, itching) are noted. In addition to the FDA-approved 5% lidocaine patch, topical treatments include capsaicin, doxepin, morphine, and isosorbide dinitrate spray. Capsaicin (Zostrix; Medicis Pharmaceuticals), a vanilloid compound isolated from chili peppers, is available in over-the-counter preparations. It is thought to elevate the pain threshold through depletion of substance P from the membranes of C nociceptive fibers and through induction of calcitonin gene-related peptide [Capsaicin Study Group; 1991] Capsaicin cream 0.075% has been evaluated in the treatment of PDN, PHN, postmastectomy pain syndrome, and complex regional pain syndrome, with some benefit demonstrated [Watson CP, 1994]. After application, there is a burning sensation with heat hyperalgesia. The burning sensation, limited pain relief, and difficulty from inadvertently spreading cream to other parts of the body cause many patients to discontinue its use. Doxepin, a TCA drug, has been available in the United Kingdom for several years as a topical formulation for the treatment of eczema [McCleane G; 2003]. Topical application of doxepin alone or in combination with capsaicin was found to be equally effective in neuropathic pain [McCleane G; 2003]. Eutectic mixture of local anesthetics (EMLA;
Astra Zeneca) cream is another topical treatment. EMLA contains lidocaine 2.5% with prilocaine 2.5%. Unlike the lidocaine patch, EMLA produces numbness in the skin over which it is applied. A prilocaine-free topical local anesthetic cream (lidocaine 4%, LMX4; Ferndale Labs) is also available in USA as an over-the-counter product [Debra B. G; 2004]. Advantages include a faster onset and no risk of methemoglobinemia. In a small pilot study, a topical cream containing a combination of amitriptyline (1%) and ketamine (0.5%), when used for 7 days, was effective in relieving neuropathic pain [Lynch ME; 2003]. Clinical trials have demonstrated analgesic efficacy when opioids are applied locally in certain situations (e.g., knee surgery, skin ulcers, and oral mucositis) with more controversial results in other situations. The analgesic efficacy of peripheral opioids appears to increase linearly with the duration of inflammation and little is known about its effect on neuropathic pain [Zhou, L; 1998]. In a pilot study, isosorbide dinitrate applied in a spray form was effective in relieving the neuropathic pain and burning sensation associated with PDN [Yuen; 2002].
1.2 SKIN

The human skin is the largest organ of the body, accounting for more than 10% of body mass, and the one that enables the body to interact most intimately with its environment. Figure 1.2.1 shows a diagrammatic illustration of the skin.

![Skin components and their function](image)

Fig 1.6: Skin components and their function.
1.2.1. The functions of the skin are as follows:

- **Protection**: Skin acts as an anatomical barrier from pathogens and protects damage between the internal and external environment as a body defense system. Langerhans cells in the skin are part of the adaptive immune system [Madison KC, 2003].

- **Sensation**: The skin contains a variety of nerve endings that react to heat and cold, touch, pressure, vibration, and tissue injury.

- **Heat regulation**: The skin contains a blood supply far greater than its requirements which allows precise control of energy loss by radiation, convection and conduction. Dilated blood vessels increase perfusion and heat loss, while constricted vessels greatly reduce cutaneous blood flow and conserve heat.

- **Control of evaporation**: The skin provides a relatively dry and semi-impermeable barrier to fluid loss. Loss of this function contributes to the massive fluid loss in burns.

- **Storage and synthesis**: Acts as a storage center for lipids and water, as well as a means of synthesis of vitamin D by action of UV on certain parts of the skin.

- **Excretion**: Sweat contains urea; however its concentration is $1/130^{\text{th}}$ that of urine, hence excretion by sweating is at most a secondary function to temperature regulation.

- **Absorption**: In addition, medicines can be administered through the skin, by ointments or by means of adhesive patches, such as the nicotine patch or iontophoresis.

- **Water resistance**: The skin acts as a water resistant barrier so that the essential nutrients are not washed out of the body.

1.2.2. Percutaneous Absorption

Although the skin is the most accessible organ of the body to superficial investigations, the direct measurement of penetrating substances has long posed major hurdles for detailed mechanistic studies. In recent decades many investigators have studied the mechanisms, routes and time curves by which drugs and toxic compounds penetrate the skin [Schnetz E; 2001]. Percutaneous absorption is a complex physicochemical and biological process. In addition to partition and diffusion processes, there are other fate of
drug entities entering the skin like irreversible binding to cutaneous proteins such as keratin, degradation by cutaneous enzymes and partition into subcutaneous fat [Venter JP; 2001]. Many \textit{in vitro} and \textit{in vivo} experimental methods for determining transdermal absorption have been used to understand and/or predict the delivery of drugs from the skin surface into the body. The skin acts as a barrier to maintain the internal milieu, however, it is not a total barrier and many chemicals have been shown to penetrate into and through the skin [Poet TS; 2002]. The release of a therapeutic agent from a formulation applied to the skin surface and its transport to the systemic circulation involves:

a. dissolution within and release from the formulation,
b. partitioning into the outermost layer of the skin, SC,
c. diffusion through the SC,
d. partitioning from the SC into the aqueous viable epidermis,
e. diffusion through the viable epidermis and into the upper dermis and
f. uptake into the local capillary network and eventually the systemic circulation.

\textbf{Fig 1.7: Schematic depiction of percutaneous absorption}

In order to rationally design formulations for cosmetic or pharmaceutical purposes, a detailed knowledge of the human skin and its barrier function is imperative [Bouwstra JA; 1997].
1.2.3. Skin and its different Layers

Anatomically, the skin can be divided into subcutis and cutis (Fig 1.8). The subcutis (tela subcutanea) is formed of small lobes of fat (panniculus adiposus) separated by septa of connective tissue. The fat is responsible for thermo-insulation, and the connective tissue incorporates lymph and blood vessels reaching into the dermis. The cutis is divided into dermis and epidermis, which are firmly bound together in the dermo-epidermal junction by hemidesmosomes on the epidermal side and by anchoring collagen fibrils on the dermal side. There are also several associated appendages: hair follicles, sweat ducts, apocrine glands, and nails [Kenneth AW; 2002].

![Figure 1.8: Schematic representation of different layers of Skin](image)

The dermis is about 1-3 mm thick, and consists of cells (fibroblasts, inflammatory cells) and fibers (collagen, elastic, reticular) embedded in an amorphous matrix consisting of mucopolysaccharides produced by the fibroblasts. Also present in the dermis are: blood and lymph vessels, free nerve endings for the perception of temperature, itching and pain, encapsulated nerve endings such as the Vater-Pacini corpuscles (sensitive to pressure and vibration) and the Meissner’s corpuscles (sensitive to touch), nerves for the vegetative innervation, and muscles (M. arrector pili, mimetic muscles). Skin appendages (hair,
sebaceous glands, sweat glands, nails) originate in the dermis or in the upper subcutis (sweat glands). Structurally, the dermis comprises the deeper situated, thicker stratum reticulare (few cells except fibroblasts, many fibers) and the stratum papillare (many cells, capillaries, nerves), located just below the epidermis [Batisse D; 2002].

The epidermis is the outermost skin layer and is a vessel-free, nerve-free, stratified, squamous epithelium with a water content of 70%. It is nourished by the underlying capillary loops of the stratum papillare. The thickness of the epidermis varies depending on the anatomical region, with mean values of 77 µm at the forearm, minimal values of 30 µm at the eye lid and maximal values of 1.6 µm at the plantar region [Batisse D; 2002]. Two kinds of cells make up the epidermis. First, the keratinocytes (90%), which are responsible for keratin production and are kept together by desmosomes. Second, the dendritic cells (10%): melanocytes (pigment cells), Langerhans cells (immunocompetent cells), and Merkel’s cells (responsible for the perception). The layers that characterize the epidermis include: the stratum basale (basal layer) with one cell layer, the stratum spinosum (prickle cell layer) with 2-5 cell layers, the stratum granulosum (granular layer) with 1-3 cell layers, the stratum lucidum (in palmar and plantar skin only), and the stratum corneum (corneal layer) with 10-20 cell layers. In a cycle of about 1 month, new keratinocytes originate in the stratum basale, differentiate in the stratum spinosum, produce keratohyalin containing granules and lipid/ enzymes-containing lamellar bodies (Odland bodies), which are then exocyted in the stratum granulosum and are finally transformed into the stratum corneum [Batisse D; 2002].

