Colloids and Surfaces B: Biointerfaces 65 (2008) 300–307

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Improvement in bioavailability of transdermally applied flurbiprofen using tulsi (*Ocimum sanctum*) and turpentine oil

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ARTICLE INFO

Article history:
Received 6 February 2008
Received in revised form 23 April 2008
Accepted 1 May 2008
Available online 8 May 2008

Keywords:
Transdermal delivery
Flurbiprofen
Histological studies
Pharmacokinetics
Terpenes

ABSTRACT

Penetration enhancing potential of tulsi and turpentine oil on transdermal delivery of flurbiprofen, a potent non-steroidal anti-inflammatory agent, was investigated. The transdermal permeation rate of flurbiprofen across the rat abdominal skin from binary solvent mixture composition of propylene glycol (PG):isopropyl alcohol (IPA) (30:70%, v/v) was 98.88 μg/cm^2/h, significantly higher than other binary solvent mixtures. The corresponding steady state plasma concentration, 0.71 μg/ml, was much lower than required steady state plasma concentration of 3–5 μg/ml. Hence influence of tulsi and turpentine oil in the optimized binary solvent mixture along with the increased drug load on the flurbiprofen permeation was evaluated. The magnitude of the flux enhancement factor with turpentine oil and tulsi oil was 2.4 and 2.0 respectively at 5% (v/v) concentration beyond which there was no significant increase in the flux. Addition of 2% (w/v) hydroxypropyl methylcellulose (HPMC), as a thickening agent, resulted in desired consistency for the fabrication of patch with insignificant effect on permeation rate of flurbiprofen. The reservoir type of transdermal patch formulation, fabricated by encapsulating the flurbiprofen reservoir solution within a shallow compartment moulded from polyester backing film and microporous ethyl vinyl acetate membrane, did not modulate the skin permeation of flurbiprofen through rat skin in case of turpentine formulations whereas flux of formulations with tulsi oil was significantly altered. The influence of penetration enhancer and solvents on the anatomical structure of the rat skin was studied. Enhancement properties exhibited by turpentine oil and tulsi oil in optimized binary solvent mixture were superior as compared to solvent treated and normal control groups with negligible skin irritation. The fabricated transdermal patches were found to be stable. The bioavailability of flurbiprofen with reference to orally administered flurbiprofen in albino rats was found to increase by 2.97, 3.80 and 5.56 times with transdermal patch formulation without enhancer, tulsi and turpentine oil formulations, respectively. The results were confirmed by pharmacodynamic studies in rat edema inflammation model.

1. Introduction

Flurbiprofen has been found to be one of the most potent members of a series of phenylalkanoic acids in various animal species in anti-inflammatory, analgesic and antipyretic tests [1]. It is highly effective for the treatment of rheumatoid arthritis in man [2]. Flurbiprofen also causes a dose dependent inhibition of collagen induced platelet aggregation in platelet rich plasma from human, rats and rabbits in vitro [3]. Flurbiprofen is at least 99% bound to human serum albumin at therapeutic concentration. Flurbiprofen may bind to red blood cells. Area under plasma drug concentration versus time curve increases with increasing dose of drug administered [4]. Upon oral administration, the most frequently reported side effects of flurbiprofen are abdominal discomfort along with other gastrointestinal effects. It has high log p (lipophilic character) value of 4.16. Also it has short elimination half-life of 3.9 h and requires frequent dosing [5]. Therefore transdermal route for administering flurbiprofen warrants investigation especially for the treatment of ailments like rheumatoid arthritis and its related disorders, which need prolonged therapy. Among several attempts to achieve this goal, transdermal preparations such as gels, ointments and creams have been intensively studied [6,7]. However, it was difficult to get an effective blood concentration by transdermal delivery of flurbiprofen due to its intrinsically poor skin permeability and solubility. Therefore it is indispensable to employ penetration enhancers to increase the skin permeation rate of flurbiprofen in order to maintain an effective blood level. One of the mechanism by which penetration enhancers increase skin permeability is to shift the solubility parameter of the skin in the direction of that of the permeant. The solubility of the permeant in the outer

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doi:10.1016/j.colsurfb.2008.05.001

layers of the skin will be increased and this in turn, improves the flux, simple solvent type molecules such as propylene glycol (PG), ethanol, transcutol® and *N*-methyl pyrollidone are thought to act in this way. For example, it is well Known that propylene glycol permeates the skin, therefore, it must be distributed in the stratum corneum [8].

The inherent barrier properties of the skin, and the fact that flurbiprofen is one of the least permeable drugs across skin among a series of lipophilic drugs [9] has inspired us to design the present study. We have reported the transdermal feasibility of flurbiprofen from a binary solvent mixture [10].

Alcohols have been found to affect skin permeability [11] and when used in combination with water increase the permeability of drugs through the skin [12,13]. Although PG:isopropyl alcohol (IPA) (30:70, v/v) binary combination provided the highest C_{max}, the desired steady state plasma concentration was still not achieved. These results led us to further investigate the transdermal delivery of flurbiprofen form PG:IPA (30:70, v/v) binary vehicle system containing varying quantities of penetration enhancers. We are reporting penetration enhancing capacity of tulsi oil (a fixed oil) obtained from Tulsi (*Ocimum sanctum*). Tulsi is a widely grown, sacred plant of India and it belongs to the labiateae family. It is used in ayurvedic system of medicine to treat various ailments. The extract of leaves is used to give relief in common cold, fever, bronchitis and cough.

