SYNOPSIS
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FORMULATION DEVELOPMENT OF NOVEL TOPICAL DRUG DELIVERY SYSTEMS FOR PAIN MANAGEMENT

SUBMITTED TO
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INTRODUCTION
Neuropathic pain has a complex and variable etiology, distinct from nociceptive or inflammatory pain. It is a chronic condition attributable to complete or partial transection of a nerve or trauma to a nerve plexus. Neuropathic pain is classified as peripheral neuropathic pain and central neuropathic pain. Among the peripheral neuropathies most widely investigated for pharmacologic response are painful diabetic neuropathy, postherpetic neuralgia, trigeminal neuralgia and painful HIV-associated distal symmetrical neuropathy. Peripheral neuropathy refers to a disorder that affects the peripheral nerves, manifested as motor, sensory, sensorimotor or autonomic neural dysfunction. Peripheral nerve damage can lead to pathological states where there is a reduction in pain threshold (alldynia), an increased response to noxious stimuli (hyperalgesia) or an increased response duration (persistent pain).

Current treatment for painful peripheral neuropathy includes the use of antiepileptic gabapentinoids (e.g., gabapentin, pregabalin), tricyclic antidepressants (e.g., amitriptyline, desipramine), topical local anesthetics, capsaicin and opioids as mono or combination therapy. Tragically there is no existing method to adequately, predictably and specifically treat established peripheral neuropathic pain. 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.

RATIONALE
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). 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. 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.

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.

**OBJECTIVES:**

- To formulate and optimize robust topical formulations of an intermediate acting amide type local anesthetic like mepivacaine and its pharmaceutically acceptable salts for alleviation of peripheral neuropathic pain.
- To increase the contact time of the formulation with the skin using different carrier systems such as gels, nanoemulsion based gels and metered dose sprays thereby providing sustained pain relief.

**PLAN OF WORK:**

The experimental work was planned as follows:

1. Selection of drug candidates
2. Standardization of drugs and excipients
3. Analytical and bioanalytical method development and validation for estimation of drug using U.V spectroscopy and HPLC.
4. Formulation development of topical drug delivery systems like film forming gels, nanoemulsion based gels, and metered dose sprays.
5. Optimization of product as well as process variables using experimental factorial design.
6. 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.
7. *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
8. Scale up and Pilot studies.
9. Anti-microbial Effectiveness Testing as per USP.
10. Skin Irritation Potential by Draize Test and Ocular Irritation Potential Test as per OECD Guidelines.
11. Bioavailability studies of the optimized formulations using dermatopharmacokinetics approach by Tape-stripping methodology
13. 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.

**EXPERIMENTAL**

**Drug Candidate**

Mepivacaine base and its pharmaceutically acceptable hydrochloride salt: Mepivicaine, 1-methyl-2, 6-pipecoloxylidide is a local anesthetic of the amide type with intermediate duration of action.

**PART I: PREFORMULATION STUDIES ON LOCAL ANESTHETICS:**

1. **Standardization of Drugs and Excipients:** Mepivacaine and its hydrochloride salt were procured from Hagzhou Verychem Co. Ltd, China. The drugs were standardized as per the Certificate of Analysis. All other excipients were standardized as per the specifications and were found to be within the Pharmacopeial/monographic limits.

2. **Drug-Excipient compatibility studies:** To investigate the stability of mepivacaine and its interaction with polymers and other excipients, infrared spectroscopy and DSC studies were carried out. Endotherms of other excipients did not overlap with the endotherm of the drug indicating compatibility.

3. **Analytical Method Development:** The analytical methods for quantitation of drug were developed both by U.V spectrometry and HPLC. The developed methods were validated and found to be reproducible, accurate and precise.

HPLC method was developed using Hypersil C18 gold column (250 X 4.6mm, 5µ) using acetonitrile: phosphate buffer pH 6.8 (60:40, v/v) as mobile phase. It was found to be sensitive in the concentration range of 0.5-5 µg/ml. The linearity was obtained with \( r^2 = 0.9998 \). The developed method was validated for linearity, precision, accuracy, LOD, LOQ and robustness.