1.2.4. **Stratum corneum, the skin barrier**

The excellent barrier property of the skin resides in the outermost layer, the stratum corneum. This unique membrane is only some 20 µm thick but has evolved to provide a layer that prevents from losing excessive amounts of water and limits the ingress of chemicals it comes into contact. It is composed of dead, flattened, keratin-rich cells, the corneocytes. These dense cells are surrounded by a complex mixture of intercellular lipids. They comprise ceramides, free fatty acids, cholesterol, and cholesterol sulphate. Their most important feature is that they are structured into ordered bilayer arrays [Jonathan H; 2002]. The outer layer of the skin, the stratum corneum, forms the rate-
controlling barrier for diffusion for almost all compounds. It was not until the 1940’s that the stratum corneum clearly emerged as the specific site of the skin barrier for both endogenous and exogenous compounds [Windsor T; 1944 Blank IH; 1952]. In the 70’s, the intercellular lipids were recognized as the primary site of the barrier [Elias P; 1975]. The qualitative and quantitative organization of the intercellular lipid lamellae is determinant for the barrier function. Several models such as the domain-mosaic [Forslind B; 1994], the sandwich [Bouwstra J; 2001], and the single-gel-phase model [Norlen L; 2001] have been proposed to explain their molecular organization [Norlen L; 2003]. The precise mechanisms by which drugs permeate the stratum corneum are still under debate but there is substantial evidence that the route of permeation is a tortuous one following the intercellular channels. However, the tortuosity alone cannot account for the impermeability of the skin. The intercellular channels contain a complex milieu of lipids that are structured into ordered bilayer arrays [Cornwell PA; 1994]. It is the combination of the nature of these and the tortuous route that is responsible. A diffusing drug has to cross, sequentially, repeated bilayers and therefore encounters a series of lipophilic and hydrophilic domains. The lipid–water partitioning characteristics of the permeant are a dominant determinant of its penetration or that mathematical models developed to predict percutaneous absorption include a term to describe partitioning. Fick’s laws of diffusion describe the diffusional step [Jonathan H; 2002].

1.2.5. Routes of penetration into the skin

There are 3 potential routes of penetration from the skin surface into the epidermis (Fig 1.9): 1) the intercellular route, 2) the transcellular route, and 3) the transappendageal route through either the eccrine (sweat) glands or the hair follicles with their associated sebaceous glands.

- **Intercellular pathway**

The intracellular SC spaces were initially dismissed as a potentially significant diffusion pathway because of the small volume they occupy. However, the physical structure of the intracellular lipids is a significant factor in the barrier properties of the skin [M. S. Roberts; 2002]. The solute remains in the lipid domains and permeates via a tortuous pathway. Within this lipid domain, the drug has to cross repetitively complete lipid bilayers. Available evidence has shown [R. H. Guy; 1992] that there is a preponderance
of support for the intercellular pathway and it has been identified as the major route of 
transport across the SC. The intra cellular route is usually regarded as a pathway for polar 
(hydrophilic) molecules, since cellular components are predominantly aqueous in nature.
Here the pathway is directly across the SC, the rate-limiting barrier being the multiple 
bilayered lipids that must also be crossed.

- **Transcellular pathway**
Transcellular diffusion mechanisms dominate over the intercellular and transappendageal 
routes during the passage of solutes through the SC [Roberts MS; 2002]. The permeant 
crosses the SC by the most direct route and repeatedly partitions between and diffuses 
through the cornified cells, the extracellular lipid bilayers, viable epidermis and papillary 
layer of the dermis, with the microcirculation provide an infinite sink [Moghimi HR; 1999]. Although the transcellular route appears most favoured on geometric grounds, 
there has been no direct evidence presented to provide support for its participation in the 
SC penetration process. However the so-called ‘protein domain’ of the SC represents a 
region into which topically applied molecules may partition and therefore act as a 
reservoir. Additionally, certain penetration enhancers (e.g., anionic surfactants and alkyl 
sulphoxides) have been shown to interact with keratin and induce protein conformational 
changes. The presence of these materials could increase the likelihood that permeates 
access the transcellular route [Roberts MS; 2002].

- **Appendageal pathway**
The penetrant transverses the SC via a ‘shunt’ pathway: e.g., a hair follicle or a sweat 
gland. These shunts are important at short times prior to steady state diffusion. The 
available diffusional area of the shunt route is approximately 0.1% of the total skin area 
and therefore the contribution to drug permeation compared to the former is significantly 
less [Barry BW; 2002, Hadgraft J; 2001]. Despite their small fractional area, the skin 
appendages may provide the main portal of entry into the subepidermal layers of the skin 
for ions and large polar molecules. The appendageal pathway has been reported to be the 
major contributor to the initial phase of SC permeation [Roberts SM; 2002].
The precise mechanisms by which drugs permeate the SC are still under debate but there is substantial evidence that the route of permeation is a tortuous one following the intercellular channels [Rathbone MJ; 2003 & Hadgraft J; 2004]. The intercellular route consists of a tortuous route along the cornified envelope-armored corneocytes through the structured intercellular lipid bilayers [Ouriemchi EM; 2000]. Although the intracellular route [Chien YW; 1987] has been identified as the major contributor to percutaneous permeation, the other pathways also contribute. The three pathways are not mutually exclusive and most molecules will pass through the SC by a combination of these routes. The existence of these pathways for permeation across skin has significant implications in the design, development and use of penetration enhancers. An enhancer that acts primarily on one pathway, e.g., by increasing the fluidity of the extracellular lipid, will have any great effect on the permeability rate of a compound whose route is primarily transcellular. Furthermore, it is entirely feasible that the presence of an enhancer may alter the thermodynamic activity of a penetrant in a formulation resulting in changes in partitioning tendencies [Hadgraft J; 2004].
1.2.6. Mathematical principles in transmembrane diffusion

1.2.6.A. Fick’s laws of diffusion

After application of a topical formulation, the active compound has to be released from the vehicle, partition between vehicle and stratum corneum, and diffuse through (and partition between) the different layers of the skin before it can exert its pharmacological action, finally being “excreted” into the systemic circulation. Diffusion is a passive kinetic process taking place along a concentration gradient from a region of higher concentration to a region of lower concentration. The diffusion through the skin can be described by Fick’s first law:

$$J = -D \frac{dc}{dx} = \frac{\dot{m}}{A} \frac{1}{dt}$$

……. Eq. 1

where $J$ is the steady state flux of the compound mass (m) through the stratum corneum per unit area (A) and unit time (t) (mg/cm²/s), D is the diffusion coefficient of the compound in the stratum corneum (cm²/s), c is the drug concentration, and x is the position. The solution of the equation with the appropriate boundary conditions gives:

$$J = \frac{K D}{h} \cdot \Delta c = k_p \cdot \Delta c$$

……. Eq. 2

where K is the partition coefficient of the compound between vehicle and stratum corneum, h is the diffusional pathlength (cm), kp is the permeability coefficient, and $D_c (= c_{appl} - c_{rec})$ is the concentration difference (mg/cm²) across the stratum corneum between applied concentration ($c_{appl}$) and concentration below the stratum corneum ($in vivo$) or in the receptor phase ($in vitro$, $c_{rec}$). Under normal circumstances, the applied concentration ($c_{appl}$) is much larger than the concentration in deeper skin layers, and $D_c$ can be replaced with $c_{appl}$. The real diffusional pathlength (h) is the tortuous pathway along the intercellular lipids, which is longer than the mere stratum corneum thickness.

