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Effect of gel properties on transdermal iontophoretic delivery of diclofenac sodium

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Abstract: The aim of the study was to develop hydrogels and investigate the suitability for transdermal delivery of diclofenac sodium (DS) using constant voltage iontophoresis (CVI). Four batches of hydrogels of DS were developed using hydroxylethyl cellulose (HEC) as matrix material and terpenes as penetration enhancers. The hydrogels displayed a viscosity of ~1500 cps at a shear rate of 250 s^{-1} that was unlikely to change on minute shift in pH or temperature so that the iontophoretic transport would be unaffected. Moreover, the hydrogels were found to possess adequate conductivity at pH 7.4 to enable iontophoretic delivery of DS. *In vitro* studies indicated that passive transport of DS across porcine skin from hydrogels was comparable ($p>0.05$) to aqueous solution. The lead hydrogel (F_1), containing geraniol was found to enhance the iontophoretic flux of DS by 5.16 fold at 1.5 V compared to passive control. *In vivo* studies in rats indicated that CVI on application of F_1 significantly suppressed ($p<0.001$) carrageenan induced edema compared to passive treatment throughout the study.

Keywords: constant voltage iontophoresis; diclofenac sodium; hydrogels; hydroxyethyl cellulose; terpenes.

1 Introduction

People throughout the world are known to suffer from pain and disability due to various musculoskeletal (MSK) disorders. MSK disorders account for nearly 3.4% and 1.7% of the total global disease burden in the developed and developing countries respectively (1). Currently, the treatment for MSK disorders involves oral or parentral administration of steroids or non steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are known to provide symptomatic relief from pain, allow quicker recovery and return to normal activity (2). Diclofenac is a NSAID that is commonly used in chronic pain management associated with various MSK disorders (3). Though the drug relieves pain by reducing prostaglandin synthesis, diclofenac undergoes extensive hepatic first pass metabolism that invariably limits the oral bioavailability to 50–60%. Therefore, the drug needs to be frequently administered at a daily dose of 150 mg in three divided doses (3). Owing to the non-selective cyclooxygenase inhibition and the frequent dosing, oral diclofenac is frequently associated with severe gastrointestinal side effects, which invariably limits its long-term use. Transdermal administration is likely to overcome hepatic first pass metabolism, improve bioavailability, allow dose reduction, reduce systemic exposure and thereby the dose-dependent adverse effects and eventually improve patient compliance. Though transdermal therapy is known to overcome the gastrointestinal adverse effects of oral diclofenac, the penetration of therapeutic amounts into underlying inflamed tissues has been a big challenge (4). Considering the limitations of these products, the current work primarily aims to enhance the transdermal delivery of diclofenac sodium (DS) from hydrogels using constant voltage iontophoresis (CVI). CVI is an electrically assisted technique that can promote the transdermal transport of ionic permeants under the influence of applied low voltage (5). Though constant current iontophoresis has been used in the past to enhance the transdermal transport of DS from aqueous solutions (6–10), no attempt has been made till date to investigate the iontophoretic transport of DS from hydrogels under constant voltage. In this context, the present study aims to formulate hydrogels of DS using generally regards as safe (GRAS) listed excipients and characterize the same to assess their suitability for transdermal iontophoretic delivery.

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2 Materials and methods

2.1 Materials

Diclofenac sodium was purchased from Yarrow Chemicals (Mumbai, India) Natrosol 250 M, a sample of

hydroxyethyl cellulose (HEC) was donated by Ashland Chemicals Ltd (Mumbai, India). Geraniol, *l*-menthol, thymol and δ-carrageenan were purchased from Sigma-Aldrich (Bangalore, India). The rest of the chemicals and reagents of analytical grade were bought from S.D. Fine Chemicals (Mumbai, India). Deionized water (resistivity~18 MΩ-cm) was used for all the preparations.

2.1.1 Animals

The *in vivo* experimental protocol was approved by institutional Animal Ethical Committee (No.06/SAK/HNSK/01/2013). Male Wistar rats weighing 200–250 g were used for all the studies.