4. **Development of Bioanalytical Method for Estimation of Drug in Rat and Rabbit Plasma.**

Analytical method was also developed for the quantification of drug in the skin and horizontal fractions of stratum corneum during ex vivo skin permeation study, tape stripping procedure and
in vivo pharmacokinetic studies respectively. In ex vivo skin permeation studies, mepivacaine was recovered from the whole skin by vortex homogenization and bath sonication in organic solvent. In dermatopharmacokinetic studies on rats, tape strips were extracted to recover and quantify the absorbed drug. Local bioavailability was assessed from the combined tape strips. HPLC method for quantification of drug in rat and rabbit plasma samples was also developed.

5. Forced degradation of the drug: Forced degradation studies using photo, acid, base, thermal and oxidation reactions were carried out on the drug. Mepivacaine was stable to all the forced treatments; no degradation of the API was seen.

PART II: DEVELOPMENT OF LOCAL ANESTHETICS TOPICAL DRUG DELIVERY SYSTEMS

Formulation Development and Evaluation of:

A) Film forming gels:

Three prototypes of clear, non greasy, semisolid gel formulations were optimized. Different gelling agents such as Carbopol 980 NF, Carbopol 974, HPMC K4M, HPMC K100M and HPC were explored at varied concentrations to achieve the optimum viscosity and drug permeation profiles. It is well established that a principal driving force for diffusion across the skin is the thermodynamic activity of the permeant in the donor vehicle. This activity is reflected by the concentration of the permeant in the donor vehicle as a function of its saturation solubility within that medium. Solvents act as penetration enhancers (PE) by increasing thermodynamic activity of the drug and/or changing the barrier property of the skin. Optimum and pharmaceutically acceptable binary vehicle systems of propylene glycol/ethanol, polyethylene glycol/ethanol or propylene glycol/isopropyl alcohol in the ratio of 1:1, 2:1 and 1:2 were explored in permeation studies of topical mepivacaine gels.

Further, penetration enhancers of various chemical classes such as fatty acids, fatty alcohol ethers, propylene glycol derivatives, nonionic surfactants, terpenes and fatty acid esters at different concentrations (0.5, 1, 1.5 and 2%, w/w) were used to investigate their effect on permeation rate of mepivacaine topical gels. The diffusion profiles of the optimized formulations were compared with extemporaneously formulated lidocaine gels. The developed gels were evaluated for drug content, pH, clarity, homogeneity, viscosity, spreadability and bloom strength (using texture analyzer). Amount of drug diffused was quantified by the developed HPLC method. The cumulative amount of drug permeated at 24 h (µg/cm²), steady-state flux Jss (µg/cm² h), lag time tL (h), permeability coefficient kp (cm/s), partition coefficient k, and diffusion coefficient D (cm²/s) were determined in order to optimize the gel formulations.
Effect of formulation pH on Flux through skin: The effect of formulation pH on transdermal flux of mepivacaine, optimized gels was investigated by various pH’s ranging from acidic to basic pH. Maximum flux was obtained with mepivacaine HCl gels formulated at pH 7. At pH above 8, the drug precipitated out in carbopol based gels however such effect was not observed in HPMC K4M based gels. The results indicated that flux of mepivacaine increased by a factor of three when the pH of gel system increased from pH 5 to pH 6 or pH 7.

B) Nanoemulsion and nanoemulsion based gels
Nanoemulsion formulations were prepared by water titration method firstly, by investigating the solubility of drug in various oils, surfactants and co-surfactants. Phase diagrams were developed using Tridraw software to obtain the nanoemulsion region. The nanoemulsion formulations were loaded with increasing concentrations of mepivacaine (1% to 5%) and its impact on globule size, polydispersity index and drug diffusion profiles were explored. The developed nanoemulsions were evaluated based on parameters like clarity, pH, stability after centrifugation, viscosity, freeze-thaw cycles and drug content. The following parameters of formulations were characterized:

- Electric conductivity is a measure of electric charge of movable particles that can carry electricity when a difference of electric potential is placed across a conductor. This data was useful for the determination of type of nanoemulsion o/w or w/o. Conductivity was measured by using conductivity meter. The phase transfer of nanoemulsion from w/o to o/w was also investigated.

