Summary
Transdermal drug delivery is gaining interest in recent times because of its non-invasive nature and broad scope of potential applications in treating human diseases. To overcome the superficial stratum corneum barrier of skin, various percutaneous enhancement technologies are being explored such as use of chemical, bio-chemical or physical enhancers. Iontophoresis, sonophoresis and hyperthermia are the physical methods of enhancing percutaneous absorption of drugs.

The present study explores the possibility of employing the above mentioned novel physical approaches for enhancing the transdermal penetration of ketorolac tromethamine (a non-steroidal analgesic and anti-inflammatory agent) for either local or systemic delivery.

In order to investigate the effect of iontophoresis, sonophoresis and hyperthermia on in vitro transport of ketorolac tromethamine, suitable diffusion cells were designed and fabricated. The fabricated cells included both horizontal and vertical two-compartment models. To achieve uniform stirring of the donor and receptor solutions and to avoid maintenance of non-sink condition, a mini magnetic stirring device was developed. The mini magnetic stirring device was found to be very useful as it provided the cost-effective alternative to conventional means of stirring.

Because of absence of suitable iontophoresis devices in Indian market, an effort was made to develop 'indigenously' portable iontophoresis unit. Initially, an iontophoresis unit powered by electrical supply from household current was developed. Further to improve the elegance and considering the safety aspects, the device was modified to a 'miniatured' iontophoresis device, powered by battery supply. This low powered transitorised circuitry made the device safe and attractive not only for experimental application but also for clinical use. The device offered variety of features for regulation of various parameters such as current density, frequency, on:off ratio and selection of pulsed mode. In the present work, this device has been used for optimisation of iontophoretic parameters, both in vitro and in vivo during experimental studies and also in clinical set up. The device becomes promising both for researchers as well as clinicians because of its robustness, miniature size and control of various features tailored to the needs of individuals. For acceptance of iontophoresis as a method of drug delivery, such
lightweight iontophoresis devices capable of providing an easy direct application to the skin is desirable.

In the initial phase, effect of iontophoresis on membrane transport of ketorolac was studied using synthetic cellulose membrane. This model permitted the determination of optimal conditions of iontophoretic mobility of ketorolac with a constant quality membrane that offers no resistance to ion movements. The parameters evaluated using synthetic membrane were - effect of current density, drug concentration, buffer composition (and pH) and viscosity of the donor solution. The results of synthetic membrane studies indicated that higher iontophoretic transport of ketorolac was obtained when no extraneous ions (such as NaCl) were present in donor solution. The flux of ketorolac increased linearly with the current density. However, a decrease in drug concentration or an increase in viscosity slowed down the iontophoretic transport of ketorolac. Comparison of the total flux under iontophoresis with sum of passive and iontophoretic contribution indicated that these two contributions could justify the value of total flux measured. Further, the electro-osmotic contribution if any, during iontophoretic transport through cellulose membrane was investigated using glucose as a neutral molecule. The application of current did not modify the flux of glucose confirming the absence of electro-osmotic flux.

In the second phase of studies, iontophoretic penetration of ketorolac through hairless rat skin was investigated. Application of iontophoresis significantly enhanced the skin penetration of ketorolac. Maximal iontophoretic transport was observed when low ionic strength McIlvaine buffer (0.06 M, pH 7.4) was used as donor solution. The choice of electrode material or diffusion cell design did not influence the iontophoretic permeation of drug. Constant DC was found be more potent than pulse DC in enhancing the penetration of drug. With pulse current, increasing the on:off ratio of pulse or frequency increased the penetration of drug. Increasing the drug load in donor compartment significantly enhanced the penetration of drug. The duration of iontophoresis was also found to be important. Flux was greater when iontophoresis was applied for 6h instead of 1 or 4h. Termination of current application did not cause the flux to return immediately to the passive control level. Pretreatment of the skin with chemical enhancer, d-limonene (5% in ethanol), significantly enhanced the iontophoretic permeation of
ketorolac. Further enhancement in flux was observed when chemical enhancer pretreatment (d-limonene/ethanol) was combined with ultrasound pretreatment.

In order to evaluate the use of pulse current modality with optimal transport conditions, a factorial design based experiment was conducted. The results of experimental design indicated the positive contribution of drug load and increasing on:off ratio of pulse current on iontophoretic transport while increasing donor ionic strength (of buffer) resulted in negative effect on flux.