2.2 Determination of partition coefficient

The partition coefficient of DS was determined in phosphate buffer (PB) of pH 7.40 and n-octanol system. Aqueous solutions of DS in PB (2 mg/ml) was equilibrated with equal volume of n-octanol for 24 h with intermittent shaking after which the resultant mixture was centrifuged at 2000 rpm for 10 min to separate the two constituent phases. Later, the amount of drug in aqueous phase was determined spectrophotometrically at 276 nm (UV-1800, Shimadzu Corporation, Kyoto, Japan) on suitable dilution. The partition coefficient was determined using equation [1] after calculating the differential amount of DS partitioned into n-octanol (11).

$$\log P = \log \frac{C_O}{C_{PB}} \quad [1]$$

Where, C_O and C_{PB} represent the DS concentration in organic phase and PB, respectively.

2.3 pH solubility profiling

The solubility of DS was determined spectrophotometrically at pH values of 1.2, 4.0, 5.0, 6.8 and 7.4 to establish the pH solubility profile of the drug. The fraction of DS ionized at different pH was calculated by as per Henderson-Hasselbach's equation [2]

$$\% \text{ Ionized} = \frac{100}{1+10^{\text{pH-pKa}}} \quad [2]$$

2.4 Gel preparation

Hydrogels of DS were prepared in dual asymmetric centrifuge (DAC 150 Speed mixer, Hauschild and Co. Waterkamp, Germany) using HEC as a matrix material. Terpenes like geraniol, l-menthol and thymol were incorporated as a penetration enhancer into hydrogels F_1, F_2, and F_3, respectively while the control gel (Fc) was devoid of any enhancer. The composition of different gel formulations are outlined in Table 1. Briefly, DS (1% w/w), terpenes (5% w/w), ethanol (40% w/w) in PB (Q.S) were taken to a mixing cup of the speed mixer and dispersed at 3000–3500 rpm and for 8 min (12). Subsequently, HEC was added to the cup and mixed for another 10 min at the same speed to obtain a clear homogeneous hydrogel free from air bubbles. The hydrogels were subjected to physicochemical characterization before further characterization.

2.5 pH measurements

The pH of the hydrogels were determined using an Orion pH meter (115/220 VAC, Thermo Scientific, India). The hydrogels were taken in separate glass beakers and the electrode was dipped 2–3 cm deep into the gel to measure the pH. If necessary, the gels were set to pH of 7.40±0.50 using 3 molar sodium dihydrogen phosphate or disodium hydrogen phosphate.

2.6 IR Spectrometry

Infrared spectra of DS and drug loaded hydrogel were recorded in region of 4000–400 cm^{-1} in an attenuated total refluctance (ATR) spectrometer (Alpha, Bruker, Billerica, MA, USA) at 20°C to identify the functional groups in DS. No special procedure was involved in sample preparation as the samples were directly exposed to the IR rays in ATR spectrometer. The spectra of the gel were compared to that of DS to establish the chemical integrity of DS in gels.

Table 1: Composition of DS hydrogels.

Ingredients	Composition (% w/w)			
	F_C	F_1	F_2	F_3
DS	1.00	1.00	1.00	1.00
Geraniol	–	5.00	–	–
l-menthol	–	–	5.00	–
Thymol	–	–	–	5.00
Ethanol	–	40.00	40.00	40.00
HEC	2.75	2.75	2.75	2.75
PB (QS)[a]	100.00	100.00	100.00	100.00

[a]QS, Quantity sufficient.

2.7 Viscosity profiling

The viscosity of DS hydrogels was determined in a modular compact rheometer (MCR-302, Anton-Paar Gmbh, Austria) using a PP50/P2 spindle. The hydrogels were loaded on to the lower peltier plate of the rheometer and the measuring head was lowered to a gap of 0.5 mm. The viscosity was recorded by varying the shear rate between 250 s^{-1} and 1 s^{-1} at a temperature of 25±0.2°C (13).

