CHAPTER 6
SUMMARY AND CONCLUSION
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Neuropathic pain comprises a mixed group of conditions delineated neither by their underlying aetiology nor anatomical location (Sindrup & Jensen, 1999). Local anaesthetics directly block transmission of pain from nociceptive afferents. Local anaesthetics can alleviate some types of neuropathic pain. Part of this effect may be related to sensitisation of the antinociceptive pain pathways that occur in the neuropathic pain state.

In view of the pharmaceutical and efficacy limitations of topical treatment of neuropathic pain, there is a need for an alternative or adjunctive treatment mode to topically applied local Lidoderm patch for the treatment of peripheral neuropathy. Therefore, the aim of the research work was to develop pharmaceutically elegant, non-greasy gel and spray formulations of Local anaesthetics like Mepivacaine and Ropivacaine that demonstrate robust stability and in vitro/ex vivo diffusion and permeability.

Topical delivery systems for local anaesthetics are composed by a diversity of formulations (viscosity inducing agents, preservatives, permeation enhancers, emollients,) and dosage forms such as semisolid (gel, creams, ointments), liquid (emulsions, dispersions), and solid (patches).

Preformulation: Chapter 3 on Preformulation studies describes the Standardization of drugs and excipients and the Analytical method development and Validation by RP-HPLC.

Extensive studies were conducted to select suitable excipients for the formulations. This study helped us in understanding the behaviour of drug and various excipients with respect to their properties and solubility that may affect the important dissolution rate of developed formulations.

Mepivacaine and Ropivacaine were standardized as per specifications and Certificate of Analysis. The drugs passed all the tests for identity. The FTIR spectra and UV spectra showed characteristic functional groups and λmax of 263nm respectively. The DSC thermogram of Mepivacaine and Ropivacaine revealed their purity and characteristics of the drug molecules. Scanning electron microscopy was performed to visualize the morphology of the drugs. Excipients utilized were standardized as per the specifications and passed all the tests for identity and were well within pharmacopoeial limits.

HPLC method of analysis was developed and validated for the analysis of Mepivacaine and Ropivacaine, using Perkin Elmer HPLC system and PDA detector. Qualisil gold C18 HPLC (4.6 x 250 X 5 µm) column was utilized. The mobile phase was Acetonitrile: Phosphate
buffer pH 6.8 (60:40 v/v). The method was successfully developed at the wavelength ($\lambda_{\text{max}}$) of 210 and retention time of 5.0 ± 0.5 min for Mepivacaine and 10.0 ± 0.5 min for Ropivacaine. Bioanalytical HPLC method development was carried out with Lidocaine as the internal standard (IS) using the developed HPLC method.

**Formulation Development of Topical Delivery Systems of Mepivacaine and Ropivacaine**

Improved efficacy of pain inhibition combined with aesthetic properties is achieved through several approaches in efficient and novel topical targeted delivery systems. Topical polymeric film forming gels as well as sprays, and lipidic nanocarriers for targeted delivery of Local Anaesthetics to the dermis have been explored in this research project. The standardized drug and excipients were used in different permutations to develop formulations like topical film forming gels and sprays, microemulsion based gels and SLN based gels

**Topical Film forming gels:**

The formulations were attempted which would be capable of forming a film on topical application, on the skin. Various solvents, polymers and humectants were assessed for their ability to give clear transparent gels which when applied over the skin, evaporate and leave behind smooth wrinkle free, transparent and shiny films. Solubility of Mepivacaine and Ropivacaine was evaluated in different solvents and ethanol was selected for further use as the solvent since it was found to be quick drying, safe (IIG and GRAS listed) and formed clear and transparent solutions of drugs as well as most polymers.

Conventional polymers like pyrrolidone (PVP), polyvinyl alcohol (PVA), HydroxyPropyl cellulose (HPC), HydroxyPropyl Methyl cellulose (HPMC), Ethyl Cellulose and various grades of Methacrylates were used. Novel gel bases like Dermaseal, Avalure and Aquatrix were assessed for their ability to form transparent gels with quick drying property to leave a transparent shiny film over the skin. HPC EF in the concentration of 15% and Methyl Gluceth 20 in the concentration of 5% were finally selected as the polymer and the humectant with desired characteristics.