- Self diffusion NMR studies were performed to determine the type and phase of nanoemulsion. Self-diffusion coefficient of a compound is inversely related to the macromolecule/aggregate radius and viscosity of the medium, and reflects the degree of structural encapsulation and association of phases. NMR measurement were performed on Bruker DRX-500 spectrometer with a BGU II gradient amplifier unit and a 5-mm BBI probe equipped with a z-gradient coil, providing a z-gradient strength (G) of up to 55 G cm⁻¹. The self-diffusion coefficients were determined using bipolar-pulsed field gradient stimulated spin-echo (BPFG-SSE). The diffusion experiments were performed by varying the gradient strength between 2% and 95% of the maximum strength in typically 8 or 16 single experiments while keeping the diffusion times and gradient lengths constant.

- DSC studies were performed for exploring the microstructures and their thermal behaviour.

- Small angle neutron scattering (SANS) studies were carried out to determine the globule shapes and size of the developed nanoemulsion.
Rheological properties of MEs were investigated using Brookfield Programmable Digital Rheometer. A cone and plate geometry was used. The viscosity was determined by torque sweep from 10 to 110%. All the measurements were performed in triplicate at 25°C.

Photon correlation spectroscopy (PCS) is a method that correlates time depended fluctuations of scattered laser light of particles in solution due to Brownian motion with their size. Polydispersity index is the measure of the size distribution of the sample. PCS (Zetasizer Nano S, Malvern Instruments) was used to determine the average globule size and polydispersity index of the nanoemulsion droplets in the presence and absence of mepivacaine.

The physical stability of the nanoemulsions containing mepivacaine was studied by observation of clarity and phase separation, droplet size, pH, refractive index, viscosity and electrical conductivity. The nanoemulsions were subjected to varying centrifugation cycles to assess the physical stability.

**Preparation of nanoemulsion-based hydrogels**

Various polymers such as carbopol 980NF, HPMC K4M and HPC at varying concentrations of 0.5, 1 and 1.5% were used to select the optimum gel matrix to prepare the nanoemulsion-based hydrogel formulations. Polymer was slowly mixed with nanoemulsion under stirring. The influence of the addition of hydrogel into nanoemulsions on the viscosity and permeation ability was investigated. The permeation rates of mepivacaine from the optimized nanoemulsion hydrogel were significantly higher than the mepivacaine HCl hydrogels.

**C) Metered dose topical sprays (MDTS)**

The objective of the work was to fabricate and evaluate patient-friendly metered dose topical sprays of mepivacaine using various film-forming polymers. A novel nanoemulsion based metered dose topical spray of mepivacaine was also developed. Solubility of various polymers such as Eudragit RL100, Eudragit L100, Eudragit S100, HPMC K4M, Lutrol F68, PVP K30 and Carbopol 980NF in varying ratios of ethanol:acetone was determined. MDTS were formulated for topical delivery of mepivacaine using different concentrations of film formers, plasticizers and solvents. A $2^3$ factorial design was employed for optimization of MDTS. The developed MDTS were filled in Aptar’s APF$^{\text{plus}}$ dispensing system. The pH, viscosity, volume of solution delivered upon each actuation, spray angle, ex vivo physical evaluation, drug content, in vitro drug diffusion from the mepivacaine film formed on spray actuation and ex vivo drug diffusion studies were carried out on the optimized mepivacaine MDTS spray formulations.
The drug diffusion profiles of all the drug delivery systems were compared with the marketed reference product, Lidoderm patch which contains Lidocaine 5%. The size of the patch is 10 cm x 14 cm. Each adhesive patch contains 700 mg of lidocaine (50 mg per gram adhesive) in an aqueous base. Hence it was used as a comparative reference product for our developed mepivacaine topical formulations.

**Statistical analysis:** All the formulations were optimized using a two-factor, three-level design and independent and dependent variables (responses) were selected. Mathematical equations and response surface plots were used to relate the dependent and independent variables. The statistical validity of the polynomials was established, and optimized formulation factors were selected by feasibility and grid search.