2.8 Conductivity measurement

The ability of the hydrogel to carry the electric current was assessed by measuring the conductivity (14). Prior to the measurement, conductivity meter (CD-4301, Ankom International, India) was calibrated using standard calibration solution (0.05% w/v NaCl solution) at 25±0.5°C. Subsequently, the conductance of the samples was measured at the same temperature by dipping the electrode into the beaker containing hydrogels.

2.9 Skin preparation

Porcine ears from the pigs of 6–8 months of age were procured from a nearby local slaughter house. Hairs from the surface of ears were removed using a custom made electrical trimmer while the whole skin was separated from the underlying cartilage using a surgical scalpel. The fat underlying the subcutaneous layer was carefully removed using a pair of surgical scissors. Later, the full thickness skin was washed with water before use (11).

2.10 Electrodes preparation

Most of the iontophoretic studies recommend the use of silver-silver chloride (Ag-AgCl) electrodes due to their stability and reversibility (15). Two silver wires having a diameter 0.8 mm were connected to the two terminals of a direct current source while the free ends were dipped in 0.1 N hydrochloric acid (HCl). AgCl was gradually deposited on the anode after passing the current for a period of 6 h. This Ag electrode thus coated with AgCl was used as a cathode for the iontophoresis studies while Ag wire was used as an anode.

2.10.1 *In vitro* passive diffusion

In vitro passive permeation studies of DS from hydrogels were conducted for a period of 8 h in a modified Franz diffusion cells (FDC) (11). The porcine skin was sandwiched between the two halves of a diffusion cell in such a way that the epidermis was facing the donor compartment. DS gels (0.5 g) were charged into the donor compartment while the receiver compartment filled with phosphate buffer saline (PBS) was maintained at 37±0.5°C and a stirring speed of 600±10 rpm in a diffusion cell apparatus. Samples (0.5 ml) from the receiver were withdrawn from sampling port of the FDC at 1, 2, 3, 4, 6 and 8 h, suitably diluted and analyzed by high performance liquid chromatography (HPLC).

2.10.2 *In vitro* iontophoretic permeation

The *in vitro* iontophoretic permeation studies of DS from hydrogels were performed under a similar set of conditions for a period of 8 h in modified FDC. However, an AgCl cathode was mounted on the donor while a pure Ag wire placed in the receiver compartment served as an anode during the iontophoretic studies. A constant voltage was applied across the electrodes during the studies and the current flowing through the circuit was constantly monitored with a digital multimeter. Samples from the receiver were withdrawn during the study, suitably diluted and analyzed by HPLC. The amount of DS diffused per unit area of the skin from the hydrogels was plotted against time and the steady state flux (J_{ss}) was obtained from the slope of the linear portion of the plot. The enhancement ratios (ER) for the hydrogel were determined using equation [3] (11).

$$\mathrm{ER}=\frac{\text{Iontophoretic flux}}{\text{Passive flux}} \quad [3]$$

The target flux (F) for DS was calculated as per equation [4] from the pharmacokinetic parameters cited in the literature (16).

$$\mathrm{F}=\frac{C_{SS}Cl_{T}W}{\mathrm{A}} \quad [4]$$

where, Cl_T stands for total body clearance of diclofenac, which is reported to be 4.2±0.9 ml/min.kg (17). The steady state plasma concentration (C_{ss}) was assumed to be 400 ng/ml that represented half of the peak concentration achieved in blood after an oral dose of 50 mg in human volunteers (18). "W" refers to the standard body weight (~60 kg), while "A" represents the area of transdermal patch.

2.11 HPLC analysis

The samples collected at each time point during *in vitro* studies were analyzed in a liquid chromatographic system

(LC-2010CHT, Shimadzu Corporation, Japan). The samples were analyzed using a UV detector at 276 nm at the oven temperature of 25±0.2°C (19). The mobile phase comprising of a mixture of acetonitrile:deionized water (70:30 v/v) was set to pH of 3.5 before elution of DS.