The optimized film forming gel formulations were selected based on the required viscosity, appearance of the gels, drying time required for film formation, appearance of the placebo films over skin and the results obtained from the in-vitro diffusion studies indicating a good balance between the viscosity of the gels and drug permeation. The parameters of the films and the gels were, rapid drying time for films, Shiny, transparent and non tacky films and Gel
like consistency with cohesive property to adhere to the skin. *In-vitro* and *ex-vivo* drug diffusion studies were investigated through the dialysis membrane and porcine ear skin respectively. It was observed that the permeation through stratum corneum, epidermal and dermal layers of the skin was significantly slower as compared to diffusion through the dialysis membrane.

The formulations were subjected to long term and accelerated stability studies as per ICH guidelines. The samples were kept under refrigeration at 4°C ± 2°C, room temperature at 25°C ± 2°C/ 60 ± 5 % RH, and accelerated stability conditions at 40°C ± 2°C / 75 ± 5 % RH as per ICH guidelines. The physicochemical attributes were assessed at various time points to confirm the stability of the formulations. The formulations were found to be robust and stable without any change in the appearance, drug content and *in-vitro* diffusion profiles.

Film forming gels of local anaesthetics, Mepivacaine and Ropivacaine, were formulated with an elegant appearance and aesthetic appeal. Topical film forming gels presented a novel platform to deliver drugs to the skin.

**Topical Film forming sprays:**

Conventional approaches like gels, ointments and creams have been used for a long time. Topical metered dose spray systems present a novel and easy platform for delivery of drugs into the skin. The three main components of formulating a topical metered dose spray are the spray pump, the solvent system and the film forming polymer. The selection of solvents for metered dose formulations depends on various criteria like the solubility of drugs and excipients, the safety and accepted pharmaceutical utilization of the solvents, regulatory status and their cost. The choice of film forming polymer depends upon its solubility in the solvent system, its ability to form smooth wrinkle free transparent films after evaporation of the solvent over the skin and compatibility with the drug and solvent. APF Plus as well as VP 6 pumps from Aptar Pharma and spray pumps from the local vendor were screened. VP 6 pump was selected as it gave the desired spray pattern. The concentration and grades of film forming polymers necessary to form a shiny and transparent film were determined by varying the concentration of polymer from 2 -10% and assessing various grades of Eudragit polymers. Different humectants and plasticizers were used to obtain wrinkle free and non flaky films with sufficient plasticity and flexibility.
The optimized spray was assessed based on parameters like appearance of spray and film formed after drying of spray (ex – *in vivo* studies). The spray pattern and spray angle, volume of solution delivered upon each actuation, viscosity, droplet size distribution, tackiness of the films formed after evaporation of solvent, Determination of rate of evaporation of ethanol and IPA by Gas chromatography, Occlusion potential of the film forming sprays and leakage test.

A transparent, clear and aesthetic looking film was formed after spraying with Eudragit S 100, however to increase the flexibility and plasticity of the film it was advisable to add a plasticizer. Propylene glycol, dibutyl Phthalate and PEG 400 all gave almost equal flexibility to the film by visual examination.

A good, circular, full cone type spray pattern with absence of any stray droplets was formed on the Whatman filter paper using APF Plus and VP6 spray pumps. The sprays formed a non-occlusive or a semi – occlusive film over the skin. Negligible leakage from the spray pumps was found to occur after 365 days of standing.

The size distribution of soray droplets and their uniformity affects the film characteristics. A spray which is coarse and not uniform does not form a continuous film over the dermal surface. Hence a fine and uniform droplet size is desired. VP6 gave a fine distribution of droplets when sprayed from a distance of 10 cms (Figure 4.2.12- 4.2.16). The formulations were stable up to a period of three months

**Microemulsions:**

A wide variety of molecules can be transported through microemulsion based delivery systems. Preparation of the microemulsion involves determining the region of spontaneous microemulsion formation using Pseudo ternary diagram software (Chemix version 3.6). A ratio of surfactants and co-surfactants to give maximum microemulsion region was determined using the phase diagram (Table 4.3.2 and 4.3.3).

Oils, surfactants and co-surfactants need to be selected according to the properties of the microemulsion desired, solubility of the drug and surfactant in the oils and surfactants. The solubility of the drugs in the oil, surfactant and co-surfactant was determined previously by saturation solubility studies. From the saturation solubility studies it was noted that Oleic acid, span 80, Tween 80, Transcutol and Labrasol were found to solubilise the drug well.
Oleic acid, tween 80, transcutol and labrasol were found to solubilise the drug well and hence for the initial trials oleic acid was selected as the oil phase while Tween 80 and ethanol were selected as the surfactant and co-surfactant.