The results of phase III studies (in vivo pharmacokinetic studies in rat) indicated that significant concentration of ketorolac could be detected in serum following iontophoresis. After, 1h of iontophoresis, 1969 ng/ml of ketorolac was detected in serum. The half-life of drug following iontophoretic delivery was found to be 6.29h with an elimination rate constant of 0.11h⁻¹. This indicates that iontophoresis can be used to deliver ketorolac tromethamine transdermally. This fact can also be utilised to achieve maximal concentration of drug in localised area for management of painful conditions such as muscle or joint pain.

Application of sonophoresis (1 MHz, 3 watts/cm²) also significantly enhanced the flux of ketorolac across hairless rat skin in vitro. The observed enhancement in flux was minimal when compared with iontophoresis. Use of lower intensities of ultrasound did not result in enhancement of flux. The increase in flux with sonophoresis was found to be primarily because of temperature rise of donor solution during sonophoresis since controlling the temperature of donor solution resulted in flux values similar to passive (with ultrasound) flux.

Combination of hyperthermia (increased temperature) and chemical enhancer pretreatment (5% d-limonene in ethanol) also resulted in enhanced flux of ketorolac across hairless rat skin in vitro. Enhancement in flux was maximal when higher temperature (42 °C) was applied in combination with chemical enhancer pretreatment. The enhanced penetration following combination of hyperthermia and enhancer pretreatment may be beneficial for better local delivery of ketorolac for management of local pain or inflammation.
In an attempt to explore the clinical applications of iontophoresis and sonophoresis, few studies were undertaken. Iontophoresis of lignocaine was examined for inducing anaesthesia to facilitate tooth extraction in children. Lignocaine iontophoresis was accomplished using special clip electrodes along with cotton gauze soaked in lignocaine hydrochloride solution. The procedure required only 5-10 minutes and was found to be an important alternative to avoid painful injection of lignocaine. Use of suitable electrodes and inclusion of penetration enhancer was found to induce rapid anaesthesia.

Another clinical application of iontophoresis was development of modified sweat chloride test for diagnosis of cystic fibrosis. The pilocarpine iontophoresis sweat test was modified suitably and was employed to screen patients susceptible of cystic fibrosis. Out of 120 patients, three patients could be diagnosed for cystic fibrosis which were further confirmed by clinical manifestations. This study is particularly important because there are very few cases of cystic fibrosis reported in India, which could be attributed to the absence of proper diagnostic tool such as sweat test.

Methotrexate iontophoresis was explored as an alternative treatment for refractory palmoplantar psoriasis (PPP) in a 46 year old male patient. The patient had the disease on both palms. Four treatments at weekly intervals were given to right palm, while left palm served as control. The right palm showed considerable improvement compared to the left palm.

Clinical application of sonophoresis was examined for treatment of localised painful conditions. Ultrasound treatment in combination with topically applied analgesics/anti-inflammatory agents was evaluated in a double blind placebo controlled clinical trial. Sonophoresis of ketorolac was found to be effective clinically in resolving soft tissue injury/pain as compared to sonophoresis of nimesulide, diclofenac or placebo gels.

In order to further explore the application of one of the above mentioned physical modality i.e. hyperthermia in drug delivery, temperature sensitive liposomes, which would release the drug only to heated area (localised hyperthermia) were formulated. The approach of combining hyperthermia and temperature sensitive liposomes may result in targeted delivery of drug and hence enhance the efficacy of drug. This is particularly
important in delivery of cytotoxic drugs to localised tumour sites. To explore this novel approach, in the present study, thermosensitive liposomes of bleomycin and plumbagin, anti-cancer agents, were prepared and the formulations were evaluated in vivo in combination with localised hyperthermia (43 °C) in mice bearing tumour. The liposomes were designed to release their contents at 41 °C using a suitable mixture of synthetic phospholipids, dipalmitoyl phosphatidylcholine and disteroyl phosphatidylcholine. The liposomes were characterized for size and entrapment efficiency. The results of in vitro release test at various temperatures indicated that the maximum release occurred at 42 °C and only a smaller fraction of drug was released at physiological temperature. When evaluated in vivo in mice bearing melanoma B16F1 in combination with localised hyperthermic treatment of tumour, thermosensitive liposomes resulted in enhanced antitumour activity as evidenced by enhanced tumour volume doubling time and growth delay. The enhanced antitumour activity might be attributed to the targeted delivery of the drug and interaction of hyperthermia and drug leading to increased cytotoxicity. These results suggest that localised hyperthermia in combination with temperature sensitive liposomes encapsulating either bleomycin or plumbagin may serve as a useful targeted drug delivery system for management of localised accumulation of tumours such as melanoma B16F1.
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