2.12 *In vivo* pharmacodynamics

Four groups of animals with six animals in a group were used for the pharmacodynamic studies. Prior to the start of the studies, animals were anesthetized using ketamine hydrochloride by intraperitoneal injection and clipped to a wooden board with the abdomen facing upwards. DS was allowed to passively diffuse after the application of F_C and F_1 hydrogels for the first and second groups of animals, respectively. CVI (1.5 V) was applied after applying hydrogel F_1 to the third group while no hydrogel was applied to the positive control group.

One hour after treatment, carrageenan solution (0.05 ml of 1% solution in normal saline) was injected into the sub-plantar region of right hind paw for all the animals to induce the inflammation (20). Subsequently, the thickness of swollen paw of all the animals was measured using a digital vernier caliper (Mitutoyo, Kawasaki, Japan) at 0, 1, 3, 5 and 8 h following the injection. The percentage inhibition of paw thickness was calculated using equation [5] (21).

$$\%\ \text{Inhibition} = \frac{(C_t - C_0)_{\text{Control}} - (C_t - C_0)_{\text{Treated}}}{(C_t - C_0)_{\text{Control}}} \times 100 \quad [5]$$

where C_t and C_0 represent the paw thickness at time "t" and "0" after injection.

2.13 Statistical analysis

The data was statistically compared by performing ANOVA in GraphPad Instat 5.0 demo version software (GraphPad Inc., CA, USA). The probability value of <0.05 was considered to be significant.

3 Results and discussion

Iontophoresis is found to be more effective compared to passive diffusion in delivering charged therapeutic agents to subdermal tissues as it is known to promote direct tissue penetration rather than systemic reabsorption. Moreover, iontophoresis is considered to be the right option to deliver NSAIDs as the technique is likely to ensure a quicker onset of action when compared to a passive transdermal patch (22). However, most often iontophoretic delivery is determined by gel properties like pH, viscosity, and conductivity (14).

3.1 Determination of partition coefficient

DS seems to be an ideal molecule for iontophoretic transdermal delivery by virtue of its low molecular weight (334.24 Da) and high charge to mass ratio (2.99×10^{-3}). The experimental value of partition coefficient of DS in n-octanol-PB system was found to be 12.907±0.256. The corresponding log P value (1.114) indicates the relative polar nature of the DS at physiological pH and therefore, justifies the selection of CVI to enhance the transdermal delivery. The experimental value of log P was found to be close to the calculated value for DS at the physiological pH (23).

The pH solubility profile of DS indicates that the solubility of the drug increases with pH at values exceeding the pKa (pKa ~3.80) (Figure 1). The pH-pK_a differential determines the fraction of ionized species and therefore the iontophoretic efficiency. Further, the majority of the soluble fraction of DS (~0.999) is likely to exist in an ionized state at the physiological pH as per equation [2] making DS a suitable candidate for cathodal iontophoresis. The diclofenac anion would be repelled away from the cathode by electrorepulsion, which acts as a primary driving force during CVI.

3.2 Gel preparation

Gels are known to be the most suited vehicles for iontophoresis as they can be easily integrated within the device

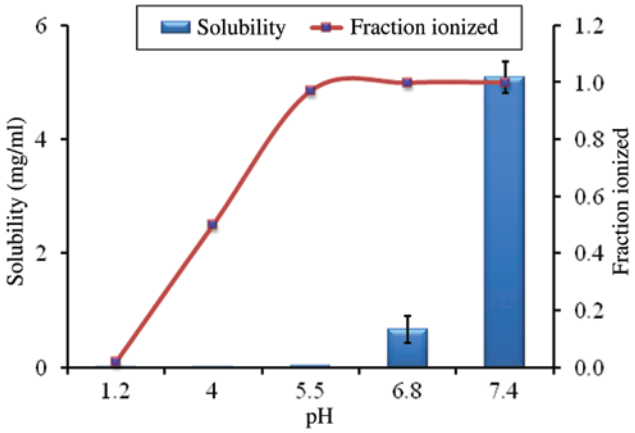

Figure 1: pH solubility profile of diclofenac sodium. Each data point represents mean±SD (n=3).