From the pseudo ternary phase diagrams it was observed that as the ratio of Tween 80:Ethanol was increased from 1:1 to 3:1 there was a significant decrease in the microemulsion region, i.e. when the concentration of Tween 80 was increased and ethanol decreased the amount of surfactant and co-surfactant required for microemulsion formation became higher. The final concentration of the oily phase was approximately 5 – 6% and S:CoS mixture concentration ranged from 40 – 45% while the rest was water.

In the final formulation 0.5% w/w of Xanthan gum was selected as the gelling agent. Addition of menthol produced a cooling sensation on the skin and it also helped to mask the slightly unpleasant odour of oleic acid in the formulation.

The microemulsions were found to be stable to centrifugation as no phase separation occurred after centrifugation. They were also stable to heating and cooling cycles which were applied and did not show any phase separation or turbidity. The mean droplet size of the microemulsion was 195nm and in the sub micron size.

Lower concentrations of the gelling agents (0.5% w/w) in the microemulsion produced a good diffusion profile with high flux values indicating high permeation into the skin.

Hence microemulsions may be considered as potential carriers of lipophilic drugs for designing of topical delivery systems.

**Solid Lipid Nanoparticles:**

SLNs are sub-micron colloidal carriers composed of physiological lipids, dispersed in water or in an aqueous surfactant solution. Formulations were developed using the solvent injection-evaporation method. Lipids like Stearic acid, Glyceryl monostearate, Compritol 888 and Precirol were explored. The solubility of the lipids was assessed in various solvents at room temperature as well as at elevated temperatures of 70-80°C. Most of the lipids were miscible in the organic phase at temperature of 70-80°C. The organic phase was selected depending upon the solubility of the drug as well as the lipid in it. Methanol, Ethanol, acetone and Isopropyl alcohol were considered as solvents for the organic phase as the drug was soluble in them and most of the selected lipids were miscible in the organic solvents at higher temperatures.

The drug along with the lipid and a surfactant was dissolved in the water miscible organic phase under heating if required. A surfactant (Poloxamer 188) was dissolved in the aqueous
phase under constant heating and stirring conditions. The organic phase was then added drop wise using a syringe to the aqueous phase under rapid stirring.

Design of experiments (Factorial design) was applied to ascertain the optimum formulation of solid lipid nanoparticles containing 0.5% of Ropivacaine. The lipid content, surfactant content and stirring rate were the independent variables. Particle size and Entrapment Efficiency were calculated and they were taken as the dependent variables. A significant difference was not observed.

Poloxamer 407, Carbopol 940 and Xanthan gum were explored in varying concentrations as the gelling agents for their ability to form smooth, non gritty, translucent to opaque gels with ease of spreading and application.

Appearance, Particle size and zeta potential analysis of SLN’s, Entrapment Efficiency (EE) of drug into lipids were investigated using suitable techniques as described in the chapter. Occlusion, Spreadability and viscosity of the SLN based gels were determined.

The in-vitro drug diffusion from the Solid lipid nanoparticles as well as the SLN based gels was higher than the ex-vivo release. This may be due to the fact that the skin being lipophilic retains the lipid nanoparticles in the epidermal and dermal layers.

DSC and XRD studies were performed to evaluate the entrapment of drug into the lipid matrix. Formation of lipid nanoparticles reduces the degree of crystallinity of the lipid as well as the drug. A separate drug peak was not observed in the SLNs and hence DSC studies indicated that the drug was entrapped. The XRD of the SLNs exhibited diminished peak of the drug. The peaks of the lipid did not show any significant change. This indicated that the drug is entrapped in the lipid shell and it has gained an amorphous nature. SEM imaging of the SLNs was attempted. The drug appeared as rod/ needle shaped crystals with a particle size of 20 -50 µm. The images of spray dried SLNs indicated that the drug entrapment in the lipid was achieved as the rod shaped drug crystals were not visible and spherical particles were present. It was seen in the TEM images that the drug was entrapped well in the lipid.

Hence Ropivacaine was successfully incorporated into the SLNs and SLN based gels were formulated for topical application for management of neuropathic pain.

Preclinical studies: The experimental protocol was approved by Institutional Animal Ethics Committee. The CPCSEA guidelines were followed.
**Dermatopharmacokinetic studies:** DPK encompasses drug concentration measurements with respect to time and provides information on drug uptake, apparent steady-state levels, and drug elimination from the stratum corneum based on a stratum corneum concentration-time curve. Tape stripping studies involved selection of tapes and solvents. Transpore™ and Millipore™ tapes were assessed for their adhesive properties, peelability, ease of extraction of drug from the tape stripes. Transpore™ was selected for further stripping. Isopropyl Alcohol, Ethanol, Acetonitrile were evaluated as solvents to determine the maximum extraction of the drug from the tapes. After tape stripping was completed, the skin was sliced into very small sections using a scalpel and drug extracted to measure the amount of drug deposited into the skin. Highest drug deposition in skin was observed by SLNs followed by microemulsions. Confocal microscopy studies were performed to visualize the retention of the drug in the skin. Topical sprays and SLN’s were studied using CLSM. The SLN based gels showed deeper and more intense fluorescence indicating higher deposition into the skin as compared to the topical sprays. This may be attributed to their highly lipophilic nature and the sub micron size which are significant in enhancing the delivery of drugs into the skin.