If the steady state is not attained, the diffusional flux can be explained by Fick’s second law, which describes the concentration change over time at a definite position, x within the membrane:
Different solutions of this equation with appropriate boundary conditions have been proposed.

### 1.2.6.6. Higuchi model

Higuchi [Higuchi WI; 1962] describes drug release as a diffusion process based on Fick’s law, square root time dependant. This relation is used to describe the drug dissolution from modified release pharmaceutical dosage forms like transdermal systems and matrix tablets with water soluble drugs. For drug release from an ointment in which the drug is initially uniformly dissolved is governed by equations 4 & 5 where \( Q \) is the amount of drug released per unit area of application, \( h \) is the thickness of layer, \( C_0 \) is the initial concentration of the drug in the ointment, \( D \) is the diffusion co-efficient of drug in the ointment, \( t \) is the time after application and \( R \) is the percent drug released [Qvist MH; 2002].

\[
Q = 2C_0 \left( \frac{Dr}{\pi} \right)^{\frac{1}{2}}
\quad \text{……Eq. 4}
\]

\[
R = 200 \left( \frac{Dt}{nh^2} \right)^{\frac{1}{2}}
\quad \text{……Eq. 5}
\]

If the rate of drug release obeys this law, the amount of drug released is a linear function of \( t^{1/2} \), and \( D \) can be calculated from the slope. The assumptions in this treatment are that the drug is the only component diffusing out of the vehicle, that sink conditions are maintained in the receptor phase and that \( D \) is constant with respect to time and position in the vehicle [Ricci EJ; 2005]. Permeation of this nature has a characteristic curved profile, exhibiting relatively high flux at early contact times which decreases as the diffusant front regresses into the bulk vehicle, away from the membrane. The path is progressively more tortuous and it takes longer for drug molecules to diffuse from the region of high concentration in the vehicle to replenish the drug molecules at the
membrane interface that have partitioned into the membrane, therefore the flux rate decreases with time [Ricci EJ; 2005].

1.2.6.C. Physicochemical parameters important in dermal absorption

The most basic diffusion equation is Fick’s 1st law which describes steady state flux per unit area (J) in terms of the partition of the permeant between the skin and the applied formulation (K); its diffusion coefficient (D) in the intercellular channels of diffusional pathlength (h); the applied concentration of the permeant in the vehicle (c_{app}) and the concentration of the permeant in the receptor phase (c_{rec})

\[ J = KD \frac{(c_{app} - c_{rec})}{h} \quad \text{………(Eq. 6)} \]

In most circumstances \( c_{rec} << c_{app} \) and Eq. (6) is often simplified to

\[ J = k_p c_{app} \quad \text{…………(Eq. 7)} \]

Where, \( k_p = KD/h \) is the permeability coefficient. This parameter (from an aqueous donor phase) may be estimated by an empirical relationship described by Potts and Guy [Potts RO; 1992]

\[ \log \left[ \frac{k_p}{(\text{cm h}^{-1})} \right] = 2.7 + 0.71 \log K_{oct} - 0.0061 \text{MW} \quad \text{………(Eq. 8)} \]

Where, \( K_{oct} \) is the octanol water partition coefficient and MW the molecular weight.

The maximum flux for a compound is when \( c_{app} \) is equal to the solubility. Important physicochemical properties affecting diffusion are partition coefficient, diffusion coefficient, and solubility. Large molecules will tend to diffuse slowly, hence the MW term in Eq. (8), molecules with good solubility in both oils and water will permeate well. These are compounds with low melting point. Eq. (6) or (8) indicate that a high partition coefficient favors a high flux, however, large values of \( K \) produce molecules that have poor solubility and in general molecules with a \( \log K_{oct} \sim 1–3 \) have the optimum partition behavior. This also fits with the notion, stated nearly half a century ago, that a balanced solubility in both oils and water is desirable [Hadgraft JW; 1956]. Many permeants are weak acids or weak bases. Permeation will depend on the degree of ionization and how ionization influences the solubility in the applied phase and its partition into the skin. There have been few studies investigating this and indicate that it is beneficial to apply the drug in its ionized form, in which state it will be much more soluble but with a lower permeability coefficient. One of the problems involved in interpreting permeation data of
ionized compounds is that the species that permeate will be a composite of the free acid (or base) of the ionized material and ion pairs that can exist with counter ions present either in the formulation or in the skin [Barker N; 1983].

1.2.7. Limitations of skin as a delivery mode

a. The Epithelial barrier

The main route of permeation through the skin for small molecules is via the intercellular pathway [Albery WJ; 1979, Bodde H; 1989]. However, there is evidence that for some compounds the intracellular domain is also important [Perkins NC; 1999] and several mechanisms might be working in parallel [Degim IT 2003]. The intercellular space contains a mixture of lipids that can be organized to provide hydrophilic as well as lipophilic domains. This organization of lipids dictates the required physicochemical properties of a molecule to ensure that it can diffuse rapidly through the skin. Generally, suitable candidates for transdermal permeation are small molecules with good water and lipid solubility. These solubility characteristics are often also indicated by the possession of a low melting point, typically < 200°C [Finnin, BC; 1999].

b. Drug-related factors: partitioning

The two drug-related properties that influence flux across the skin are the concentration gradient of drug within the skin and the diffusivity, in accordance with Fick’s Law. The concentration gradient is influenced by the ability of the drug to partition into the skin [Surber, C; 2000] and its ability to partition out of the skin into the underlying tissues [Müller, B; 2003]. The octanol–water partition coefficient is used to predict this partitioning behaviour within the skin. Thus, there is a parabolic relationship between the octanol–water partition coefficient as expressed by log P and the penetration rate [Kim MK; 2000]. Compounds with low log P exhibit low permeability because there is little partitioning into the skin lipids. However, compounds with high log P also give low permeability due to their inability to partition out of the stratum corneum. The generally accepted range of log P for maximum permeation is between 1 and 3 [Guy RH; 1987 & 1988]. The partitioning of the drug into the skin will also be influenced by its thermodynamic activity in the application vehicle. This can be improved by increasing the concentration of drug in the applied vehicle or by manipulating the vehicle to reduce
drug solubility. The diffusivity of the drug can be enhanced by using permeation enhancers [Aungst BJ; 1990] or by physical enhancement methods such as iontophoresis. Whereas most work with enhancers has focused on mildly lipophilic drugs, a combination of the enhancers, propylene glycol and lauric acid has demonstrated the potential to enhance the permeability of highly lipophilic drugs [Funke AP; 2002]. This is attributed to their synergistic lipid fluidizing activity within the stratum corneum.

c. Drug-related factors: diffusivity

The chemical structure of the drug also influences the diffusivity [Katz M; 1965, Scheuplein RJ; 1965], due to interactions between the polar head groups of the intercellular lipids with hydrogen-bond forming functional groups present in the drug structure [Du Plessis J; 2002]. Although the skin represents a suitable target for drug delivery, the functional properties that enable it to act as an excellent barrier also serve to limit the access of drugs into and across the epidermis. A closer examination of the skin surface reveals a complex combination of a range of cell types. The outer layer, the stratum corneum, is a membrane ~20 μm thick, which is the main contributor to the skin’s impermeability.

d. Occlusion

To improve the efficiency of TDD systems, traditional TDD products relied mainly on their occlusive nature to increase the permeability of the drug candidates. Although the mechanism by which occlusion increases the diffusivity of many drugs is not known, some of the effects of occlusion include: water accumulation within the skin leading to increased water content and swelling of the corneocytes and increased water content of the intercellular matrix [Tsai TF; 1999]; increase in skin temperature and decreased evaporative loss of co-solvents [Taylor LJ; 2002]. However, occlusion causes an increased propensity for skin irritation at the application site due to the affects of the accumulated water or to trapped sweat. This is a major hurdle to the patient acceptance of occlusive TDD systems and recent efforts have focused on the development of newer generation products with less potential for this reaction [Matsumara H; 1995].
1.3 TRANSDERMAL/TOPICAL DRUG DELIVERY SYSTEMS