Chemical penetration enhancers modify barrier properties of the stratum corneum and hence increase drug permeability across skin. Ideally, the effects of the penetration enhancer on the skin should be reversible, non-toxic, non-allergenic, compatible with drugs and excipients and non-irritating. Many enhancers exhibit these attributes, e.g. azone and its analogues [14], fatty acids [15,16], alcohols [17], pyrrolidones [18], sulfoxides [19], 6-*O*-ascorbic acid alkanoates [20], urea and its analogues and terpenes [21].

Due to their systemic and localized toxicity many effective permeation enhancers have not been explored yet [22]. Hence natural products have increasingly been used as enhancers due to their better safety profile. Terpenes are essential oils, which are used as fragrance, flavourings, and medicines. They have been found effective penetration enhancers for a number of hydrophilic and lipophilic drugs [23,24]. Terpenes are highly lipophilic due to their isoprene (C_5H_8) units [21]. They are generally recognized as safe (GRAS) by the FDA [25]. They increase the drug diffusivity in the stratum corneum for hydrophilic drugs and they enhance partitioning of drug into the stratum corneum for lipophilic drugs, besides causing increased diffusivity [26,27].

2. Materials and methods

2.1. Materials

Flurbiprofen and ibuprofen were kindly gifted by Sun Pharmaceutical Industries Ltd. (Mumbai); acetophenone was supplied by SD fine chemicals (Mumbai). Hydroxypropyl methylcellulose (HPMC) was gifted by Wings Pharmaceuticals (New Delhi). Turpentine and tulsi oil was purchased from Nice Pharmaceuticals (New Delhi). All other chemicals were commercially available and used as such.

2.2. In vitro skin permeation studies

A number of animal models have been reported for performing in vitro skin permeation studies, e.g. rat [28], rabbit [29], hairless mouse [28], shed snake skin [30] and human cadaver skin [31]. Although human cadaver skin may be the best choice, as a skin model for transdermal permeability experiments for the transdermal therapeutic systems to be used in humans, it is not easily available. Hence in the present study, the rat abdominal skin was used after removing the hair. The protocol for the preparation of whole skin was approved by Institutional Animal Ethical Care Committee. The protocol number 032/2002-3 was assigned to the study. Rats were sacrificed by excess ether inhalation. The abdominal skin of animal was shaved with the help of animal hair clipper and full thickness skin was surgically removed from each rat. The skin specimen was cut into appropriate size sections after carefully removing subcutaneous fat and washing with isotonic phosphate buffer (IPB) of pH 7.4, wrapped in aluminum foil and stored at −20 °C till it was used. In vitro skin permeation across rat skin was conducted with vertical Keshary Chien diffusion cell. The freshly excised full thickness skin was mounted on the diffusion cell with the stratum corneum side facing the donor compartment. The area of diffusion for all in vitro experiments was 8.86 cm^2 and the capacity of the receiver compartment was 40 ml. The skin was equilibrated for 6 h with the receptor medium. A blank sample of 1 ml was withdrawn from the receptor compartment and analyzed to ensure that the diffusion cells did not have any residual absorbance at 247 nm (due to interfering components from the skin). The buffer solution was replaced after every 30 min. The 6 h, sample showed no absorbance indicating the complete stabilization of the skin. The receptor solution (IPB of pH 7.4) was then introduced into the magnetically stirred receptor compartment maintained at 37 °C by an electric water bath. The donor compartment was maintained at the ambient temperature of 25 ± 2 °C. The donor compartment, which faced the stratum corneum surface, contained a 1% (w/v) solution (5 ml) of flurbiprofen in the vehicles with or without skin permeation enhancer (formulations N1–N12) and was covered with para film. Samples (1 ml) were withdrawn from receptor compartment for 24 h at regular intervals and analyzed for drug content by UV spectrophotometery method at 247 nm using IPB of pH 7.4 as blank. The receptor volume was immediately replaced with fresh receptor medium. Sampling port was covered with parafilm to prevent the evaporation of receptor medium.

2.3. Preparation of flurbiprofen formulation

A reservoir type transdermal delivery system of flurbiprofen was developed. Transdermal system of flurbiprofen comprised of 3 basic components; drug impermeable backing membrane, drug reservoir viscous solution consisting of flurbiprofen in PG:IPA (30:70, v/v) co-solvent system containing a skin permeation enhancer and thickening agent and a rate controlling membrane. The formulations were prepared by soaking method. Penetration enhancer and drug were solubilized in the co-solvent system at 25 °C by gentle stirring in magnetic stirrer. The influence of penetration enhancers on rheological property was insignificant. Then the polymer was added slowly with constant stirring and weight was made by addition of sufficient quantity of co-solvent system. The system was kept overnight for complete swelling of polymer. HPMC gel formulations with 1% (w/w) of HPMC were too thin whereas HPMC gel formulations with 3% (w/w) HPMC were too thick. HPMC (2%, w/w) gel formulations were found to possess optimum consistency and were easy to handle during the fabrication of the patch. The composition of the formulation is given in Table 1.

2.4. In vitro drug permeation studies of medicated viscous system

The formulations were subjected to in vitro permeation studies across the rat skin using Keshary Chien diffusion cells as mentioned in in vitro skin permeation studies section, except that 4 g flurbiprofen viscous system was used as donor phase in place of flurbiprofen

Table 1
Composition of viscous formulations of flurbiprofen

Ingredient	Amount in grams					
	A1	A2	A3	B1	B2	B3
Flurbiprofen	0.1	0.1	0.1	0.1	0.1	0.1
Methocel	0.04	0.08	0.12	0.04	0.08	0.12
Tulsi oil	0.25	0.25	0.25	–	–	–
Turpentine oil	–	–	–	0.25	0.25	0.25
PG:IPA[a] (30:70%, v/v) quantity sufficient	4	4	4	4	4	4

[a] Sufficient quantity was added such that the final weight was 4 g.

solution. The formulations were given codes as A1, A2, A3, B1, B2 and B3. Formulations A1, A2 and A3 contained tulsi oil as penetration enhancer and 1, 2 and 3% (w/w) HPMC, respectively. Similarly formulations containing turpentine oil B1, B2 and B3 were prepared with 1, 2 and 3% (w/w) HPMC, respectively. The receptor medium (0.2 ml) was withdrawn at different time intervals and replaced immediately with an equal volume of fresh IPB of pH 7.4. Samples were mixed with equal volume of internal standard, acetophenone, followed by dilution with 20 volumes of mobile phase. 20 μl of the resulting solution was injected into the HPLC system [32] after filtering through 0.45 μm membrane filter (Millipore).