**Ex vivo skin penetration study through porcine ear and rat abdominal skin:** The drug penetration behavior of the optimized topical mepivacaine formulations was investigated on porcine ear skin, rat abdominal skin and human cadaver skin using Franz diffusion cells. At the end of 24 h, the amount of drug in the receptor compartment, the drug remaining in the skin and the drug retained in the skin layers were determined by a validated HPLC method after extraction of the drug in a suitable organic solvent.

**In-house method to study Drug-excipient decomposition:** An In-house method was used to study the decomposition of drug and excipients in the gel formulations. Drug and all the excipients were individually mixed in appropriate ratios in a stoppered glass vial and exposed to 45°C ± 2°C/75% RH ± 5% RH for 1 month. The mixtures were analyzed by the developed HPLC method of analysis for formation of any degradation product.

**Results and Discussion:**
Drug delivery systems such as film forming gels, nanoemulsions and metered dose sprays were loaded with 5% and 10% of mepivacaine as base and as its hydrochloride salt and formulations were compared in terms of drug diffusion profiles. The incorporation of cosolvents like propylene glycol and ethanol significantly increased skin penetration of the drug. However, permeation rate achieved with this solvent system was only modest and hence, it was necessary to further improve the permeation rate of mepivacaine using suitable penetration enhancers.

With the addition of penetration enhancer, the permeation of mepivacaine was remarkably enhanced compared with that of the control (without penetration enhancer). All the enhancers
provided concentration dependent increase in flux value from 0.5% to 2% w/v during *in vitro* and *ex vivo* studies. Among the enhancers used, oleic acid, terpene oil and transcutol showed significant enhancement in flux as compared to extemporaneously formulated lidocaine gels using the same composition. The cumulative amount of mepivacaine increased linearly progressively with time after certain lag time. Because penetration enhancers need time to diffuse from the vehicle to the skin and also need time for penetration through skin and interact with the stratum corneum, the lag time was increased for the penetration-enhanced mepivacaine gels. The optimized clear, transparent, stable topical mepivacaine gel formulations containing novel permeation enhancers resulted in enhanced permeation of the drug across the skin to provide neuropathic pain relief.

Nanoemulsions of mepivacaine base 5% were formulated using oil 5%, surfactant: cosurfactant (1:1) at 42% concentration, water 48% and carbopel 980NF at 1%. The drug diffusion of nanoemulsion based gels formulated containing mepivacaine base 5% showed enhanced permeation as compared to film forming gels. The reason attributed could be (i) the base form of mepivacaine, and/or (ii) the nanoemulsion nature of the dosage form.

The optimized metered dose topical spray formulations of mepivacaine base 5% containing Eudragit® RL100, Lutrol® F68 and PVP K-30 yielded mucoadhesive and flexible film on the human skin. The pH of formulations ranged from 6.5 to 7. The volume of solution delivered upon each actuation was 120μl ±0.05 μl and spray angle was 79.8°. The film was formed within a minute after actuation on human volunteer skin. It passed the desired criteria of film formation. Dermal adhesion, water washability and flexibility of films were moderate. Feeling of warmth and subsequent cooling sensation were perceived after application of spray (around 10 min) because of coolant in the formulation. None of the placebo formulations resulted in irritation, rashes and itching in any of the volunteers.

Comparison of *in vitro* and *ex vivo* studies: The permeation fluxes of mepivacaine were measured *ex vivo* using skin excised from three species; porcine ear skin, rat abdominal skin and human cadaver skin. In both the rodent species, the penetration flux obtained following the application of the nanoemulsion based gels was significantly higher than those obtained by application of the hydrogel. The permeability of drug across the skin was of the order; rat abdominal skin > porcine ear skin > human cadaver skin.

Testing of Salt v/s Base Hypothesis: Mepivacaine HCl and base were formulated at 5% and 10% concentrations using various topical drug delivery systems like film forming hydrogels, nanoemulsion thickened with neutralised carbopel 980NF gels and a propellant free, metered dose sprays. Increasing the mepivacaine concentration from 5%w/w to 10% showed insignificant
increase in the permeation flux of drug. Mepivacaine base delivered from nanoemulsion system and metered dose spray exhibited significantly enhanced rate of permeation as compared to that attained from a mepivacaine HCl hydrophilic gel. Thus permeation studies demonstrated superiority of the base over the hydrochloride form in terms of flux through the skin.