The skin offers several advantages as a route for drug delivery. In clinical drug therapies, topical application allows localized drug delivery to the site of interest. This enhances the therapeutic effect of the drug while minimizing systemic side effects. The problems associated with first-pass metabolism in the GIT and the liver are avoided with TDD and this allows drugs with poor oral bioavailability to be administered once a day and this may result in improved patient compliance. Transdermal administration avoids the vagaries of the GIT milieu and does not shunt the drug directly through the liver. The circumvention of the drug from the hostile environment of the GIT minimizes possible gastric irritation and chemical degradation or systemic deactivation of the drug [Naik A; 2000, Finnin BC; 1999 & Guy RH; 1996, Thomas BJ; 2004, Langer R; 2004]

![Schematic diagram showing sites of action when the skin is exposed to different molecules](image)

Fig 1.10: Schematic diagram showing sites of action when the skin is exposed to different molecules [Yuri G. A; 2013]

As with the other routes of drug delivery, transport across the skin is also associated with several disadvantages. For transdermal delivery, as a rule of thumb, the maximum daily dose that can permeate the skin is of the order of a few milligrams. This further underscores the need for high potency drugs. As evidence of this, all of the drugs presently administered across the skin share constraining characteristics such as low molecular mass (< 500 Da), high lipophilicity (log P in the range of 1 to 3), low melting point (< 200°C) and high potency (dose is less than 50 mg per day) [Boucaud A; 2004 Prausnitz MR; 2004]. The smallest drug molecule presently formulated in a patch is nicotine (162 Da) and the largest is oxybutinin (359 Da). Opening the transdermal route
to large hydrophilic drugs is one of the major challenges in the field of TDD. The required high potency can also mean that the drug has a high potential to be toxic to the skin causing irritation and/or sensitization. Other difficulties encountered with TDD are the variability in percutaneous absorption, the precision of dosing, the reservoir capacity of the skin, heterogeneity and inducibility of the skin in turnover and metabolism, inadequate definition of bioequivalence criteria and an incomplete understanding of technologies that may be used to facilitate or retard percutaneous absorption [Morganti P; 2001].

1.3.1. Factors affecting Skin Permeability:

A. Factors associated with the vehicle:

**Contact with body surface:** The prime function of a semisolid dermatological vehicle is to ensure close contact with the skin, thus providing protection and facilitating penetration of medicament. Drug penetration is enhanced if the vehicle easily covers the skin surface, mix readily with the sebum and brings the drug into contact with the tissue cells.

**Hydration of stratum corneum:** Increased hydration of the stratum corneum appears to open up dense closely packed cells and increases its porosity that leads to enhanced skin penetration. An occlusive layer of a dermatological product, by reducing evaporation of water from the skin into the atmosphere, increases hydration of the horny layer and therefore, promotes penetration of the drug [Zhai H; 2002].

**Excipients:** Common solvents and surfactants can affect penetration of drugs through the skin.

**Penetration enhancers:** Increase skin permeability by reducing the diffusional resistance of the stratum corneum, by reversibly damaging it, or by altering its physic-chemical nature (Barry 1983; Martin 1993). Some of the examples are dimethyl sulfoxide, dimethyl acetamide, dimethyl formamide, phosphine oxides, propylene glycol and ethanol.

B. Factors associated with the skin

**Skin age:** Skin of the fetus, infant, young and the elderly is more permeable than the adult tissue.
**Skin condition:** The permeability is affected by disease, climate and injury. A combination of abnormal cell membrane phospholipids and abnormal stratum corneum increases skin permeability.

**Regional skin sites:** Drug penetration varies with body site. Variations in cutaneous permeability will depend on the thickness of stratum corneum, its nature and to some extent on the density of skin appendages.

**Skin metabolism:** The biotransformation of compounds in the skin produces inactive metabolites, but sometimes active compounds can be formed. Oxidation, reduction, hydrolysis and conjugation are kinetic processes that compete with the transport of drugs across the skin.

**Circulatory effects:** Theoretically changes in the peripheral circulation, or blood flow through the dermis could affect percutaneous absorption. Thus an increased blood flow could raise the concentration gradient across the skin.

**Hydration of the horny layer:** The permeability of a drug depends on the hydration of the stratum corneum, the higher the hydration the greater the permeability [Smith WP; 1982].

**Species differences:** Mammalian skin from different species display wide differences in anatomy in such characteristics as the thickness of the stratum corneum, the numbers of sweat glands and hair follicles per unite surface area. Laboratory animals such as rats, mice and rabbits have more hair follicles than human skin and they lack sweat glands, also there are biochemical differences between human and animal skin. Skins of laboratory animal’s guinea pigs, rat and rabbit are more permeable than human skin, rabbit skin being the most permeable to topically applied compounds. Skin from the monkey and the pig is closest to that of man.

**Skin temperature:** Rise in temperature increases the permeability which may be attributed to the thermal energy required, diffusivity and the solubility of the drug in the skin tissue [Barry BW; 1983]. Increasing the temperature of the skin has been shown to increase the rate of penetration by a direct effect on the diffusion within the skin [Scheuplein RJ., 1965]. Temperature can also affect the structure of the stratum corneum, particularly the crystalline structure of the lipid bilayers [de Jager MW; 2004], which can lead to higher permeability. Clinically, skin temperature increases in diseased states as
under occlusive dressings as sweat cannot evaporate, also heat cannot radiate as rapidly resulting in rise in surface temperature by a few degree with consequent increase in permeability.

C. Factors associated with the drug

**Partition co-efficient:** Membrane partition coefficient increases exponentially as the length of the lipophilic alkyl chain increases. Permeability coefficient shows a linear dependency on the partition coefficient. The drug should possess some degree of solubility in both lipid and water and also have greater affinity towards the skin which is essential for effective percutaneous absorption. The drug substance should have a greater physicochemical attraction to the skin than to the vehicle in which it is presented in order for the drug to leave vehicle in favour of the skin. Thus partition coefficient influences the rate of transport across the absorption site.

**Solubility of the drug in stratum corneum:** Follicular penetration is favoured by high lipid solubility while penetration directly through the epidermis is facilitated by balanced lipid and water solubilities.

**Concentration of the drug:** Drug concentration plays an important role in penetration through the skin. Fick’s law of diffusion states that the driving force responsible for the transfer of substances is proportional to the concentration gradient. Thus the amount of drug percutaneously absorbed per unit surface area per unit time increases as the concentration of the drug in the vehicle is increased.

**Particle size:** Reducing the particle size of a poorly soluble drug in formulation improves the therapeutic activity by increasing the dissolution rate and therefore, the release from the vehicle (Carter et.al., 1975).

**pH and Dissociation constant:** Application of solution with very high or low pH values can be destructive to the skin. The pH conditions of the skin surface and of the drug delivery system affect the extent of dissociation of ionogenic drug molecules and their skin permeability. pH of the dermal formulation must be between 5.5 – 6.6. According to the pH partition hypothesis, only the unionized form of the drug is able to cross the lipoidal membranes in significant amounts. Therefore penetration of the ionic drugs is influences by its dissociation constant and pH of its surrounding.
**Binding of drug to the skin:** The skin may act as a reservoir for some drugs that are able to bind to macromolecules. The drug fraction bound is not able to diffuse and thus binding hinders the permeation rate of molecules. The interaction between a drug and the skin can be expected to range from weak physical attractions of the van der Waals type to strong chemical bonding.