2.5. Fabrication of the patch

Transdermal patches of flurbiprofen were fabricated by encapsulating the flurbiprofen reservoir viscous solution within a shallow compartment moulded from a drug impermeable backing and a rate controlling membrane. The ethylene vinyl acetate (EVA) (3M, USA) rate controlling membrane was placed over on the release liner fluropolymer coated polyester film (3M, USA) and then polyester film laminate (3M, USA) backing membrane was placed on it. The composite was heat sealed and cut to appropriate size. The flurbiprofen reservoir viscous solution was dispersed into the device using a syringe. The device was heat sealed again to close the unsealed side of the device ensuring no reservoir leaking out of the device. Then each patch was packed in laminated aluminum foil. In vitro permeation study of patch formulations were carried out using the same experimental set up as mentioned above except that a transdermal patch was placed in intimate contact with rat skin secured with the help of Micropore adhesive (3M, USA) which was placed over backing membrane. The samples were analyzed by HPLC.

2.6. Histological studies

Transdermal patch formulations were applied for 24 h on the excised skin mounted on the diffusion cell. The transdermal patch formulations were removed, the rat skin was wiped off with tissue paper and fixed with 10% (v/v) formalin solution in saline for at least 72 h before routine processing. The skin was sectioned vertically and each section was dehydrated and embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. All sections were then examined under a microscope (10×). Skin not treated with any formulation served as a control.

2.7. Pharmacokinetic studies

The study had been approved by local animal ethics committee (ICPCA). Project number 94 was assigned to the animal requisition form. Albino rats weighing between 250 ± 10 g were used for this study. The rats were lightly anesthetized with ether prior to dosing. The hair of the dorsal area was removed carefully with clipper. The transdermal patch was applied on the dorsal area of 9 cm^2 gently with the help of micropore adhesive. After the application of the patch, blood sample of 250 μl was collected at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post dose from the tail vein of the rat. The blood was collected in polypropylene tubes containing EDTA and centrifuged at 3000 rpm for 5 min to obtain plasma. The plasma was stored at −20 °C prior to analysis by HPLC method developed in our laboratory [33].

2.8. Pharmacodynamic studies (carrageenin paw edema inflammation model)

The topical anti-inflammatory efficacy was evaluated by measuring the change in paw volume with a Plethysmometer (Ugo Basile, Italy) using carrageenin induced inflammation edema model in rats weighing between 250 ± 10 g. Left hind paws of each rat were marked, just beyond tibiotarsal junction, so that every time the paw is dipped up to the fixed mark to ensure constant paw volume. Animals were divided into five groups, each group comprising of 4 rats, initial paw volume of each rat was noted. Formulations were applied on the dorsal area of 9 cm^2 gently with the help of micropore adhesive, 1 h prior to the carrageenin injection. The paw volume was noted at 0, 1, 2, 4, 6, 8 and 24 h. Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) carrageenin solution in the sub plantar region of the left hind paw 1 h after treatment with drug.

The percentage swelling in paw was calculated by the following formula.

$$\%\text{age swelling} = \frac{(b-a)\times 100}{a}$$

where a is the paw volume before producing the edema; b is the paw volume measured hourly after producing the edema.

The steady state flux was determined from the slope of the linear portion of a cumulative amount permeated versus time plot. The lag time (T_{lag}) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa. Permeability values were calculated by the following equation;

$$\text{Permeability} = \frac{J}{C_d}$$

where J is the steady state flux (μg/cm^2/h); C_d is the concentration in donor compartment.

Enhancement ratio of the flux (E_{pen}) can be expressed as;

$$E_{pen} = \frac{P_{treatment}}{P_{control}}$$

where $P_{treatment}$ is the flux of transdermal patch containing enhancer and $P_{control}$ is the flux of control group, i.e. transdermal patch without enhancer.

Statistical analysis was performed using t test and analysis of variance (ANOVA) followed by Students–Newman–Keuls multiple comparison tests.

Steady state plasma concentration was theoretically calculated by using following equation;

$$C_{ss} = \text{flux} \times \text{surface area of patch elimination rate constant} \times \text{volume of distribution.}$$

3. Result and discussion

3.1. In vitro preliminary skin permeation studies

In the present study, the effects of solvents isotonic phosphate buffer of pH 7.4, isopropyl alcohol and propylene glycol and their

Table 2
Permeation parameters of 1% flurbiprofen in various vehicles through excised rat skin