**Pharmacodynamic studies:** The latency period in reaction of the rats (response) to heat as the noxious stimulus was measured after application of topical formulations. Statistical Analysis of data obtained from the pharmacodynamic studies performed all the optimized formulations were carried out. It was compared to the conventional Lidocaine gel and with the negative control. The onset of analgesia started within 2 hours and was seen to decline after 6 hours in the tail flick test and at the end of 7 hours in the Hot Plate test. The maximum analgesic response time in the hot plate test was recorded at the end of 5 hours, with the optimised microemulsion formulation as 45.94 %, probably due to the submicron size of the particles. Percent Maximum possible response of 54.35% was observed at the end of 2 hours in the rats with application of optimised SLN based gels by tail flick test as seen in Table 5.5-5.8.

**Skin Irritation and Acute Dermal Toxicity Studies:** Acute dose toxicity studies were conducted for 14 days and as per OECD guidelines with slight modifications. Wistar rats were used as animal models. Animals were observed for presence of tremors, convulsions, salivation, diarrhoea, lethargy, body weights and food intake. Draize Patch Test was conducted to assess the irritation potential of the formulations. All the developed formulations also did not show any erythema or edema in rats on intact skin at the end of 1, 24h and 72 h. All the scores of Primary irritation index (PII) recorded after a 24 hour application of the six
formulations were observed to be 0.00 (< 2), suggesting that the formulations may be considered as non-irritating.

**Conclusion:**

Topical delivery of Mepivacaine and Ropivacaine via various formulation approaches like film forming gels and sprays as well as submicron sized lipid based delivery systems was investigated in the present research work. Novel film forming gels and metered dose topical film forming spray formulations of Mepivacaine and Ropivacaine were developed using various film forming gelling agents and polymers. Film forming gels were developed to provide prolonged delivery into the skin in a hassle free manner without the application of visible films or patches. Metered dose spray formulations were developed with spray pumps to provide propellant free delivery and cover the contours of the skin for effective and uniform coverage of the pain area. The film forming gels and metered dose topical sprays of Mepivacaine and Ropivacaine may provide the basis of an alternative to conventional topical delivery for relief of neuropathic pain.

The SLN’s and Topical sprays were compared for their drug deposition into the skin by confocal microscopy. Although the SLN’s yielded the highest drug deposition into the skin, the sprays offered fast and economical scale-up techniques and ease of preparation. From the economic point of view the cost of the spray pumps may be considered negligible if purchased in bulk. Film forming sprays also offer metered dose delivery, hence the amount of product discharged per application is constant. The film forming topical sprays could be considered as highly novel and patient compliant delivery system of all the formulations prepared.

The shortcomings of the conventional Lidocaine gel and the Lidoderm topical patches could be reduced by film forming deliveries to achieve the same target. Occlusion and irritation caused by patches was overcome to a great extent. The research presented in this thesis is a small step towards a plethora of further development the field of topical and transdermal delivery systems. The developed formulations showed aesthetic appeal, physico-chemical stability, enhanced permeation, improved cutaneous bioavailability and anti-nociceptive activity. In addition to this, the developed drug delivery systems were non toxic and nonirritant.
Future Scope of the Research Work:

Novel penetration enhancers and other Terpenes like Eugenol, Carvones etc, could be explored for permeation enhancement from film forming delivery of gels and sprays. Pilot plant scale up studies can be taken up for the nano-carrier based formulations. The delivery of Microemulsions and SLN’s sprays could be explored from metered dose spray pumps. The Nerve Conduction Velocities (NCV) after application of the formulations and after application of drug solution could be explored for enhancement if any could be observed. Efficacy studies using other established pain models such as Chronic Constriction Injury, formalin test for neuropathic pain and Spinal Cord Ligation in animals may add value to the study and confirm the effects of pain management through local anaesthetics. Further Clinical studies and repeat dose toxicity studies are recommended to prove the therapeutic efficacy and safety in humans.