**D. Other factors**

**Area of application:** More amount of drug is absorbed if the drug substance is applied to a larger surface area.

**Amount of rubbing in / inunctions:** The longer the period of inunctions, the greater the absorption.

**Time of contact:** If the medicated preparation is permitted to remain in contact with the skin for longer period of time, the absorption is enhanced.

**Application frequency:** Multiple applications dosing or dosing the same site more than once a day rather than single application can increase the drug absorption.

**1.3.2. Approaches to overcoming the barrier**

A number of techniques have been developed to enhance and control transport across the skin, and enlarge the range of drugs delivered. These involve chemical and physical methods, based on two strategies: increasing skin permeability and/or providing driving force acting on the drug [Foldvari M; 2000]. There have been many ingenious technologies developed to enhance TDDS for therapeutic and diagnostic purposes ranging from chemical enhancers to iontophoresis, electroporation, and pressure waves generated by ultrasound effects or the synergistic mixtures of both the mechanism. The fate of effectiveness of TDD system lies on the drug's ability to invade the skin barrier and how its reaches the targeted site [Prausnitz MR; 2004].

**A. Physical Approaches—Active Penetration Enhancement Technique**

Physical enhancement methods have been studied that involve the use of an energy source to overcome the barrier properties of the skin. These methods rely on providing a reservoir of drug on the skin surface, from which the required levels of delivery can be achieved. The most notable advancements in product development are approaches such as iontophoresis and electroporation, typically for the delivery of large molecular weight or highly potent compounds. Low-frequency sonophoresis using ultrasound has also
demonstrated the capacity to enhance drug delivery [Mitragotri S; 2000]. An alternative approach involves the use of microfabricated microneedles, which can be inserted into the skin, thereby producing a channel for drug transport across the stratum corneum [Henry S; 1998]. The microneedles have been designed to penetrate only the outer layers of the skin, that is, the stratum corneum. The nerves reside much deeper within the skin and consequently this method provides painless administration. Radio-frequency has also been used as a means of enabling the transdermal delivery of lipophilic drugs, through the generation of microchannels across the stratum corneum [Henry S; 1998]. Sintov et al. [Sintov, AC; 2003] have described significant in vivo enhancement of skin permeability for both of the poorly penetrating drugs granisetron and diclofenac after pretreatment of rat skin with radiofrequency electrodes. Powderject’s technology [powderject.com] takes a slightly different approach to breaching the barrier by the use of high velocities to force particles across the stratum corneum [Sarphie DF; 1997].

**B. Chemical Approaches**

Chemical penetration enhancers have provided the basis for considerable research in the TDD area. These are compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant. Penetration enhancers are incorporated into a formulation to improve the diffusivity and solubility of drugs through the skin that would reversibly reduce the barrier resistance of the skin. Thus allow the drug to penetrate to the viable tissues and enter the systemic circulation [Amit A; 2012].

![Fig 1.11: Schematic representation of various penetration strategies through the human skin. [Mark RP; 2004].](image)

- **a** | Transdermal diffusion in the presence of a chemical enhancer,  
- **b** | Low-voltage electrical enhancement by iontophoresis,  
- **c** | High-voltage enhancement by electroporation,  
- **d** | Microneedles and thermal poration
The ideal candidate would provide a reversible reduction in the skin’s barrier properties without long-term damage to the viable cells. Effort has focused on the identification of chemicals or combinations of chemicals capable of acting as penetration enhancers and the prediction of their efficacy [Yu B; 2002 & 2003]. Few compounds have been successfully incorporated into marketed products, partly because of the difficulty in predicting in vivo behaviour under conditions of use from the in vitro permeation tests is used as screens for enhancement. It has also proved a difficult task to balance the formulation characteristics to ensure that the drug retains its tendency to partition from the vehicle in the presence of the permeation enhancer. Permeation enhancers fall into two major categories: those that impact diffusion across the stratum corneum and those that alter partitioning into the stratum corneum. The former class generally comprises a long alkyl chain capable of intercalating with the long chains of the intercellular lipids, in addition to a polar head group that is capable of interacting with the lipid polar head groups [Walters KA 1989]. This serves to disrupt the ordered nature of the skin lipids, increasing the fluidity and hence assisting permeation of the drug. The latter class of permeation enhancers works by affecting the solubility properties of the skin, thereby increasing the solubility of the drug within the stratum corneum. Some of the examples of the widely used classical enhancers involve various classes that include, hydrocarbons, alcohols, acids amines, amides, esters, surfactant terpenes, terpenoids and essential oil, sulfoxides, lipids and miscellaneous such as cyclodextrin derivatives, chitosan etc [Amit A; 2012].

The rationale for development of topical drug delivery systems is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery systems or by modifying the physiological/ physiochemical parameters inherited in the selected drug delivery systems.

Development of TDDS is multidisciplinary activity that encompasses fundamental feasibility studies starting from the selection of drug molecule to the demonstration of sufficient drug flux in an ex vivo and in vivo model followed by fabrication of a drug delivery system that meets all the stringent needs that are specific to the drug molecule.
(physicochemical and stability factors), the patient (comfort and cosmetic appeal), the manufacturer (scale up and manufacturability) and most important the economy.

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes [Ansel H.C, 2000]. Although some unintended drug absorption may occur, it is sub therapeutics quantities and generally of minor concern. Topical dosage forms, an alternative to conventional formulations, are becoming popular because of their unique advantages. Various novel topical/transdermal dosage forms currently developed as novel drug delivery systems include nano-gels, sprays and patches.

C. Passive penetration enhancement techniques

Recent technologies which are currently under investigation, ranging from chemical enhancers which either increase the diffusivity across the skin or increase the drug solubility in the skin [Moser K; 2001] to newer innovative approaches which involve the extension of this concept to the design of super loaded formulations, micro emulsion [Zhao X; 2006] and vesicular systems.

i. Transdermal/Topical Patch

TDDS or skin patch is used for the delivery of a controlled dose of a drug through the skin over a period of time [Meenakshi B; 2010, Ajay B; 2012]. The components of TDDS are liners, adherents, drug reservoirs, drug release membrane etc. [Wokovich AM; 2006] that play an imperative role in the release of the drug through the skin. It is considered that a well-designed TDDS can supply the drug at a rate, to sustain the required therapeutic plasma concentration without much fluctuation that may cause basic manifestation or therapeutic inefficacy [Thomas BJ; 2004]. A topical patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the bloodstream. The first commercially available topical prescription patch was approved by the U.S. Food and Drug Administration in December 1979, namely scopolamine patch for motion sickness [Segal M; 2007]. The adhesive is covered by a release liner, which needs to be peeled off before applying the patch on the skin. Designing and development of topical patches has been described as state of the art.
ii. Inclusion complexes
Cyclodextrins influence the percutaneous absorption basically by the two mechanisms. Firstly by solubilizing the drug thereby increasing its accessibility at the absorption site, and secondly by an interaction with the free lipids present in the SC [Swartzendruber DC; 2004]. Thus, it stabilizes the drug and also reduces drug irritancy.

iii. Eutectic mixtures
Transformation of solid drugs into a highly concentrated oily state at ambient temperatures exhibits improved skin permeability due to their high thermodynamic activity in the vehicle. Melting point of a drug is inversely proportional to its solubility and lipophilicity in lipids. As a consequence, lowering the melting point exhibits increased transdermal permeation. These systems are interesting since they serve two mechanisms by which skin permeation of an active drug across skin can be enhanced. First is the formation of a low-melting mixture with the drug which improves its partitioning across the skin. Second is the direct disruption of the skin structure which further enhances drug permeation. This synergy in mechanism can be exploited by selecting the right permeation enhancer or enhancers to be combined with the drug.