Formulation code	Solvent mixture	Flux (μg/cm^2/h)	Permeability coefficient (cm/h)	Lag time (h)	E_{pen}
N1 (control)	100:0[a]	32.6	5.8×10^{-3}	0.9	1
N2	70:30[a]	29.9	5.3×10^{-3}	1.1	0.9
N3	50:50[a]	37.7*	6.7×10^{-3}	2.2*	1.2
N4	30:70[a]	98.9*	0.0175	4.6*	3.0
N5 (control)	0:100[b]	15.3	2.7×10^{-3}	7.7	1
N6	70:30[b]	20.5*	3.6×10^{-3}	6.8*	1.3
N7	50:50[b]	41.3*	7.3×10^{-3}	3.9*	2.7
N8	30:70[b]	60.8*	0.0108	3.2*	4.0
N5 (control)	0:100[c]	15.3	2.7×10^{-3}	7.7	1
N9	100:0[c]	11.9	2.1×10^{-3}	1.6*	0.8
N10	70:30[c]	8.6	1.5×10^{-3}	1.4*	0.6
N11	50:50[c]	29.1*	5.2×10^{-3}	1.3*	1.9
N12	30:70[c]	11.1*	2.0×10^{-3}	2.5*	0.7

IPB: Isotonic phosphate buffer; IPA: isopropyl alcohol; PG: propylene glycol two-tailed *t* test was used to determine the significant difference in flux and lag time of flurbiprofen between control and treatment groups.

* $p < 0.05$.

[a] PG:IPA.

[b] PG:IPB.

[c] IPA:IPB.

binary combinations on the in vitro skin permeability of flurbiprofen was investigated in order to optimize the solvent system to develop a reservoir type of transdermal drug delivery system of flurbiprofen. The effect of different solvent systems on in vitro permeation of flurbiprofen through rat skin is shown in Table 2. The permeation rate of flurbiprofen through rat skin increased as the amount of IPA increased from 30 to 70% in PG:IPA binary solvent system, however significant ($p < 0.05$, *t* test) drop in permeation rate of flurbiprofen was observed when IPA was used alone (formulation N9). The results are summarized in Table 2. Dehydration of the skin by IPA causes significant loss of plasticity of keratins [34] and hence might have triggered this drop in drug flux.

The lag time of flurbiprofen in the PG/IPA binary combination ranged from 0.9 to 4.6 h. The lag time of formulation N4 was significantly higher than the control formulation N1. IPA enhanced the E_{pen} (enhancement ratio of the flurbiprofen skin permeation rate) of flurbiprofen (formulation N4) by 3.0 times. PG has been shown to enhance sorption by its co-solvency effect and by carrier mechanism [35]. This was confirmed by the significant ($p < 0.05$, *t* test) increase in flurbiprofen flux as the amount of PG increased from 0 to 30% with penetration enhancement of 4.0. At higher concentration, PG extracts water from the lipid bilayer of stratum corneum and thereby leading to an increase in the barrier property of stratum corneum [36]. Hence decrease in flux was observed on increasing the concentration of PG further. The lag time of flurbiprofen in the PG:IPB binary solvent mixture ranged from 0.9 to 7.7 h. The lag time of formulation N8 was significantly less than that in the purified IPB (T_{lag} of 7.7 h).

The increase in the penetration enhancement of 1.9 times was obtained when amount of IPA was increased from 0 to 50% in IPA:IPB solvent mixture. However a sharp decrease in the skin flux was observed when concentration of IPA was further increased. Also a significant decrease ($p < 0.05$, *t* test) in the lag time was observed with the addition of IPA in IPA:IPB binary solvent mixture. The increase in flux at lower concentration of IPA in IPA:IPB solvent mixture may be due to the fact that at lower concentration only lipoidal pathway gets affected. Flurbiprofen being a lipophilic compound, its flux increases at lower concentration of IPA. Among the three binary solvent mixtures, PG:IPA (30:70, v/v) showed the highest permeation rate of 98.9 μg/cm^2/h. The flux of formulation N4, N8 and N11 were compared by Students–Newman–Keuls Multiple comparison test. It was found that the flux of formulation N4 was significantly higher ($p < 0.05$) than N8 and N11 formulations. Thus N4 formulations (PG:IPA; 30:70, v/v) were selected for further studies.

3.2. Permeation of flurbiprofen from the PG:IPA (30:70, v/v, N4 formulations) solvent mixture containing turpentine and tulsi oil

Tulsi and turpentine oil where added to PG:IPA (30:70%, v/v) solvent mixture in different concentrations and the resultant formulations were named as: P1, P2, P3 and P4 containing 1, 3, 5, and 7% tulsi oil and P5, P6, P7 and P8 containing 1, 3, 5 and 7% turpentine oil respectively.

Co-application of terpenes in PG/water and PG/IPA solvent systems have synergistic action on drug flux [37] due to high diffusion and increased lipid disruption in the stratum corneum. As shown in Fig. 1, an additive effect on the skin permeation rate of flurbiprofen in binary solvent mixture was observed when tulsi and turpentine oil were added to the optimized co-solvent mixture, separately. Both the enhancers caused further enhancement of transdermal permeation rate when added to binary solvent mixture. The magnitude of the flux enhancement factor with tulsi and turpentine

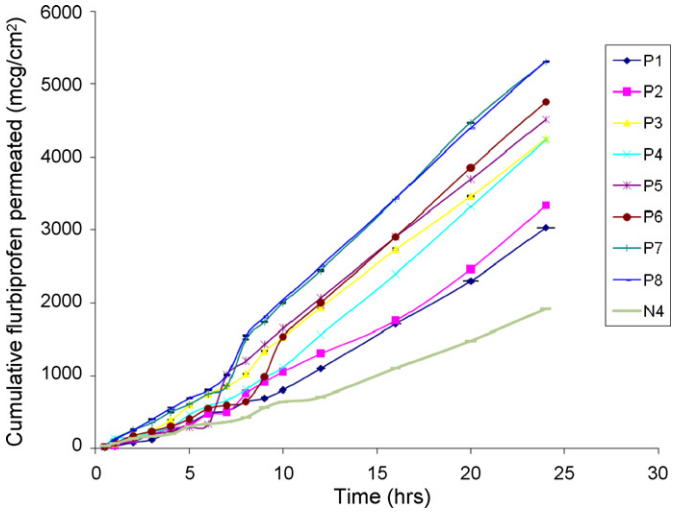

Fig. 1. Effect of tulsi and turpentine oil in PG:IPA (30:70%, v/v) solvent mixture on the in vitro permeation of flurbiprofen through rat skin. *Key*: (Curves P1–P4) 1, 3, 5, and 7% tulsi oil; (Curves P5–P8) 1, 3, 5, and 7% turpentine oil.