(14). Bioadhesive gels are advantageous compared to liquids in that they adhere well to the skin conforming to the skin contours thereby preventing the material from flowing over the skin (24). Water was used as a solvent in the gels due to its biocompatibility, high dielectric constant and its ability to render the gels more conductive. Bearing in mind the low pK_a value of DS, the gels were buffered to a pH of 7.40 so as to ensure that the major fraction of the drug existed in the ionized state. Moreover, the pH selected is less prone to cause skin irritation. Considering the permeation issues of DS, terpenes were used as a permeation enhancer in the hydrogels (Table 1). Terpenes are GRAS listed under natural transdermal penetration enhancers that are non-toxic and non-irritating (25). As the terpenes were insoluble in the aqueous phase, ethanol (40% w/w) was used as a co-solvent to facilitate its dissolution in the hydrogels. HEC is a nonionic polymer that is GRAS listed and approved by the USFDA for topical applications owing its nonirritating and nonsensitizing nature. HEC was used as a matrix due to its good bioadhesion, high polarity, biocompatibility and nonionic nature. Being non-ionic, HEC avoids the possible drug-polymer interaction and active ionic competition during CVI so that the iontophoretic transport of DS remains unaffected. HEC is used as a matrix material in the design of the Fentalis, a marketed transdermal iontophoretic patch of Fentanyl (26).

3.3 FTIR studies

The IR spectra of DS displayed distinctive peaks at 3380.20 cm^{-1}, 1570.12 cm^{-1} and at 747.35 cm^{-1} due to the NH stretching of the secondary amine, -C=O stretching of the carboxylate ion and -C-Cl stretching, respectively. The spectra of HEC portrayed the -C-OH stretching peak at 3444 cm^{-1}, while the SP^3 stretching vibrations of $-CH_2$ group were seen at 2923 cm^{-1} and 1020 cm^{-1}. In the spectra of the gels, the characteristic peaks of diclofenac appeared at 1568.24 cm^{-1} (C=O, carboxylate) and at 744.56 cm^{-1} (-C=Cl, stretching) proving the chemical integrity of DS in hydrogels. The IR spectral observations rule out the possible interference in the iontophoretic transport due to chemical interaction.

3.4 Viscosity profiling

The viscosity profiles of the gels were found to overlap with each other indicating that the concentration of HEC in the gel is likely to determine the gel viscosity rather than the composition of the dispersion media (Figure 2). The observations were consistent with the earlier reports that suggest that viscosity of hydrogels were a function of HEC concentrations (27). The non-Newtonian nature of the gels was clearly evident as the viscosity was found to drop at high rates of shear. Gels of comparable viscosities have been used in the past for iontophoretic delivery of DS (28).

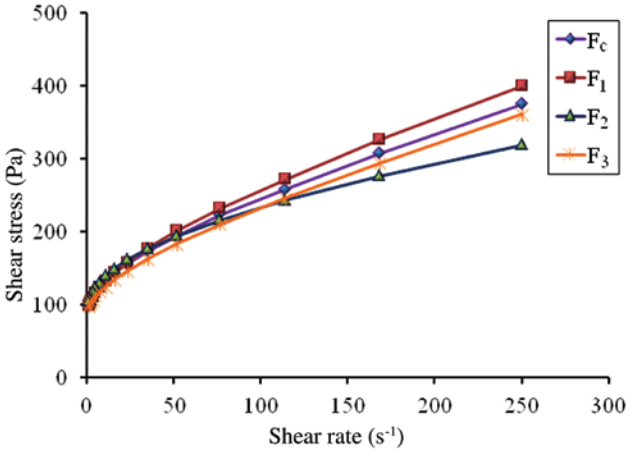

Figure 2: Rheograms representing the rapid viscosity profiles of hydrogels.