iv. Nanoparticles
Nanoparticles possess inimitable properties of promoting drug absorption, allowing sustained drug release for prolonged time period and protecting the encapsulated substance from chemical degradation hence they have the potential in effectively delivering drugs across the skin. It is also an alternative system not requiring the permeation enhancers or temporary skin digestion, both of which can increase the possibility of irritation. Nanoparticles for pharmaceutical applications range from the size and shape of a (spherical) micelle through to 1 μm. In a suspension they are always much longer lived than micelles. This can be a consequence of polymeric conjugation (e.g. in polymeric nanoparticles, such as latex, or in the special purpose polymer particles [M. Green; 2009]); poor solubility of the components that form nanoparticles core is the other common reason (e.g. in the solid (ordered phase) lipid particles sized between 50 nm and 1000 nm [Siekmann B; 1998 Dingler A; 1999]). Deliberate epicutaneous use of nanoparticles dates back at least to the introduction of the first modern sun-blockers several decades ago. Epicutaneous pharmacological applications that are all based on
organic particles sometimes focus on minimizing the depth of cutaneous penetration as well [Jenning V; 2000 Müller RH; 1998], but otherwise seek to enhance skin permeation, mainly via a (semi)occlusive superficial film formation.

v. Microemulsions

They are optically clear, and must consequently contain only structures smaller than ~300 nm. Droplets in simple microemulsions resemble (mixed) micelles, except that they contain an extra oily component. With decreasing water or increasing oil content the originally (quasi)globular structures transform into bicontinuous microemulsions that at some point become turbid. The characteristic structural length is in either case composition- and temperature-dependent and broadly proportional to relative oil-concentration in the system. The suspension structure is controlled by the average surface curvature, or tension. A well balanced microemulsion therefore lacks spontaneous curvature and has a very low interfacial tension [Peter U; 2001]. Microemulsions, being akin to traditional dermal preparations such as lotions, ointments and creams, have been in use for transdermal drug delivery for a long time; their popularity for dermal applications is second only to liposomes. Recently other continuous mesophases (cubic, hexagonal, sponge) have attracted attention as they can ensure an unusually intimate contact between the epicutaneous drug reservoir and skin lipids [Kreilgaard M; 2002].

vi. Vesicles

One of the most divisive methods is the use of vesicle formulations as skin delivery systems. The justification for using vesicles in dermal and transdermal drug delivery is many folds. Vesicles might (a) act as drug carriers to deliver entrapped drug molecules into or across the skin; (b) act as penetration enhancers following the penetration of the individual lipid components into the SC and subsequently the alteration of the intercellular lipid lamellae within this skin layer; (c) serve as a depot for sustained release of dermally active compounds; and (d) serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system. Vesicles can be prepared by a wide variety of lipids and surfactants. Most commonly, they are composed of phospholipids or non-ionic surfactants such as span 80 and are referred to as liposomes and niosomes and phospholipids, and ethanol and water in case of ethosomes. Liposomes are the most popular nano-sized drug carrier aggregates.
They are always vesicular in structure, i.e. comprise of one or several lipid bilayer(s) without surface tension enclosing an aqueous core. Conventional pharmaceutical liposomes have stiff bilayers (to prevent undesirable drug leakage), are nearly spherical (due to bilayer elastic energy far exceeding the thermal activation threshold), and normally have an average diameter above 75 nm.

Micelles form spontaneously near and above amphipaths solubility limit. Polar surfactants in water or relatively apolar surfactants in oil thus take a variety of shapes (cylindrical or thread-like, disk-like, spheroidal, spherical micelle) but at least in one direction exhibit sizes (3–10 nm) in the nano-range. Micelles are smaller than liposomes. A micelle has either a fatty core separated from an aqueous solvent by the polar heads (normal micelle) or else an aqueous core separated by the polar heads from a fatty solvent (inverse micelle).

vii. Topical gels

Gels are transparent to opaque semisolids containing a high ratio of solvent to gelling agent. Gels are created by entrapment of large amounts of aqueous or hydroalcoholic liquids in a network of colloidal solid particles, consisting of inorganic substances, such as aluminum salts or organic polymers of natural or synthetic origin. Depending upon the nature of colloidal substance and the liquid in the formulation, the gels range in appearance from clear transparent to opaque. Most topical gels are prepared with organic polymers, such as carbomers, that impart an aesthetically pleasing, clear, sparkling appearance to the product, and are easily washed off the skin with water [Nayank SH; 2004]. The type of base used in formulating a topical gel greatly influences its effectiveness. Bases containing large amounts of oleaginous substances provide an emollient effect to dry, irritated skin [Mehta RN; 2000]. More importantly, bases made up of non-volatile oleaginous substances (e.g., hydrocarbon bases) can form an occlusive barrier on the skin that prevents escape of moisture from the skin into the environment. As a result, moisture accumulates between the skin and the gel layer that causes hydration of the stratum corneum. Hydration of stratum corneum allows ‘opening up’ of intra- and inter-cellular channels and pathways for easier passage of drug molecules. Additionally, the moisture layer provides a medium for dissolution of the drug. Since only the dissolved drug presented to the skin as an individual molecular entity is able to
enter the stratum corneum, skin occlusion generally results in enhanced percutaneous drug absorption [Changez M; 2006]. Polymeric gels do not provide an occlusive barrier to the skin and, thus, allow moisture to escape from its surface. Some well-formulated gels have been successful in facilitating greater drug permeation into the skin, when compared with ointments and creams, in which the drug may be dispersed as fine particles, but dissolution is inadequate because of their limited water content. Gels have a higher aqueous component that permits greater dissolution of drugs, and also permits easier migration of the drug through a vehicle that is essentially a liquid compared with ointment or cream bases. In addition; many gels contain penetration enhancers, such as alcohol, in the formulation. Gel formulations provide faster drug release compared with ointments and creams, and are superior in terms of use and patient acceptability [Ammar HO; 2007].

viii. **Topical sprays**

Metered Dose Topical Sprays (MDTS) deliver drug to the surface in the form of mist/film of the skin and the drug is absorbed into the circulation on a sustained basis. The drug is delivered by a device placed gently against the skin and triggered; causing it to release a spray containing a proprietary formulation of the drug that quickly dries on the skin to form an invisible drug depot. As it would from a transparent patch, the drug is absorbed steadily for a predetermined amount of time.

Metered-Dose Transdermal Sprays (MDTS) [Rathbone J; 2004]: Are classified, as enhanced, passive TDD systems. They contain a topical solution made up of a volatile cum non-volatile vehicle containing the drug dissolved as a single-phase solution. A finite metered - dose application of the formulation to intact skin results in subsequent evaporation of the volatile component of the vehicle, leaving the remaining non-volatile penetration enhancer and drug to rapidly partition into the stratum corneum during the first minute after application, resulting in a stratum corneum reservoir of drug and enhancer. The system developed is a rapid-drying solution containing a volatile component that enables the volume per area of application to be precisely defined. This component also enables the formulation to have uniform distribution on the skin over a defined area after application, without leaving excess vehicle. Hence, this ensures that the dose can be administered in a precise and highly reproducible manner and that aesthetic
and transference issues are avoided. The evaporation of some of the vehicle leads to an increase in concentration of the active drug and hence enhanced partitioning into the stratum corneum. The non-volatile component prevents the drug from precipitating from solution as the volatile solvent component evaporates. The physicochemical properties of the non-volatile component are selected so that it partitions rapidly into the stratum corneum and aids partition of the drug into the stratum corneum, as well as serving to disrupt the ordered intercellular lipids and enhance permeation. Hence, this type of delivery system creates an invisible depot of drug and enhancer in the stratum corneum from which the drug can be slowly absorbed into the systemic circulation (Fig 1.12). This can provide delivery over prolonged period following a single application and sustained steady-state serum levels with chronic, once daily application. This system also has the flexibility to cater for a dial-a-dose concept, whereby the user can alter the dose delivered from a multi-dose product. Drug candidates for this concept would require a suitable safety and dose–response profile. Drug products using this technology are being developed in a range of therapeutic areas.