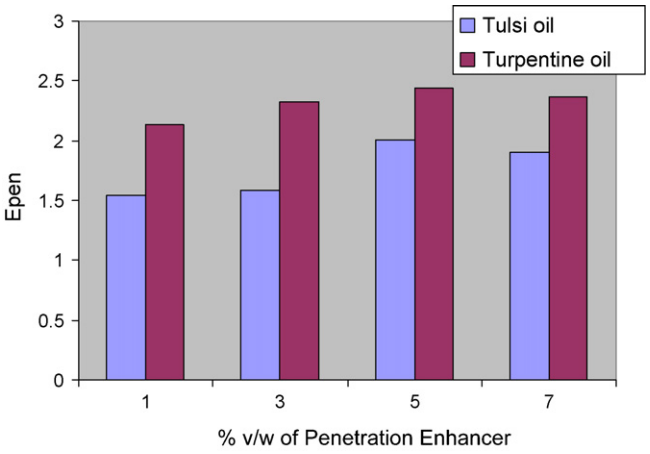

Fig. 2. Relationship between E_{pen} to the percent fraction of penetration enhancer in PG:IPA (30:70%, v/v).

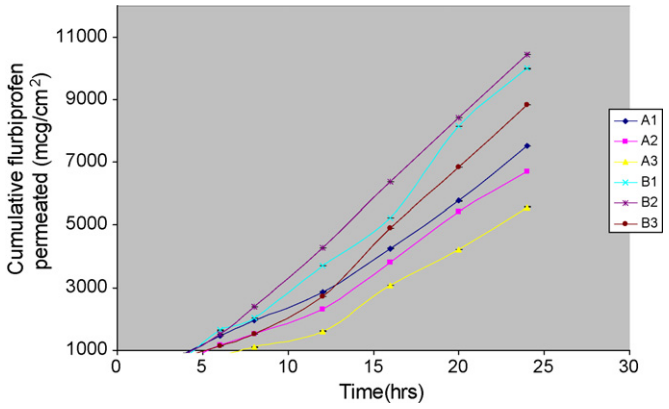

Fig. 3. Effect of HPMC in PG:IPA (30:70%, v/v) solvent mixture, containing 5% tulsi and turpentine oil, on the in vitro permeation of flurbiprofen through rat skin. *Key*: (Curves A1–A3) 1, 2 and 3% HPMC in PG:IPA (30:70%, v/v) containing tulsi oil; (Curves B1–B3) 1, 2 and 3% HPMC in PG:IPA (30:70%, v/v) containing turpentine oil.

oil was 2.0 and 2.4 at 5% (v/v) concentration, respectively, significantly ($p < 0.001$) higher than binary solvent mixture. At higher concentrations the effect on flux and lag time was insignificant ($p > 0.05$, Students–Newman–Keuls test). The corresponding lag time at 5% (v/v) concentration was 2.0 and 1.9 h, significantly ($p < 0.001$, Students–Newman–Keuls test) lower than the binary solvent vehicle.

The maximum transdermal permeation rate of 198.88 μg/cm^2/h was obtained with 5% tulsi oil in PG:IPA (30:70%, v/v) solvent mixture implying the ability of tulsi oil to modify the barrier properties of stratum corneum, leading to an increase in drug diffusion. Among the two different penetration enhancers, turpentine oil was significantly ($p < 0.001$, Students–Newman–Keuls test) most effective, increasing the flux from 98.88 to 241.37 μg/cm^2/h at a concentration of 5% with an E_{pen} of 2.4 (Fig. 2). Based on the above results, formulations P3 and P7 were selected for further studies.

3.3. Effect of thickening (gelling agent)

The target steady state concentration of flurbiprofen was calculated using the available pharmacokinetic data (C_{ss} −4 μg/ml, Cl = 0.35 ml/min/kg) assuming the surface area of transdermal device as 9 cm^2 and body weight of the patient as 60 kg. The steady state plasma concentration (theoretical) corresponding to the flux 198.88 and 241.37 μg/cm^2/h was 1.42 and 1.72 μg/ml for tulsi (P3) and turpentine oil (P7) formulations respectively. Since the achieved steady state plasma concentrations are less than the required steady state plasma concentration of flurbiprofen 3–5 μg/ml [2] the permeation rate of the drug needed further enhancement. In an ideal system there is a linear relationship between the rate of diffusion and the concentration of diffusant. The maximum flux occurs when the concentration of the diffusant reaches the solubility limit [38]. Thus drug load to be incorporated in transdermal patch was increased to 100 mg, intended to release drug for 24 h with an objective of achieving steady state drug blood plasma levels comparable to oral therapy with reduced number of application and improved bioavailability.