**Product Package compatibility Study:** To confirm the package suitability, the gel formulations were packaged both in aluminum collapsible tubes and Lamitubes and stability was assessed. Metered dose sprays were filled in Aptar’s APFplus containers and their compatibility was investigated. Stability studies included testing the attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. These included the pH, consistency, homogeneity and drug content. Validated analytical procedures were applied for determination of the above attributes.

**Results:** The formulations were found to be stable in all the packaging systems used for topical mepivacaine formulations.

**PART III: SCALE UP AND PILOT STUDIES**

The optimized gels, nanoemulsion based gels and MDT spray formulations were scaled up to five times of the original batch size. The reproducibility of manufacturing process was validated by preparing the same batch in triplicate. Effect of various formulation parameters such as stirring speed, temperature, globule size, viscosity, pH and drug diffusion profiles were assessed. The developed formulations were found to be stable and reproducible during the pilot plant study without any significant changes in physicochemical parameters.

**PART IV: LONG TERM AND ACCELERATED STABILITY STUDIES AS PER ICH GUIDELINES**

The optimized gel formulations (film forming gels and nanoemulsion based gels) were filled in both aluminum collapsible tubes and lamitubes of 10 g capacity and optimized spray formulation in APFplus dispensing systems. All the formulations were subjected to stability studies at temperature and humidity conditions as per ICH Guidelines:

**Stability Conditions:**

1. Storage in refrigeration 2-8°C ± 2°C; sample withdrawn at 1, 3 and 6 Months.
2. 25°C ± 2°C/60% ± 5% RH for 12 months; sample withdrawn at 1, 2, 3, 6, 9 and 12 Months.
3. 30°C ± 2°C/65% ± 5% RH for 6 months; sample withdrawn at 1, 3 and 6 Months.
4. $40^\circ C \pm 2^\circ C/75\% \pm 5\%$ RH for 6 months; sample withdrawn at 1, 2, 3 and 6 Months.

The gels, spray and patches showed no appreciable changes in appearance, colour, % drug content, pH, viscosity, drug diffusion profiles and the formulations were stable at all storage conditions at the end of 12 months

**PART V: ANTI-MICROBIAL EFFECTIVENESS TESTING AS PER USP**

Antimicrobial preservatives are substances added to non-sterile dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. Antimicrobial effectiveness, whether inherent in the product or whether produced because of the addition of an antimicrobial preservative, must be demonstrated for multi dose topical products containing antimicrobial preservatives.

The optimized film forming and nanoemulsion based gels were subjected to anti-microbial preservative efficacy testing as per USP. There was no recovery of *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. niger* culture at the end of 28 days. There was no increase from the initial count of *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. niger* at the end of 14th and 28th day. The results indicate that the topical formulations complied with the acceptance criteria for the Antimicrobial Effectiveness testing. The formulations remained stable over the entire observation period showing no signs of any microbial contamination.

**PART VI: ASSESSMENT OF SKIN IRRITATION POTENTIAL BY DRAIZE TEST**

This study evaluated the dermal irritancy potential after 4 hr and 24 hr of optimized mepivacaine topical dosage forms in two species, Albino rabbits and Wistar rats. Dermal reaction to each test and vehicle control was recorded up to 72 hr post-gauze removal, using the Draize scale as per the protocol approved by IAEC. There were no evident toxic symptoms during observation period.

The gels did not exert any significant irritant effect on rabbit or rat skin. Histopathological evaluation of encoded skin samples showed no significant gross or microscopic changes.

**PART VII: OCULAR IRRITATION POTENTIAL TEST AS PER OECD GUIDELINE NO # 405 AND US EPA GUIDELINES**

Since there is potential for inadvertent eye contact, the study evaluated ocular irritancy potential of optimized formulations in rabbit eye. The objective was to assess the potential of test articles to produce irritation (reversible changes in eye) or corrosion (irreversible tissue damage) when applied to eye of rabbits. Corneal lesion, iris lesion, conjunctival redness and chemosis scores were recorded at 1, 24, 48 and 72 hours. The total ocular lesion score (sum of all scores) was
derived at each time point. There were no evident toxic systemic symptoms observed in the animals during observation period and all animals survived until study completion. The instillation of mepivacaine into the rabbit eye did not produce any significant long lasting changes in ocular lesion scores. Based on current EC guidelines, the tested mepivacaine gels may be considered to be "not irritating" to the rabbit eye.