Iontophoresis is likely to induce a slight change in the pH or temperature depending on the electrodes used, voltage applied and the nature of the permeant. Such changes in pH or temperature are likely to induce a potential change in the gel viscosity and therefore the drug delivery from a pH-sensitive or a thermo-sensitive polymer making them a poor choice for iontophoretic delivery. However, gels of HEC are less prone to viscosity changes due to minute shifts in the pH or temperature making them the materials of choice for iontophoretic delivery. HEC solutions undergo small changes in viscosity in the pH range of 2–12 while they exhibit greatest viscosity stability in the pH range of 6.5–8.0 (29).

3.5 Determination of conductivity

The conductivity of the control gel containing DS was found to be 384.4±1.20 μSi/cm that was significantly higher ($p<0.0001$) than the value obtained for the placebo gel (162.1±2.5 μSi/cm). The increase in conductivity on the addition of DS indicated the potential of the drug to ionize at pH 7.40. Further, since the conductivity of PB containing DS (398.4±5.48 μSi/cm) was comparable ($p>0.05$) to the value obtained for the control gel, it was obvious that the increase in gel viscosity failed to restrict the ionic

mobility and therefore is unlikely to hamper the iontophoretic transport of DS. Solutions having similar conductivities have enabled iontophoretic transport of therapeutic agents (14, 24).

3.5.1 *In vitro* passive diffusion

The passive steady state flux of DS from PB and the control gel was found to be 0.099±0.011 and 0.102±0.012 μmol/cm²/h, respectively. Though the gel viscosity was higher compared to the aqueous solution, no significant difference ($p>0.05$) in the passive steady state flux of DS was evident for the control gel when compared to the solution. The comparable values of fluxes indicated that the gel viscosity did not substantially restrict the mobility of the diclofenac anion. These observations imply that diffusion of the drug through the skin was the rate determining step in the permeation of DS rather than the diffusion through the gel matrix. An earlier study has shown that diffusion of ionic permeants from gels were not different from aqueous solutions (11).

The enhancement with different penetration enhancer for DS was found to increase in the following order: geraniol>*l*-menthol>thymol. Of all the hydrogels, F_1 emerged as the most efficacious formulation with an enhancement of ~2 fold compared to the passive control (Figure 3). Geraniol is an unsaturated acyclic monoterpene having a trans confirmation with two double bond. Generally, acyclic terpenes by virtue of the definitive hydrocarbon tail with a polar head are said to be structurally well suited to disrupt the stratum corneum lipids. Geraniol at a concentration of 1% in gels is reported to enhance the passive permeation of DS by 19-fold (30). The better efficacy of geraniol compared to *l*-menthol could be due to the fact that geraniol being a liquid terpene has a lesser tendency to form hydrogen bonds with the ceramides and cholesterol of the skin lipids compared to solid counterparts. Further, the average number of hydrogen bonds that could be formed by liquid and solid terpenes is reported to be 1.1 and 2.0, respectively (31). However, of the three terpenes, thymol was not as effective as other terpenes in accelerating the *in vitro* transport of DS.

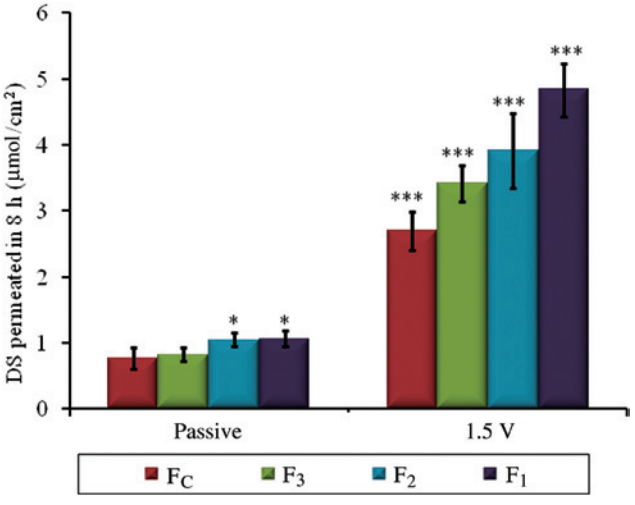

Figure 3: *In vitro* passive and iontophoretic transport of diclofenac sodium.
Each data point represents mean±SD (n=3), (***$p<0.0001$, *$p<0.05$), compared to passive control (F_C).