HPMC was used as thickening agent for ease of fabrication of the patch. HPMC has been used extensively as viscosity building agent in the design of reservoir systems for testosterone [39] and hydrocortisone [40]. Being less hydrophilic than methylcellulose, HPMC could be easily solubilized in PG:IPA (30:70%, v/v) solvent mixture. Various concentrations of HPMC were added to the optimized formulations P3 and P7. There was no significant difference in flux ($p > 0.05$ Students–Newman–Keuls test) of the flurbiprofen at 2 and 3% (w/w) HPMC in P7 formulations (Fig. 3). However there was a considerable difference in the cumulative percentage of drug permeated at two concentration levels of HPMC. At the end of 24 h, 88.61, 92.52 and 78.31% of the initial drug loading permeated from the turpentine formulations (P7) containing HPMC in 1, 2 and 3% concentration respectively. Lag time was increased significantly ($p < 0.01$, Students–Newman–Keuls test) at 3% (w/w) concentration. P7 formulations containing 2% (w/w) HPMC were easy to handle during the fabrication of the patch. The cumulative amount of flurbiprofen permeated across the rat skin from P3 formulations with 1, 2 and 3% HPMC over the period of 24 h was 7525.1, 6717.8 and 5570.2 μg/cm^2 respectively. The corresponding permeation rate of flurbiprofen was 362.3, 389.2 and 329.7 μg/cm^2/h respectively (Fig. 3). There was a significant ($p < 0.05$) drop in flux in formulations containing 3% (w/w) of HPMC as compared to formulation 2% (w/w). As mentioned in Section 2.3, the 3% (w/w) HPMC formulations were too thick due to increased viscosity which led to sharp decrease in transdermal flux of flurbiprofen.

3.4. In vitro skin permeation of flurbiprofen encapsulated within a shallow compartment moulded from a drug impermeable backing and a rate controlling membrane (patch formulation)

Two formulations containing 5% tulsi oil (A2) and 5% turpentine oil (B2) in the PG:IPA (30:70%, v/v) with 2% (w/w) HPMC were encapsulated in a shallow compartment moulded from a drug impermeable polyester backing film laminate and EVA rate controlling membrane. After incorporation into patch formulation, A2 and B2 were called T1 and T2 respectively.

The flux of flurbiprofen across EVA membrane/skin composite was found to be 323.6 and 523.2 μg/cm^2/h from formulations T1 and T2 respectively. The flux and lag time of the flurbiprofen was not significantly altered in turpentine patch formulations (T2) ($p > 0.05$, Students–Newman–Keuls test) as compared to viscous formulations (B2) (Fig. 4). In contrary the flux of Tulsi oil containing patch formulation (T1) was significantly lower than A2 formulation. The decrease in permeability rate might have occurred due to the low vinyl acetate content of the EVA membrane which may cause decreased water vapour permeability [38]. However there was no significant effect of EVA membrane on the transdermal permeation rate of flurbiprofen from T2 across the rat skin possibly due to the higher enhancing potential of turpentine oil. On storage significant portion of the drug migrates from the reservoir system to the membrane.

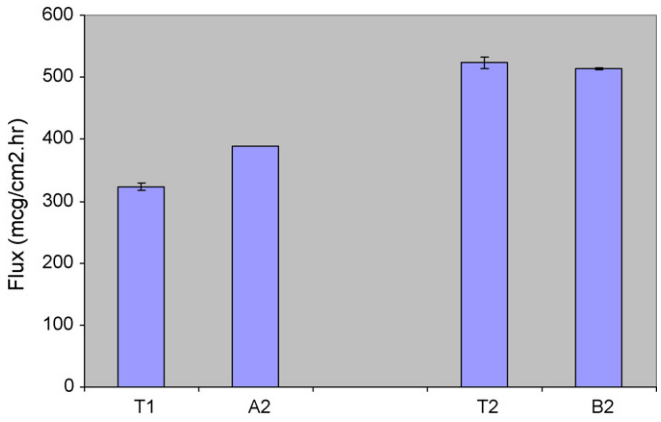

Fig. 4. Effect of EVA membrane on the in vitro permeation of flurbiprofen through rat skin.

In vitro permeation data were fitted to different equations and kinetic models to explain the release kinetics of flurbiprofen from patch formulations. The best fit with the highest coefficient of correlation $r = 0.9894$ and 0.9932 and coefficient of determination $r^2 = 0.9790$ and 0.9864 was shown by zero order drug release for tulsi oil (T1 formulation) and turpentine oil (T2 formulation) containing formulations respectively. The facts were further confirmed by the coefficient of variation, which were lesser for zero order rate constant.

3.5. Histological studies

The influence of penetration enhancer and solvents on the anatomical structure of the rat skin is discussed with the aid of light microscopic findings. Fig. 5a section of untreated control group shows normal rat skin with uniformly layered stratum corneum, a 2 cell thick epidermis and loosely textured collagen in dermis. No significant change in epidermal thickness was seen in biopsies from the skin section treated with PG:IPA (30:70, v/v), although the section shows a clear disruption of stratum corneum organization confirming the reported enhancing capacity of these solvents which accelerate the penetration of drug (Fig. 5b). The microscopic appearance of rat skin treated with 5% turpentine oil in PG:IPA (30:70, v/v) solution shows disruption of normal stratification, epidermis is not thickened. Dermis has a mildly increased number of inflammatory cells (Fig. 5c). Skin treated with 5% tulsi oil in PG:IPA (30:70%, v/v) shows extensive disruption of stratum corneum with condensation of the normally stratified corneal layers and a definite increase in epidermal thickness from normal 2–3 to 4–5 cells. Dermis does not show significant change (Fig. 5d).

3.6. Stability studies

Stability studies were carried out according to ICH guidelines [41] to establish the structural integrity of the reservoir formulation. The HPLC chromatograms showed neither any additional or interfering peaks nor any change in the retention time of flurbiprofen indicating that the drug is stable in the formulations. The studies revealed no significant changes in the physical appearance of the reservoir formulations. The degradation constant of transdermal turpentine and tulsi oil containing patch formulations was found to be 2.15×10^{-4} and 2.38×10^{-4} day^{-1} respectively (Fig. 6) and the corresponding amount of drug present after 3 months of accelerated stability studies was 98.1 and 97.6%. Since there was no significant change in drug content (less than 5%), a tentative shelf life of 24 months may be given to the formulations.