PART VIII: PRE CLINICAL STUDIES
The pharmacokinetic and pharmacodynamic studies were conducted as per the animal study protocol approved by the Institutional Animal Ethics Committee (CUP/IAEC/015/2010).

a) *In vivo* Dermatopharmacokinetic Studies: The analgesic response to mepivacaine is related to the amount of drug that reaches the target site, i.e. the nociceptors located in the dermis. The local concentration of drugs in the dermis is not easy to determine. Drug concentration into the stratum corneum which is the main barrier to drug transport across the skin, is related to the amount penetrated across the skin, hence mepivacaine flux can be taken as a rough estimate of the amount of drug reaching the target site. Drug from the tape strips were analyzed by using validated HPLC method to investigate the amount of drug permeated in the stratum corneum. The amount of drug permeated in the epidermis and dermis was also estimated by extraction procedure. The study was performed on developed formulations according to the draft guidance released by the FDA in 1998 using Wistar rats as animal model.

b) *In vivo* skin retention using rat as animal model and quantitation of drug in different skin layers: Weighed quantity of formulation was applied on the delineated shaven skin of Wistar rats covering an area of approximately 2 cm x 2 cm. After 24 hrs, the animals were sacrificed and the entire dosing area was collected. The stratum corneum was removed by tape stripping. The epidermis was separated from the dermis using heat separation technique. The drug accumulated in various skin layers was extracted using suitable organic solvent and quantified using validated HPLC method.

c) Visualization of skin penetration in vivo using Confocal Scanning Laser microscopy studies
In this study, confocal scanning laser microscopy (CSLM) was used to visualize the distribution of fluorescent formulations across rat skin and human cadaver skin using a suitable lipophilic dye, Nile red after topical application in rats. Fluorescence pictures were taken (X 40 magnification) using Laser microscope LSM 510 META-Carl Zeiss, argon laser. Qualitative evaluation of passive permeation after 24 h treatment with nile red loaded metered dose spray and nanoemulsion based gels resulted in significant mepivacaine permeation into all skin strata compared to hydrogels. The permeation of mepivacaine delivered via propylene glycol:ethanol
from hydrogel was mostly restricted to the superficial epidermal layers, predominantly the stratum corneum. Viscous solvents such as propylene glycol contribute to increased stratum corneum localization with decreased permeability and solute diffusion into the deeper skin. Incorporation of mepivacaine into nanoemulsion enhanced its permeation from the stratum corneum, through the epidermis finally to the dermis.

d) **Pharmacokinetic studies:** Pharmacokinetics of the developed formulations was studied in Albino rabbits as per the animal protocol approved by IAEC. The rabbits were anaesthetized and at different time intervals the blood was collected and concentration of drug in blood was estimated by developed bioanalytical method using HPLC. Various pharmacokinetic parameters like $C_{\text{max}}$, $T_{\text{max}}$, AUC, bioavailability and $t_{1/2}$ were determined.

e) **Pharmacodynamic Studies:**

Experimental conditions: Wistar rats of either sex (equally distributed among groups) weighing 250±20g maintained on standard laboratory diet having free access to tap water were employed. Rats were housed in the departmental animal house and were exposed to 12 hr light and dark cycle and animals were divided in vehicle control group and formulation group. The analgesic activity of developed topical mepivacaine formulations was investigated using the following pain models.