3.5.2 *In vitro* iontophoretic permeation

The three pathways utilized by permeants to pass through the stratum corneum of the skin are transcellular, paracellular and follicular pathways. Of these, the paracellular lipid and follicular shunt pathways have been identified to be the key pathways involved in the iontophoretic transport of DS (9). Due to higher current intensities observed at higher voltages (3.0 V and 4.5 V) iontophoretic trials were carried out only at voltages of 1.5 V. Current strengths exceeding 0.5 mA/cm² often cause skin irritation, skin damage or uncomfortable electrical stimulation during iontophoresis (32). CVI significantly increased ($p<0.0001$) the amount of DS permeated through the skin in 8 h, compared to their passive counterparts (Figure 3). Of all the hydrogels, F_1 emerged as the leading formulation with an enhancement factor of 5.16 compared to the passive control. The better efficacy of terpenes can be attributed to the presence of hydroxyl groups which are known to strongly interact and disrupt the hydrogen bonds prevailing between ceramide head group of the stratum corneum under the applied electric filed (11). The target flux of DS deduced based on equation (4) was found to be 19 μmol/h. Considering the iontophoretic flux of the F_1 (0.526±0.041 μmol/cm²/h) at 1.5 V, a patch having a dimension of 36 cm² would be able to meet the target flux of DS.

3.6 *In vivo* pharmacodynamics

CVI following F_1 treatment suppressed the carrageenan-induced edema as early as 1 h and completely inhibited the paw swelling for 8 h indicating a faster and long lasting action (Figure 4). Moreover, with F_1, a significantly better inhibition ($p<0.001$) was seen with CVI compared to

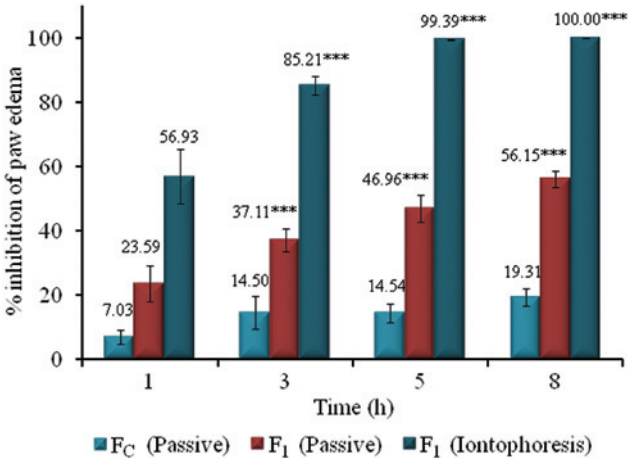

Figure 4: Percentage inhibition of paw thickness as a function of time.
Each data represents mean±SEM (n=3), (***p<0.0001, *p<0.05), compared with passive control (F_C).

passive treatment of the same hydrogel at all time points. Likewise, on passive treatment the percentage inhibition by F_1 at all the time points were significantly higher ($p<0.05$) compared to the control (F_C). However, the effect of CVI was found to be more pronounced compared to the efficacy of geraniol alone. The *in vivo* studies conclusively demonstrated the superior efficacy of F_1 in inhibiting the paw edema following the carrageenan challenge. Due to the bioadhesive nature, the gels were well retained till the end of the study maintaining excellent contact with the skin.

4 Conclusion

Hydrogels of DS were developed using GRAS listed penetration enhancers and matrix material. By virtue of the unique composition, the hydrogels developed are likely to be robust enough to resist viscosity changes despite minute changes in the pH or temperature so that the iontophoretic delivery would be unaffected. The *in vitro* studies indicated that the amount of DS delivered from hydrogels was comparable to those delivered from aqueous solutions. The *in vivo* studies suggested that the hydrogel F_1 was able to safely deliver therapeutic amounts of DS by transdermal iontophoresis.

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