3.7. Pharmacokinetic and pharmacodynamic studies

The plasma concentration of flurbiprofen in albino rats following oral administration and transdermal application of flurbiprofen formulations is shown in Fig. 7. Transdermal patches of 9 cm^2 area were applied on the dorsal side of the rats. Patch formulation containing no enhancer acted as the control. The time to reach maximum was 8 h for all the formulations. As shown it Table 3,the C_{max} of the patch formulations T1 and T2 were 17.86 and 30.82 μg/ml respectively. The C_{max} of all the formulations was significantly ($p < 0.01$, ANOVA followed by Dunnet's t test) higher than the control patch formulation ($C_{max} = 13.08$). The C_{max} of the T1 and T2 was 1.37 and 2.36 times higher than the control patch formulation, respectively. The area under the plasma concentration time curve (AUC) of the patch formulations T1 and T2 was 1.28 and 1.87 times higher than the control patch formulation respectively.

The area under the plasma concentration time curve (AUC) decreased in the following order T2 ≫ T1 > control > oral. The pharmacokinetic parameters of flurbiprofen after the oral administration were significantly different from the parameters obtained after the transdermal application of patch formulations. After the oral administration of flurbiprofen the C_{max} of the drug reached within 1 h and a sharp decline was observed. The increase in the $AUC_{(0-\alpha)}$ of flurbiprofen after application of transdermal patch formulations was significantly higher than orally administered flurbiprofen, which indicates the improved bioavailability of flurbiprofen through transdermal drug delivery route. The bioavailability of flurbiprofen with reference to orally administered flurbiprofen was increased by 2.97, 3.80 and 5.56 times when transdermal patch formulations; plain patch formulation, T1 and T2 respectively, were applied. The results were consistent with in vitro skin permeation studies were T2 provided higher flux and cumulative percentage drug permeation.

Flurbiprofen plasma levels of greater than 5 μg/ml were achieved immediately and were maintained till the last sample. It has been previously reported that plasma levels of approximately 5 μg/ml were achieved in albino rats after 2 h of application of transdermal patch containing 2 mg of drug applied over 14 cm^2 area and were maintained till 10 h [42]. In other previously reported study flurbiprofen levels of 43.9 μg/ml were achieved in rats at 1.8 h after the application of 500 μl of 1% flurbiprofen containing 5% of oleic acid and 5% urea in propylene glycol, on the dorsal area of 9 cm^2 [9]. However the plasma levels of the drug dropped

Table 3
Mean (±S.D.) pharmacokinetic parameters of flurbiprofen after transdermal application and oral administration in albino rats

Formulation code	C_{max} (μg/ml)	T_{max}	$AUC_{(0-\alpha)}$	Relative bioavailability
Oral administration	26.26 ± 6.1	1.0	83.61 ± 28.2	–
Plain patch formulation (control)	13.08 ± 1.6	8.0	248.09 ± 13.8	2.97
T1	17.86 ± 0.4*	8.0	317.66 ± 12.6**	3.80
T2	30.82 ± 2.1**	8	464.98 ± 11.9**	5.56

*$p < 0.05$. **$p < 0.01$, ANOVA followed by Dunnet's t test was applied to compare T1 and T2 with control formulation.