**Fig 1.12:** Schematic representation of time course of events with the metered-dose transdermal system (MDTS®; acrux.co.au).

Surface spray is applied to the stratum corneum (SC). (b) The ‘forced partitioning’ concept, involving the rapid evaporation of the volatile vehicle and then the partitioning of the drug and enhancer into the SC. (c) The drug and enhancer form a reservoir within the SC that is lipid in character and water resistant.

Developments in transdermal drug delivery have been incremental, focusing on overcoming problems associated with the barrier properties of the skin, reducing skin irritation rates and improving formulation aesthetics [Beverley JT; 2004].
1.4. RATIONALE

Topical anesthetics (TAs) have been used since the late 1800s when topical cocaine was discovered to have anesthetic properties. Since then, many ester- and amide based anesthetics have been developed, and several are now readily available in topical forms. Some of the available agents include benzocaine, butamben, dibucaine, lidocaine, oxybuprocaine, pramoxine, proparacaine, proxymetacaine, and tetracaine. Many of these drugs are often used in topical combinations such as a mixture of tetracaine, adrenaline, and cocaine; a mixture of lidocaine, epinephrine, and tetracaine; topical lidocaine and prilocaine; liposome-encapsulated lidocaine; topical benzocaine, butamben, and tetracaine; and various other preparations [Brad AY; 2009]. The use of topical anesthetics was only to help alleviate superficial pain and minimize the pain associated with minor medical procedures, including arterial and venous punctures, lumbar punctures, intramuscular and intraleisional injections, laser treatments, laceration repairs, skin and mucous membrane biopsies, and prenumbing prior to infiltration with a local anesthetic for pain management with deeper procedures [Zilbert A; 2002, Chen BK; 2001].

A limited number of treatment options are available for the therapy of painful diabetic neuropathy and only two drugs (pregabalin and duloxetine) have been approved by the Food and Drug Administration (FDA) for the treatment of this condition. Even for effective and/or approved drugs, pain relief is often suboptimal and few patients obtain a complete response. Importantly, these pharmacologic agents can have troublesome side effects, an important issue given the co-morbid pathology in many patients with diabetes. There are no data from placebo controlled randomized clinical trials demonstrating the efficacy of any local anesthetic application to the skin for the treatment of painful diabetic neuropathy, [EP 2557924 A1]. To date no drugs have been approved for painful HIV-associated neuropathy. Indeed, in the only placebo controlled randomized clinical trial of topical lidocaine, there were no significant efficacy differences from placebo. Three drugs are approved in the United States for the management of postherpetic neuralgia: (i) topical lidocaine patch (Lidoderm™); (ii) oral gabapentin (Neurontin™) and (iii) oral pregabalin (Lyrica™). A number of drugs, including the opioid OxyContin®, tricyclic antidepressants and tramadol have demonstrated efficacy in postherpetic neuralgia and are used “off-label” [Watson CP; 1998].
Tragically there is no existing method to adequately, predictably and specifically treat established peripheral neuropathic pain. Since neuropathic pain has been shown to be sensitive to systemic delivery of anesthetics [Chabal C; 1992] and is related in part to neural signals arising at the level of the skin [Sato J; 1991, Campbell JN; 2006 & 2001], a clinical and scientific rationale exists for directing therapy directly to the skin. Topically applied local anesthetics hold promise for the treatment of neuropathic pain by virtue of their action as sodium channel blockers at the peripheral sites of nerve dysfunction. In order to provide analgesia following application to the skin, a local anesthetic must be able to reach the epidermis and dermis where the cutaneous nerve endings are located, and once there, it must be capable of blocking the generation and/or propagation of aberrant or ectopic impulses in sensory nerve fibers. The former property depends on the pharmacokinetic behavior of the local anesthetic, i.e., how efficiently it can penetrate the stratum corneum (the outermost layer of the skin), and how long it dwells in the deeper skin layers before diffusing into underlying tissue. The latter property depends on the pharmacologic activity of the local anesthetic, i.e., its ability to bind to and inhibit specific ion channels essential for the generation and/or propagation of the aberrant or ectopic impulses in sensory nerve fibers [EP 2557924 A1].

Although, topical anesthetics efficacy and onset is limited by difficulty in drugs penetrating the skin barrier, optimized drug delivery systems can dramatically improve the effectiveness and onset time of topical anesthetics and allow the use of safe, effective compounds.

Lidocaine is the first local anesthetic that has been evaluated topically for treatment of peripheral neuropathic pain. Topical lidocaine, in the form of a lidocaine patch (Lidoderm® 5% [USA], Versatis® 5% [EU]) is one of the three approved treatments in the United States for the management of postherpetic neuralgia (along with gabapentin and pregabalin). Application of the Lidoderm patch to the affected hands and feet is impractical due to the: (i) absence of a single, contiguous flat surface to assure patch adhesion and continuous contact with the skin; (ii) presence of significant interdigital spaces; (iii) need for dynamic interdigital space for activities of daily living involving the use of feet and hands (e.g., walking, washing); (iv) need for wearing socks and shoes.
(which are part of the advice provided to patients with diabetes) over any such patch; (v) need to have a clear and unobstructed view of the feet to allow the recommended daily inspection of the feet; (vi) poor bioavailability due to the reduced surface area and reduced efficiency of absorption from application over the feet and hands; (v) poor bioavailability due to the reduced circulation in patients with painful diabetic neuropathy. In addition to its poor efficacy, Lidoderm patch provides extremely poor adhesion, even over flat skin surfaces, and unacceptable adhesion over highly contoured surfaces or over hairy skin. Furthermore, the bioavailability of the drug from Lidoderm patch is quite poor; less than 5% of the lidocaine in the patch is absorbed over the dosing interval. Recently, the UK National Institute of Health and Clinical Excellence (NICE) issued clinical guidelines on neuropathic pain and stated that there is a “lack of evidence for the efficacy of topical lidocaine for treating neuropathic pain” and that topical lidocaine should be considered as “third line” treatment for neuropathic pain [guidance.nice.org.uk/CG9]. Another important practical and therapeutic barrier to the use of Lidoderm patch relates to the nature of neuropathic pain. The skin over which the patch is applied is frequently hyperesthetic and allodynic. Consequently, patches that would be acceptable for application to non-neuropathic skin, with a high level of adhesion and application to rotated skin sites are not viable for use in painful peripheral neuropathy where a high level of adhesion can make patch removal painful and the need to apply to the same skin site can result in skin friability.

There is a need, therefore for new local anesthetic pharmaceutical compositions for application to the skin that afford painless, safe application and methods for the treatment of peripheral neuropathic pain, in particular-diabetic neuropathic pain, HIV associated peripheral neuropathic pain and postherpetic neuralgia (PHN), that have an optimal safety profile. Mepivacaine has a number of potential pharmacologic advantages over lidocaine, including (i) an intrinsic vasoconstrictor effect which would be expected to reduce the rate at which drug is cleared away from peripheral (skin) sites of pain generation, and (ii) the lowest potential for neurotoxic effect on developing or regenerating primary cultured neurons (iii) among the local anesthetics lidocaine, bupivacaine, mepivacaine, and ropivacaine, it is reported that lidocaine has the highest neurotoxic potential. Compared
with lidoderm patch, mepivacaine topical dosage forms such as gels and sprays may have several advantages, including improved bioavailability and greater suitability for distal neuropathies, such as painful diabetic neuropathy and HIV neuropathy which primarily involve the feet. To our knowledge, with the possible exception of application by skin infiltration, there are no: (i) recommendations on mepivacaine application to the skin for the management of neuropathy or neuropathic pain; (ii) no public data on mepivacaine application to the skin for the management of neuropathy or neuropathic pain; (iii) no working examples of topical mepivacaine for application to the skin for the treatment of neuropathy or neuropathic pain (iv) no approved mepivacaine product for application to the skin for the management of neuropathy or neuropathic pain; and (v) no mepivacaine products for application to the skin for the treatment of neuropathy or neuropathic pain in development, regulatory review or on the market any major market.