i. **Cutaneous Trunci Muscle Reflex (CTMR):** There is pathological evidence that cutaneous branches of sensory nerves are affected in peripheral neuropathic pain. The objective of the study was to assess the potential of the topical mepivacaine formulations to provide long lasting cutaneous analgesia after topical application of mepivacaine gels in rats. This was assessed by Cutaneous Trunci Muscle Reflex method. The cutaneous trunci muscle reflex (CTMR) which is characterized by reflex movement of the skin over the back produced by twitches of lateral thoracispinal muscles in response to local dorsal cutaneous stimulation was studied as a reaction to noxious pinprick. The percentage maximum possible effect (M.P.E.) was calculated using the number of stimuli to which the animals failed to respond to the Semmes-Weinstein Monofilament. Data obtained from the study was statistically analyzed using one-way ANOVA followed by Tukey’s multiple range test as post-hoc analysis. A value of $p<0.05$ was considered to be statistically significant. Topical Mepivacaine formulations tested in the present study demonstrated a robust, long-lasting and statistically significant analgesia, when compared to vehicle control gel. The duration of activity of the cutaneous analgesic effect of the topical mepivacaine formulations was found to range between at 5 to 7 hours post-treatment initiation.

ii. **Tail Flick Test:** Wistar rats were used in which the ventral surface of tail was immersed into a glass cylinder containing water adjusted to 52°C and the latency of the first reaction. (Lick,
shake, jump) was recorded (cut off for the first trial 30 seconds). To determine whether the test formulations have analgesic properties, the latency responses of treated and control animals were compared.

PART IX: TOXICITY STUDIES
To prove the safety of the developed topical Mepivacain formulations, acute dermal and repeated dose dermal toxicity studies were carried out as per OECD guidelines # 402 and 410 respectively. The potential of developed formulations to induce toxicity was investigated using Wistar rats as animal model. The studies were conducted for fourteen days and twenty eight days respectively to evaluate the toxicity of single and repeated doses. Various hematological, biochemical and urinary parameters were assessed. Histopathological examination of vital organs such as skin, liver, kidney and heart were performed.
No mortality was observed. There were no significant changes in any of the biochemical and histological parameters. The results of histopathological studies indicated that the developed formulations were safe for topical application.

PART X: ASSESSMENT OF AMOUNT OF MEPIVACAINE GEL FORMULATION A PATIENT WOULD NEED TO APPLY TO SKIN
The developed topical formulations are intended to be applied to patients suffering from peripheral neuropathic pain, in particular, post herpetic neuralgia which spreads over a larger surface area. Since the formulations were developed with a view to commercialize the product in the market, an estimate of how much of the formulation a patient has to apply to achieve therapeutic effect needs to be predicted. In order to undertake the Clinical Phase I study, an estimate of the quantity of formulations in a defined area is essential. Studies were undertaken on rabbit skin and human skin to measure the amount of gel required to spread over a defined area of the skin, 10 cm X 14 cm. Number of grams of gel required to cover this area was noted. This exercise was repeated on three occasions each to the back and to the abdomen. Based on the results, a hypothesis could be derived as to the quantity of formulations recommended to a patient for the relief of post herpetic neuralgia.
CONCLUSIONS:

- The study examined three delivery systems as potential vehicles for cutaneous drug delivery of mepivacaine.
- Topical drug delivery formulations of mepivacaine base and mepivacaine HCl including film forming hydrogels, nanoemulsion based gels and metered dose topical sprays were developed. They were evaluated for physico chemical parameters like *in vitro* and *ex vivo* drug diffusion, pH, viscosity and globule size. The optimized topical gel and spray formulations exhibited desired *in vitro* and *ex vivo* diffusion profiles over a period of 8-12 hrs.
- The optimized formulations were stable throughout the long term and accelerated stability conditions.
- The absorption and penetration of mepivacaine through the skin is mainly by passive diffusion. Nanoemulsion based gel and metered dose spray systems provide relatively high topical fluxes in *vitro* as well as high cutaneous drug penetration and distribution of the local anesthetic in *vivo*.
- The *in vivo* efficacy of the developed topical mepivacaine formulations tested in CTMR study demonstrated a robust, long-lasting and statistically significant analgesia, when compared to vehicle control.
- Histopathological studies confirmed that mepivacaine formulations were non irritating, non toxic and safe for topical administration.
- The developed mepivacaine topical formulations exhibited desired physico chemical parameters and have been found to provide robust efficacy and thus have an enormous clinical relevance for the treatment of patients suffering from peripheral neuropathic pain.

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KEY REFERENCES:


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