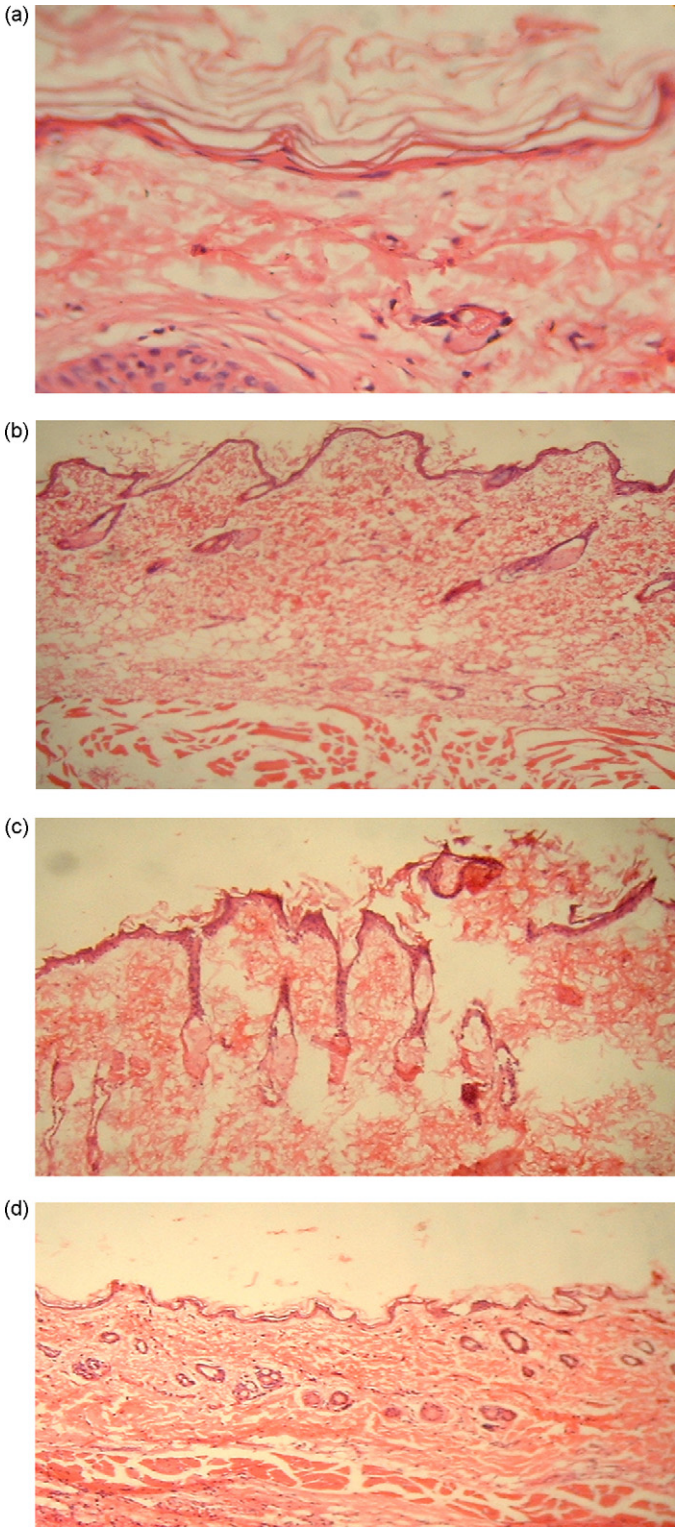

Fig. 5. Histological findings on skin biopsies from albino rats treated with (a) normal control, (b) PG:IPA (30:70, v/v), (c) 5% turpentine oil in PG:IPA (30:70, v/v) and (d) 5% tulsi oil in PG:IPA (30:70, v/v).

sharply and at 12 h less than 10 μg/ml of the drug was detected in the plasma. In present study, due to the application of skin penetration enhancers in binary solvent mixture, higher plasma levels of flurbiprofen were achieved with the application of transdermal patches.

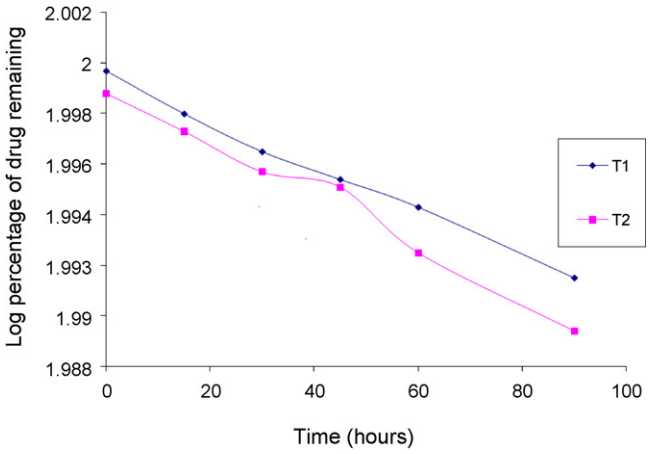

Fig. 6. Log percent of drug remaining versus time plot for optimized formulation kept at 40 °C/75%RH.

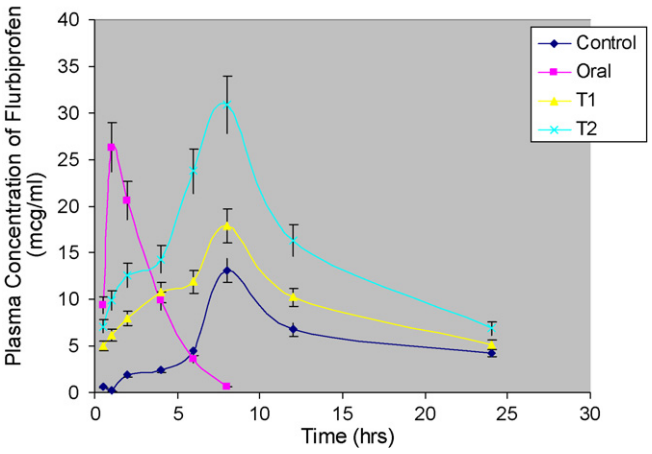

Fig. 7. Flurbiprofen plasma concentration time profile.

The tested formulations showed significantly improved anti-inflammatory activity as compared to the control formulation as shown in Fig. 8. Loganathan et al., reported 73.15% inhibition of edema with flurbiprofen gel formulations containing 1% (w/w) flurbiprofen with 15% DMSO [43]. In present study, edema was completely treated due to higher drug plasma levels.

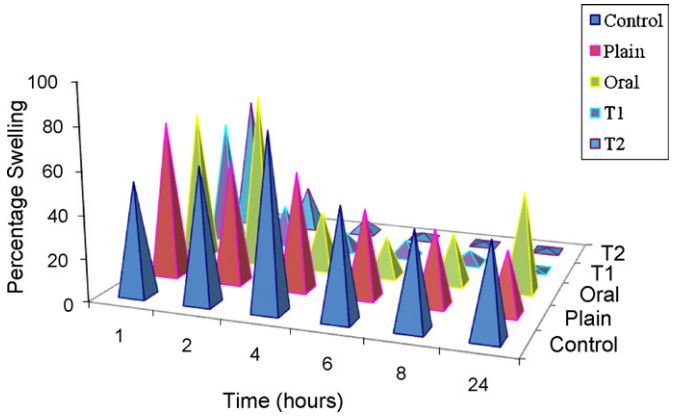

Fig. 8. Effect of flurbiprofen on the swelling edema in hind paw produced by carrageenin injection.

In conclusion, the turpentine oil showed superior absorption enhancing properties on rat skin as compared to the tulsi oil treated, solvent treated and normal control groups due to increased disruption of stratum corneum with negligible skin irritation. Glycols cause increased partitioning of drug into the stratum corneum and terpenes cause increased disruption of stratum corneum. This may be the primary reason for higher penetration enhancing behaviour shown by the formulations containing turpentine oil.

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