Considering the above, an attempt was made to design and develop topical delivery systems of an intermediate acting local anesthetic like mepivacaine in physiologically acceptable topical vehicles in an amount sufficient to provide an anti-neuropathic response.

The required concentration of local anesthetics in a topical formulation depends, among other things, on intrinsic potency, ability to penetrate the stratum corneum and retention within skin structures, metabolism in skin tissue and the nature and efficiency of the dosage form. The relative retention in the skin will also affect the dosing frequency and duration of effect. In general, it is medically and commercially more desirable to apply a topical product less frequently. By way of reference, for lidocaine, there are established 5% topical products in the USA, but none are approved for the treatment of peripheral neuropathic pain. In the case of mepivacaine, there are no topical products anywhere to be considered as reference. Now that lidocaine has 2 to 3 fold greater octanol partitioning although once at the effector site, lidocaine and mepivacaine are essentially equipotent in their ability to block Na\(^+\) channels. This difference in potency is related to their binding in the pore of the Na\(^+\) channels. Hence 5-10% w/w loading of mepivacaine in topical formulations was selected.
OBJECTIVES

- To formulate and optimize robust topical formulations such as gels, nanoemulsion based gels and metered dose sprays of an intermediate acting amide type local anesthetic like mepivacaine and its pharmaceutically acceptable hydrochloride salt for alleviation of peripheral neuropathic pain.
- To investigate the targeting potential of developed DDS to cutaneous receptors by ex vivo and in vivo studies and establish its efficacy for neuropathic pain relief.

1.5. DRUG PROFILE

Mepivacaine (1-methyl-2',6'-pipecoloxylidide) [AHFS Drug Information® (2008)]

Although local anesthetic use has been dominated by the amidoamide group, experiments with the piperidine ring within cocaine led to the combination of this structure within the xylidine component of lidocaine, resulting in the pipecoly xylidine family. Mepivacaine is the prototype of this family. Mepivacaine was introduced in the year 1957 as the first member of the amide pipecolyl xylidine family [Tetzlaff CP; 2001].

Mepivacaine hydrochloride (Molecular formula: C₁₅H₂₂N₂HCl and Chemical Name: N-(2,6-dimethylphenyl)-1-methylpiperidine-2-carboxamide) is an intermediate-acting local anesthetic of the amide type with a molecular mass of 282.82. Mepivacaine hydrochloride occurs as a white, crystalline solid and is freely soluble in water. The pKa of mepivacaine hydrochloride has been reported as 7.6 and 7.8. Commercially available solutions of mepivacaine have a pH of 4.5-6.8; pH is adjusted with sodium hydroxide or hydrochloric acid. Multiple dose vials of mepivacaine hydrochloride injection contain methylparaben as a preservative. Mepivacaine hydrochloride solutions containing levonordefrin (Neo-Cobefrin®) have a pH of 4.5-6.8.

Uses: Mepivacaine hydrochloride is used for infiltration anesthesia and for peripheral or sympathetic nerve block and epidural (including caudal) block. The drug is not used for spinal anesthesia.

Mechanism of action: blocks the generation and the conduction of nerve impulses, presumably by increasing the threshold for electrical excitation in the nerve, by slowing the propagation of the nerve impulse, and by reducing the rate of rise of the action potential.
Adverse effects: Reactions to Mepivacaine are characteristic of those associated with other amide-type local anesthetics. A major cause of adverse reactions to this group of drugs is excessive plasma levels, which may be due to over dosage, inadvertent intravascular injection, or slow metabolic degradation.

Contraindication: Mepivacaine is contraindicated in patients with a known hypersensitivity to it or to any local anesthetic agent of the amide-type or to other components of Mepivacaine solutions.

Pharmacokinetics: Mepivacaine produces less vasodilation and has a more rapid onset and longer duration than does lidocaine. When used for epidural block, the onset of action of a 2% solution of mepivacaine hydrochloride is about 7-15 minutes and the duration of anesthesia is 115-150 minutes. When used for caudal block, the duration of action of a 1-2% solution is about 105-170 minutes. When used for dental anesthesia, mepivacaine hydrochloride has an onset of action of about 0.5-2 minutes in the upper jaw and 1-4 minutes in the lower jaw. When used for dental anesthesia, 0.7-1 mL of a 3% mepivacaine hydrochloride solution provides pulpal analgesia of 10-17 minutes duration and soft-tissue anesthesia for approximately 60-100 minutes. Duration of anesthesia of mepivacaine may be prolonged by the addition of levonordefrin or epinephrine. After absorption into the blood, 60-85% of mepivacaine has been reported to be bound to plasma proteins. Mepivacaine is metabolized mainly in the liver where it undergoes N-demethylation to produce 2′,6′-pippecoloxylidide and aromatic hydroxylation to produce 1-methyl-4′-hydroxy-2′,6′-pippecoloxylidide and 1-methyl-3′-hydroxy-2′,6′-pippecoloxylidide, both of which undergo conjugation with glucuronic acid. Mepivacaine is excreted in the urine as its metabolites and small amounts (about 5-10%) of unchanged drug. Up to 5% of a dose may be metabolized to carbon dioxide which is eliminated via the lungs. More than 50% of a dose is distributed into bile as metabolites and probably undergoes enterohepatic circulation; only a small percentage of a dose is excreted in feces. Although neonates may have limited ability to metabolize mepivacaine, they are able to eliminate the unchanged drug. It is marketed under the trade name of Carbocaine® Hydrochloride, Polocaine®.
1.6. PLAN OF WORK

The experimental work was planned as follows:

a. Literature Review
b. Selection and procurement of drug candidate
c. Selection of carrier systems/excipients
d. Preformulation studies of drugs and excipients
e. Analytical and bioanalytical method development and validation for estimation of drug using U.V spectroscopy and HPLC.
f. Formulation development of topical drug delivery systems like film forming gels, nanoemulsion based gels, and metered dose sprays.
g. Optimization of product as well as process variables using experimental factorial designs.
h. Characterization and Evaluation of developed formulations including parameters like spectral analysis, compatibility studies, drug degradation, long term and accelerated stability studies and container-closure suitability testing.
i. *In vitro* and *ex-vivo* diffusion studies through synthetic membranes and animal skins respectively using Franz diffusion cell to investigate parameters like permeability co-efficient, steady state flux and enhancement ratio
j. Anti microbial Effectiveness Testing as per USP.
k. Skin Irritation Potential by Draize Test and Ocular Irritation Potential Test as per OECD Guidelines.
l. Bioavailability studies of the optimized formulations using dermatopharmacokinetic approach by Tape-stripping methodology
m. Confocal Laser Scanning Microscopy to assess penetration behavior of drug into skin.

n. *In vivo* pre-clinical studies including skin retention potential and quantitation of drug in different skin strata, toxicological studies, pharmacokinetic and pharmacodynamic studies followed by histopathology of vital organs and evaluation of hematological parameters, biochemical parameters and urinary parameters to estimate toxicity potential of developed formulations.
The preliminary studies including selection of excipients and compatibility studies were initiated based on available resources. Drug and excipients are procured by contacting the companies manufacturing or marketing the required ingredient for gift samples. Mepivacaine base and its pharmaceutically acceptable hydrochloride salt were purchased from Hangzhou Verychem Science and Technology Co. Ltd, China. All other ingredients and excipients were sourced locally.

The research work carried out as per the above mentioned plan is presented in the following chapters of the thesis. Experimental details, results, interpretation of data along with discussions and conclusion are summarized with an objective to develop novel topical drug delivery systems of mepivacaine for the treatment of peripheral neuropathic pain.